Committee for Risk Assessment

RAC

Annex 1

Background document

to the 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

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

Adopted

13 June 2019

CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

Substance Name:

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

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

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

Index Number: Not applicable

Contact details for dossier submitter: Danish Environmental Protection Agency

Version number: 4 **Date: May 2018**

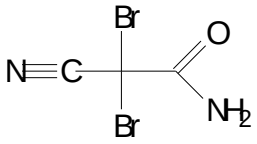
ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 2,2-DIBROMO-2-CYANOACETAMIDE; [DBNPA]

CONTENTS

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	*2,2-Dibromo-2-cyanoacetamide*
Other names (usual name, trade name, abbreviation)	Dibromonitrilopropionamide 2,2-Dibromo-3-nitrilopropionamide DBNPA
ISO common name (if available and appropriate)	*Not applicable*
EC number (if available and appropriate)	*233-539-7*
EC name (if available and appropriate)	*2,2-Dibromo-2-cyanoacetamide*
CAS number (if available)	*10222-01-2*
Other identity code (if available)	*Not applicable*
Molecular formula	$C_3H_2Br_2N_2O$
Structural formula	
SMILES notation (if available)	O=C(N)C(C#N)(Br)Br
Molecular weight or molecular weight range	*241.9 g/mol*
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	*Not applicable*
Description of the manufacturing process and identity of the source (for UVCB substances only)	*Not applicable*
Degree of purity (%) (if relevant for the entry in Annex VI)	*98.66 – 100% w/w*

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**
2,2-Dibromo-2-cyanoacetamide *EC: 233-539-7*	*98.66 – 100% w/w*	**No current CLH**	**Acute Tox. 3; H301** **Acute Tox. 2; H330** **Skin Irrit. 2; H315**

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
CAS: 10222-01-2			**Eye Dam. 1; H318 Skin Sens. 1B; H317 Aquatic Acute 1; H400 Aquatic Chronic 2; H411**

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
Confidential				

The impurities do not affect the classification of DBNPA. The impurities are considered confidential and are therefore not given in this report.

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
Not applicable					

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5: Proposed classification and labelling of DBNPA in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	International Chemical Identification	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	not available	2,2-Dibromo-2-cyanoacetamide; [DBNPA]	233-539-7	10222-01-2	Acute Tox. 2 Acute Tox. 3 Skin Irrit. 2 Eye Dam. 1 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 2	H330 H301 H315 H318 H317 H400 H411	GHS06 GHS09 GHS05 Dgr	H330 H301 H315 H318 H317 H410		inhalation: ATE = 0.275 mg/L (dust and mist) oral: ATE = 167 mg/kg bw M = 1	

Table 6: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	*data conclusive but not sufficient for classification*	Yes
Flammable gases (including chemically unstable gases)	*hazard class not applicable*	No
Oxidising gases	*hazard class not applicable*	No
Gases under pressure	*hazard class not applicable*	No
Flammable liquids	*hazard class not applicable*	No
Flammable solids	*data conclusive but not sufficient for classification*	Yes
Self-reactive substances	*data conclusive but not sufficient for classification*	Yes
Pyrophoric liquids	*hazard class not applicable*	No
Pyrophoric solids	*data conclusive but not sufficient for classification*	Yes
Self-heating substances	*data conclusive but not sufficient for classification*	Yes
Substances which in contact with water emit flammable gases	*data conclusive but not sufficient for classification*	Yes
Oxidising liquids	*hazard class not applicable*	No
Oxidising solids	*data conclusive but not sufficient for classification*	Yes
Organic peroxides	*hazard class not applicable*	No
Corrosive to metals	*data conclusive but not sufficient for classification*	Yes
Acute toxicity via oral route	*harmonised classification proposed*	Yes
Acute toxicity via dermal route	*data conclusive but not sufficient for classification*	Yes
Acute toxicity via inhalation route	*harmonised classification proposed*	Yes
Skin corrosion/irritation	*harmonised classification proposed*	Yes
Serious eye damage/eye irritation	*harmonised classification proposed*	Yes
Respiratory sensitisation	*No data*	No
Skin sensitisation	*harmonised classification proposed*	Yes
Germ cell mutagenicity	*data conclusive but not sufficient for classification*	Yes
Carcinogenicity	*data conclusive but not sufficient for classification*	Yes
Reproductive toxicity	*data conclusive but not sufficient for classification*	Yes
Specific target organ toxicity-single exposure	*data conclusive but not sufficient for classification*	Yes
Specific target organ toxicity-repeated exposure	*data conclusive but not sufficient for classification*	Yes

Hazard class	Reason for no classification	Within the scope of public consultation
Aspiration hazard	*hazard class not applicable*	No
Hazardous to the aquatic environment	*harmonised classification proposed*	Yes
Hazardous to the ozone layer	*data conclusive but not sufficient for classification*	Yes

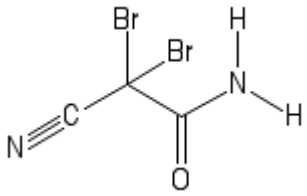
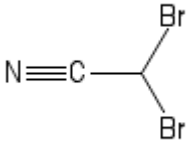
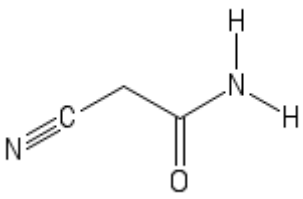
3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

No previous harmonised classification and labelling proposal submitted. This current proposal is submitted for the first time.

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**

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

The substance is an active substance in the meaning of Regulation (EU) No 528/2012 and shall normally be subject to harmonised classification and labelling, therefore a justification is not required (Article 36 CLP Regulation).

5 IDENTIFIED USES

The active substance is used in biocidal products for the the purpose of disinfection (PTs 2 and 4) and preservation (PTs 6, 11, 12 and 13).

6 DATA SOURCES

Draft Competent Authority Report (dCAR) for 2,2-dibromo-2-cyanoacetamide prepared under Regulation 528/2012.

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	crystalline solid at room temperature	[redacted] A3.1.1./03 Reliability 1	Visual inspection
Melting/freezing point	124.5 °C	[redacted] A3.1.1/03 Reliability 1	measured
Boiling point	Decomposition > 201 °C	[redacted] A3.1.1/02 Reliability 1	measured
Relative density	Bulk density: 1.356 g/cm^3 at 25 °C	[redacted] A3.1.1/02 Reliability 1	measured
Vapour pressure	1.19 x 10^{-3} Pa at 19.2 °C	[redacted] A3.2/01 Reliability 1	measured
Surface tension	72.2 ± 0.6 mN · m^{-1} at 25.0 ± 0.5 °C	[redacted] A3.1.1/03 Reliability 1	measured
Water solubility	10.8 (pH 5, 10 °C) 14.4 (pH 5, 20 °C) 20.2 (pH 5, 30 °C) 11.5 (pH 7, 10 °C) 14.1 (pH 7, 20 °C) 18.6 (pH 7, 30 °C) 19.9 (pH 9, 20 °C)	[redacted] A3.5/03 Reliability 1	measured
Partition coefficient n-octanol/water	pH 5: log Pow = 0.80 (Kow = 6.24) pH 7: log Pow = 0.80 (Kow = 6.31) pH 9: log Pow = 0.82 (Kow = 6.61) all at 20 – 21 °C	[redacted] A3.9/01 Reliability 1	measured
Flash point	Not applicable to solids	-	-
Flammability	Non-flammable	[redacted] A3.1.1/03 Reliability 1	measured

Property	Value	Reference	Comment (e.g. measured or estimated)
Explosive properties	Not explosive	[redacted] A3.1.1/02 Reliability 1	measured
Self-ignition temperature	The test substance did not ignite before melting.	[redacted] A3.1.1/03 Reliability 1	measured
Oxidising properties	Not oxidising based on structure	-	-
Granulometry	Sieve: 27.1% below 100 µm 16.7% below 53 µm Cascade Impactor: 0.818% below 10 µm 0.142% below 5.5 µm	[redacted] B3.12/02	measured
	Sieve: 17% below 100 µm 1.48% below 45 µm Cascade Impactor: 3.05% below 10 µm 0.754% below 5.5 µm	[redacted] B.3.12/03	measured
Stability in organic solvents and identity of relevant degradation products	Not applicable. Neither the active substance nor the biocidal product include an organic solvent.	-	-
Dissociation constant	spectrophotometric method: pKa = 8.3 ± 0.3 titrimetric method: pKa 8.24 ± 0.05	[redacted] A3.6/01 Reliability 2	measured
Viscosity	Not applicable to solids	-	-

8 EVALUATION OF PHYSICAL HAZARDS

8.1 Explosives

Table 8: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
DSC EPA OPP 63-16	DBNPA is not impact sensitive.	No hazardous physico-chemical properties identified	[redacted] A3.1.1/02

8.1.1 Short summary and overall relevance of the information provided on explosive properties

DBNPA was tested for explosive properties in accordance with a guideline GLP EPA OPP method.

8.1.2 Comparison with the CLP criteria

Predicted negative based on the result of the study for explosive properties.

8.1.3 Conclusion on classification and labelling for explosive properties

Based on the absence of an effect that implies explosive properties, classification of DBNPA in accordance with Regulation (EC) No 1272/2008 is not required.

8.2 Flammable gases (including chemically unstable gases)

Table 9: Summary table of studies on flammable gases (including chemically unstable gases)

Method	Results	Remarks	Reference
Not applicable as DBNPA is a solid			

8.2.1 Short summary and overall relevance of the provided information on flammable gases (including chemically unstable gases)

Not applicable as DBNPA is a solid.

8.2.2 Comparison with the CLP criteria

Not applicable as DBNPA is a solid.

8.2.3 Conclusion on classification and labelling for flammable gases

Not applicable as DBNPA is a solid.

8.3 Oxidising gases

Table 10: Summary table of studies on oxidising gases

Method	Results	Remarks	Reference
Not applicable as DBNPA is a solid			

8.3.1 Short summary and overall relevance of the provided information on oxidising gases

Not applicable as DBNPA is a solid.

8.3.2 Comparison with the CLP criteria

Not applicable as DBNPA is a solid.

8.3.3 Conclusion on classification and labelling for oxidising gases

Not applicable as DBNPA is a solid.

8.4 Gases under pressure

Table 11: Summary table of studies on gases under pressure

Method	Results	Remarks	Reference
Not applicable as DBNPA is a solid			

8.4.1 Short summary and overall relevance of the provided information on gases under pressure

Not applicable as DBNPA is a solid.

8.4.2 Comparison with the CLP criteria

Not applicable as DBNPA is a solid.

8.4.3 Conclusion on classification and labelling for gases under pressure

Not applicable as DBNPA is a solid.

8.5 Flammable liquids

Table 12: Summary table of studies on flammable liquids

Method	Results	Remarks	Reference
Not applicable as DBNPA is a solid			

8.5.1 Short summary and overall relevance of the provided information on flammable liquids

Not applicable as DBNPA is a solid.

8.5.2 Comparison with the CLP criteria

Not applicable as DBNPA is a solid.

8.5.3 Conclusion on classification and labelling for flammable liquids

Not applicable as DBNPA is a solid.

8.6 Flammable solids

Table 13: Summary table of studies on flammable solids

Method	Results	Remarks	Reference
EC method A.10	Not a flammable solid	No hazardous physico-chemical properties identified	[redacted] A3.1.1/03

8.6.1 Short summary and overall relevance of the provided information on flammable solids

Flammability (solids) was determined by measuring the burning rate of test material prepared as a pile of set dimensions. Testing was conducted using Method A.10 of Commission Directive 92/69/EEC (which constitutes Annex V of Council Directive 67/548/EEC).

8.6.2 Comparison with the CLP criteria

DBNPA was determined to not be a flammable solid as it did not propagate combustion.

8.6.3 Conclusion on classification and labelling for flammable solids

DBNPA does not require classification as a flammable solid under the terms of Regulation (EC) No 1272/2008.

8.7 Self-reactive substances

Table 14: Summary table of studies on self-reactivity

Method	Results	Remarks	Reference
Not applicable – chemical groups associated with explosive or self-reactive properties absent			
EC method A.16	DBNPA did not ignite before melting.	No hazardous physico-chemical properties identified	[redacted] A3.1.1/03

8.7.1 Short summary and overall relevance of the provided information on self-reactive substances

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 A6.2 of the UN Recommendations on Transport of Dangerous Goods, Manual of Tests and Criteria.

Testing for auto-flammability was conducted using Method A.16 of Commission Directive 92/69/EEC (which constitutes Annex V of Council Directive 67/548/EEC).

8.7.2 Comparison with the CLP criteria

CLP classification procedures for self-reactive substances need not be applied if there are no chemical groups present in the molecule that are associated with explosive or self-reactive properties as specified in Tables A6.1 and A6.2 of the UN Recommendations on Transport of Dangerous Goods, Manual of Tests and Criteria.

8.7.3 Conclusion on classification and labelling for self-reactive substances

DBNPA does not require classification as a self-reactive substance under the terms of Regulation (EC) No 1272/2008.

8.8 Pyrophoric liquids

Table 15: Summary table of studies on pyrophoric liquids

Method	Results	Remarks	Reference
Not applicable as DBNPA is a solid.			

8.8.1 Short summary and overall relevance of the provided information on pyrophoric liquids

Not applicable as DBNPA is a solid.

8.8.2 Comparison with the CLP criteria

Not applicable as DBNPA is a solid.

8.8.3 Conclusion on classification and labelling for pyrophoric liquids

Not applicable as DBNPA is a solid.

8.9 Pyrophoric solids

Table 16: Summary table of studies on pyrophoric solids

Method	Results	Remarks	Reference
Not applicable as DBNPA does not ignite spontaneously in air.			

8.9.1 Short summary and overall relevance of the provided information on pyrophoric solids

DBNPA does not possess self-heating properties. Experience in manufacturing or handling shows that DBNPA does not ignite spontaneously on coming into contact with air at normal temperatures (i.e. DBNPA is known to be stable at room temperature for prolonged periods of time (days)).

8.9.2 Comparison with the CLP criteria

No pyrophoric properties is expected of DBNPA on basis of the chemical structure. Furthermore, experience in manufacturing or handling shows that the substance does not ignite spontaneously on coming into contact with air at normal temperatures (i.e. the substance is known to be stable at room temperature for prolonged periods of time (days)).

8.9.3 Conclusion on classification and labelling for pyrophoric solids

DBNPA does not require classification as a pyrophoric solid under the terms of Regulation (EC) No 1272/2008.

8.10 Self-heating substances

Table 17: Summary table of studies on self-heating substances

Method	Results	Remarks	Reference
EC method A.16	DBNPA did not ignite before melting.	No hazardous physico-chemical properties identified	[redacted] A3.1.1/03

8.10.1 Short summary and overall relevance of the provided information on self-heating substances

Testing for auto-flammability was conducted using Method A.16 of Commission Directive 92/69/EEC (which constitutes Annex V of Council Directive 67/548/EEC) , which showed that DBNPA it is not an auto-flammable material.

8.10.2 Comparison with the CLP criteria

DBNPA is not flammable, explosive or oxidising. The molecular structure of DBNPA does not include any functional groups that indicate a hazard for self-heating. ECHA guidance on the application of CLP criteria states that the substance should not be considered for classification as self-

heating because the melting process is endothermic and the substance-air surface is drastically reduced. Furthermore, the melting point for the solid substance is < 160 ºC.

8.10.3 Conclusion on classification and labelling for self-heating substances

DBNPA does not require classification as a self-heating solid under the terms of Regulation (EC) No 1272/2008.

8.11 Substances which in contact with water emit flammable gases

Table 18: Summary table of studies on substances which in contact with water emit flammable gases

Method	Results	Remarks	Reference
Not applicable as DBNPA is known to form a stable mixture when dissolved in water			

8.11.1 Short summary and overall relevance of the provided information on substances which in contact with water emit flammable gases

DBNPA does not contain metals or metalloids: Experience in production or handling shows that DBNPA does not react with water, and is known to form stable aqueous solutions.

8.11.2 Comparison with the CLP criteria

The CLP classification procedure for substances which in contact with water emit flammable gases need not be applied because the chemical structure of DBNPA does not contain metals or metalloids, experience in production or handling shows reaction with water does not take place and DBNPA is known to form stable aqueous solutions.

8.11.3 Conclusion on classification and labelling for substances which in contact with water emit flammable gases

DBNPA does not require classification under the terms of Regulation (EC) No 1272/2008 as substance that will emit flammable gases when in contact with water.

8.12 Oxidising liquids

Table 19: Summary table of studies on oxidising liquids

Method	Results	Remarks	Reference
Not applicable as DBNPA is a solid			

8.12.1 Short summary and overall relevance of the provided information on oxidising liquids

Not applicable as DBNPA is a solid.

8.12.2 Comparison with the CLP criteria

Not applicable as DBNPA is a solid.

8.12.3 Conclusion on classification and labelling for oxidising liquids

Not applicable as DBNPA is a solid.

8.13 Oxidising solids

Table 20: Summary table of studies on oxidising solids

Method	Results	Remarks	Reference
Oxidizing properties EC Method A17	Predicted to be negative based upon structure	No hazardous physico-chemical properties identified	

8.13.1 Short summary and overall relevance of the provided information on oxidising solids

The structure of DBNPA was assessed for chemical groups that imply oxidising properties in accordance with Method A.17 of Commission Directive 92/69/EEC (which constitutes Annex V of Council Directive 67/548/EEC).

8.13.2 Comparison with the CLP criteria

Based on the chemical structure of DBNPA, the result for oxidising properties was predicted negative.

8.13.3 Conclusion on classification and labelling for oxidising solids

Based on the absence of chemical groups that imply oxidising properties, classification of DBNPA in accordance with Regulation (EC) No 1272/2008 is not required.

8.14 Organic peroxides

Table 21: Summary table of studies on organic peroxides

Method	Results	Remarks	Reference
Not applicable as DBNPA is not an organic peroxide			

8.14.1 Short summary and overall relevance of the provided information on organic peroxides

Not applicable as the organic peroxide bond grouping is not present in the chemical structure of DBNPA, hence DBNPA is not an organic peroxide.

8.14.2 Comparison with the CLP criteria

Not applicable as DBNPA is not an organic peroxide.

8.14.3 Conclusion on classification and labelling for organic peroxides

Not applicable as DBNPA is not an organic peroxide. Classification of DBNPA in accordance with Regulation (EC) No 1272/2008 is not required.

8.15 Corrosive to metals

Table 22: Summary table of studies on the hazard class corrosive to metals

Method	Results	Remarks	Reference
Not applicable as DBNPA is a solid that is not expected to become liquid during transport.			

8.15.1 Short summary and overall relevance of the provided information on the hazard class corrosive to metals

UN Transport Test C.1 is intended to determine the corrosive properties of liquids and solids that may become liquid during transport. However, DBNPA is a solid that is supplied dry and is therefore not expected to materially damage or destroy metals. No incidences of damage to metals have occurred during manufacture and use. DBNPA is a stable organic molecule with no functional groups that infer strongly acidic or basic properties, and it is considered not to be corrosive to metals.

8.15.2 Comparison with the CLP criteria

Dissolution of DBNPA in water in order to perform UN Transport Test C.1 is inappropriate because DBNPA is a solid that is supplied 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. Classification is therefore not required in the absence of a hazard.

8.15.3 Conclusion on classification and labelling for corrosive to metals

Based on the physical state of DBNPA (solid), classification as corrosive to metals under the terms of Regulation (EC) No 1272/2008 is not required.

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**.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 23: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
Absorption, metabolism and excretion of DBNPA Reliability = 2	After oral administration DBNPA is rapidly and efficiently absorbed and excreted. The vast majority is excreted within one day (>85% of radioactivity administered), almost exclusively in the urine. After the total collection time of 7 days the total recovery was 93% of radioactivity applied. The amount in faeces was 5.6%. It is thus assumed that DBNPA is completely bioavailable after oral administration (oral absorption 100%).	DBNPA is considered to be equally distributed over the blood circulation to all tissues and organs.	[redacted] A6.2/01
Absorption, metabolism and excretion of DBNPA Reliability = 2			[redacted] A6.2/02

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

The absorption, metabolism and excretion of DBNPA were investigated in rats ([redacted], A6.2/01; and [redacted], A6.2/02).After oral administration DBNPA is rapidly and efficiently absorbed and excreted. The vast majority is excreted within one day (>85% of radioactivity administered), almost exclusively in the urine. After the total collection time of 7 days the total recovery was 93% of radioactivity applied. The amount in faeces was 5.6%. It is thus assumed that DBNPA is completely bioavailable after oral administration (oral absorption 100%).
Based on the hydrophilic properties of DBNPA, there is no potential for bioaccumulation. DBNPA is considered to be equally distributed over the blood circulation to all tissues and organs.
It was shown that the excretion pattern for bromide and DBNPA related radioactivity was different indicating a breakage of the carbon-bromine-bond, most likely in a spontaneous, non-biological, non-enzymatic reaction.

In [redacted] A6.2/01, 2,2-Dibromo-3-nitrilopropionamide-2-^{14}C (DBNPA) was administered to male rats as a single oral dose of 12 mg/kg bw. Urine, feces and expired air were collected and analysed for ^{14}C radioactivity. The concentration of bromide in urine was also determined.

The results indicate that the ^{14}C-labeled DBNPA is rapidly and completely absorbed and excreted.

About 87 % of radioactivity was excreted within one day, almost exclusively in the urine. At the end of the 7 day sampling period 93 % of radioactivity were recovered. The amount of radioactivity in the feces was 5.6 % showing that the potential for bioaccumulation is limited.

The excretion pattern for bromide was different compared to the radioactivity: 25.0% of the bromide was excreted on the first day. Within 7 days 98.5% of the bromide given as DBNPA were excreted via urine. This finding indicates that the carbon-bromine bond is readily broken in vivo resulting in mono-brominated and un-brominated degradation products. Bromide is liberated in this spontaneous non-biological, non-enzymatic degradation.

In [redacted] A6.2/02, 6 rats (3 animals per sex) were treated via oral gavage with radiolabeled ^{14}C-DBNPA. The animals received a single dose of 12 mg/kg bw. Urine excreted during the first 24 hours after administration was sampled and analysed by thin layer chromatography, radioautography, mass spectroscopy, and liquid scintillation counting techniques for possible metabolites.After oral application of DBNPA cyanoacetamide is the major metabolite (77.6%) excreted in the urine. Cyanoacetic acid (2.1%) and oxalic acid (16.5%) were also found. No sex difference was observed.

The lack of brominated metabolites is probably due to a non-enzymatic degradation which was already suggested based on the results of the kinetic study (please refer to A6.2/01 [redacted]).

The study authors suggest that the metabolites identified in this study might be considered as successors of the “early” degradation products/metabolites monobromonitrilopropionamide, dibromoacetamide and dibromoacetic acid.

These products are also produced in a non-biological aqueous environment. Therefore, considering the different pH conditions in the stomach and intestine, the degradation of DBNPA *in vivo* may be nonenzymatic, and, thus , non-biological degradation products of DBNPA might occur in the animals.

The metabolism of DBNPA consists of enzymatic and non-enzymatic steps. The kinetic study suggests a break of the carbon-bromine bond.

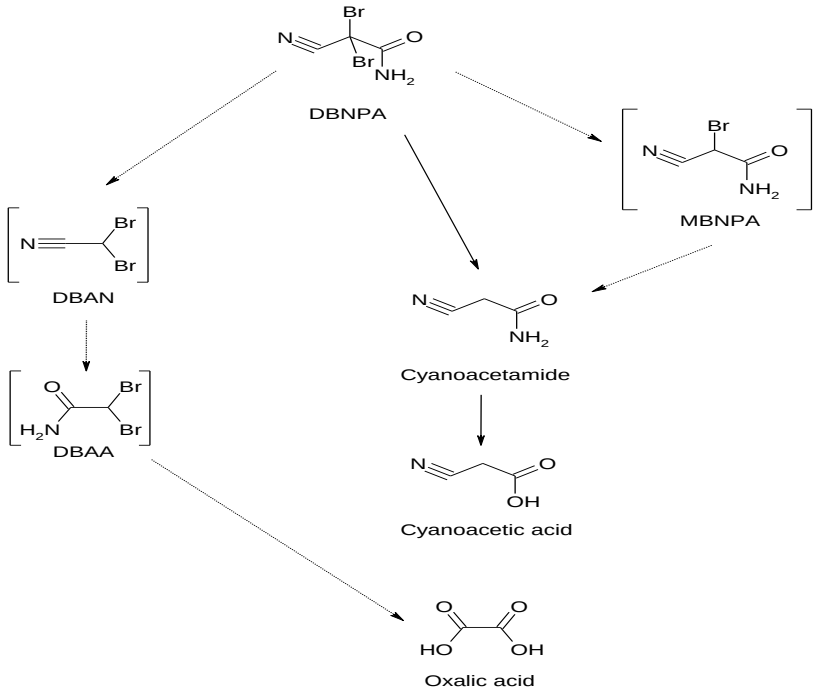
DBNPA is excreted exclusively as debrominated molecule, i.e. cyanoacetamide (~80 % of dose applied), cyanoacetic acid (~ 2 % of dose applied) and oxalic acid (~ 16 % of dose applied) as smallest molecule. No sex difference was observed.

The proposed metabolic pathway consists of two parts and is described in the following;

DBNPA is debrominated to the major metabolite Cyanoacetamide (eventually via monobromonitrilopropionamide, MBNPA). Subsequently the amide is hydrolysed to cyanoacetic acid which represents only a minor part of the radioactivity found. A further hydrolysis of the nitrile group to malonic acid was not observed. A conversion from malonic acid to oxalic acid is not likely as malonic acid is a stable molecule. In the case of an enzymatic decarboxylation, e.g. by malonyl-CoA decarboxylase the resulting acetyl-CoA would be incorporated into the carbon-pool of the organism and not oxidised to oxalic acid.
This means that the high amount of oxalic acid in the urine of rats dosed with DBNPA is due to an alternative pathway. This pathway leads via hydrolysis and decarboxylation of DBNPA to the oxalic acid. The intermediates dibromoacetonitrile (DBAN) and dibromoacetamide (DBAA) might have been formed.

Two metabolic pathways are present (Figure 9.1-1): The debromination and subsequent deamination of DBNPA lead to cyanoacetamide and cyanoacetic acid, accounting for 77.6, and 2.1% of radioactivity, respectively, whereas desamination and decarboxylation of the amide function result in oxalic acid (~16.5% of radioactivity). Malonic acid, dibromoacetonitrile (DBAN) and dibromoacetamide (DBAA) were not found in rat urine although the study authors suggested the formation of these molecules.

Figure 9.1-1 Proposed metabolic pathway of DBNPA



In the environment, cyanoacetamide, cyanoacetic acid and malonic acid were found to be the degradation products of a degradation pathway associated with reaction with nucleophiles rather than via hydrolysis. This degradation pathway is in compliance with the main metabolic route occurring in the rat. As these molecules account for about 80% of the radioactivity applied in the rat metabolism study, the toxicity of these molecules is considered to be covered by the studies conducted with the parent DBNPA.

A dermal penetration study is not available for DBNPA. Based on the almost complete oral bioavailability and the difference between $NOAEL_{systemic}$ obtained in oral and dermal studies, the dermal penetration rate is probably lower than 50%. However, a default penetration rate of 25 % is used for the human health risk assessments according to the EFSA guidance document on dermal absorption, as the representative product presented in doc IIB contains 20 % DBNPA.

Absorption via inhalation is assumed to be 100 % and equivalent to oral absorption.

For further details on the toxicokinetic studies, please refer to annex II to this report.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Table 24: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Reference
OECD 401, EPA 81-1 EPA TSCA Oral (gavage) Reliability = 1	Rat CD Male/female 5/group	DBNPA Constant application volume as required by OECD; additional dose group allows more reliable estimation of the LD50	133, 265, 529, 1056 mg/kg bw Single treatment	375 mg/kg bw for males 284 mg/kg bw for females 308 mg/kg bw for combined	[redacted] A6.1.1/01 ***Key study***
OECD 401 EPA 81-1 MAFF EEC B.1 Oral (gavage) Reliability = 1	Rat Fischer 344 Male/female 5/group	DBNPA Variable application volume in different dose groups	50, 100, 500 mg/kg bw Single treatment	224 mg/kg bw for males 167 mg/kg bw for females	[redacted] A6.1.1/02 ***Key study***
Non-guideline, non-GLP Oral Reliability = 3	Rat Female 2 / group Strain not reported	DBNPA 10% suspension in corn oil	126, 252, 500, 1000, 2000 mg/kg bw Single dose	Not reported, No animals died at lowest dose. All other died.	[redacted] A6.1.1/03
Non-guideline, non GLP Oral (gavage) Reliability = 3	Sherman rat Male/female 5/group. Hartley guinea pigs Female, 5/group. 2 groups dosed 126mg/kg bw	DBNPA 10% suspension in corn oil	Rat (male): 126, 252, 500 mg/kg bw Rat (female): 126, 252, 500 mg/kg bw Guinea Pig: 31, 63, 126, 126, 252, 500 mg/kg bw	235 mg/kg bw for male rats 178 mg/kg bw for female rats 118 mg/kg bw for female guinea pigs 118 mg/kg bw for rabbits	[redacted] A6.1.1/04

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Reference
	NZ white rabbit (mixed sex population) 5/group.		Rabbit: 63, 126, 252, 500mg/kg bw Single dose		
Non-guideline, non-GLP Oral (gavage) Reliability = 3	Rat Strain not reported Female 2/group	DBNPA –supply change sample 5% suspension in corn oil	63, 126, 252, 500, 1000 mg/kg bw Single dose	Reported: Acute oral lethality of the sample is the same as the previously tested.	[redacted] A6.1.1/05
Non-guideline, non-GLP Oral (gavage) Reliability = 3	Rat Sprague Dawley Female 5/group	DBNPA 10% suspension in water pH adjusted to 4.	126, 252 mg/kg bw Single dose	Reported as 177 mg/kg bw.	[redacted] A6.1.1/06

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

The acute oral toxicity of DBNPA has been investigated in a total of 6 studies, Two studies were selected as key-studies (A6.1.1/01 and A6.1.1/02[redacted]), due to the reliability scoring and level of detail in the reports.

The study A6.1.1/01 [redacted] (non-GLP) was performed to assess the acute oral toxicity of DBNPA to the rat (OECD guideline 401, 1983).

Charles River CD rats (5/sex/group),received a single oral dose of the DBNPA prepared in corn oil by gavage at doses of 133, 265, 529 or 1056 mg/kg bw. The test material was administered at a constant volume-dosage of 5 mL/kg bw.

Death occurred at the three highest dose levels between 30 minutes and 2 days of dosing.

Clinical signs were decreased motor activity and diarrhoea, hunching, pigmented stain of snout, ataxia and tremor. In surviving animals all clinical sign of reaction to treatment had resolved by the second day after dosing (Day 3).

Necropsy findings were mucoid or haemorrhagic intestinal content with mucosal congestion associated with erosions in the stomach, dark, stained spleen, petechial haemorrhages in the thymus, and oedematous and haemorrhagic lungs associated with presence of frothy fluid in the trachea and pale areas on liver lobes adjacent to stomach. In a few surviving rats of the low and intermediate dosage groups there were signs of gastric perforation associated with adhesion with the liver.

LD_{50} values of 375 and 284 mg/kg bw for males and females, respectively were observed (combined: 308 mg/kg bw). The inclusion of a fourth dose group increased the reliability of the results as indicated by the confidence intervals of 298-470 and 218-370 mg/kg bw for males and females, respectively.

The LD_{50} values were 375 and 284 mg/kg bw for males and females, respectively (combined: 308 mg/kg bw).

Table 25: Summary of results in acute oral toxicity study (A6.1.1/01 [redacted])

Dose [mg/kg bw]	Number of dead / number of investigated	Time of death (range)	Observations
133	0/5 (F) 0/5 (M)	-	Most animals showed no abnormalities, in single cases signs of decreased motor activity and hunching and signs of gastric perforation with adhesion with the liver
265	3/5 (F) 0/5 (M)	2 hrs – day 3	Decedent animals showed decreased motor activity, ataxia, tremor, hunching. surviving animals showed signs of gastric perforation associated with adhesion with the liver.
529	5/5 (F) 5/5 (M)	2 hrs – 6 hrs	Decedent animals showed decreased motor activity, Diarrhoea, pigmented stain of snout and Urogenital wetness. The gastro-intestinal contents were mucoid and haemorrhagic with mucosal congestion associated with erosions, dark stained spleen, petechial haemorrhages in the thymus, and oedematous and haemorrhagic lungs associated with presence of frothy fluid in the trachea and pale areas on liver lobes adjacent to stomach
1056	5/5 (F) 5/5 (M)	2 hrs – 6 hrs	Decedent animals showed decreased motor activity, Diarrhoea, pigmented stain of snout and signs of muzzle staining and salivation. The gastro-intestinal contents were mucoid and haemorrhagic with mucosal congestion associated with erosions, dark stained spleen, petechial haemorrhages in the thymus, and oedematous and haemorrhagic lungs associated with presence of frothy fluid in the trachea and pale areas on liver lobes adjacent to stomach.
LD_{50} value	males: 375 mg/kg bw females: 284 mg/kg bw males + females: 308 mg/kg bw		

The study A6.1.1/02 [redacted] was performed to assess the acute oral toxicity of DBNPA to the rat (OECD guideline 401, GLP compliant)

Fischer 344 rats(5/sex/group) received single oral doses by gavage of DBNPA at 50, 100 or 500 mg/kg bw as a 2.5 % suspension in 0.5 % Methocel in water. All rats administered 500 mg/kg bw died on test day one, and one female rat administered 100 mg/kg bw died by test day two.

Clinical signs were lacrimation, decreased activity, and urine soiling in the perineal area. Non-surviving animals also were observed with lateral recumbency.

The necropsy showed findings in the digestive tract and lungs.
The LD_{50} values were 224 and 167 mg/kg bw for males and females, respectively.

Table 26: Summary of results in acute oral toxicity study (A6.1.1/02[redacted])

Dose [mg/kg bw]	Number of dead / number of investigated	Time of death (range)	Observations
50	0/5 (F) 0/5 (M)	(F) no deaths (M) no deaths	-
100	1/5 (F) 0/5 (M)	(F) day 2 (M) no deaths	(F) decreased activity, laterally recumbent; Rats that died had watery contents in the digestive tract. Hemolyzed blood in the digestive tract was consistent with a stress-induced alteration. (M) decreased activity, lacrimation;
500	5/5 (F) 5/5 (M)	(F) day 1 (M) day 1 (within 2.5 hrs)	(F) + (M) deceased activity, lacrimation and laterally recumbent Rats that died had watery contents in the digestive tract. One dead rat had mottled lungs. Six rats had hemolyzed blood in the digestive tract, which was consistent with a stress-induced alteration.
LD_{50} value	Males: 224 mg/kg bw Females: 167 mg/kg bw		

In three other non key-studies a LD_{50} values was 235 mg/kg bw for males and 177-178 mg/kg bw for females.

For further details on the studies, please refer to annex II to this report.

10.1.2 Comparison with the CLP criteria

Classification for acute oral toxicity is required for substances that exhibit an LD_{50} for systemic effects at doses less than 2000 mg/kg bw. The lowest LD_{50} value reported is 167 mg/kg bw (females) and this result meets the criteria for Acute oral toxicity (Category 3). In addition, an Acute Toxicity Estimate (ATE) is proposed for the oral route to allow industry to classify and label mixtures properly. ATE value should be based on the LD_{50} and LC_{50} (See Table 3.1.1 and its Notes in the CLP Regulation) or, if not available, conversion values from Table 3.1.2. Based on the results of the acute oral toxicity studies, the proposed ATE value for DBNPA is 167 mg/kg bw.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

DBNPA is classified as Acute Tox. 3; H301: Toxic if swallowed according to the criteria given in Regulation (EC) No 1272/2008 and subsequent amendments to that legislation with an ATE (oral) of 167 mg/kg bw.

10.2 Acute toxicity - dermal route

Table 27: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Value LD_{50}**	**Reference**
OECD 402 EPA 81-1 MAFF	Rabbit New Zealand White	DBNPA (98.2% purity)	Limit test: 2000 mg/kg bw 24 h, semi-occlusive	> 2000 mg/kg bw No systemic toxicity observed	A6.1.2/01 ***Key study***

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Value LD_{50}	Reference
EEC B.1 (Dermal) GLP Reliabilty = 1	Male/female 5/group	10% DPNPA in 0.5 % Methocel® in water			
OECD 402 EPA Subpart F, 1981 Reliability = 2	Rabbit Albino Male/female 5/group	DBNPA Dry substance	2000mg/kg bw 24hr semi-occlusive	> 2000 mg/kg bw No systemic toxicity observed	A6.1.2/02

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

The acute dermal toxicity of DBNPA has been investigated in 2 studies, One study was selected as key-study ([redacted] A6.1.2/01), due to the reliability scoring and level of detail in the report.

This study was performed to assess the acute dermal toxicity of DBNPA to rabbit (OECD guideline 402,GLP). Groups of 5 male and female rabbits received a single dermal application of DBNPA at 2000mg/kg bw.

A dose of 2000 mg/kg bw was tolerated by male and female rabbits without clinical signs indicative of systemic toxicity. Erythema and edema were observed on all animals, immediately after test material removal. Burns were observed on all animals, except two immediately after test material removal. The edema was resolved in all animals by test day fifteen. Erythema persisted on eight of the rabbits, and burns persisted on four of the rabbits through the remainder of the study. Eight of ten rabbits had scabs noted at the application site beginning at either test day eight or nine, which persisted through the remainder of the study on four rabbits. Scaling was observed at the application site on two female rabbits beginning either test day seven or eight and persisted through the remainder of the study. Fissures were observed on two male rabbits from test day thirteen through the remainder of the study. At necropsy, six of ten rabbits were observed with scales at the application site and all rabbits were observed with a pale yellow discoloration on the fur at the application site. One male rabbit had an abrasion noted at necropsy.

The LD_{50} was greater than 2000 mg/kg bw.

Table 28: Summary of results in acute dermal toxicity study (A6.1.2/01[redacted])

Dose [2000 mg/kg bw]	Number of dead / number of investigated	Time of death (range)	*Observations*
2000 (male)	0/5	-	Erythema (duration: 2d - 15d), edema (duration: 2d – 14d) and burns (duration: 2d – 15d) were observed immediately after test material removal. Formation of scabs on day 8, which persisted till day 15. Fissures were observed from day 13.

2000 (female)	0/5	-	Erythema (duration: 2d - 15d), edema (duration: 2d – 12d) and burns (duration: 2d – 15d) were observed immediately after test material removal. Formation of scabs on day 8, which persisted till day 15. Scaling was observed, beginning at day 7.
LD_{50} value	> 2000 mg/kg bw		

10.2.2 For further details on the studies, please refer to annex II to this report. Comparison with the CLP criteria

Classification for acute dermal toxicity is required for substance that exhibits a LD_{50} for systemic effects at doses less than 2000 mg/kg bw. The LD_{50} in the rabbit is > 2000 mg/kg bw and does not meet the criteria for classification. No ATE value is proposed to classify and label mixtures containing DBNPA.

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

Classification for acute dermal toxicity is not proposed.

10.3 Acute toxicity - inhalation route

Table 29: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance, , form and particle size (MMAD)**	**Dose levels, duration of exposure**	**Value** **LC_{50}**	**Reference**
OECD 403 EPA 81-3 MAFF EEC B.2 (Inhalation) GLP Reliability = 1	Rat Fischer 344 Male/female 5/group	DBNPA (98.3%)	0.10, 0.37, 0.68 mg/L 4 hr	0.31 mg/L for males 0.24 mg/L for females 0.275 mg/L combined	[redacted] A6.1.3/01 ***Key study***
OECD 403 GLP Reliability = 2	Rat Sprague Dawley Male/female 5/group	DBNPA (98%) Respirable dust,	Mean analytical concentration - 0.088, 0.041, 0.42mg/L Whole body, 1hr	0.13 mg/L for both sexes	[redacted] A6.1.3/02

10.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

The acute inhalation toxicity was investigated in two studies. The study A6.1.3/01 [redacted] was performed to assess the acute toxicity of DBNPA via the inhalation route (OECD guideline 403). Fisher F344 rats (5/sex/group) were treated with DBNPA at dosages of 0.10, 0.37 and 0.68 mg/L over a period of 4 hours followed by an observation period of 14 days post-exposure.

All rats died during or following the exposure to 0.68 mg/L DBNPA, three male and four female rats died from exposure to 0.37 mg/L and all rats survived the exposure to 0.10 mg/L.

Labored breathing and deep respiration persisted for up to six days post-exposure. This was associated with noisy respiration of nasal origin, clear nasal discharge, sneezing and decreased activity in most surviving rats for up to a week after exposure.

Necropsy findings were generalized visceral congestion while some rats had pulmonary congestion and edema and clear fluid in the trachea. In addition, most rats that died from the 0.68 mg/L exposure group had treatment-related congestion/hyperemia of the nasal tissues. Some rats from the 0.37 mg/L exposure level had erosions and/or ulcers of the glandular mucosa of the stomach and gas in the digestive tract or stomach, likely due to reduced feed consumption and mouth breathing, respectively. Furthermore, dead rats showed wet and soiled haircoats, facial soiling with porphyrin-colored secretions and perineal soiling.

At necropsy, there were no treatment-related effects associated with exposure to technical grade DBNPA in any of the surviving rats.

The administration of DBNPA by inhalation according to provisions of OECD 403 guideline with respirable particles resulted in an LC_{50} of 0.31 and 0.24 mg/L for males and females, respectively (combined: 0.275 mg/L).

Table 30: Summary results of acute inhalation toxicity study (A6.1.3/01 [redacted]

Dose [mg/L]	Sex	Number of dead/number of investigated	Time of death	Clinical observations (number of animals per group)
0.10	male	0		During exposure: labored breathing (5/5) Post-exposure: respiration noisy (5/5), mouth breathing (5/5), decreased activity (3/5), Sneeze (4/5) Pathology: none
0.10	female	0		none
0.37	male	3/5	day 1 (2 animals during 4 hrs exposure) day 2 (1animal post-exposure)	During exposure: labored breathing/mouth breathing (3/5) Post-exposure: respiration noisy (3/5), mouth breathing (3/5), decreased activity (2/5), facial soiling (1/5), sneeze (1/5), Pathology: gas in the stomach (1/5),soiled haircoat (2/5), visceral congestion (3/5), Lungs with edema (1/5) and fail to collapse (1/5), decreased size of testes (1/5), trachea with clear, frothy fluid (3/5)
0.37	female	4/5	day 1 (2 animals during 4 hrs exposure, 2 animals post-exposure)	During exposure: labored breathing/mouth breathing (3/5) Post-exposure: respiration noisy (1/5), mouth breathing (1/5) Pathology: Gas in the digestive tract (1/5), erosions and/or ulcers of the glandular mucosa of the stomach (1/5), perineal soiling (1/5), soiled haircoat (3/5), visceral congestion (4/5), trachea with clear, frothy fluid (4/5)
0.68	male	5/5	day 1 (4 animals during 4 hrs exposure; day 2 (1 animal post-exposure)	During exposure: labored breathing/mouth breathing (4/5) Post-exposure: incoordinated (1/5), respiration noisy (1/5), decreased activity (1/5), mouth breathing (1/5); Pathology: visceral congestion (4/5), congestion of the nasal tissues (3/5), Lung with edema (1/5) and dark colour (1/5), Hemorrhage (1/5), facial soiling (3/5), perineal soiling (3/5), wet haircoat (4/5)
0.68	female	5/5	day 1 (5 animals during 4 hrs exposure)	During exposure: labored breathing/mouth breathing (4/5) Pathology: visceral congestion (5/5), congestion of the nasal tissues (3/5), hyperemia (2/5), facial soiling (4/5), perineal soiling (4/5), wet haircoat (4/5)
LC_{50} value	Male: 0.31 mg/L Female: 0.24 mg/L			

10.3.2 For further details on the studies, please refer to annex II to this report. Comparison with the CLP criteria

Classification for acute inhalation toxicity is required for substance that exhibits a LC_{50} for systemic effects at doses less than 5.0 mg/L for dust/mist. The LC_{50} in the rat is 0.275 mg/L for males and females and meets the criteria for Acute inhalation toxicity (Category 2). In addition, an ATE is proposed for the inhalation route to allow industry to classify and label mixtures properly. Based on the results of the acute inhalation toxicity studies, the proposed ATE (inhalation) for DBNPA is 0.275 mg/L (dust and mist).

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

DBNPA is classified with Acute Tox. 2; H330: Fatal if inhaled according to the criteria given in Regulation (EC) No 1272/2008 with an ATE (inhalation) of 0.275 mg/L (dust and mist).

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 in 2 studies, one of which (A6.1.2/01) was selected as the key study. As there was no mortality in this study at the limit dose of 2000 mg/kg bw, the DS proposed no classification.

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 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	Rat: m: 235 mg/kg bw f: 178 mg/kg bw Guinea pig: f: 118 mg/kg bw Rabbit: m+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; 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).**

10.4 Skin corrosion/irritation

Table 31: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
OECD 404 EPA 81-5 EEC B.4GLP Reliability = 1	Rabbit 3/sex	DBNPA 98.2%	500 mg administered /patch Exposure period 4 hours 15 day observation period	Average erythema score (24, 48, 72 h) = 3.3 Average edema score (24, 48, 72 h) = 2.1	[redacted] A6.1.4/02 ***Key study***
Non-guideline Non-GLP Reliability = 3	Rabbit Strain, sex and no/group not reported	DBNPA Dry/wet substance or 10% solution in Dowanol DPM	Repeated application 1-10 times on abraded or intact skin Single application 15min - 4.4 hr. Dose not reported	Repeated applications –Slight hyperemia, edema and necrosis followed by scabs and healing with slight scar. Single application – no reation to moderate hypermia, slight edema and slight necrosis, depending on exposure duration.	[redacted] A6.1.4/04b
Non-guideline Non-GLP Reliability = 3	Albino rabbits, 3/group	DBNPA	1-2 g occlusive Intact skin: 5 d/ week, for 2 weeks Abraded skin: 3d exposure duration	Intact skin: Moderate etrythema end slight edema after 1 application. Prolonged exposur – sligt or moderate necrosis, which healed slowly with a slight or moderate scab. 1 rabbit formed a scar the other 2 no scar. Abraded skin: Moderate erythema, edema and necrosis, whereafter the test was stopped. Subsequently they all formed	[redacted] A6.1.4/05b

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
				moderate scars.	
Non-guideline, Non –GLP Reliability = 3	White rabbits 1/group	DBNPA Wetted paches	1-5 applications to intact skin 1-3 applications to abraded skin Single application: 1hr-1,5hrs	Moderate hyperemia, moderate edema and slight necrosis followed by questionable scab and no to moderate scarring. Single application produced no scarring.	[redacted] A6.1.4/06b
OECD 404 EPA 81-5 EPA TSCA Reliability = 2	Albino rabbits Number and sex not reported	DBNPA, slightly moistened	Single dermal application of 0.5g, semi occusive 4hr duration	Draize scores: Erythema 2 or 3, accompanied by oedema 1-3. Five days after exposure, exfoliation was observed on all rabbits.	[redacted] A6.1.4/03

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

Skin irritation was investigated in five studies. The study A6.1.4/02[redacted] was performed to assess acute dermal irritation effects of DBNPA in rabbits (OECD guideline 404).

3 animals per sex received a single application of the test substance (500 mg) to a shaved skin area.

DBNPA produced slight to severe erythema and oedema in all rabbits within 30 minutes of test material removal. The erythema was resolved in all but one rabbit, by the end of the study. Oedema was resolved on all animals by test day eight. Blisters were observed at the application site on five of the six rabbit and persisted through test day seven. Scaling was observed on two animals on test day eight. One of these rabbits had scaling through test day fourteen. Scabs were noted at the application site on four of the six rabbits beginning either at test day eight or nine and persisting on one rabbit through the remainder of the study.

Table 32: Average scores for skin irritation study (A6.1.4/02[redacted])

Score (average animals investigated)	time	Erythema	Edema
Average score Draize scores (0 to maximum 4)	30 min	3.7	3
	24 h	3.5	2.3
	48 h	3.3	2.2
	72 h	3.2	1.8
Average score	24 h, 48 h, 72 h	3.3	2.1

Other times	d 7 d 8 d 9 d 10 d 11 d 14 d 15	2.7 2.7 2.7 2.3 2.3 0.5 0.3	1.5 0 0 0 0 0 0
Reversibility: *		c (except for one animal)	c
Average time for reversibility (days)		11.4	6.8
* c : completely reversible			

Table 33: Individual scores for skin irritation study (A6.1.4/02[redacted])

Animal no.	Degrees of erythema after:						Degrees of oedema after:						Ø 24/48/72h ≥ 2.3?	
	0.5h	24h	48h	72h	7d	15d	0.5h	24h	48h	72h	7d	15d	Erythema	Oedema
1	2	4*	4*	4*	2#	0	3	3	2	2	1	0	Yes	Yes
2	4	4	4*	4*	3*	0	3	3	3	2	2	0	Yes	Yes
3	4	4*	4*	4*	4*	0	3	3	3	2	2	0	Yes	Yes
4	4	3*	3*	3*	3*	0	3	2	2	2	2	0	Yes	No
5	4*	2	1	0	0	0	3	0	0	0	0	0	No	No
6	4	4*	4*	4*	4*	1Δ	3	3	3	3	2	0	Yes	Yes

*Blisters at application site, #Scaling at application site, Δ Scabs at application site

A Health and Safety report A6.12.2 [redacted] (presented in more detail in section 10.7 Skin sensitization), following accidential dermal worker exposure to a solution 20% DBNPA, showed erythema muliform-like lesions, which appeared as subepidermal blisters associated with many necrotic or apoptotic keratinocytes and dense lymphocytic infiltration in the epidermis. Most of the infiltrating cells in the epidermis were CD8-positive T lymphocytes expressing cytotoxic molecules. The reaction is likely linked to the individuals being sensitized to DBNPA, The indivudals recovered after treatment with cyclosporine.

10.4.2 For further details on the studies, please refer to annex II to this report. Comparison with the CLP criteria

Classification for skin irritation is required for a substance where there is a mean score of ≥ 2.3 for erythema or edema in at least four out of six animals at 24, 48 and 72 hours after patch removal. There was no report of necrosis or corrosive responses to justiy classification for corrosion, however the results meet the criteria for classification under Skin irritation (Category 2).The accident report supports the classification of Skin irritation (category 2), which due to the high concentration and the sensitizing potential of DBNPA, was very severe.

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

DBNPA is classified as Skin Irrit. 2; H315: Causes skin irritation according to the criteria given in Regulation (EC) No 1272/2008.

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**.

10.5 Serious eye damage/eye irritation

Table 34: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results - Observations and time point of onset - Mean scores/animal - Reversibility	Reference
OECD 405 EPA Reliability = 1	Rabbit 3 animals /sex	DBNPA	100 mg DBNPA instilled Exposure period 1 h	One hour after instillation of the test material, eyes were monitored. DBNPA resulted in opacity, discharge and chemosis of the highest grades of severity and conjunctival ulceration. Due to these findings, animals were killed humanely	[redacted] A6.1.4/01 ***Key study***
Non-guideline, Non –GLP Reliability = 3	Rabbit 1 female	DBNPA	Undiluted, dose not reported	Moderate conjunctival redness and severe corneal injury, which resulted in extensive impairment and possible loss of vision.	[redacted] A6.1.4/04a
Non-guideline, Non –GLP Reliability = 3	3 albino rabbits	DBNPA	0.1g 30 seconds exposure, followed by 2 min wash in one treated eye.	Initially: Moderate degree of pain and slight inflammationof the conjunctival membranes. 1 hour post application: Severe inflammation of the membranes, slight to moderate corneal injury. The inflammation increased for the remaining 7 observation days. Washing did not result in a significant lessening of eye irritation.	[redacted] A6.1.4/05a
Non-guideline, Non –GLP Reliability = 3	1 animal	DBNPA	30 seconds exposure, followed by 2 min wash, one eye not washed	Severe pain, severe conjunctival inflammation, and severe corneal injury.	[redacted] A6.1.4/06a

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

Eye irritation was investigated in four studies. In the study A6.1.4/01 [redacted] acute toxicity (eye irritation) was investigated for DBNPA. DBNPA (100 mg) was instilled into the right eye of six healthy rabbits (male, female). Ocular irritation was evaluated by the method of Draize.

Instillation of 100 mg DBNPA to the eye resulted in opacity, discharge and chemosis of the highest grades of severity and conjunctival ulceration at the examination one hour after instillation of the test material. Due to these findings, the rabbits were killed humanely.

DBNPA produced severe and irreversible eye effects.

Table 35: Average scores for eye irritation study (A6.1.4/01 [redacted])

	Cornea	**Iris**	**Conjunctiva**	
			redness	**chemosis**
Score (average of animals investigated)	0 to 4	0 to 2	0 to 3	0 to4
60 min	4	0	3	4
Area effected	¾ to whole eye			
Reversibility*	n	n	n	n
*n :not reversible, the reversibility of the reaction was not assessed properly due to the euthenation of the test animals of humane reasons.				

Table 36: Individual scores for eye irritation study (A6.1.4/01 [redacted])

Region of eye	**Response**	**Grade of response at time after application after 1 hour in the individual animals**					
		Animal 1	**Animal 2**	**Animal 3**	**Animal 4**	**Animal 5**	**Animal 6**
Cornea	Opacity	4	4	4	4	4	4
	Area	4	4	4	4	4	4
	Ulcaration	-	-	-	-	-	-
	Stippling	-	-	-	-	-	-
Iris	Value	0	0	0	0	0	0
Conjuctiva	Redness	3	3	3	3	3	3
	Chemosis	4	4	4	4	4	4
	Discharge	3	3	3	3	3	3
	Necrosis	-	-	-	-	-	-
	Ulceration	*	*	*	*	*	*

*Present

For further details on the studies, please refer to annex II to this report.

10.5.2 Comparison with the CLP criteria

Classification for serious eye damage is required for a substance when there is a mean score of ≥ 3 for corneal opacity and/or ≥ 1.5 for iritis in at least four out of six animals at 24, 48 and 72 hours after instillation of test material and/or effects on the cornea, iris or conjunctiva are not reversible within 21 days. The classification can also be considered if the pH ≤ 2 or ≥ 11. The pKa value of DBNPA is 8.24 + 0.05, and thus is not expected to contribute to the eye-damaging effects at physiological pH (7.4).

The mean corneal score was 4 for all six animals and the severity of the reactions caused the animals to be humanely killed one hour after instillation of the test material. The reversibity was not assessed, but not expected, due to the reportedly obvious corrosive effect of the test material. The substance therefore meets the criteria for Irreversible effects on the eye (Category 1).

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

DBNPA is classified as Eye Dam. 1; H318: Causes serious eye damage according to the criteria given in Regulation (EC) No 1272/2008.

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**.

10.6 Respiratory sensitisation

Table 37: Summary table of animal studies on respiratory sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Results	Reference
No data available					

Table 38: Summary table of human data on respiratory sensitisation

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data available				

Table 39: Summary table of other studies relevant for respiratory sensitisation

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data available				

10.6.1 Short summary and overall relevance of the provided information on respiratory sensitisation

No data are currently available to asses the respiratory sensitization potential of DBNPA; however given a lack of clinically diagnosed cases of occupational asthma associated with exposure to DBNPA, it is considered highly unlikely that DBNPA is able to cause respiratory sensitization.

10.6.2 Comparison with the CLP criteria

No data available.

10.6.3 Conclusion on classification and labelling for respiratory sensitisation

No data available.

10.7 Skin sensitisation

Table 40: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results	Reference
Comparable to OECD 406 (Buehler test) Reliability = 2	Dunkin Hartley Guinea pig 10/sex test item group 5/sex control	DBNPA (purity not given)	Induction: 2% w/v in acetone Days 1, 8 and 15 0.4 ml applied to test site for 6 hours Challenge: 0.5% w/v in acetone Day 29 0.4ml applied to challenge site for 6 hours	24 hours: Following challenge, patchy or slight erythematous responses were observed in 6 animals (males) 48 hours: Erythema persisted in 6 animals (4 males and 2 females) . Patchy or slight erythema also noted in 1 control animal	[redacted]) A6.1.5/01
OECD 406 EPA 81-6 (Buehler test) GLP Reliability = 1	Hartley Guinea pig Males 10/test item group 5/control	DBNPA (98.2%)	Induction: 25% w/v in 0.5% Methocel ® Days 1, 8 and 15. 0.4ml applied to test site for 6 hr. Challenge: 0.5% or 5% w/v in 0.5% Methocel ® Positive control with DER 331 epoxy resin.	The challenge with 0.5% DBNPA in Methocel ®, did not result in erythema or edema in any of the 10 animals. The challenge with 5% resulted in positive response, which was contributed to the dermal irritation potential.	[redacted] A6.1.5./03

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results	Reference
Non-guideline Non-GLP Reliability =3	Guinea pigs Males 10/test item 10/control	DBNPA (purity not given)	5% w/v in 9:1 mixture of DOWANOL DPM and Tween 80 Induction : Twice a week for three weeks Challenge: One application after two weeks Positive control with 15% DER 331 epoxy resin, in same solvent.	7/10 positive responses to test material.	[redacted] A6.1.5./02

Table 41: Summary table of human data on skin sensitisation

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Human Repeat Insult Patch test	DBNPA 125 ppm (0.0125%)	26 healthy subjects, 10 inductions over 3 weeks	3 out of 26 subjects showed only mild irritation. None of the subjects developed an allergic contact dermatitis as a result of the test. DBNPA is not a strong skin sensitizer.	[redacted] A6.12.6/01
Health & Safety report	DBNPA 20% (liquid)	Two industrial workers from a paper mill, accidentally exposed on a single occasion for an unknown duration of time.	Generalized erythema multiforme (EM)-like lesions 17 and 10 days after accidental exposure. The EM-like lesions appeared as subepidermal blisters associated with many necrotic or apoptotic keratinocytes and dense lymphocytic infiltration in the epidermis. Most of the infiltrating cells in the epidermis were CD8-positive T lymphocytes expressing cytotoxic molecules. Steroid pulse therapy failed to suppress the development of the lesions sufficiently, but ciclosporin 2.5-3 mg/kg successfully controlled this. DBNPA-induced, generalized erythema multiforme-like eruptions may be mediated by cytotoxic T-lymphocytes.	[redacted] A6.12.2
Cumulative Irritancy with Delayed 21 Day Challenge	DBNPA 20% (liquid), 0, 500, 750, 1000, 1250, 1500 and	27 healthy subjects (24 for challenge), 21 inductions (5 pr week), single challenge after 10 days.	Results indicated concentration-dependent skin responses at concentrations of 1250 ppm and greater in the cumulative irritation phase . The challenge at 500 ppm at the 48 and 72 hours post-application readings	[redacted] A6.12.6/02

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
	2000ppm DBNPA Challenge 500ppm DBNPA		suggests that 13 subjects were probably sensitized.	
ICDRG (International Contact Dermatitis Research Group)	DBNPA 20% (liquid) 0, 1, 2, 4, 8, 16, 31, 63, 125, 250 and 500 ppm DBNPA	6 subjects which submitted positive reactions in previous study (Maibach 2002a). Single exposure of each concentration simultaneously for 48h.	Subject 1 was clearly positive to 4 ppm and equivocal at the next two dilutions. Subject 2, 3, 4 and 5 were clearly positive to 16 ppm. Subject 6 had an equivocal response even at the highest concentration. The positive results from the first assay could be verified. Sensitization was observed down to the low concentration range.	[redacted] A6.12.6/02
Human Repeat Insult Patch test	DBNPA 500ppm and vehicle control	26 healthy subjects 21 inductions (3 times pr. Week), 2 challenges after 14 days.	The test sample was of low irritancy potential at 500 ppm DBNPA. 7/26 subjects were deemed to have developed allergic contact dermatitis to the test sample. 2 out of this 7 subjects had a response to the vehicle believed to represent the Excited Skin Syndrome.	[redacted] A6.12.6/02
Modified Draize Skin Sensitization Probe Study by Mazulli and Maibach (1977)	DBNPA 20% (liquid, sample QK) or DBNPA 20% in Tetraethylene glycol (sample P) or control with destilled water. Re-test: 500ppm DBNPA Re challenge: 1, 5, 50, 125, 250, 500 ppm DBNPA	5 subjects which submitted positive reactions in previous study (Maibach 2002c). Retest Phase: one time application Rechallenge Phase: one time application, 48 hr	In the retest period, in every subject the test material elicited a skin response. In the rechallenge period skin responses to Sample QK and Sample P appeared identical. For at least one subject, a concentration of 125 ppm of both samples elicited a skin response. Exposure to concentrations of 50 ppm or less did not cause a skin response in any of the five sensitized subjects. The identical skin responses for DBNPA prepared from both Sample QK and Sample P, suggest that the production sample of DBNPA (Sample QK) did not contain any contaminants specific to its production that might cause a skin response. Exposure to the rechallenge concentrations of 50 ppm or less did not cause a response in any of the 5 subjects previously sensitized to higher concentrations.	[redacted] A6.12.6/02

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Modified Draize Skin Sensitization Probe Study by Mazulli and Maibach (1977)	DBNPA 20% (liquid) 250ppm DBNPA. Starting test materialdiluted with distilled water	26 healthy subjects (25 completed) 21 days induction (3 times pr. week), for 48-72 hours per application. 72h challenge after 2 weeks.	Of the 25 subjects, only one demonstrated any skin response during the induction phase. This response was equivocal (0.5) and occurred a single time over the 3-week-period. During the challenge period, a different subject elicited a mild skin response (0.5) to the challenge patch. When the challenge was repeated with the same concentration of test material 10 days later, the same subject had no skin response to the test material.	[redacted] A6.12.6/02

Table 42: Summary table of other studies relevant for skin sensitisation

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data available				

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

Three tests for dermal sensitization in guinea pigs are available for assessment. In the study A6.1.5/01 [redacted], 10 male and 10 female guinea pigs received topical applications of 0.4 ml of 2% w/v DBNPA in acetone on days 1, 8 and 15 and were challenged with 0.4 ml 0.5% w/v in acetone on day 29 in a protocol similar to OECD 406/Buehler test. Slight erythema was observed in 6/10 males at 24 hours following challenge and in 4 males and 2 females at 48 hours from challenge. Slight erythema was observed in 1 control animal at 48 hours from challenge. Purity of the test material was not provided and classification of the skin reaction was not in accordance with OECD 406; however, data indicate that DBNPA is a weak sensitizer.

Table 43: Average dermal responses following induction and challenge of DNBPA (A6.1.5/01[redacted])

Inductions	**Buehler test**		**Observations/Remarks**
	day of treatment	Application	
Induction 1	1	Topical	Slight patchy erythema in 14 (7 males and 7 females) of 20.
Induction 2	8	Topical	A patchy or slight (5 males, 3 females) to slight but confluent or moderate patchy erythema (2 males, 3 females) in 13 of 20 animals.
Induction 3	15	Topical	A patchy or slight (5 males, 6 females) to slight but confluent or moderate patchy erythema (4 males, 4 females) in 19 of 20 animals.
Challenge	29	Topical	
Scoring 1	24 hrs after challenge application		Patchy or slight erythema in 6 (males) of 20 animals. No reactions in control animals
Scoring 2	48 hrs after challenge application		Patchy or slight erythema in 6 (4 males, 2 females) of 20 animals. Patchy or slight erythema in 1 female of 10 in the control group

Table 44: Individual dermal responses to induction and challenge of DBNPA (A6.1.5/01[redacted])

Group and sex	**Animal no.**	**Phase of study**				
		Induction			**Challenge**	
		Day 2	**Day 9**	**Day 16**	**Day 30**	**Day 31**
Test –males	1	±	1	±	±	0
	2	±	0	1	0	±
	3	±	±	1	±	0
	4	0	±	±	±	0
	5	0	±	±	0	0
	6	±	1	1	±	0
	7	±	±	±	±	±
	8	0	0	1	±	0
	9	±	±	0	0	±
	10	±	0	±	0	±
Test –females	1	±	1	1	0	±
	2	0	0	1	0	0
	3	±	1	1	0	±

	4	0	1	1	0	0
	5	±	0	±	0	0
	6	±	±	±	0	0
	7	±	±	±	0	0
	8	±	0	±	0	0
	9	±	0	±	0	0
	10	0	±	±	0	0
Control – males	1	-	-	-	0	0
	2	-	-	-	0	0
	3	-	-	-	0	0
	4	-	-	-	0	0
	5	-	-	-	0	0
Control – females	1	-	-	-	0	0
	2	-	-	-	0	0
	3	-	-	-	0	0
	4	-	-	-	0	0
	5	-	-	-	0	±

Grade system: 0 -no response, ± - patchy or slight erythema, 1 –slight but confluent or moderate patchy erythema, 2 - moderate erythema, 3 –severe erythema with or without oedema.

In the study A6.1.5/03 [redacted] performed according to the Buehler method (OECD 406), 10 male guinea pigs per group received topical applications of 0.4 ml of 25% w/v DBNPA technical (98.2% a.i.) in 0.5% Methocel® on days 1, 8 and 15, and challenge applications of 0.5% or 5% w/v DBNPA in 0.5% Methocel®. The challenge with 5% DBNPA resulted in a positive response in 10/10 animals; however, 5/5 naive control animals also reacted to the 5% w/v challenge dose. As such, the response was attributed to dermal irritation. The challenge concentration of 0.5% DBNPA, which was the highest non-irritating dose in the preliminary irritation screen, did not produce erythema or edema in any of the exposed guinea pigs. As such, under the conditions of this study, DBNPA technical material was considered negative for sensitization to guinea pigs.

In the study A6.1.5./02 [redacted] 5% w/v DBNPA in a 9:1 mixture of Dowanol DPM and Tween 80 was applied to 10 male guinea pigs twice a week for 3 weeks during the induction phase and two weeks later during the challenge phase. Seven animals (70%) responded at challenge. However, purity of test material and severity of skin reactions are not provided.

Human repeat insult patch test (HRIPT) data are available for DBNPA. In the study A6.12.6/01 [redacted] Dow Antimicrobial 7287, a 20% DBNPA formulation, was tested in 26 volunteers. Three of 26 subjects showed mild irritation during the induction phase of the study, but none developed evidence of allergic contact dermatitis as a result of exposure to 125 ppm DBNPA. In studies A6.12.6/02 [redacted] Dowicil Qk-20, a 20% DBNPA formulation, was tested at various induction and challenge concentrations according to a number of irritancy and sensitization protocols. In the first study, which followed a Cumulative Irritancy Protocol with Delayed Challenge, subjects received 21 induction applications of 0, 500, 750, 1000, 1250, 1500, and 2000 ppm and a single challenge application of 500 ppm after 10 days. Thirteen of the 24 subjects who received the challenge application were considered probably sensitized. However, the cumulative irritancy protocol appears to involve simultaneous application of patches at the aforementioned doses. Considering this and the absence of information on the area exposed, no dose per area can be determined for induction or challenge applications. In 26 subjects tested according to the HRIPT test methodology of A6.12.6/02 [redacted] with 500 ppm DBNPA, 7 subjects were suggested to have developed allergic contact dermatitis. Two of these subjects also reacted to vehicle. Five of these subjects later underwent a one-time 500 ppm re-test followed 48 hours later by a one-time re-challenge at 1, 5, 50, 125, and 500 ppm DBNPA. All subjects reacted during the re-test phase.

At re-challenge, at least one subject reacted to 125 ppm DBNPA, while no subjects reacted to 50 ppm. In a modified Draize Skin Sensitization Probe Study, 250 ppm did not produce sensitization reactions in any of 25 healthy subjects.

For further details on the studies, please refer to annex II to this report.

10.7.2 Comparison with the CLP criteria

In an investigation equivalent to the Buehler test (A6.1.5/01 [redacted]), six out of 20 of animals were sensitised (30%) and the substance therefore meets the criteria for Skin sensitiser (Category 1). Further, according to the CLP regulation (EC) No 1272/2008, substances can be subcategorised according to potency based on the number of animals responding at a given induction concentration.

According to Table 3.4.4 of the CLP regulation, sub-category 1B is applicable if in the Buehler assay >15% to < 60% of guinea pigs respond at > 0.2% to < 20% topical induction dose. In the study A6.1.5/01 [redacted], the topical induction dose was 2% DBNPA and 30% of animals showed a response at challenge. As such, animal data indicate that DBNPA falls within category 1B. The study A6.1.5./03 [redacted] the only study performed according to GLP and where purity of the test material has been identified, did not show sensitization potential and, alone, would not suggest a need for any labelling. The study A6.1.5/02 [redacted] would suggest Category 1A, as ≥ 60% response was observed at a topical induction dose of 5% (> 0.2% to ≤ 20%). However, no information on test material purity is provided. The weight of evidence based on these conflicting animal data indicate that DBNPA technical may have the inherent hazard potential to induce sensitization reactions; however, based on the conflicting outcomes these data do not allow for appropriate sub-categorization.

Together the available HRIPT data appear to indicate a sensitization induction threshold between 250 and 500 ppm. However, sample sizes were small in each study; only formulations of DBNPA in polyethylene glycol or tetraethylene glycol were tested, rather than DBNPA technical; purity and impurity information were not provided for the test materials; and no surface area of exposure was provided in any study to allow for calculation of dose to surface area. As such, a quantitative evaluation of the sensitization potential for DBNPA technical to humans cannot be performed from the available human data. Moreover, the CLP Guidance recommends that the size of the exposed population and the extent and frequency of exposure be considered. Only one case of sensitization in response to DBNPA exposure has been reported, wherein two industrial workers from a paper mill were accidentally exposed on a single occasion to 20% DBNPA for an unknown duration and later developed generalized erythema multiform that was successfully controlled with cyclosporine (A6.12.2[redacted]). This indicates either low hazard potential for DBNPA related sensitization reactions, adequate protection in the form of PPE in the potentially exposed work force, or both. The low frequency of sensitization occurring in response to DBNPA exposure would in accordance with guidance support sub-categorization as 1B rather than 1A.

In accordance with CLP regulation sub-categorization should only occur, if data are sufficient to allow appropriate assignment to a sub-category. Based upon a weight of evidence approach, the available animal and human data are not sufficient for sub-categorization. Conflicting results have been observed in guinea pig studies and even negative results obtained for the technical material. Human repeat insult patch test data are available only for formulated DBNPA, in the absence of purity and impurity information, and with insufficient detail to allow for dose per surface area calculation. Further only a single clinical case of dermal sensitization has been reported in over 45 years of use indicating DBNPA to be of poor dermal sensitization potential. As these data do not provide sufficient evidence to sub-categorize DBNPA, Category 1 is the appropriate classification for DBNPA and as such a generic concentration limit of 1.0% should also be applied.

10.7.3 Conclusion on classification and labelling for skin sensitisation

DBNPA is classified as Skin Sensitiser 1 H317: May cause an allergic skin reaction, according to the criteria given in Regulation (EC) No 1272/2008.

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	**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

	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)	
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	**Negative** 3 subjects reacted during the induction phase None of the subjects reacted to challenge

	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	
Cumulative irritancy with delayed challenge A6.12.6/02, Study I and II 2002	Study I 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 Study II 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	Study I **Positive** 13 subjects were probably sensitized (according to the DS) Irritancy was observed at ≤ 1250 ppm Study II 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	Study III 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 Study IV 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	Study III **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 Study IV *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 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	**Negative** 1 mild reaction during the induction phase None of the subjects reacted to challenge (including the subject with a reaction during induction)
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: Slight pain after exposure. Superficial skin necrosis occurred 3 days later, eruptions subsided after treatment with a topical steroid. 17 days after the incident,

	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	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**.

10.8 Germ cell mutagenicity

Table 45: Summary table of mutagenicity/genotoxicity tests in vitro

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Gene reverse mutation in bacteria OECD 471 Reliability = 2	DBNPA (100%)	*S. typhimurium* TA1535, TA1537, TA98, TA100, TA1538 -S9: 0.25 to 25 µg/plate +S9: 0.125 to 25 µg/plate	+S9 = negative -S9 = negative Absence of colonies was observed at the highest concentration without S9 mix.	[redacted] (A6.6.1/01)
Gene mutation in bacteria Ames test – comparable to OECD 471 Reliability = 3	DBNPA (20%)	*S. typhimurium*, TA 98, TA 100, TA 1537, TA1535	+S9 = negative -S9 = negative A marginal induction in one tested strain, TA 100, at doses near toxic levels. No effects were found in other strains. The study author concluded that it cannot be concluded based on the results from TA 100 that the samples contain mutagens.	[redacted] A6.6.1/02
Genemutation in bacteria microsome reverse mutation assay US EPA (1998), EEC (2000) OECD (1997) Reliability = 1	DBNPA (99.4%)	S. *Escherichia coli* +S9: 0.333 to 100 µg/plate -S9(S. *Escherichia coli)*: 0.0333 to 10 µg/plate -S9 (WP2*uvr*A): 0.100 to 33.3 µg/plate	+S9 = negative -S9 = negative DPNPA did not induce a positive increase in the mean number of revertants per plate with any of the tester stains either in the presence or absence of microsomal enzymes induced rat liver (S9).	[redacted] A6.6.1/03
Cytogenetic assay OECD 473 EU B.10 EPA Reliability = 1	DBNPA (98%)	Human lymphocytes -S9: 25 to 50 µg/mL +S9: 25 to 50 µg/mL	+S9 = negative -S9 = negative Reduction of mitotic index at high dose.	[redacted] (A6.6.2/01)
Cytogenetic assay/SCE Reliability = 2	DBNPA (20%)	CHO cells ±S9: 0.5 to 50 µg/mL	+S9 = negative -S9 = negative A slight increase of SCE induction was observed in the absence of S9 mix. However, the study author concluded that the finding has no biological relevance. The reliability of the test is questionable due to the various methodological deficiencies.	[redacted] (A6.6.2/02)

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Cytogenicity in mammalian cells OECD No 473 Reliability= 3	DBNPA (100%)	Chinese Hamster Ovary cells (CHO) +S9: 3.33, 10.0, 33.3 µg/mL -S9:0.33, 1.0, 3.33 µg/mL	+S9 = negative -S9 = negative None of the tested DBNPA concentrations between 0.33 and 33.3µg/ml produced a statistically significant increase in the frequencies of chromosome aberrations at any fixation time. Positive controls produced a clear increase in the incidence of chromosome aberrations..	[redacted] A6.6.2/03
Gene mutation in mammalian cells (HPGRT) OECD 476 Reliability = 1	DBNPA (95%)	Chinese Hamster Ovary cells (CHO) -S9: 10 to 400 µM +S9: 25 to 400 µM	+S9 = negative -S9 = negative Distinctly reduced survival at high doses	[redacted] (A6.6.3/01)
UDS OECD 482 EPA Reliability = 1	DBNPA (98%)	Primary hepatocytes of rats 0.08 to 7.67 µg/mL	+S9 = negative -S9 = negative Severe toxicity at top concentration	[redacted] (A6.6.3/02)
UDS Comparable to OECD 482 Non –GLP Reliability = 3	DBNPA (95%)	Primary hepatocytes of rats 4 x 10^{-6}, 1.26x10^{-5}, 4x10^{-5}, 1.26x10^{-4}, 4x10^{-4}, 1.26 x10^{-3} and 4 x10^{-3}M in 0.1%(v/v) DMSO Positive control: 2-acetylaminoflourene	DBNPA failed to elicit UDS at any contration tested. Apparent cytotoxicity at 1.26x10^{-5}M.	[redacted] A6.6.3/03
UDS Comparable to OECD 482 GLP Reliability =3	DBNPA (98%)	Primary hepatocytes of rats 1, 3, 5, 10, 15, 20µg/mL Positive control: 4-Nitroquinolineoxide (4-NQO), 7, 12-Dimethylbenzanthracene (DMBA)	DBNPA was found to respond negatively in the DNA repair assay. Cytotoxicity at top concentration level.	[redacted] A6.6.3/04

Table 46: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells in vivo

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Micro-nucleus assay OECD 474	DBNPA (97.1%)	Mouse CD-1 (ICR) BR	No increase in frequency of micronucleated polychromatic erythrocytes in dose groups.	[redacted] (A6.6.4/01)

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
EU B.12 EPA Reliability = 1		males, females 5/group Single application Dose levels 30, 60, 120 mg/kg bw Sampling times 24, 48, 72 h Cyclophosphamide as positive control	One mid dose male (48 h) died prior to scheduled sacrifice time. Four deaths in high dose (two males and two females). Two of these deaths were in the extra group animals (one male and one female). No clinical signs were observed. DBNPA was considered to be negative in the mouse bone marrow micronucleus test.	***Key study***
Micro-nucleus assay Comparable to OECD 474 Non-GLP Reliability = 3	DBNPA in destilled water	Mouse CD-1 (ICR) BR males, females 5/group Single oral gavage Dose levels 9, 30, 90 mg/kg bw Sampling times 24, 48h Cyclophosphamide as positive control	No increase in the frequencies of micronucleated bone marrow polychromatic erythrocytes, compared with negative controls. DBNPA was considered to be negative in the mouse bone marrow micronucleus test.	[redacted] (A6.6.4/02)

Table 47: Summary table of human data relevant for germ cell mutagenicity

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data available				

10.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

In vitro

The mutagenic potential of DBNPA was investigated in the Ames test (A6.6.1/01[redacted]) using the histidine auxotroph *S. typhimiurium* strains TA98, TA100, TA1535, TA1537, and TA 1538, all with and without metabolic activation by rat liver S9 mix from Phenobarbital sodium/3methylcholanthrene induced male CD rats. DBNPA was tested at concentrations of 25, 12.5, 6.25, 1.25 and 0.25 µg/plate without metabolic activation and 25, 12.5, 6.25, 3.125, 1.25, 0.625, 0.25 and 0.125 µg/plate with metabolic activity. The solvent used for the test substance was DMSO. No statistically significant increases in revertant colony numbers over control counts were obtained with any tester strains following exposure to DBNPA at any tested concentration in the presence and absence of S9 mix. Toxicity was seen following exposure to DBNPA at 25 µg/plate without metabolic activation.

Cytogenicity in mammalian cells was investigated in A6.6.2/01 [redacted] who investigated the mutagenic potential of DBNPA in human blood lymphocytes in vitro both with and without metabolic activation. Human lymphocytes were obtained from a healthy, male donor. The study was performed with an incubation time of 2 h, followed by two fixation periods (24 and 48 h after start of treatment). Three hours before harvesting, cells were treated with colchicine in order to arrest cells in the metaphase. The mitotic index of each culture was determined by counting the number of metaphases per 1000 cells. At least 100 metaphase chromosome spreads per culture were examined by light microscopy. Only metaphases containing 46 centromeres were analysed.

In the absence and presence of metabolic activation, the concentrations tested were 25, 37.5 and 50 µg/mL (24 h fixation period) and 37.5 µg/mL (48 h fixation period) without S9 mix and 5, 25 and 50 µg/mL (24 h fixation period) and 37.5 µg/mL (48 h fixation period) with S9 mix.

In the absence of S9 mix the mitotic index was reduced by 32 % (24 h fixation period) at a dose level of 50 µg/mL and reduced by 18 % (48 h fixation period) at a dose level of 37.5 µg/mL. In the presence of S9 mix the mitotic index was reduced by 50 % (24 h fixation period) at a dose level of 50 µg/mL and reduced by 40 % (48 h fixation period) at a dose level of 37.5 µg/mL.
Both in the absence and presence of S9 mix DBNPA did not induce a statistically and biologically significant increase in the number of cells with chromosome aberrations. [redacted]

The number of cells with chromosome aberrations found in the solvent control cultures fell within the laboratory historical control data range (i. e. 1.3 ± 1.2 aberrant cells per 100 metaphases (with/without S9 mix, gaps excluded)). Both positive control compounds, mitomycin C and cyclophosphamide, caused large, statistically significant increases in the counts of aberrant cells demonstrating the sensitivity of the test system. DBNPA is not clastogenic under the experimental conditions of the test system employed.

DBNPA was investigated in A6.6.2/02 [redacted], for its potential to cause Sister Chromatid Exchanges in CHO cell line in vitro both with and without metabolic activation.

The CHO cells were incubated with DBNPA concentrations of 0, 0.5, 1.5, 5.0, 15.0 and 50.0 µg/mL with and without S9 mix. Cytotoxicity investigations were conducted during dose range finding studies. After incubation time of 1 h the medium was replaced by medium containing BrdU and postincubation continued in absence of light for a further 21 h. Then colchicine was added in order to arrest cells in the metaphase and three hours later the cells were harvested. Staining of chromosomes was conducted using Hoechst 33285 and Giemsa (Merck). Two slides were prepared from the contents of each experimental point. The numbers of SCE in 20 metaphases were counted for each experimental point using light microscopy.

No cytotoxicity was observed from exposure of CHO cells to polyethylene glycol (0.55 %) and DBNPA at concentrations of 50 µg/mL or below for one hour in the absence of S9 mix. According to the study author a statistically significant increase in the number of SCE in the absence of S9 mix was found. However, there was no description in the study report about the way of statistical analysis. In addition, it was not stated whether a statistically significant increase in the number of SCE was observed or whether the dose-response relationship was statistically significantly different to control. The author considered this increase to be not biologically relevant due to the small increase. The notifier is also of the opinion that, besides the other methodological deficiencies, the increases observed were so small that they could not be indicative for a relation to treatment. In addition, it should be noted that only one culture was investigated in one single experiment.

DBNPA was considered to induce no SCE in CHO cells in the presence and absence of metabolic activation. However, it should be noted that the reliability of the test is questionable.

[redacted] A6.6.3/01 tested DBNPA for its potential to induce forward mutation in mammalian CHO cells using the HPRT-locus in the presence and absence of S9-Mix. Negative solvent controls and positive controls (EMS and MCA) were concurrently used. Five replicate dishes per concentration were used each in the presence and absence of metabolic activation. In the absence and presence of metabolic activation, cells were exposed to the test substance for 4-5 hours.

The positive controls (EMS and MCA) clearly induced an increase in mutation frequency as expected and proved the suitability of the test system.
DBNPA was tested at 10, 12.5, 15, 17.5, 20 and 25 µM in an initial assay without activation and at 25, 50, 75, 100, 150, 200 and 400 µM in the final non-activated assay. It was also evaluated at 25, 50, 75, 100, 150, 200 and 400 µM with metabolic activation.

DBNPA produced in the absence and presence of metabolic activation neither a statistically significant dose-related increase in mutation frequency nor a statistically significant and reproducible increased mutation frequency at high toxicity in this study. In the final non-activated assay, survival progressively decreased from 100 % at 25 µM to app. 7 % of that of control cultures at 100 µM DBNPA. DBNPA concentrations greater than 100 µM were completely toxic to the cultures as indicated by the fact that no cell remained viable

after treatment with the test substance.
With metabolic activation, survival decreased from 100 % at 25 µM to app. 0.1 % of that of the control cultures at 100 µM DBNPA. In presence of S9 mix DBNPA was toxic to the cell cultures at concentrations higher than 100 µM such that no cells remained viable after treatment with DBNPA.

DBNPA was not mutagenic in the CHO/HGPRT assay, with or without metabolic activation, indicating a lack of genotoxic activity in cultured mammalian somatic cells.

[redacted] A6.6.3/02 looked at the potential of DBNPA to induce unscheduled DNA synthesis. This was assessed in freshly isolated primary rat hepatocytes in vitro. 5 different DBNPA concentrations or the positive controls (MMS, 2-AAF) and negative/solvent controls (culture medium) were added together with 3HTdR. Suitable test substance concentrations were selected on the basis of the results of a pre-experiment. Each concentration was tested in triplicate and two independent experiments were conducted.

Following termination of an exposure period of 18 hours, cells were processed by coating produced slides with a photographic emulsion and storage in the dark at 4°C for 7 days. Thereafter, slides were developed, fixed and stained. Slides were evaluated microscopically by counting sliver grains over the nucleus and silver grains in the cytoplasm area adjacent to the nucleus. Two slides per concentration and 50 cells per slides were evaluated. Increased net grain counts (number of nuclear grains subtracted from number of cytoplasmic grains) which are based on enhanced nuclear grains counts are considered relevant.

Criteria for a test-article-related positive response:

Statistically significant concentration-related increase in nuclear and net grains counts

Statistically significant increase of nuclear grain counts for at least one of the test points.

Five adequately spaced concentrations were used in this study. The suitability of the concentrations was confirmed in a pre-experiment based on cell viability/alteration of cell morphology. In two independent experiments, no reproducible concentration dependent increase in the number of nuclear and net grain counts were observed up to the highest test substance concentration tested. The positive controls 2-AAF and MMS showed a reproducible, distinct increase in the number of nuclear and net grain counts.

In vivo

In the in vivo micronucleus study (A6.6.4/01[redacted]) groups of CD-1 mice were orally administered a single dose of DBNPA at concentrations of 90, 120, 150, and 300 mg/kg bw in a dose range-finding test. In the micronucleus test doses of 0, 30, 60, and 120 mg/kg bw were administered. The animals were killed by cervical dislocation 24, 48 and 72 hours, respectively, after the administration and bone marrow cells were prepared. 1000 polychromatic erythrocytes were examined from each animal and the number of micronucleated polychromatic erythrocytes was recorded. The ratio of polychromatic(PCE)/normochromatic(NCE) erythrocytes in the bone marrow was determined by examining 1000 erythrocytes. The ratio was expressed as PCEx100/PCE+NCE.

Dose Range-Finding Test: Mortality

Table 48: Dose Range Finding Test: Mortality (A6.6.4/01[redacted])

Dose mg/kg bw	Animals dosed (Female/Male)	No. of Dead (Female/Male) Day					Dead/Treated
		1[a]	2	3	4	7	
90	4/4	0/0	0/0	0/1	0/1	0/0	2/8
120	4/4	0/0	1/0	0/0	0/0	0/0	1/8
150	4/4	1[b]/0	1/1	0/0	0/0	0/0	3/8
300	4/4	2/1	2/3	-	-	-	8/8

[a] Day 1 is the day of dosing.

[b] The female died immediately after dosing. Gross necropsy revealed pulmonary lesions that were consistent with gavage error therefore, this female was excluded from the calculation of LD_{50}=159 mg/kg bw.

Mammalian

Table 49: Summary of results: Mammalian Erythrocyte Mouse Micronucleus Test (A6.6.4/01 [redacted])

MALES									
Treatment	**Dose[b]**	**24 h Sacrifice**			**48 h Sacrifice**			**72 h Sacrifice**	
	mg/kg bw	**N[c]**	**MN**	**%PCE**	**N**	**MN**	**%PCE**	**N**	**MN**	**%PCE**
negative control[d]	0	5	1.8 1.3	72.0 5.9	5	0.6 0.5	76.5 7.0	5	1.6 1.8	69.2 11.2
Positive Control[e]	120	5	38.0 19.7	36.8 7.7	ND[f]	- -	- -	ND[f]	- -	- -
DBNPA	30	5	0.4 0.5	66.6 5.6	5	0.2 0.4	64.4 6.9	5	0.6 0.5	73.1 6.2
DBNPA	60	5	1.4 1.7	67.6 2.8	4	1.0 1.4	65.3 6.8	5	1.4 2.1	68.8 7.8
DBNPA	120	5	0.6 0.9	69.3 4.2	5	1.0 0.7	68.0 4.9	5	1.6 1.3	70.0 3.5
FEMALES									
Treatment	**Dose[b]**	**24 h Sacrifice**			**48 h Sacrifice**			**72 h Sacrifice**	
	mg/kg bw	**N[c]**	**MN**	**%PCE**	**N**	**MN**	**%PCE**	**N**	**MN**	**%PCE**
negative control[d]	0	5	1.2 1.1	69.2 3.8	5	1.6 0.9	68.0 9.5	5	2.2 1.6	62.9 13.2
Positive Control[e]	120	5	35.4 4.9	41.9 13.3	ND[f]	- -	- -	ND[f]	- -	- -
DBNPA	30	5	2.8 0.8	64.9 7.0	5	0.8 0.8	71.8 12.8	5	1.6 0.9	63.3 10.3
DBNPA	60	5	0.8 1.3	63.7 15.2	5	1.4 1.3	71.8 5.7	5	1.0 0.7	70.4 7.1
DBNPA	120	5	0.8 0.8	52.2 8.0	5	0.6 0.5	60.9 9.1	5	0.2 0.4	67.5 7.4

[a] Data are means and standard deviations.

[b] Doses are expressed in mg/kg body weight.

[c] N is the number of animals per dose group. 1000 PCE were examined/animal for MN incidence.

[d] Mice were dosed with the vehicle (distilled water).

[e] The MN and %PCE values are significantly different from the negative control ($p \leq 0.01$).

[f] Not done.

There were no statistically significant increases in the frequencies of micronucleated polychromatic erythrocytes in groups treated with the test material as compared to negative controls. The positive control mice showed significant increases in micronucleated polychromatic erythrocytes. Under the experimental conditions used, the test material was considered to be negative in mouse bone marrow micronucleus test.

For further details on the studies, please refer to annex II to this report.

Summary

DBNPA was not genotoxic in vitro up to and including cytotoxic concentrations in Salmonella typhimurium bacteria or in Chinese hamster CHO cells. DBNPA did not induce unscheduled DNA synthesis in primary rat hepatocytes. There was no indication of cytogenetic activity in human lymphocytes and CHO cells. DBNPA did not induce micronuclei in mice bone marrow up to and including the MTD. DBNPA is therefore not regarded to be a genotoxic substance.

10.8.2 Comparison with the CLP criteria

Substances producing a positive mutagenic or clastogenic effect in vivo are subject to classification. DBNPA did not exhibit a positive result either *in vitro* or *in vivo*.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

Classification for germ cell mutagenicity is not proposed for DBNPA.

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	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

	Sampling time 24h, 48h and 72h (positive control 24h) Top dose chosen based on mortalities in a dose range-finding experiment	
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.**

10.9 Carcinogenicity

Table 50: Summary table of animal studies on carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
USEPA OPPTS 870.4300 (1998), OECD, Guideline 453 (1981), EEC, Part B (1988), JMAFF, Combined Chronic Toxicity/Oncogenicity Study (2000) Rat, F344/DuCrl Male & female 60/sex Oral, dietary Reliability = 1	DBNPA 0, 3, 20, or 150 mg DBNPA/kg body weight/day (mg/kg/day, mkd) targeted dose levels; 1.4, 9.551, 71.32 mg/kg bw/d actual dose levels in diet 104 weeks	Based on treatment-related hyperplasia of the thyroid follicular cells in males given 20 or 150 mg/kg/day, the no-observed-effect level (NOEL) for males was 3 mg/kg/day (actual dose of 1.431 mg/kg/day). Females given 150 mg/kg/day had treatment-related lower body weights and feed consumption, and histopathologic thyroid and liver effects. The only effect of treatment in females given 20 mg/kg/day was slightly higher serum bromide concentration (recorded as chloride) at the 3-month time point. This observation was interpreted to be a non-adverse marker of exposure to DBNPA Therefore, females had a no-observed-adverse effect level (NOAEL) of 20 mg/kg/day and a NOEL of 3 mg/kg/day (actual doses of 9.551 and 1.434 mg/kg/day).Confidential No treatment-related increase in neoplasms was observed in either male or female rats at any dose level, indicating that DBNPA did not have an oncogenic potential under the conditions of this study.	[redacted] (A6.4.2/01)

Table 51: Summary table of human data on carcinogenicity

Type of data/report	**Test substance,**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
No data available				

Table 52: Summary table of other studies relevant for carcinogenicity

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data available				

10.9.1 Short summary and overall relevance of the provided information on carcinogenicity

A 2-year combined repeat oral dose and carcinogenicity study in F344 rats is available.

Groups of 60 male and 60 female F344/DuCrl rats were fed diets formulated to provide 0 (control), 3, 20, or 150 mg DBNPA/kg body weight/day for up to two years (actual dosages accounting for test material degradation in the diet were 1.4, 9.5 and 71.3 mg/kg bw/d).

Males and females given 150 mg/kg/day had treatment-related statistically identified lower mean body weights and feed consumption during the latter portion of the study. At study termination, the body weight gains of males and females given 150 mg/kg/day were 15.1% and 12.8% lower than controls, respectively. There were no treatment-related effects on body weights or feed consumption of males or females given 3 or 20 mg/kg/day.

The only treatment-related clinical observation was an increased incidence of perineal urine soiling in males given 150 mg/kg/day. The urine soiling may have been caused by decreased grooming of the perineal region in response to the presence of excretory by-products of DBNPA in the urine.

Hematologic alterations in males given 150 mg/kg/day consisted of slightly lower reticulocyte counts at 3, 6, 12, 18, and 24 months, and higher red blood cell counts, hemoglobin concentrations, and hematocrits at 18 and 24 months. These alterations were interpreted to be of minimal toxicologic significance since there was no associated anemia or adverse clinical signs such as dehydration. There were no hematologic effects in males given 3 or 20 mg/kg/day, or in females at any dose level.

Males and females given 150 mg/kg/day had treatment-related higher serum chloride concentrations throughout the study, whereas males and females given 20 mg/kg/day had slightly higher chloride concentrations only at 3 months. The higher chloride concentrations were attributed to interference from elevated serum bromine (associated with release of bromide ion from the test material following administration) with the specificity of the chloride electrode of the clinical chemistry analyzer to detect chlorine as well as bromine.

There was a general tendency for males given 150 mg/kg/day to have slightly higher urine pH than controls at 3, 6, 12, and 18 months. This alteration was interpreted to be treatment related, likely due to the presence of cyanoacetamide, the major excretory product in the urine of rats given DBNPA. Males and females given 150 mg/kg/day had a general tendency to have higher urinary ketones levels than controls at 3, 6, 12, 18, and 24 months. The higher ketone levels were associated with significantly lower body weights in males and females given 150 mg/kg/day, and may be the result of a secondary effect on lipid catabolism to meet energy needs.

The primary treatment-related histopathologic effect in animals from the 24-month sacrifice consisted of statistically-identified increases in the incidence of very slight or slight diffuse follicular cell hyperplasia in the thyroid glands of males and females given 150 mg/kg/day, and in males given 20 mg/kg/day. Treatment-related thyroid follicular hyperplasia was also present at the 12-month sacrifice in males and females given 150 mg/kg/day. The hyperplasia did not appreciably worsen in the males during the final 12 months of the study and only slightly increased (e.g., more females with slight versus very slight) in female rats. This effect was interpreted to be caused by the bromide released in-vivo from DBNPA. Thyroid follicular cell hyperplasia is a commonly recognized effect of exposure to exogenous compounds containing bromine attributable to bromide ion competing with iodine uptake into thyroid cells. The very slight to slight, diffuse thyroid hyperplasia did not progress to more significant or severe thyroid pathology or to thyroid tumors.

While thyroid changes were observed in both the 90 day dog study and 2 year cancer study in rats, the increased thyroid weights and follicle dilation observed in dogs did not result in histopathological evidence of injury indicating that no organ dysfunction is evident. In rats after a chronic exposure during 2 years the increase in incidence of follicular cell hyperplasia of the follicular epithelium was very slight. During the two years the change was not associated with increased

cancer risk and the genotoxicity studies were negative. According to a Commission group of specialized experts1 the rat is a more sensitive species with regards to changes of the thyroid and development of epithelial thyroid tumors after long term exposure to non-genotoxic agents and the changes should not be considered representative for humans. It is therefore concluded that the effects seen in the 2 year rat study and in the 90 dog study is not sufficient to trigger classification as carcinogenic.

Females given 150 mg/kg/day had a treatment-related statistically-identified increase in the incidence of slight vacuolization (consistent with fatty change) of individual hepatocytes. The fatty change of the liver may have been secondary to lower feed consumption and body weights of this dose group during the final months of the study. Male rats did not have a similar effect on liver vacuolization at any dose level. In addition, hepatotoxicity was not evident since serum liver enzymes (ALT and AST) were not elevated with respect to the normal range at 24 months in female rats.

Males given 150 mg/kg/day had statistically-identified decreases in the incidence of large granular lymphocyte (LGL) leukemia, adenomas of the pars distalis of the pituitary gland, and moderate or severe chronic progressive glomerulonephropathy of the kidneys compared to controls. The decreased incidence of these findings was interpreted to be related to the lower feed consumption of rats from this dose group for the majority of the two-year dosing period.

No treatment-related increase in neoplasms was observed in either male or female rats at any dose level, indicating that DBNPA did not have an oncogenic potential under the conditions of this study.

A full summary of the chronic toxicity parts of the study can be found in Section 10.12 of this document.

Table 53 (part 1/9): Tumour summary of Two year chronic/Carcinogenicity/Chronic neurotoxicity study (A6.4.2/01[redacted]).

Observations: Neo-Plastic	MALES				FEMALES			
Removal Reasons: All of those SELECTED	0 MKD	3 MKD	20 MKD	150 MKD	0 MKD	3 MKD	20 MKD	150 MKD
Number of Animals on Study :	50	50	50	50	50	50	50	50
Number of Animals Completed:	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
ADRENAL GLANDS;								
Examined	(49)	(24)	(21)	(50)	(50)	(6)	(15)	(50)
Within Normal Limits	12	4	4	28	28	3	2	25
Not Examined: MISSING	1	0	0	0	0	0	0	0
Adenoma; cortex; benign; primary	0	0	0	0	0	0	1	2
Ganglioneuroma; medulla; benign; primary	1	0	0	0	0	0	0	0
Pheochromocytoma; medulla; benign; primary	4	3	2	2	0	0	1	1
Pheochromocytoma; medulla; malignant with metastasis; primary	1	0	0	1	0	0	0	0
Pheochromocytoma; medulla; malignant without metastasis; primary	0	1	0	0	0	0	1	0
Pheochromocytoma; two; medulla; benign; primary	0	0	1	0	0	0	0	0
AORTA;								
Examined	(49)	(21)	(20)	(50)	(50)	(6)	(12)	(50)
Within Normal Limits	48	21	20	49	50	6	12	50
Not Examined: CANNIBALISM	1	0	0	0	0	0	0	0
AUDITORY SEBACEOUS GLAND;								
Examined	(1)	(0)	(0)	(1)	(1)	(1)	(1)	(1)
Within Normal Limits	0	0	0	0	0	0	0	0
Carcinoma; malignant without metastasis; primary	1	0	0	1	0	1	1	1
BONE;								
Examined	(50)	(21)	(20)	(50)	(50)	(6)	(13)	(50)
Within Normal Limits	49	21	19	50	50	6	11	48
Osteoma; calvarium; benign; primary	0	0	1	0	0	0	0	0
Osteosarcoma; tibia; malignant without metastasis; primary	1	0	0	0	0	0	0	0
BONE - JOINT;								
Examined	(50)	(21)	(20)	(50)	(50)	(6)	(12)	(50)
Within Normal Limits	50	21	20	50	50	6	12	50
BONE MARROW;								
Examined	(50)	(21)	(20)	(50)	(50)	(6)	(12)	(50)
Within Normal Limits	41	16	16	47	46	4	9	46
Sarcoma; poorly differentiated; malignant; secondary	0	0	1	0	0	0	0	0
BRAIN;								
Examined	(50)	(24)	(22)	(50)	(50)	(9)	(16)	(50)
Within Normal Limits	39	11	9	41	39	5	10	34
Astrocytoma; malignant without metastasis; primary	0	2	0	0	0	0	0	0
Carcinoma; malignant; secondary	1	0	1	0	1	0	0	0
Medulloblastoma; benign; primary	0	0	0	0	0	0	0	1

no statistical differences from controls by Yates' Chi-Square Alpha=0.05, two sided.

1 Summary record – Commission group of specialised experts in the fields of Carcinogenicity, mutagenicity and reprotoxicity, ECBI/49/99, 1999, excerpt of agenda item 3.1

Table 54 (part 2/9): Tumour summary of Two year chronic/Carcinogenicity/Chronic neurotoxicity study (A6.4.2/01[redacted]).

Observations: Neo-Plastic	MALES				FEMALES			
Removal Reasons: All of those SELECTED	0 MKD	3 MKD	20 MKD	150 MKD	0 MKD	3 MKD	20 MKD	150 MKD
Number of Animals on Study :	50	50	50	50	50	50	50	50
Number of Animals Completed:	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
BRAIN; (continued)								
Mixed Glioma; malignant without metastasis; primary	0	1	0	0	0	0	0	0
Sarcoma; meninges; malignant without metastasis; primary	0	1	0	0	0	0	0	0
CECUM;								
Examined	(50)	(21)	(20)	(50)	(50)	(6)	(12)	(49)
Within Normal Limits	50	21	20	50	50	6	12	49
Not Examined: CANNIBALISM	0	0	0	0	0	0	0	1
CERVIX;								
Examined	(-)	(-)	(-)	(-)	(50)	(7)	(12)	(50)
Within Normal Limits	-	-	-	-	49	6	12	46
Not Examined: MISSING	-	-	-	-	0	0	1	0
Adenocarcinoma; malignant with metastasis; primary	-	-	-	-	0	0	0	1
Stromal Cell Sarcoma; malignant without metastasis; primary	-	-	-	-	1	0	0	2
Lymphosarcoma; malignant with metastasis; primary	-	-	-	-	0	0	0	1
COAGULATING GLAND;								
Examined	(50)	(21)	(20)	(50)	(-)	(-)	(-)	(-)
Within Normal Limits	49	20	20	50	-	-	-	-
COLON;								
Examined	(50)	(21)	(20)	(50)	(50)	(6)	(12)	(49)
Within Normal Limits	50	21	20	50	50	6	12	49
Not Examined: CANNIBALISM	0	0	0	0	0	0	0	1
CRANIAL NERVE;								
Examined	(0)	(0)	(0)	(0)	(0)	(1)	(0)	(0)
Within Normal Limits	0	0	0	0	0	1	0	0
CRANIAL NERVE - OPTIC;								
Examined	(50)	(23)	(20)	(50)	(50)	(11)	(17)	(50)
Within Normal Limits	47	20	18	45	49	5	15	48
DUODENUM;								
Examined	(50)	(21)	(20)	(50)	(50)	(6)	(12)	(50)
Within Normal Limits	49	21	19	49	50	5	12	50
Adenocarcinoma; malignant without metastasis; primary	0	0	0	1	0	0	0	0
EPIDIDYMIDES;								
Examined	(50)	(21)	(20)	(50)	(-)	(-)	(-)	(-)
Within Normal Limits	2	2	4	4	-	-	-	-

no statistical differences from controls by Yates' Chi-Square Alpha=0.05, two sided.

Table 55: (part 3/9): Tumour summary of Two year chronic/Carcinogenicity/Chronic neurotoxicity study (A6.4.2/01[redacted]).

Observations: Neo-Plastic	MALES				FEMALES			
Removal Reasons: All of those SELECTED	0 MKD	3 MKD	20 MKD	150 MKD	0 MKD	3 MKD	20 MKD	150 MKD
Number of Animals on Study :	50	50	50	50	50	50	50	50
Number of Animals Completed:	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
ESOPHAGUS;								
Examined	(49)	(21)	(20)	(50)	(50)	(6)	(12)	(50)
Within Normal Limits	48	20	19	49	49	6	10	49
Not Examined: CANNIBALISM	1	0	0	0	0	0	0	0
EYE;								
Examined	(50)	(25)	(20)	(50)	(50)	(30)	(33)	(50)
Within Normal Limits	1	3	8	0	9	3	3	3
HEART;								
Examined	(49)	(21)	(21)	(50)	(50)	(7)	(12)	(50)
Within Normal Limits	4	1	3	4	7	2	1	9
Not Examined: CANNIBALISM	1	0	0	0	0	1	0	0
Mesothelioma; atrium; malignant without metastasis; primary	1	0	0	0	0	0	0	0
Schwannoma; benign; primary	0	0	1	1	0	1	0	0
ILEUM;								
Examined	(50)	(21)	(20)	(50)	(50)	(6)	(12)	(49)
Within Normal Limits	50	21	20	50	50	6	12	49
Not Examined: CANNIBALISM	0	0	0	0	0	0	0	1
JEJUNUM;								
Examined	(50)	(21)	(20)	(50)	(50)	(6)	(12)	(49)
Within Normal Limits	48	21	20	50	49	6	12	49
Not Examined: CANNIBALISM	0	0	0	0	0	0	0	1
Adenocarcinoma; mucinous; malignant without metastasis; primary	2	0	0	0	0	0	0	0
Liposarcoma; malignant without metastasis; primary	0	0	0	0	1	0	0	0
KIDNEYS;								
Examined	(50)	(50)	(50)	(50)	(50)	(49)	(50)	(50)
Within Normal Limits	0	1	1	2	6	4	2	1
Not Examined: MISSING	0	0	0	0	0	1	0	0
Renal Mesenchymal Tumor; malignant without metastasis; primary	0	0	1	0	0	0	0	0
LACRIMAL/HARDERIAN GLAND;								
Examined	(50)	(21)	(20)	(50)	(50)	(6)	(12)	(50)
Within Normal Limits	45	19	19	49	32	4	11	34
LARYNX;								
Examined	(49)	(21)	(20)	(50)	(49)	(6)	(12)	(50)
Within Normal Limits	43	17	16	49	49	6	11	48
Not Examined: CANNIBALISM	1	0	0	0	0	0	0	0

no statistical differences from controls by Yates' Chi-Square Alpha=0.05, two sided.

Table 56: (part 4/9): Tumour summary of Two year chronic/Carcinogenicity/Chronic neurotoxicity study (A6.4.2/01[redacted]).

Observations: Neo-Plastic	MALES				FEMALES			
Removal Reasons: All of those SELECTED	0 MKD	3 MKD	20 MKD	150 MKD	0 MKD	3 MKD	20 MKD	150 MKD
Number of Animals on Study :	50	50	50	50	50	50	50	50
Number of Animals Completed:	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
LARYNX; (continued)								
Not Examined: MISSING	0	0	0	0	1	0	0	0
LIVER;								
Examined	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Within Normal Limits	0	0	0	0	0	1	0	0
Adenoma; hepatocyte; benign; primary	4	5	0	0	0	2	1	0
Adenoma; cystic; bile duct; benign; primary	1	0	0	0	0	0	0	0
Carcinoma; hepatocyte; malignant without metastasis; primary	0	1	0	0	0	0	0	0
LUNG;								
Examined	(50)	(21)	(22)	(50)	(50)	(5)	(12)	(50)
Within Normal Limits	39	14	16	32	32	3	9	30
Not Examined: CANNIBALISM	0	0	0	0	0	1	0	0
Adenoma; bronchiolo - alveolar; benign; primary	2	1	1	0	1	0	0	0
Carcinoma; malignant; secondary	0	1	1	0	0	0	0	1
Sarcoma; poorly differentiated; malignant; secondary	0	0	1	0	0	0	0	0
Pheochromocytoma; malignant; secondary	1	0	0	1	0	0	0	0
LYMPH NODE - MEDIASTINAL;								
Examined	(49)	(21)	(20)	(50)	(50)	(6)	(12)	(50)
Within Normal Limits	49	21	19	49	50	6	12	50
Not Examined: CANNIBALISM	1	0	0	0	0	0	0	0
Sarcoma; poorly differentiated; malignant; secondary	0	0	1	0	0	0	0	0
LYMPH NODE - MESENTERIC;								
Examined	(50)	(21)	(19)	(50)	(50)	(6)	(12)	(50)
Within Normal Limits	50	20	18	50	50	6	11	50
Not Examined: MISSING	0	0	1	0	0	0	0	0
Sarcoma; poorly differentiated; malignant; secondary	0	0	1	0	0	0	0	0
Stromal Cell Sarcoma; malignant; secondary	0	0	0	0	0	0	1	0
LYMPH NODE - MISCELLANEOUS;								
Examined	(2)	(2)	(0)	(1)	(0)	(1)	(0)	(1)
Within Normal Limits	0	0	0	0	0	0	0	0
Adenocarcinoma; malignant; secondary	0	0	0	0	0	1	0	1
Carcinoma; malignant; secondary	0	1	0	0	0	0	0	0
LYMPH NODE - SUBMANDIBULAR;								
Examined	(0)	(0)	(0)	(2)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0	0	0	0

no statistical differences from controls by Yates' Chi-Square Alpha=0.05, two sided.

Table 57: (part 5/9): Tumour summary of Two year chronic/Carcinogenicity/Chronic neurotoxicity study (A6.4.2/01[redacted]).

Observations: Neo-Plastic	MALES				FEMALES			
Removal Reasons: All of those SELECTED	0 MKD	3 MKD	20 MKD	150 MKD	0 MKD	3 MKD	20 MKD	150 MKD
Number of Animals on Study :	50	50	50	50	50	50	50	50
Number of Animals Completed:	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
OVARIES; (continued)								
Within Normal Limits	-	-	-	-	50	5	10	46
Not Examined: CANNIBALISM	-	-	-	-	0	1	0	0
Not Examined: MISSING	-	-	-	-	0	0	1	0
Adenocarcinoma; malignant; secondary	-	-	-	-	0	0	0	1
Gonadal Stromal Tumor; benign; primary	-	-	-	-	0	0	0	1
OVIDUCT;								
Examined	(-)	(-)	(-)	(-)	(50)	(5)	(11)	(50)
Within Normal Limits	-	-	-	-	50	5	11	50
Not Examined: CANNIBALISM	-	-	-	-	0	1	0	0
Not Examined: MISSING	-	-	-	-	0	0	1	0
PANCREAS;								
Examined	(50)	(21)	(20)	(50)	(50)	(5)	(12)	(50)
Within Normal Limits	26	9	15	16	34	3	10	37
Not Examined: MISSING	0	0	0	0	0	1	0	0
Adenoma; islet cell; benign; primary	4	2	2	8	0	0	0	0
Carcinoma; duct; malignant without metastasis; primary	0	0	1	0	0	0	0	0
PARATHYROID GLAND;								
Examined	(49)	(20)	(19)	(48)	(49)	(6)	(12)	(50)
Within Normal Limits	49	20	19	48	49	6	12	49
Not Examined: CANNIBALISM	1	0	0	0	0	0	0	0
Not Examined: MISSING	0	1	1	2	1	0	0	0
PENIS;								
Examined	(0)	(0)	(0)	(1)	(-)	(-)	(-)	(-)
Within Normal Limits	0	0	0	0	-	-	-	-
PERIPHERAL NERVE;								
Examined	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(1)
Within Normal Limits	0	0	0	0	0	0	0	0
PERIPHERAL NERVE - TIBIAL;								
Examined	(50)	(21)	(20)	(50)	(50)	(6)	(12)	(50)
Within Normal Limits	3	1	1	4	0	1	2	3
PITUITARY GLAND;								
Examined	(50)	(35)	(33)	(50)	(50)	(36)	(38)	(50)
Within Normal Limits	9	5	7	22	7	1	1	16
Adenoma; pars distalis; benign; primary	31	24	23	14*	21	32	28	21

* statistically different from controls by Yates' Chi-Square Alpha=0.05, two sided.

Table 58: (part 6/9): Tumour summary of Two year chronic/Carcinogenicity/Chronic neurotoxicity study (A6.4.2/01[redacted]).

Observations: Neo-Plastic	MALES				FEMALES			
Removal Reasons: All of those SELECTED	0 MKD	3 MKD	20 MKD	150 MKD	0 MKD	3 MKD	20 MKD	150 MKD
Number of Animals on Study :	50	50	50	50	50	50	50	50
Number of Animals Completed:	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
PITUITARY GLAND; (continued)								
Adenoma; pars intermedia; benign; primary	0	0	0	0	1	0	0	0
Carcinoma; pars distalis; malignant without metastasis; primary	1	0	1	0	1	0	0	0
PREPUTIAL/CLITORAL GLAND;								
Examined	(6)	(8)	(8)	(9)	(1)	(3)	(2)	(3)
Within Normal Limits	0	0	0	0	0	0	0	0
Carcinoma; malignant with metastasis; primary	0	1	0	0	0	0	0	0
Carcinoma; malignant without metastasis; primary	6	3	5	5	1	1	2	2
PROSTATE;								
Examined	(50)	(21)	(21)	(50)	(-)	(-)	(-)	(-)
Within Normal Limits	9	8	4	10	-	-	-	-
Adenocarcinoma; malignant without metastasis; primary	1	0	0	3	-	-	-	-
RECTUM;								
Examined	(50)	(21)	(20)	(50)	(50)	(6)	(12)	(50)
Within Normal Limits	50	21	20	50	50	6	12	50
SALIVARY GLAND;								
Examined	(49)	(21)	(19)	(50)	(49)	(6)	(12)	(50)
Within Normal Limits	49	20	19	48	49	6	12	49
Not Examined: CANNIBALISM	1	0	0	0	0	0	0	0
Not Examined: MISSING	0	0	1	0	1	0	0	0
Fibroma; benign; primary	0	1	0	0	0	0	0	0
SEMINAL VESICLE;								
Examined	(50)	(21)	(20)	(50)	(-)	(-)	(-)	(-)
Within Normal Limits	49	21	20	49	-	-	-	-
SKELETAL MUSCLE;								
Examined	(50)	(21)	(20)	(50)	(50)	(6)	(12)	(50)
Within Normal Limits	49	20	20	50	50	5	12	49
SKIN AND SUBCUTIS;								
Examined	(50)	(29)	(31)	(50)	(50)	(16)	(15)	(50)
Within Normal Limits	38	15	13	36	41	4	8	46
Adenoma; sebaceous gland; benign; primary	0	0	0	1	0	0	0	0
Basal Cell Adenoma; benign; primary	0	0	0	1	0	0	0	0
Basal Cell Carcinoma; malignant without metastasis; primary	0	0	0	1	0	0	0	0
Carcinoma; sebaceous gland; malignant without metastasis; primary	0	0	1	0	0	0	0	0
Fibroma; abdominal; benign; primary	1	0	1	1	0	0	0	0

no statistical differences from controls by Yates' Chi-Square Alpha=0.05, two sided.

Table 59 (part 7/9): Tumour summary of Two year chronic/Carcinogenicity/Chronic neurotoxicity study (A6.4.2/01[redacted]).

Observations: Neo-Plastic	MALES				FEMALES			
Removal Reasons: All of those SELECTED	0 MKD	3 MKD	20 MKD	150 MKD	0 MKD	3 MKD	20 MKD	150 MKD
Number of Animals on Study :	50	50	50	50	50	50	50	50
Number of Animals Completed:	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
SKIN AND SUBCUTIS; (continued)								
Fibroma; axillary; benign; primary	0	1	1	0	0	0	1	1
Fibroma; hind foot; benign; primary	0	0	1	0	0	0	0	0
Fibroma; hindlimb; benign; primary	0	0	0	0	0	0	1	0
Fibroma; inguinal; benign; primary	0	1	0	0	1	0	0	0
Fibroma; thoracic; benign; primary	0	0	1	0	0	0	0	0
Fibrosarcoma; axillary; malignant without metastasis; primary	0	0	0	0	0	0	1	0
Fibrosarcoma; back; malignant without metastasis; primary	1	0	0	0	1	1	0	0
Fibrosarcoma; head; malignant without metastasis; primary	0	0	1	0	0	0	0	0
Fibrosarcoma; tail; malignant without metastasis; primary	0	0	1	0	0	0	1	0
Hemangiosarcoma; tail; malignant without metastasis; primary	0	0	1	0	0	0	0	0
Keratoacanthoma; abdominal; benign; primary	1	0	1	0	0	1	0	0
Keratoacanthoma; axillary; benign; primary	0	1	0	0	0	0	0	0
Keratoacanthoma; back; benign; primary	2	2	0	3	0	1	0	0
Keratoacanthoma; digit; benign; primary	0	0	0	1	0	0	0	0
Keratoacanthoma; inguinal; benign; primary	0	1	1	0	0	0	0	0
Keratoacanthoma; tail; benign; primary	0	1	0	0	0	0	0	0
Lipoma; axillary; benign; primary	0	1	0	0	0	0	0	0
Lipoma; thoracic; benign; primary	0	0	1	0	0	0	0	0
Neurofibroma; eyelid; benign; primary	0	0	0	0	0	1	0	0
Neurofibrosarcoma; pinna; malignant without metastasis; primary	1	0	0	0	0	0	0	0
Papilloma; ear canal; benign; primary	0	0	1	0	0	0	0	0
Papilloma; hind foot; benign; primary	0	0	1	0	0	0	0	0
Papilloma; hindlimb; benign; primary	0	0	1	0	0	0	0	1
Papilloma; muzzle; benign; primary	0	0	1	0	0	0	0	0
Papilloma; nose; benign; primary	0	1	0	1	0	0	0	0
Sarcoma; poorly differentiated; abdominal; malignant without metastasis; primary	0	1	0	0	0	0	0	0
Sarcoma; poorly differentiated; back; malignant without metastasis; primary	0	0	0	0	1	0	0	0
Squamous Cell Carcinoma; head; malignant without metastasis; primary	0	1	0	0	0	0	0	0
Squamous Cell Carcinoma; inguinal; malignant without metastasis; primary	0	0	1	0	0	0	0	0
Lymphosarcoma; subcutis; malignant with metastasis; primary	0	1	0	0	0	0	0	0
SPINAL CORD - CERVICAL, THORACIC AND LUMBAR;								
Examined	(50)	(21)	(20)	(50)	(50)	(6)	(12)	(50)
Within Normal Limits	2	10	8	3	3	2	1	2
Meningioma; lumbar; benign; primary	1	0	0	0	0	0	0	0
SPLEEN;								
Examined	(50)	(30)	(30)	(50)	(50)	(7)	(19)	(50)
Within Normal Limits	24	7	6	42	32	0	3	41
Sarcoma; poorly differentiated; malignant with metastasis; primary	0	0	1	0	0	0	0	0
Sarcoma; poorly differentiated; malignant without metastasis; primary	0	0	0	0	0	0	0	1

no statistical differences from controls by Yates' Chi-Square Alpha=0.05, two sided.

Table 60 (part 8/9) Tumour summary of Two year chronic/Carcinogenicity/Chronic neurotoxicity study (A6.4.2/01[redacted]).

Observations: Neo-Plastic	MALES				FEMALES			
Removal Reasons: All of those SELECTED	0 MKD	3 MKD	20 MKD	150 MKD	0 MKD	3 MKD	20 MKD	150 MKD
Number of Animals on Study :	50	50	50	50	50	50	50	50
Number of Animals Completed:	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
SPLEEN; (continued)								
Leukemia; large granular lymphocyte (fischer rat); malignant; primary	17	16	13	4*	11	4	7	4
STOMACH;								
Examined	(50)	(21)	(20)	(50)	(50)	(6)	(12)	(50)
Within Normal Limits	40	14	11	46	46	4	8	46
Adenocarcinoma; malignant; secondary	0	0	0	0	0	0	0	1
Papilloma; nonglandular mucosa; benign; primary	0	1	0	0	0	0	0	0
TESTES;								
Examined	(50)	(48)	(49)	(50)	(-)	(-)	(-)	(-)
Within Normal Limits	0	0	0	2	-	-	-	-
Interstitial Cell Adenoma; unilateral; benign; primary	8	3	3	8	-	-	-	-
Interstitial Cell Adenoma; bilateral; benign; primary	36	41	42	38	-	-	-	-
THYMUS;								
Examined	(49)	(21)	(20)	(50)	(50)	(6)	(12)	(50)
Within Normal Limits	3	2	0	4	2	1	0	1
Not Examined: CANNIBALISM	1	0	0	0	0	0	0	0
THYROID GLAND;								
Examined	(49)	(50)	(50)	(50)	(49)	(50)	(50)	(50)
Within Normal Limits	31	27	33	13	34	40	39	26
Not Examined: CANNIBALISM	1	0	0	0	0	0	0	0
Not Examined: MISSING	0	0	0	0	1	0	0	0
Adenoma; follicular cell; benign; primary	1	3	0	0	1	0	0	1
Adenoma; parafollicular cell; benign; primary	5	13	5	3	8	8	6	4
Adenoma; two; parafollicular cell; benign; primary	3	0	0	0	0	0	1	0
Carcinoma; follicular cell; malignant without metastasis; primary	0	0	1	0	0	0	0	0
Carcinoma; parafollicular cell; malignant without metastasis; primary	1	3	1	1	1	2	0	0
TONGUE;								
Examined	(0)	(0)	(0)	(1)	(2)	(0)	(1)	(0)
Within Normal Limits	0	0	0	0	0	0	0	0
Papilloma; benign; primary	0	0	0	0	1	0	0	0
Squamous Cell Carcinoma; malignant without metastasis; primary	0	0	0	1	1	0	1	0
TRACHEA;								
Examined	(49)	(21)	(20)	(50)	(50)	(6)	(12)	(50)
Within Normal Limits	48	21	17	48	50	6	12	50
Not Examined: CANNIBALISM	1	0	0	0	0	0	0	0

* statistically different from controls by Yates' Chi-Square Alpha=0.05, two sided.

Table 61(part 9/9): Tumour summary of Two year chronic/Carcinogenicity/Chronic neurotoxicity study (A6.4.2/01[redacted]).

Observations: Neo-Plastic	MALES				FEMALES			
Removal Reasons: All of those SELECTED	0 MKD	3 MKD	20 MKD	150 MKD	0 MKD	3 MKD	20 MKD	150 MKD
Number of Animals on Study :	50	50	50	50	50	50	50	50
Number of Animals Completed:	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
URETER;								
Examined	(1)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
Within Normal Limits	0	0	0	0	0	0	0	0
URINARY BLADDER;								
Examined	(50)	(21)	(20)	(49)	(50)	(5)	(11)	(50)
Within Normal Limits	46	20	18	49	49	5	10	50
Not Examined: CANNIBALISM	0	0	0	0	0	1	0	0
Not Examined: MISSING	0	0	0	1	0	0	1	0
Papilloma; benign; primary	0	0	0	0	0	0	1	0
UTERUS;								
Examined	(-)	(-)	(-)	(-)	(50)	(17)	(22)	(50)
Within Normal Limits	-	-	-	-	30	2	7	37
Not Examined: CANNIBALISM	-	-	-	-	0	1	0	0
Not Examined: MISSING	-	-	-	-	0	0	1	0
Adenocarcinoma; malignant with metastasis; primary	-	-	-	-	0	1	0	0
Adenocarcinoma; malignant without metastasis; primary	-	-	-	-	2	1	1	1
Adenoma; endometrium; benign; primary	-	-	-	-	1	0	0	1
Endometrial Stromal Polyp; benign; primary	-	-	-	-	12	10	7	7
Endometrial Stromal Polyp; two; benign; primary	-	-	-	-	1	1	4	2
Endometrial Stromal Polyp; three; benign; primary	-	-	-	-	2	1	0	0
Sarcoma; poorly differentiated; malignant with metastasis; primary	-	-	-	-	1	0	0	0
Stromal Cell Sarcoma; malignant with metastasis; primary	-	-	-	-	0	1	1	0
Stromal Cell Sarcoma; malignant without metastasis; primary	-	-	-	-	0	0	1	0
VAGINA;								
Examined	(-)	(-)	(-)	(-)	(50)	(6)	(11)	(50)
Within Normal Limits	-	-	-	-	50	6	11	46
Not Examined: MISSING	-	-	-	-	0	0	1	0
VASCULAR SYSTEM;								
Examined	(0)	(0)	(0)	(0)	(1)	(0)	(0)	(1)
Within Normal Limits	0	0	0	0	0	0	0	0

no statistical differences from controls by Yates' Chi-Square Alpha=0.05, two sided.

For further details on the toxicokinetic studies, please refer to annex II to this report.

Table 62: Compilation of factors to be taken into consideration in the hazard assessment

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
Not applicable								

10.9.2 Comparison with the CLP criteria

Substances producing a positive response in relation to development of tumours or non-neoplastic lesions are subject to classification and labelling for carcinogenicity. Based on available data, DBNPA does not meet the criteria for classification as carcinogenic.

10.9.3 Conclusion on classification and labelling for carcinogenicity

In the absence of evidence for carcinogenic effects, classification and labelling is not proposed.

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.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 63: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
OECD 416 EPA Reliability = 1	DBNPA Rat Sprague Dawley males, females 28/group (F0)	The high dose level was reduced from 40 to 35 to 30mg/kg bw/d over 21 days owing to severe toxicity including dyspnoea, swollen abdomen, piloerection, hunched posture and deaths. Some signs persisted at 30 mg/kg/day and dyspnoea was attributed to local irritation from the dosing formulation by gavage, supported by necropsy findings of distension of the stomach/ intestines	[redacted] (A6.8.2/02)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
	26/group (F1) Exposure period 10 weeks before mating (F0); 11 weeks before mating (F1) Dose 0, 5, 15, (40/35/) 30 mg/kg bw/day Gavage	and oedema in the lungs. In total, 4 males and 7 females of the F_0 generation died and 2 males and 5 females in the F_1. Dyspnoea was also observed, at a lower incidence, at 15 mg/kg/day. At the high dose inthe F_0 generation transient individual periods of weight loss was accompanied by clinical signs described above. The body weight gain of males at the high dose was 90% of the control value. Body weight and body weight gain of other dosing groups and in females were comparable to controls. Food consumption in dosing groups were similar to controls. There were no adverse effect on weight gain in the F_1 generation. There were no treatment-related findings on organ weights or at histopathology of group 1 and 4, including qualitative and quantitative examination of the testes. There were no significant effects of treatment on mating, fertility, litter size, pup weight, growth or development in both generations, including reproductive function of the F_1. NOAEL = 15 mg/kg bw/day (parental) NOAEL = $\geq$ 30 (reproduction) NOAEL $\geq$ 30 (development)	

10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

In the multigeneration study A6.8.2/02 [redacted], animals were randomised into 4 groups each containing 28 males and 28 females. Dosing was performed orally by gavage, once daily, using the dose levels of 0, 5, 15 and 30 mg/kg bw/d. The high dose level was initially set at 40 mg/kg bw/d, based on a low incidence of dyspnoea in a range-finding study but, after unexpectedly severe reactions to treatment and one death, was decreased to 35 mg/kg bw/d after 11 days and to 30 mg/kg bw/d after a further 10 days. The F_0 animals were dosed for a total of 10 weeks prior to mating and throughout the mating, gestation, and lactation periods until all the F_1 pups were weaned. Selected F_1 animals (26 males and 26 females per group) were dosed from ~4 weeks of age (day 25) throughout mating, gestation and lactation periods until all the F_2 pups were weaned.

All animals were monitored for clinical signs and changes in body weight and food consumption throughout the study.

At termination of the study, animals were necropsied and examined for any external and internal abnormalities. In adults, liver, kidneys, adrenal glands, oesophagus, tongue, gastrointestinal tract (stomach, duodenum, jejunum, lieum, caecum, colon, rectum), trachea, pituitary gland and reproductive organs (uterus, cervix, vagina or testes, prostate, epididymides, seminal vesicles and coagulating gland) were retained and fixed. Testes, epididymides and prostate were weighed. Histological analysis was limited to the reproductive organs of the high dose and control groups and included assessment for effects on spermatogenesis, including enumeration of cauda epididymal sperm reserve and sperm morphology. F_1 and F_2 pups found dead before Day 14 were examined for externally visible abnormalities and after Day 14 subjected to a gross necropsy.

Additionally, 2 males and 2 females from each litter of F_1 and F_2 pups were examined macroscopically after necropsy.

Statistical analysis was applied to determine the statistical significance of differences from control.

Dyspnoea, swollen abdomen, piloerection and hunched posture accompanied by transient weight loss were observed in many animals in the F_0 generation at the high dose level.Dyspnoea was attributed to local irritation from gavage dosing of test material and this was supported by necropsy findings of distension of the stomach and/or intestines and oedema in the lungs. Dyspnoea was still observed after the dose level was reduced to 30 mg/kg bw/d. In total 20 animals of this dose group died throughout the study (F_0: 4 males and 7 females, F_1: 2 males and 7 females).

At the intermediate dose level (15 mg/kg bw/d) dyspnoea was observed, not as frequently as in high dose animals, but at a greater incidence than in the control group, particularly among F_1 females. One male from this group died in week 4, having shown no previous signs of toxicity: necropsy showed congested/oedematous lungs but these may have been post-mortem changes and this single occurrence could not conclusively be related to treatment.

At the low dose (5 mg/kg bw/day) the incidence of clinical signs was similar to controls. The premature death of one female was attributed to dystocia.

At the high dose transient individual periods of weight loss accompanied by clinical signs described above. The body weight gain of males at the high dose was 90% of the control value. Body weight and body weight gain of other dosing groups and in females were comparable to controls.Food consumption in dosing groups were similar to controls.Mating performance and fertility of males and females were similar in all groups in each generation.

The mean total number of pups born per litter was marginally lower in DBNPA-treated groups than in controls. Taking into account the mean control range for litter size at birth from 8 control groups in 4 similar studies the value for F_0 generation at 30 mg/kg bw/day in this study was only just outside the historical background range. The difference was considered to be too small to be treatment-related.
Except at 15 mg/kg bw/day in the F_0 generation the incidence of total litter loss in this study was within the historical background control range. The loss of 4 litters in this group was not considered to be treatment-related in the absence of a similar increase at the high dose level (2 litter losses).
The loss of 2 litters in the F_1 generation at 30 mg/kg bw/day was within the historical control range. In one of these cases the female showed dyspnoea which may have been associated with the litter loss.

The nature and distribution of histopathology findings for the reproductive organs in Groups 1 (Control Group) and 4 (High dose Group) was considered comparable, so the remaining groups were not examined. There was no effect on the weight of male reproductive organs and qualitative and quantitative assessment of the testes in Groups 1 and 4 did not indicate and differences between the groups.

Table 64: Multigeneration Reproduction Toxicity Study in Rats: Parental effects on sexual function, fertility, development and/or lactation.

Parameter	Generation	**Dose level [mg/kg bw/day]**									
		0		**5**		**15**		**(40/35/30)**		Dose-response +/-	
		m	**f**	**M**	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Number of animals per group		**28 (F_0) 26 (F_1)**	**28 (F_0) 26 (F_1)**	**28 (F_0) 26 (F_1)**	**28 (F_0) 26 (F_1)**	**28 (F_0) 26 (F_1)**	**28 (F_0) 26 (F_1)**	**28 (F_0) 26 (F_1)**	**28 (F_0) 26 (F_1)**		
Clinical Observations											
Dyspnoea	F_0	0	0	0	0	2	3	16	12	+	+
	F_1	0	0	0	1	2	7	13	20	+	+
piloerection/subdued/dull eyes/hunched/cold/ encrustations around nose/mouth	F_0	0	2	1	2	0	3	7	7	+	+
	F_1	0	0	0	1	1	0	7	17	+	+
abdomen swollen/firm	F_0	0	0	0	0	0	0	2	4	+	+
	F_1	0	0	0	0	0	0	1	4	+	+
Mortality	F_0	0	0	0	0	1	0	4	7	+	+
	F_1	0	0	0	1	0	0	2	5	+	+
Body weight g	F_0										
Week 0		226	168	229	172	293	167	278	166		
Week 2 (40 mg/kg/day to Day 11)		346	213	354	220	347	218	330	223	+	-
Week 3 (35 mg/kg/day to Day 21)		387	232	386	240	393	238	370	223	+	+
Week 10 pre-mating		563	304	571	315	559	318	535	312	+	-
Week 16		635		647		625		598		+	
Day 20 gestation			462		471		466		453		-
Week 0 (nominal age 4 weeks)	F_1	112	101	119	109	106	100	116	102	-	-

Parameter	Generation	**Dose level [mg/kg bw/day]**									
		0		**5**		**15**		**(40/35/30)**		Dose-response +/-	
		m	**f**	**M**	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Number of animals per group		**28 (F_0) 26 (F_1)**	**28 (F_0) 26 (F_1)**	**28 (F_0) 26 (F_1)**	**28 (F_0) 26 (F_1)**	**28 (F_0) 26 (F_1)**	**28 (F_0) 26 (F_1)**	**28 (F_0) 26 (F_1)**	**28 (F_0) 26 (F_1)**		
Week 10, pre-mating		515	311	534	316	533	310	539	311	-	-
Week 22		621		653		636		649		-	
Day 20 of gestation			465		488		455		465		-
Body weight gain g [percentage of control]	F0										
Week 0-2		120	45	125 [104]	48 [107]	120 [100]	51 [113]	101 [84]	42 [93]	+	+
Week 2-3		41	19	42 [102]	20 [105]	46 [112]	20 [105]	40 [98]	15 [70]	-	+
Week 4-10		134	54	136 [101]	57 [106]	129 [96]	60 [111]	125 [93]	65 [120]	-	-
Week 0-10		409	136	418 [102]	143 [105]	398 [97]	151 [111]	369 [90]	146 [107]	+	-
Day 0-20 gestation			157		158 [101]		149 [95]		148 [94]		-
Week 0-10 (Week 0= nominal age 4 weeks)	F_1	418	210	430 [103]	207 [98]	442 [106]	210 [100]	442 [106]	209 [100]	-	-
Week 0-17		509		534 [105]		530 [104]		533 [105]		-	-
Day 0-20 gestation			155		166 [107]		155 [155]		153 [98]		-
Histology											
perivascular lymphocytic cuffing/	F_0	4	-	-	-	-	-	1	-	-	
segmental atrophy epididymides	F_1	0	-	-	-	-	-	0	-	-	
testicular tubular atrophy	F_0	4	-	-	-	-	-	6	-	-	

Parameter	Generation	**Dose level [mg/kg bw/day]**									
		0		**5**		**15**		**(40/35/30)**		Dose-response +/-	
		m	**f**	**M**	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Number of animals per group		**28 (F_0) 26 (F_1)**	**28 (F_0) 26 (F_1)**	**28 (F_0) 26 (F_1)**	**28 (F_0) 26 (F_1)**	**28 (F_0) 26 (F_1)**	**28 (F_0) 26 (F_1)**	**28 (F_0) 26 (F_1)**	**28 (F_0) 26 (F_1)**		
	F_1	1	-	-	-	-	-	4	-	-	
Focal lymphocytic infiltration/	F_0	4	-	-	-	-	-	2	-	-	
inflammation of prostate	F_1	3	-	-	-	-	-	4	-	-	
Uterine dilatation/dilated cystic	F_0	5						4			-
Glands	F_1	2						4			-
Duration of gestation	F_0										
21 days		-	3	-	6	-	4	-	1		-
22 days		-	23	-	19	-	19	-	13		-
23 days		-	1	-	2	-	4	-	7		-
mean duration		-	21.9	-	21.9	-	22.0	-	22.3		-
Number of pregnant females/number of females paired [fertility index %]	F_0	-	27/28 [96]	-	27/28 [96]	-	27/27 [100]	-	21/21 [100]		-
	F_1	-	24/26 [92]	-	24/26 [92]	-	22/26 [85]	-	20/23 [87]		-
Number males siring a pregnancy/paired [fertility index %]	F_0	27/28 [96]		26/28 [93]		24/27 [89]		20/21 [95]		-	
	F_1	23/26 [88]		22/26 [85]		21/26 [81]		19/23 [83]		-	
Mean number of implantation sites	F_0		16.9		16.2		16.3		16.5		-
	F_1		16.9		17.2		14.5		14.6		-
Mean number of pups born per litter[2]											
F_0 (F_1 production)	F_0	-	15.8	-	15.0	-	15.0	-	14.0	-	-
F_1 (F_2 production)	F_1	-	15.4	-	15.1	-	14.5	-	14.6	-	-
Total litter loss	F_0	-	1	-	1	-	4	-	2	-	-
	F_1	-	0	-	0	-	0	-	2	-	-
Viability index [%] days 0-4	F_1	90 (93[2])		93 (96[2])		83 (97[2])		85 (94[2])		-	

Parameter		**Dose level [mg/kg bw/day]**									
	Generation	**0**		**5**		**15**		**(40/35/30)**		Dose-response +/-	
		m	**f**	**M**	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Number of animals per group		**28 (F_0) 26 (F_1)**	**28 (F_0) 26 (F_1)**	**28 (F_0) 26 (F_1)**	**28 (F_0) 26 (F_1)**	**28 (F_0) 26 (F_1)**	**28 (F_0) 26 (F_1)**	**28 (F_0) 26 (F_1)**	**28 (F_0) 26 (F_1)**		
	F_2	97 (97[2])		92 (92[2])		97 (97[2])		83 (93[2])		-	
Lactation index [%] days 4-21	F_1	99		94		99		98		-	
	F_2	99		99		97		99		-	
Mean pup weight M/F - Day 1	F_1	7.0/6.5		7.0/6.6		7.0/6.7		7.2/6.5		-	
	F_2	6.7/ 6.3		7.1/6/6		6.7/6.5		7.3/6.8		-	
- Day 21	F_1	46.3/43.3		48.0/45.0		46.4/45.8		49.4/47.1		-	
	F_2	44.3/42.0		47.1/44.9		43.6/41.8		50.5/47.3		-	

[2]excludes litters with total litter loss

*statistically significantly different from controls ($p < 0.05$)

10.10.3 **Comparison with the CLP criteria**

No specific effects on fertility were reported as the result of DBNPA administration to rats. Therefore classification is not required.

10.10.4 **Adverse effects on development**

Table 65: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
EPA Oral (gavage) Reliability = 1	DBNPA Rabbit New Zealand white female 14/group Exposure period gestation day 7-19 Dose 0, 2, 10, 30, 60 mg/kg bw/day	Excessive maternal toxicity and high mortality at 60 mg/kg bw/d resulted in only 7 litters remaining for evaluation and this dose level is therefore unsuitable for classification purposes. At 30 mg/kg/day (a dose group initated in response to deaths at the higher level) mild toxicity was observed, characterised by reduced body weight gain and food intake at the start of treatment. There was no effect on litter size, placental weight, fetal weight or crown/rump length and no fetal malformations. Slight increases over concurrent control in the incidence of incomplete ossification of some skeletal elements were within the historical control range and considered unrelated to treatment. NO(A)EL = 10 mg/kg bw/day (maternal animals) LO(A)EL ≥ 30 mg/kg bw/day (maternal animals) NO(A)EL ≥ 30 mg/kg bw/day (offspring)	[redacted] (A6.8.1/01)
OECD 416 EPA Reliability = 1	DBNPA Rat Sprague Dawley males, females 28/group (F0) 26/group (F1) Exposure period 10 weeks before mating (F0); 11 weeks before mating (F1) Dose 0, 5, 15, (40/35/) 30 mg/kg bw/day Gavage	Clinical signs (dyspnoea, swollen abdomen, piloerection, hunched posture) in the high dose (F_0, F_1), even at 30 mg/kg bw/day and in the F_1 generation, Similar but less frequent findings at the mid dose (F_0). Dyspnoea was considered to be related to local irritation associated with gavage administraton of DBNPA, supported by ulcerative gastritis/gastroenteritis at necropsy of decedents. Weight gain in F_0 males and females was lower in Weeks 0-3 (higher dosages) and in males was 90 % of control at the end of the pre-mating period. There were no significant effects of treatment on mating, fertility, litter size, pup weight, growth or development in both generations, including reproductive capacity of the F_1 NOAEL = 15 mg/kg bw/day (parental) NOAEL = ≥ 30 (reproduction) NOAEL ≥ 30 (development)	[redacted] (A6.8.2/02)

10.10.5 Short summary and overall relevance of the provided information on adverse effects on development

In the developmental toxicity study in rabbits (A6.8.1/01[redacted]), four groups of 14 mated HY/CR New Zealand White rabbits were dosed by gavage with DBNPA in water, at dosages of 2, 10, 30 or 60 mg/kg bw/d, daily from Day 7 to Day 19 post coitum, consistent with concurrent EPA guidance to cover the period of organogenesis although current OECD guidance recommends extending the dosing period until the day before caesarian kill. The 30 mg/kg bw/d dose group was initated in response to excessive mortality at the 60 mg/kg/day dose level, and consisted of 15 females as one dead animal (dose administration error) was replaced. Dose volume was 10 mL/kg bw. A fifth group of 14 additional animals served as a vehicle control. The group size was consistent with concurrent EPA guidance although lower than current OECD recommendations (>16 females with implantation sites). Given the high pregnancy rate (≥12 pregnant/group) is the number of animals adequate for evaluation, with the exception of the 60 mg/kg/day dose level where mortality/euthanasia of 6/14 females resulted in insufficient litters (7) for evaluation. This, and current advice in OECD TG 414 suggesting termination of any dose group showing excessive toxicity, render this group invalid for the purposes of classification and labelling.

Test compound formulations (solutions in distilled water) were tested for stability and achieved concentration.

Maternal body weight was recorded on Days 0, 3, 22, 25, 29 of gestation and daily during the treatment period, food intake was recorded at gestation day intervals of not more than 4 days and all animals were examined for clinical signs daily. On Day 29 of gestation, all females were subject to caesarean kill. The uterine contents were examined and all live fetuses were subsequently examined for external, soft tissue (including brain transverse-section) and skeletal abnormalities. Differences among experimental groups were analysed for statistical significance.

Animals dosed at 60 mg DBNPA/kg bw/d showed severe maternal toxicity and an increased mortality (6 out of 14 animals). The surviving animals had a reduced food consumption and reduced body weight gain with associated reduction in quantity of faeces. From all other groups (0, 2, 10 and 30 mg/kg bw/d) at least 12 animals were pregnant at scheduled kill (day 29 post coitum). Necropsy of the surviving fetuses from the high dose group revealed slightly smaller mean fetal weights, mean crown-rump lengths and proportions of fetuses defined as “small” relative to controls. However, the study author considered these findings as not toxicologically relevant. At necropsy, all dams showed gastric abnormalities of ulcerative /haemorrhagic gastritis or gastroenteritis and two had pulmonary oedema. Food consumption was statistically significantly reduced among surviving animals in the 60 mg/kg bw/d group. Body weight gain during the dosing period in the 60 mg/kg bw/d group was statistically significantly lower than controls

Since current OECD TG 414 states ‘the highest dose should be chosen with the aim to induce some developmental and/or maternal toxicity (clinical signs or a decrease in body weight) but not death or severe suffering’ this dose level is considered invalid for the assessment of developmental toxicity. Nevertheless, despite such severe maternal toxicity, developmental effects were limited to a sligth increse in the number of small fetuses (< 30.0 g) as well as marginal reductions in fetal weight and crown rump length. There was an associated increase in the incidence of fetuses with incomplete ossification (eg hyoid, sternebra 5, long bone epiphyses), but only at an incidence which was within the historical control range for the laboratory. There was no adverse effect on litter size, post-implantation loss or fetal malformations. One fetus was found to have a cardiac syndrome (rudimentary pulmonary artery, enlarged aortic arch and agenesis of the interventricular cardiac septum) which was also found in one low dose fetus, but historical control data demonstrated that similar defects occurred spontaneously in control animals from this strain before and after this study. However, a definitive assessment of developmental toxicity at this dosage is not possible owing to the small number of litters/foetuses available for evaluation.

In contrast, treatment at 30 mg/kg/day produced minimal maternal toxicity, typical as a high dose for this type of study. Although no statistically significant changes were apparent in the body weight intervals analysed, toxicologically relevant changes occurred: Body weight gain was reduced, particularly in the first few days of treatment and mean weight gain for the treatment period (Days 7 to 19 of gestation) was 56% of control values (42g vs 75g). All females showed some weight loss.After treatment ceased, mean weight gain at 30

mg/kg bw/d showed some recovery. Food intake was lower than pre-treatment values once dosing commenced and was approximately 90% of control values over the treatment period. Food intake was comparable to controls immediately after dosing ceased but declined to approximately 80% of control values prior to termination.

One female died owing to accidental administration of test material into the lungs and was replaced. There were no treatment-related deaths and there were no significant clinical signs. One female aborted on Day 27 of gestation and was killed. The report suggests the timing of the abortion was unrelated to treatment owing to the duration off-dose but, as all foetuses had died in utero some time before the abortion, this explanation is questionable. However, a single occurrence of abortion in the group is unlikely to be a treatment effect.

Despite these maternal effects, at caesarian examination, there was no effect on litter size, post-implantation loss, placental weight, fetal weight or crown/rump length. No malformations were observed but there was a slightly increased incidence (compared to the concurrent control) of small foetuses and foetuses with incomplete ossification of some skeletal elements (eg sternebra 5 and/or xiphisternum, long bone epiphyses) and/or articulation of the ilium from the first to the second sacral vertebra. No direct effect of treatment was concluded, as many of these findings were observed in the litters of females which lost weight over the treatment period and all group incidences were within the laboratory historical control range for the strain.

There was no effect on treated females or their offspring at 2 or 10 mg/kg bw/d. A slight increase in the incidence of foetuses with bilateral lumbar rib at 10 mg/kg/d was considered incidental as values were within the range of spontaneous variation demonstrated by the historical control range for the strain and laboratory.

Table 66: Maternal effects in developmental toxicity study (A6.8.1/01[redacted])

DBNPA	**Groups concentration**	**historical control data**	**1**	**2**	**3**	**5**	**4**	**dose-response +/-**
	targeted dose [mg/kg bw/d]	**0**	**0**	**2**	**10**	**30**	**60**	
Number of animals/group		-	14	14	14	15	14	
Clinical signs		-						
no abnormality detected			8	7	12	11	2 (↓ *)	-
few or no faeces			0	0	0	2	7 (↑ **)	+
nasal discharge			5	6	1	0 (↓ *)	1	-
Mortality (and killed in extremis)		-	0	0	0	1[a]	6 (↑ **)	+
Mean Food consumption (g)		-						-
Days 8-10 p.c.			185	191	174	169	67 (-63.78 %)***	
Days 11-14 p.c.		-	175	186	171	156	68 ***	+
Days 15-19 p.c.		-	180	186	188	162	111***	+
Mean Body weight gain (g)		-						
Days 7-19 p.c.			75	108	82	42 (↓)	-93 (↓ **)	+
Days 7-13 p.c.		-	-16	48	-2	-33	-131	+
Days 13-19 p.c.		-	91	61	84	75	38	+
Days 19-29 p.c.		-	149	185	198	173	223	-
Number Pregnant (includes decedents)		-	13	14	14	14	11	-
Number Aborted		-	0	1	0	1	0	-
Number of litters for evaluation		-	13	13	14	12	7	+
Mean weight of gravid uterus (g)		-	498.9	609.8 (↑ *)	572.8	525.2	449.0	-

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

p.c. post coitum

[a] mortality due to lung-dosing

Table 67: Developmental effects – Examination of foetuses/litter distribution (A6.8.1/01[redacted])

Number of foetuses affected (%)/**number of litters affected**								
DBNPA	**Groups** **Concentration**	**historical control data**	**1**	**2**	**3**	**5**	**4**	**dose-response +/-**
	targeted dose [mg/kg bw/d]	**0**	**0**	**2**	**10**	**30**	**60**	
Number of foetuses/litters examined		1316/149[A]	108/13	138/13	137/14	107/12	58/7	
Necropsy Small fetus <30.0 g			6 (5.6 /5	9 (6.5)/5	6 (4.4)/3	9 (8.4)/4	7 (12.1)/4	-
Visceral malformations: Cardiac syndrome (rudimentary pulmonary artery, enlarged aortic arch and agenesis of the interventricular cardiac septum)		0-0.9[1]	0	1	0	1	0	-
Hydrocephaly, brachygnathia, arthrogryposis		NR	1	0	0	0	0	-
Skeletal anomalies: Incomplete ossification of frontal bone			0 (0)	0 (0)	0 (0)	1 (0.9)	0 (0)	-
Incomplete ossification of parietal bone			1 (0.9)	0 (0)	0 (0)	0 (0)	0 (0)	-
Incomplete ossification of interparietal bone			1 (0.9)	1 (0.7)	3 (2.2)/**2**	3 (2.8)/**2**	0 (0)	-
Bilateral lumbar rib		43.0-65.7 %[4]	47 (43.5)/**12**	61 (44.2)/**13**	97(70.8)/**14*****	62 (57.9)/**12***	28 (48.3)/**6**	-
Incomplete ossification of sternebra 5 and/or xiphisternum		0.0-36.8 %[2]	20 (18.5)/**6**	29 (21.0)/**10**	35 (25.5)/**11**	43 (40.2)/**12*****	20 (34.5)*/**5**	-
Ileum articulating with 1st or 1st+2nd sacral vertebra bilateral		11.6-57.7 %[5]	57 (52.8)/**12**	70 (50.7)/**12**	50 (36.5)*/**10**	39 (36.4)*/**12**	23 (39.7 %)/**7**	-
Incomplete ossification of long bone epiphyses		10.7-35.9 %[3]	22 (20.4)/**7**	1 (22.5)/**10**	17 (12.4)/**7**	37 (34.6)* /**9**	18 (31.0)/**7**	-
Incomplete ossification of hyoid bone		NR	21(19.4)/**9**	21 (15.2)/**9**	20 (14.6)/**6**	19 (14.0)/**7**	19 (32.8)/**4**	-
Mean placental weight (g)		-	5.5 ± 0.8	5.2 ± 0.6	5.3 ± 1.0	4.7 ± 0.8	5.2 ± 0.8	-

Fetal weight (g)	-	42.2	41.7	42.7	42.4	40.0	-
Crown/rump length (mm)	-	93.5	92.7	94.1	95.5	90.9	-
Live fetuses	-	8.3	9.9	9.8	8.9	8.3	-
Early resorptions	-	0.5	0.9	0.1	0.9	0.6	-
Late resorptions	-	1.1	0.4	0.7	0.9	0.6	-
Total resorptions	-	1.6	1.2	0.8 (↓ *)	1.5	0.7	-
Post-implantion loss	-	15.9	10.9	7.5	8.0	14.0	-

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$ $^{\Delta}$ Inlcuding 108/13 from present study, data obtained Jan 1985 –Jul 1988 at the same laboratory.

NR – not reported

[1] Historical control incidence of “major cardiac malformation”, Addendum 1

[2] Historical control incidence of “reduced or incomplete ossification of sternebrae 5 and/or xiphisternum”, Addendum 1

[3] Historical control incidence of “reduced ossification of long bone epiphyses”, Addendum 1

[4] Historical control incidence of “lumbar rib present bilaterally”, Addendum 1

[5] Historical control incidence of “ileum articulating with 1st or 1st + 2nd sacral vertebra bilaterally”, Addendum 1

As the developmental effects seen in the rabbit development study were minimal, even at a dose eliciting severe maternal toxicity, and as the rabbit is likely to be the most sensitive species, a developmental toxicity study in rats was considered unjustified.

In the rat multigeneration study, litter size was slightly smaller than controls in the F_0 generation but comparable to the historical control range. There were no adverse effects on pup weight, viability, growth or development in either generation, including reproductive capacity of the F_1.

For further details on the studies, please refer to annex II to this report.

10.10.6 Comparison with the CLP criteria

The highest dose level (60 mg/kg bw/d) for the rabbit developmental toxicity study is considered invalid for classification and labelling purposes as the maternal reaction to treatment, including mortality/morbidity and weight loss over the treatment period, was too severe to provide meaningful assessment of developmental effects and as the number of litters/foetuses from surviving females was too low for full fetal evaluation.

At 30 mg/kg/day, the maximum feasible dose, minimal maternal toxicity was demonstrated by reduced weight gain and food intake. There were, however, no effects on litter size, post-implantation loss, placental weight, fetal weight or crown/rump length. There were no malformations and slight increases over control in the incidence of incomplete ossification of some skeletal elements were considered unrelated to treatment.

Developmental effects following DBNPA administration to rabbits were within the historical control range and considered unrelated to treatment.No effects on development were noted after administration of DBNPA to rats. Therefore, classification is not required.

10.10.7 Adverse effects on or via lactation

No adverse effects identified.

10.10.8 Short summary and overall relevance of the provided information on effects on or via lactation

The two generation reproduction study did not identify any effects on pup viability which would suggest an adverse effect on or via lactation.

10.10.9 Comparison with the CLP criteria

Classification for effects on or via lactation can be assigned on the basis of human evidence, results from one or two generation animal studies, or absorption, metabolism, distribution and excretion studies.

10.10.10 Conclusion on classification and labelling for reproductive toxicity

In the absence of evidence for reproductive or developmental effects at dose levels not inducing severe maternal toxicity, classification and labelling are not required.

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.

Additional key elements

A summary of an additional PNDT study in the rat (A6.8.1/03) that was omitted in the CLH report is provided below.

PNDT study in the rat		
Type of study; Reference; Year	**Method**	**Observations**
PNDT study A6.8.1/03 2013	OECD TG 414 GLP Substance: DBNPA, purity 99.87% Rat, strain Crl:CD(SD) Doses: 0, 3, 10, 30 mg/kg bw/d Gavage Dosing GD 6-21 24 females/group Toxicokinetic investigations: blood levels of cyanoacetamide, bromide and chloride in dams and foetuses; blood collected at terminal sacrifice (1-2 hours post-dosing)	<u>Maternal toxicity</u> 30 mg/kg bw/d: • Mortality 2/24 (1 spontaneous, 1 sacrificed due to laboured respiration) – both animals had multifocal congestion of the lung at necropsy; noisy respiration 4/24 ≤ 10 mg/kg bw/d: no adverse effects <u>Developmental toxicity</u> ≤ 30 mg/kg bw/d: no adverse effects <u>Toxicokinetic investigations</u> A dose-related increase in cyanoacetamide and bromide levels in both dams and foetuses; no effect on chloride levels

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**.

10.11 Specific target organ toxicity-single exposure

Table 68: Summary table of animal studies on STOT SE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
Not applicable based on the available data			

Table 69: Summary table of human data on STOT SE

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference
No data available				

Table 70: Summary table of other studies relevant for STOT SE

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

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

Relevant data for DBNPA are presented in the acute oral, dermal and inhalation toxicity studies, discussed in the sections 10.1-10.3. No acute neurotoxicity studies are available.

10.11.2 The acute effects of DBNPA at doses below the LD50 value, when administered orally, include decreased motor activity, diarrhoea, hunching and signs of gastric perforation with adhesion with the liver. The acute effects of DBNPA at doses below the LC50 value following inhalation include laboured breathing (during administration), noisy respiration, mouth breathing, decreased activity and snezzing. No pathological effect were noted at these dose levels. *Confidential*Comparison with the CLP criteria

Classification with STOT SE is appropriate for substances showing clear evidence of toxicity to a specific organ following a single exposure, especially where this is seen in the absence of lethality. Specific target organ toxicity (single exposure) is defined as specific, non-lethal target organ toxicity arising from a single exposure (Section 3.8.1.1 of Annex I of the CLP Regulation).

The effects following single oral exposure indicate a generalized toxic event rather than specific target organ toxicity by single exposure and the effects following single inhalation exposure are not severe enough to warrant classification of STOT SE 3 (RTI), although some irritation is indicated.

Criterias for classification as STOT SE are not met according to Regulation (EC) No 1272/2008. Classification of the substance as Acute Tox 3 (oral), Acute Tox 2 (inhal), Eye Dam 1, Skin Irrit 2 is considered to cover and communicate specific toxicological effects.

10.11.3 Conclusion on classification and labelling for STOT SE

Classification for specific target organ toxicity by single exposure is not required under the terms of Regulation (EC) No 1272/2008 and subsequent amendments to that legislation, based on the available data.

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**.

10.12 Specific target organ toxicity-repeated exposure

Table 71: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
28 d oral (dose range finder) Reliability = 2	DBNPA Rat CD male, female 5/group Dose 0, 1, 5, 10, 25, 50 mg/kg bw/d Daily	Dyspnoea was observed among rats of the high dose groupdue to the irritating properties of DBNPA when administrated via gavage. Rats in medium and low dose groups showed no treatment-related findings. LO(A)EL = not relevant NO(A)EL = not relevant	[redacted] (A6.3.1/01)
28 d oral (dose range finder) Reliability = 2	Dog Beagle male, female 2/ group 0, 0.03%, 0.15% (dietary application, equivalent to 0, 3.8, 17.5 and 0,3.5, 15.9 mg/kg bw/d for males and females, respectively) Continuous	In clinical chemistry parameters an increase in aspartate aminotransferase (AST) in males and females given 0.15% DBNPA was noted. However, the slight increase in AST value was not considered adverse because the value was only slightly above the historical control, there was no histopathologic correlate to the higher AST values and no elevated AST values was observed in 90-day study in dog.LO(A)EL = not relevant NO(A)EL = not relevant	[redacted] (A6.3.1./02)
90 d oral Reliability = 1	Dog Beagle male, female 4/group Target dietary concentrations: 0, 0,045, 0.09, 0.15%; Target dose: 0, 15, 30, 50 mg/kg bw/d achieved intake: males: 0, 5.9, 11.6, 18.3 mg/kg bw/d; females: 0, 6.1, 10.7, 18.3 Continuously	Males and females given 0.09 and 0.15% DBNPA in the diet had treatment-related, statistically significant higher absolute and relative thyroid weights. No dose relationship was found in females. In males given 0.15% DBNPA and in females given 0.09% or 0.15% DBNPA an increase in the incidence of very slight dilatation of thyroid follicles could be observed, at microscopic pathology. These alterations were characterized by a very slight increase in the amount of colloid in most of the follicles of the thyroid gland. No evidence of degradation, inflammation, necrosis, or a proliferative response of the thyroid follicular epithelium or parafollicular cells was observed in any animal from the treatment or control groups. LO(A)EL = 0.09% (male: 11.3 mg/kg bw/d female):10.7 mg/kg bw/day NO(A)EL = 0.045% (male: 5.9 mg/kg bw/d; female: 6.1 mg/kg bw/d)	[redacted] (A6.4.1/01)

90d oral Reliability = 1	Rat Fischer 344 male, female 10/group Target dietary doses: 0, 3, 10, 100, 300, 600, 1000 mg/kg bw/ achieved intake: males: 0, 1.22, 4.6, 45, 133, 245, 388 mg/kg bw/d; females: 0, 1.17, 4.3, 44, 130, 251, 392 mg/kg bw/d Continuously	Reduced body weight (gains) in males receiving 300, 600, 1000 mg/kg bw/d and females receiving 600, 1000 mg/kg bw/d (indicating an exceedance of the maximum tolerated dose). Sacrifice of males (600, 1000 mg/kg bw/d) and females (1000 mg/kg bw/d). Reduced feed consumption for males given ≥ 300 mg/kg bw/day and females given ≥ 600 mg/kg/day. Clinical signs in males and females given 600, 1000 mg/kg bw/day and females receiving 300 mg/kg bw/d. Increased Hb concentration in males at 100 or 300 mg/kg bw/d. In males at 300 mg/kg bw/d lower RBC count and higher MCV. Splenic erythroid extramedullary hematopoiesis and erythroid hyperplasia of the bone marrow in males at 300 mg/kg/d. Altered Hgb and RBC count values were within or very close to the historical control range. Increase in urinary ketones in males and females given ≥ 100 mg/kg bw/d. Increased relative weight of the spleen in males at 100, 300 mg/kg bw/d. Increased relative spleen weight in females at 300, 600 mg/kg bw/d. Increased absolute spleen weight in females at 100 and 300 (but not 600) mg/kg bw/d. Increased eosinphilic staining of the hepatocellular cytoplasm in females at 600 mg/kg bw/d. Increased red blood cell production in the bone marrow and spleen of males at 300 mg/kg bw/d and females at 100 mg/kg bw/d and above. The bone marrow of males at 300 mg/kg bw/d and females at 600 mg/kg bw/d had erythroid hyperplasia. Erythrocytic extramedullary hematopoiesis of the spleen in all males at 300 mg/kg bw/d and all females at 600 mg/kg bw/d. Erythrocytic extramedullary hematopoiesis of the spleen in four females at 100 mg/kg bw/d and nine females at 300 mg/kg bw/d. Atrophy of thymus, ovaries, cervix and uterus occurred in females at 600 mg/kg bw/d. LO(A)EL = 300 mg/kg bw/d (equivalent to 133 and 130 mg/kg bw/d for males and females, respectively) NO(A)EL = 100 mg/kg bw/d (equivalent to 45 and 44 mg/kg bw/d for males and females, respectively)	[redacted] (A6.4.1./02)

90d oral Reliability = 1	Mice CD-1 male, female 10/group Target dietary doses: 0, 3, 10, 100, 300, 600, 1000 mg/kg bw/ achieved intake: males: 0,1.58, 4.4, 44, 133, 267, 447 mg/kg bw/d; females: 0, 1.57, 4.5, 45, 137, 269, 450 mg/kg bw/d Continuously	Decreased body weight (gains) in males at 600, 1000 mg/kg bw/d, in females at 300, 600, 1000 mg/kg bw/d. Decreased RBC count, Hb and Hct in males at 1000 mg/kg bw/d were secondary to reduced body weight and body weight gain. LO(A)EL = 600 mg/kg bw/d (males, equal to 267 mg/kg bw/d) 300 mg/kg bw/d (females, equal to 137 mg/kg bw/d) NO(A)EL = 300 mg/kg bw/d (males, equal to 133 mg/kg bw/d) 100 mg/kg bw/d (females, equal to 45 mg/kg bw/d)	[redacted] (A6.4.1/03)
90d oral Reliability = 1	Rat CD male, female 20/group 0, 5, 13, 30 mg/kg bw/d daily gavage application	Reduced body weight in high dose males and females during the first days. Dyspnoea in high dose males and females and in one mid dose female. Macroscopic and histopathological findings associated with dyspnoea. Dyspnoea was considered to be related to dosing of DBNPA by gavage. Increased relative and absolute adrenal weight in high dose females. LO(A)EL = 30 mg/kg bw/d NO(A)EL = 13 mg/kg bw/d	[redacted] (A6.4.1/04)
90d oral Reliability = 2	Rat Sprague Dawley male, female 10/group 0, 20, 100, 500 ppm in drinking water at pH 4: intake males: 0, 2.4, 11, 49 mg/kg bw/d; females: 0, 3.7, 17.3, 75.8 mg/kg bw/d at pH 8: intake males: 0, 2.3, 10.8, 42 mg/kg bw/d; females: 0, 3.5, 15.9, 63.3 mg/kg bw/d continuously	Reduced water consumption in mid (pH 8) and high (pH 4, 8) dose male and high dose (pH 4, 8) female rats. Increased relative kidney weights in high dose females at pH 8. Increased relative liver weights in mid and high dose males and high dose females at pH 8. Increased incidence of minimal cytoplasmic swelling and vacuolization of renal tubular epithelial cells in high dose females at pH 4 and 8. LO(A)EL = 500 ppm (equal to 42-76 mg/kg bw/d) NO(A)EL = 100 ppm (equal to 10.8-17.3 mg/kg bw/d)	[redacted] (A6.4.1/05)
28d dermal (dose range finder) Reliability = 2	Rat Fischer 344 male, female 5/group 0, 2.5, 5, 10, 20 % in 2 mL vehicle (week 1-2, resulting in 0, 57, 115, 241, 515 mg/kg bw/d) or 4 mL vehicle (week 3-4, resulting in 0, 115, 233, 482, 1030 mg/kg bw/d) 6 h/d, 5 d/week	Some rats in the 5, 10 and 20 % dosing groups showed a slight and inconsistent dermal irritation at the application site consisting of very slight to well-defined erythema, very slight to moderate oedema, scabbing and scarring for males, and only very slight erythema for females. In general males were more affected at the application site than females. Organ weights were comparable to controls. There were decreased body weights in all dose groups but not in a dose dependent manner. LO(A)EL = not relevant NO(A)EL = not relevant	[redacted] (A6.3.2/01)

90d dermal Reliability = 1	Rat Fischer 344 male, female 10/group Sham control, vehicle control (TEG, 3 mL/kg bw/d), 0.4, 1.2, 4 mL/kg bw/d (equal to 103, 309, 1031 mg/kg bw/d) 6 h/d, 5 d/week (20 % DBNPA in vehicle)	Topical response limited to the application site characterised by erythema, edema and scabs/crust in high-dose males and females. Only sporadic and incidental findings in the mid and low dose not related to treatment. Histopathological findings (epidermal hyperkeratosis, inflammation and erosion/ulceration plus dermal inflammation and necrosis) in high dose males and females. Signs of systemic toxicity were observed in the female high dose group as reduced weight vs. vehicle control. LO(A)EL = Local: 1031 mg/kg bw/d Systemic: 1031 mg/kg bw/d NO(A)EL = Local: 309 mg/kg bw/d Systemic:309 mg/kg bw/d	[redacted] (A6.7)
Diet (2 year) Reliability = 1	Rat F344 Male, female 60/group 3,20, 150 mg/kg bw/d targeted dose levels; 1.4, 9.551, 71.32 mg/kg bw/d actual dose levels in diet for 2-years	Treatment-related very slight to slight, diffuse hyperplasia of the thyroid follicular cells was present in males and females given 150 mg/kg/day, and in males given 20 mg/kg/day. Treatment-related thyroid follicular hyperplasia was also present at the 12-month sacrifice in males and females given 150 mg/kg/day. The hyperplasia did not appreciably worsen during the final 12 months of the study. Females given 150 mg/kg/day had treatment-related lower body weights and feed consumption and liver effects (slight, fatty vacuolization). The 24 month liver changes were however not accompanied by increases in serum ALT and AST. LO(A)EL = 20 mg/kg bw/d (males, equal to 9.6 mg/kg bw/day). 150 mg/kg bw/d (females, equal to 71.3 mg/kg bw/day) NO(A)EL = 3mg/kg bw/d , (males, equal to 1.4 mg/kg bw/day). 20 mg/kg bw/d , (females, equal to 9.551 mg/kg bw/day)	[redacted] (A6.4.2/01)

Diet (12 months) neurotoxicity Reliability = 1	Rat F344 Male, female 10/group 3,20, 150 mg/kg bw/d targeted dose levels; 1.4, 9.551, 71.32 mg/kg bw/d actual dose levels in diet for 2-years.	There was a statistically significant, treatment-related decrease in body weight of males given 150 mg/kg/day (10%) as well as a statistically significant, treatment-related decrease in hindlimb grip performance of males and females given 150 mg/kg/day when compared to controls (<20%). All other FOB and neurotoxicity endpoints were no different in treated animals versus controls. Due to the small maginitude of change in grip performance (<20%), the non-specific nature of this endpoint (i.e., influenced by neuromuscular function, general systemic well being etc.), and the lack of correlating findings in other neurobehavioral and neuropathological endpoints it is unclear whether the effect on hindlimb grip performance represented a specific effect on the nervous system. LO(A)EL = 150 mg/kg bw/d (actual dose 71.3 mg/kg bw/d) NO(A)EL = 20 mg/kg bw/d (actual dose 9.6 mg/kg bw/d)	[redacted] (Section A6.5)

Table 72: Summary table of human data on STOT RE

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference
No data available				

Table 73: Summary table of other studies relevant for STOT RE

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

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

In the following sections, the studies summarised in table 47 are described in relation to relevance for classification as STOT RE according to CLP criteria.

Oral route

Short-term studies
In a 28-day dose range-finding rat study, 5 animals/sex/dose were treated with 0, 1, 5, 10, 25 and 50 mg/kg bw/day DBNPA adminitered by gavage. Animals were observed daily for clinical signs and mortality. Food and water consumption were determined weekly, whereas body weight was noted on the first day of treatment, twice weekly thereafter and at termination. At termination, all surviving rats were killed and subjected to necropsy. Liver , spleen, kidneys, and heart were weighed.

Dyspnoea occurred among rats of the high dose group of 50 mg/kg bw/d. This reaction was observed at two males after 4-5 days of treatment. One of these rats was found dead three days after the first recording of these signs. Three females of the same dosewas also found dead after 18 to 22 days of treatment. All surviving males of the high dose group were sacrificed on day 7 of the study, whereas surviving females were sacrificed on day 22.

At necropsy, the macroscopy of the animals found dead showed lesions that were characterized by tympanism of the gastrointestinal tract and histopathological findings in the gastrointestinal tract and lungs that are associated with the dyspnoea. According to these findings, it was concluded that dyspnoea was not a substance-related finding, but is attributed to the irritating properties when adminstered via gavage dosing. These finding occurred only in gavage studies; dermal, feeding and drinking water studies show no comparable signs, even with higher dose levels. Rats in medium and low dose groups showed no treatment-related findings.

Therefore, it is concluded that the dyspnoea is related to the irritating properties of DBNPA when administered via gavage. Thus this finding should not be considered for characterisation of human health effect and is not relevant for NOAEL deduction in the respective studies.

Table 74: Mortality and clinical signs (A6.3.1/01 [redacted])

Dosage group (50 mg/kg bw/day)	Animal	Clinical signs	Day of death	Circumstances of death
Female	1/5	Dyspnoea (day 4-5)	6	Killed in extremis
	1/5	Dyspnoea (day 5-6)	7	Found dead in cage
	3/5	No abnormalities detected	7	Killed on humane grounds
Male	3/5	Dyspnoea (day 18, 19 and 22)	19 and 22	Killed in extremis
	2/5	No abnormalities detected	22	Killed on humane grounds

A 4-week study A6.3.1./02 [redacted] was performed with 2 Beagle dogs/sex/dose treated with DBNPA (0, 0.075 and 0.15 % in the diet corresponding to dose levels of approximately 0, 3.8, or 17.5 mg/kg bw/day in males and 0, 3.5, or 15.9 mg/kg bw/day in females after applying a test material degradation factor). Mean feed consumption was initially reduced in males and females from both dose groups, but was similar to control animals by day four. There were no treatment-related findings on clinical appearance, ophthalmologic

examinations, body weights, haematology, urinalysis, organ weights, or gross and histopathologic examinations. In clinical chemistry parameters an increase in aspartate aminotransferase (AST) in males and females given 0.15% DBNPA was noted. This value was only slightly above the historical control values and there was no histopathologic correlate to the higher AST values.

Table 75: Mean Aspartate Aminotransferase (AST) Data (A6.3.1./02[redacted])

	Dose (%)			
Parameter	**0**	**Historical controls[1]**	**0.03**	**0.15**
AST (u/l) males pre-Study	31	29-40	38	35
AST (u/l) males 4 weeks	34	33-46	44	54
AST (u/l) females pre-Study	37	27-42	34	31
AST (u/l) females 4 weeks	42	32-45	49	53

AST Aspartate; Statistical significance not performed

[1] Historical controls group mean range from eleven dietary studies with pre-study data and six 28-day dietary studies done since 1999.

Sub-chronic studies

A total of five oral 90-day studies were presented (feeding dog (A6.4.1/01[redacted]), feeding rat (A6.4.1./02[redacted]), feeding mice (A6.4.1/03[redacted]), gavage rat (A6.4.1/04[redacted]) and drinking water rat ([redacted] A6.4.1/05)).

In the 90-day feeding study in rats (A6.4.1./02[redacted]), eight groups of Fischer 344 rats (10/sex/dose) were treated for 90 days with DBNPA in the diet. The targeted doses were 0, 3, 10, 100, 300, 600, or 1000 mg DBNPA/kg bw/day. Test material intake based on body weights, feed consumption and dietary concentrations corrected for test material degradation were as follows: 0,1.22, 4.6, 45, 133, 254, and 388 mg/ kg bw/day for males and 0, 1.17, 4.3, 44, 130, 251, and 392 mg/kg bw/day for females.

Parameters evaluated included in-cage observations, detailed clinical observations, ophthalmologic examinations, body weight, feed consumption, haematology, clinical chemistry, urinalysis, organ weights, gross and histopathological examinations.

The maximum tolerated dose was exceeded for males given 300, 600, or 1000 mg/kg bw/day and females given 600 or 1000 mg/kg bw/day. based on > 22 % decrement in body weight gains and adverse clinical signs. The male and female 1000 mg/kg bw/day and male 600 mg/kg bw/day dose levels were terminated due to these decrements. No statistically significant decreases in body weight or body weight gain were noted in females, given 0, 3, 10, 100, and 300 mg/kg bw/gain and in males given 0, 3, 10 and 100 mg/kg bw/day.

On the contrary, females in the 100 mg dose group have a significantly increased weight from day 37 and onwards without a corresponding increase in feed intake. In the female 10 mg and 300 mg dose groups at occasional time points weight is significantly increased over controls. Weight gain means have not been statistically compared to controls but for female animals in the following dose groups (3, 10, 100 and 300 mg) weight gain is markedly increased over controls (14-24%).The effect was however not assessed as dose-response related as these observations was only made at occasional time points. NOAEL is not established on basis of this effect due to the non adverse nature of the effect when comparing to the effects observed in other repeated dose feeding studies.The effect observed in the 2 year study was lowered body weight and food consumption at 150 mg/kg further indicating the non adverse nature of the weight increase effect seen in this study. ConfidentialMean feed consumption values for males given ≥ 300 mg/kg bw/day and females given ≥ 600 mg/kg/day were statistically significant lower than controls throughout the study. Mean feed consumption values of 3, 10 and 100 mg/kg bw/day dose groups were equal to control groups.

In males and females given 1000 mg/kg bw/day, signs of toxicity included an abnormal gait, thin and ungroomed appearance, piloerection, periocular and perineal soiling. Males showed additional signs consisting of decreased muscle tone, decreased responsiveness to touch, decreased activity, decreased feces, and dehydration. Females occasionally displayed decreased thrust response, unilateral lacrimation, decreased muscle tone, decreased responsiveness to touch, and decreased activity. In females and males given 600 mg/kg bw/day periocular soiling was observed. Occasionally, males from this dose group showed decreased thrust response, bilateral lacrimation and dehydration, whereas signs of toxicity included ungroomed appearance and lacrimation in females. In females given 300 mg/kg bw/day periocular soiling was observed throughout the study andperineal soiling occurred towards the end of the study. Clinical observations noted in males given ≤ 300 mg/kg bw/day or females given ≤ 100 mg/kg bw/daywere infrequent and sporadic and thus not toxicologically relevant.

Periocular soiling was increased in females given ≥600 mg/kg bw/day and males given ≥100 mg/kg bw/day during the terminal ophthalmascopic examination on day 87 and was attributed to exposure to DBNPA. Males given 100 or 300 mg/kg bw/day had statistically significantly higher haemoglobin concentrations and males given 300 mg/kg bw/day had a statistically significantly reduced RBC count than controls, but both were similar to the historical control range. Males exposed to 300 mg/kg bw/day and females exposed to 600 mg/kg bw/day showed increased mean corpuscular volume (MCV) suggested to reflect normal variability in this paramter. Increased erythroid production in the bone marrow and spleen (microscopic splenic erythroid extramedullary hematopoiesis and erythroid hyperplasia of the bone marrow) indicate a treatment-related effect of minimal toxicological significance.

These findings in haematology and spleen histopathology are considered to be of minimal toxicological relevance due to the following reasons:

- in the gavage and drinking water studies in rats comparable haematological findings or histopathological findings in spleen and bone marrow were not observed at similar dose levels (30-76 mg/kg bw/d).

- in the mouse feeding study red blood cell count and haematocrit were decreased at the high dose males only (447 mg/kg bw/d). In contrast to the findings in the rat feeding study the haemoglobin concentration was decreased instead of increased.

- the dog study showed no haematological findings or histopathological findings in the spleen.

The WBC count of females given 600 mg/kg bw/day was statistically significantly higher than the concurrent and historical controls This finding was attributed to inflammation of the Harderian gland noted in females of this dose group, and was interpreted to be not toxicologically relevant.

In males given 300 mg/kg bw/day, blood urea nitrogen (BUN), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) activities were significantly reduced. Toxicologically significant alterations of BUN, ALT, and AST are characterized by increases rather than decreases, indicating that these findings were not toxicologically relevant. Chloride (Cl) was significantly increased in males given 100 and 300 mg/kg bw/day; these increases were similar to historical control values. This difference was attributed to interference of plasma bromine, associated with test material administration, with the chloride electrode.

In females given 600 mg/kg bw/day, alkaline phosphatase (ALP) and cholesterol (Chol) were significantly increased, but within the historical control range and therefore not treatment related. Decreased creatinine in 600 mg/kg bw/day exposed females was not toxicologically relevant as toxicity is associated with increased creatinines. In females given 100, 300, and 600mg/kg bw/day, chloride values were statistically increased compared to control values; however, these increases were similar to historical control values. Increases in total protein and albumin in 300 mg/kg bw/day exposed females were considered not treatment relatedas no increase was observed in the 600 mg/kg bw/day dose group.

Males given 100 or 300 mg/kg bw/day had a higher incidence of ++ urinary ketones than the control or lower level rats. The incidences were outside of recent historical control values and were interpreted to be treatment related.

Females given 100, 300, or 600 mg/kg bw/day had a higher incidence of +, ++ and/or +++ urinary ketones, compared to the concurrent controls and lower dose levels, The incidence was within the range of historical controls for 100, but not 300 and 600 mg/kg bw/day exposed females. .

An interference of the ketone test with DBNPA or its metabolites is possible.

The final body weight of males given 300 mg/kg bw/day was statistically significantly lower (-14 %) than the control. The reduced absolute weights of heart, kidneys, liver, brain, epididymides and testes were attributed to the significantly lower body weight of these rats and did not reflect a primary target organ effect. This interpretation was supported by the absence of microscopic findings in these tissues. The relative weight of the spleen was increased in males given 100 or 300 mg/kg bw/day. This may have been treatment-related as extramedullary hematopoiesis was increased in the spleens of these animals.

The final body weight of females given 100 or 600 mg/kg bw/day were significantly increased (10%) and decreased (9%), respectively. In 600 mg/kg bw/day females, reduced absolute weights of adrenal glands, heart, kidneys, brain, ovaries, uterus and thymus were observed. In females given 300 mg/kg bw/day, significantly lower relative brain weights and higher absolute kidney, liver, and spleen weights were noted. In females given 100 mg/kg bw/day the weight of the kidneys (absolute), liver (absolute) and spleen (absolute and relative) were significantly higher than controls, whereas the relative brain weights were statistically significantly lower than controls. Reduced organ weight was attributed to the reduced animal weights. The lack of histopathological findings in these organs supported the interpretation that these changes were not treatment-related. However, the increased degree of extramedullary hematopoiesis in these animals indicated that differences in spleen weight may have been the result of DBNPA treatment.

At gross pathology bilateral periocular soiling occurred in seven of ten females given 600 mg/kg bw/day and was interpreted to be treatment-related. All other gross pathologic observations were interpreted to be spontaneous alterations (liver foci and dilatation of ovarian bursa) or were iatrogenic changes (aspirated blood secondary to decapitation) and not associated with treatment.

At histopathology females given 600 mg/kg bw/day showed an increase in slight eosinophilic staining of the hepatocellular cytoplasm. This alteration was probably due to an alteration in the proportion of subcellular organelles required to metabolize and excrete the test material. This finding is considered to be an adaptive change and not an indicator of toxicity.

Increased red blood cell production was noted in the bone marrow and/or spleen of males given 300 mg/kg bw/day and females given 100 mg/kg bw/day and above. The bone marrow of males given 300 mg/kg bw/day and females given 600 mg/kg bw/day had erythroid hyperplasia, which was very slight (males) or slight (females) in degree. This finding was accompanied by an erythrocytic extramedullary hematopoiesis of the spleen of a very slight (males) or slight (females) degree in all males given 300 mg/kg bw/day and all females given 600 mg/kg bw/day. A very slight or slight erythrocytic extramedullary hematopoiesis of the spleen was also noted in four females given 100 mg/kg bw/day and nine females given 300 mg/kg bw/day.

Very slight atrophy of the thymus occurred in the majority of females given 600 mg/kg bw/day and was considered secondary to the reduced body weight and body weight gain in these animals. The ovaries, uterus, and cervix of almost all females given 600 mg/kg bw/day were much smaller than the control tissues and were interpreted to reflect an atrophy of these organs due to the significant decrements in body weight and body weight gain. In this dose group 7 of 10 females showed inflammatory reactions in the Harderian glands. This finding was observed in the control and all lower dose groups with wide variability among the groups indicating that this was not a treatment related effect.

The maximum tolerated dose was exceeded in males and females when administering ≥300and ≥600mg/kg bw/d, respectively, on the basis of severe weight loss and clinical signs. Primary treatment related effects consisted of increased cytoplasmic eosinophilia of the hepatocytes of females given 600 mg/kg bw/day, a slight regenerative response of the bone marrow and/or spleen to produce RBS in males given ≥ 300 mg/kg bw/day and females given ≥100 mg/kg bw/day, and an increase in urinary ketones in males and females given ≥ 100 mg/kg bw/day. The NOAEL was 100 mg/kg/day (equivalent to 45 mg/kg bw/day in males and 44 mg/kg bw/day in females) based on the determination that effects at the 100 mg/kg bw/day dose level were minor and not considered adverse.

In the rat gavage study (A6.4.1/04 [redacted]) male and female Charles River CD rats (twenty/sex/dose) were treated with 0, 5, 13 and 30 (35 up to fifth day of study) mg/kg bw/day DBNPA (Biobrom C-103) by gavage according to OECD Guideline No. 408, (adopted 1981). DBNPA was solved and diluted in double distilled water daily and administered at a dose-volume of 10 mL/kg. Treatment continued for 13 consecutive weeks prior to sacrifice.

Animals were observed twice daily for clinical signs and mortality. Body weight was determined at the first day of treatment and twice weekly thereafter. Food consumption was calculated weekly by each cage and food conversion ratios were calculated for each week. Water consumption was evaluated daily by visual inspection of the drinking bottles and measured during week 2 and 10 of the study. Ophthalmic examination was made pre-exposure; rats of high dose and control group were re-examined during week 12. Clinical examinations (haematology, blood chemistry and urinalysis) were conducted during weeks 12-13.

Organ weights were examined and gross pathological and histopathological examinations were conducted at the scheduled necropsy.

Body weights, feed and water consumption, clinical examinations, absolute and relative organ weights were statistically analysed using Student´s t-test.

Body weight reduction and dyspnoea appeared in the first week of treatment with 35 mg/kg bw/day in one male and four females in the high dose group and was related to gavage application. One of the females died during the following week. Reduction of the dose in this group led to discontinuation of dyspnoea but at later stages of the study it was present in two males and seven females in the high dose groups and one female of the intermediate dose group. This resulted in the death of two females from the high dose group. The one female from intermediate dose group developed dyspnoea in week 14, considerably later than all females from the high dose group. For this animal no abnormality was detected in all organs examined. Females seem to be more sensitive under the conditions given in this study as no male from the intermediate dose group developed dyspnoea. Dyspnoea was considered to be related to application by gavage but not related to the test substance due to the fact that dyspnoea was not detected in any dietary study conducted with DBNPA.

Organ weights were not changed with the exception of statistically significantly increased absolute and relative adrenal weight in females of the high dose group. This response may be an adaptive response secondary to stress-induced dyspnea and associated gastrointestinal pathology as a result of gavage administration of DBNPA. This response is not considered relevant to human exposure circumstances and is not a reliable predictor of human hazard with respect to DBNPA. Effects on the adrenals was not seen in any of the other (sub)chronic studies or in other species, indicating that the adrenals are not a target organ of DBNPA toxicity.

DBNPA-treatment-related macroscopically detectable pathological alterations were confined to decedent animals. Signs of emaciation and meteorism of stomach, small and large intestine of one male and two female in the high dose group were observed and associated with mucosal congestion. Lungs were haemorrhagic and trachea occasionally filled with foamy fluid in four out of six decedent rats.

Histopathological changes were also limited to the decedent rats of the high dose group. One male showed an acute tracheitis associated with proteinaceous and cellular exudate in lumen and in one female the trachea was filled with proteinaceous and cellular exudate while in the lungs alveolar oedema was observed. The high dose female that died showed increased incidence of slight basophilic tubules in the kidney at necropsy.

No clinical findings were observed in the one decedent female from the intermediate dose group. Histological examinations were not conducted due to autolysis and cannibalism. Therefore decease of this animal can not be related to treatment.

The NOAEL in this study is 13 mg/kg bw/day based on the changes of absolute and relative adrenal weight in females seen at the LOAEL. The application-related finding dyspnoea should be not be considered for LOAEL/NOAEL derivation as this finding was only observed in gavage studies, but not in dietary studies even at higher dose levels.

In a 90 day-study feeding study in dogs (A6.4.1/01[redacted]) conducted according to OECD Guideline No 409, male and female beagle dogs (four/sex/dose) were fed diets formulated to provide 0, 0.045, 0.09, or 0.15% DBNPA. These concentrations corresponded to dose levels of approximately 0, 5.9, 11.6, and 18.3 mg/kg bw/day in males, and 0, 6.1, 10.7, and 18.3 mg/kg bw/day in females.

Evaluation included daily observations, detailed clinical observations, opthalmologic examination, body weight, feed consumption, water consumption, prothrombin time, hematology, clinical chemistry, urinalysis, selected organ weights, and gross and histopathologic examination.

There were no treatment-related findings in daily cage-side observations, clinical observations, ophthalmologic examinations, body weights, feed and water consumption, prothrombin times, haematology, clinical chemistry, urinalysis, or gross examinations at any dose level.

Males given 0.15% DBNPA had statistically significant lower absolute testicular weights, relative to controls. These findings were interpreted to be not treatment-related because the relative testicular weights of the high dose males were not statistically significantly altered, the absolute testicular weights were close to the historical control range, and there were no histopathological testicular alterations.

Males and females given 0.09 and 0.15% DBNPA had treatment-related statistically significant higher absolute and relative thyroid weights. A dose response relationship was found for absolute and relative thyroid weight in males, but not in females. The relative thyroid weight of high dose females was well within historical control range whereas the absolute thyroid weight of mid and high dose females and the relative thyroid weight of mid dose females was slightly above the historical control range. In males given 0.15% DBNPA and in females given 0.09% or 0.15% DBNPA a treatment-related increase in the incidence of very slight dilatation of thyroid follicles could be observed at microscopic pathology. These alterations were characterized by a very slight increase in the amount of colloid in most of the follicles of the thyroid gland. No evidence of degradation, inflammation, necrosis, or a proliferative response of the thyroid follicular epithelium or parafollicular cells in any animal from the treatment or control groups.

Considering the treatment related effects on the thyroid weight in combination with the very slight increase in the amount of colloid in most of the follicles of the thyroid gland, the effects could not be disregarded when establishing the NOAEL for the study at 5.9 mg/kg/day to ensure that the effects are covered by the risk assessment performed for the substance. However, the effect is only seen in the 90 days dog study and in the 2 year rat study, where the effect did not progress over time (see summary below), but not in other species or subchronic studies. The changes to the thyroid weight were within or close to the historical control ranges and no evidence of degradation, inflammation, necrosis or a proliferative response of the thyroid follicular epithelium or parafollicular cells was present at any dose level, indicating that no organ dysfunction is evident. It is therefore the conclusion of the RMS that there is not enough evidence to justify a classification as STOT RE on basis of the effects seen in the 90 day dog feeding study.

Table 76: Mean absolute and relative thyroid weight data (A6.4.1/01[redacted])

	absolute thyroid weight	**relative thyroid weight**	**absolute thyroid weight**	**relative thyroid weight**
	males		**females**	
Dose (%)	g	g/100	g	g/100
Historical[1]	0.6808-0.8103	0.0063-0.008	0.6245-0.7465	0.0084-0.0110
0	0.6818	0.0072	0.5768	0.0078

0.045	0.7123	0.0075	0.6600	0.0093
0.09	0.7510*	0.0082*	0.8768*	0.0116*
0.15	0.8993*	0.0097*	0.7525*	0.0094*
Dose response +/-	+	+	-	-

*statistically significantly different from controls, Dunnett´s test, α = 0.05

[1] Historical controls group mean range from four 13-week dog studies from 1999-2003

Table 77: Incidence of dilatation of thyroid follicles (A6.4.1/01)

	males	**females**
Dose (%)	**Thyroid Gland: Dilatation, Follicle, Diffuse, very slight**	
0	0	1
0.045	0	1
0.09	0	3
0.15	2	3

A6.4.1/05 performed a 90-day subchronic study, where drinking water containing 0, 20, 100 or 500 ppm DBNPA at pH 4 or pH 8 was administered to male and female Sprague-Dawley rats of Spartan strain (twenty/sex/dose). Corresponding doses at pH 4 were 0, 2.4, 11 and 49 mg/kg bw/day for males and 0, 3.7, 17.3 and 75.8 mg/kg bw/day for females. At pH 8 doses accounted for 0, 2.3, 10.8 and 42 mg/kg bw/day for males and 0, 3.5, 15.9 and 63.3 mg/kg bw/day for females. Treatment continued for 90 consecutive days prior to sacrifice.
Animals were observed weekly twice for appearance and behaviour. Animals were also observed for mortality. Body weight was determined twice weekly during the first month and weekly thereafter. Water consumption was measured twice weekly and food consumption weekly. Clinical examinations (haematology, blood chemistry and urinalysis) were conducted on day 86.

Organ weights were examined and gross pathologic and histopathologic examinations were conducted at the scheduled necropsy.

Body weights, feed and water consumption, clinical examinations, absolute and relative organ weights were statistically analysed using Student´s t-test.

There were no treatment-related and toxicologically relevant findings in general observations and food consumption. Increases of 7-8% in body weight in females in the 100 ppm pH 8 group were seen.This effect was however not dose-response related.The water consumption of male and female rats receiving water containing 500 ppm DBNPA at pH 4 and 8 was statistically significantly lower than that of controls throughout the experiment. Additionally, the water consumption of male rats receiving water containing 100 ppm DBNPA at pH 8 was statistically significantly lower than that of controls on several test days later in the study.

There were no significant differences between groups of either sex for any hematologic parameters measured. Changes in clinical chemistry parameters were variable and not dose-related and, therefore, considered to be not treatment-related.

The relative kidney weights but not absolute kidney weights of males maintained on water containing 20 and 100 ppm DBNPA at pH 4 were statistically significantly higher than that of controls, but not at 500 ppm DBNPA, indicating that this is not a treatment-related effect.

Slightly increased relative kidney weights but no increased absolute kidney weights were observed in females maintained on water containing 500 ppm DBNPA at pH 8.

The relative liver weights but not the absolute liver weights of males maintained on water containing 100 and 500 ppm DBNPA at pH 8 were statistically significant greater than that of controls. The same finding was observed for females from the 500 ppm pH 8 group. No corresponding findings were found at pH 4. In the absence of histological and physiological findings correlating with the increased relative liver weights these findings were considered to be incidental and not toxicologically relevant or adverse.

The gross and histopathological examinations revealed no compound related lesions in any of the rats maintained on water containing DBNPA at pH 4. At pH 8 the only lesion attributed to the compound or its degradation products was observed in the kidney of female rats receiving 500 ppm DBNPA. Histopathological observation of the renal tubular epithelial cells of these animals showed minimal cytoplasmic swelling and vacuolization. This response was not seen in the dietary studies (rats, mice and dogs) and may be of minimal significance for a response that is a frequent age-related change in rats. The response is not considered relevant for humans or for classification purposes.

The results of this study indicate that rats can tolerate 100 ppm DBNPA (corresponds to 10.8 to 17.3 mg/kg bw/day) or its degradation products in drinking water for 90 days without adverse toxicological findings. Only slight toxicological effects were observed at 500 ppm DBNPA (corresponds to 42 to 76 mg/kg bw/day) or its degradation products. The NOAEL is established at 100 ppm (equal to 10.8-17.3 mg/kg bw/d).

In the 90-day feeding study in the mouse (A6.4.1/03[redacted]), male and female CD-1 mice (ten/sex/dose) were treated with targeted doses of 0, 3, 10, 100, 300, 600 and 1000 mg/kg bw/day DBNPA according to OECD Guideline No. 408, (adopted 1998). Achieved (feed consumption, body weight and test material stability respected) DBNPA intake was 0, 1.58, 4.4, 44, 133, 267, or 447 mg/kg bw/day for males, and 0, 1.57, 4.5, 45, 137, 269, or 450 mg/kg bw/day for females.

Animals were observed daily twice for clinical signs and mortality. Body weight and food consumption were determined twice in week 1 and thereafter weekly. Ophthalmic examination were made pre-exposure, and prior to necropsy. Blood samples for haematology and clinical chemistry were collected at the scheduled necropsy.

Organ weights were examined and gross pathologic and histopathological examinations were conducted at the scheduled necropsy.

Body weight and body weight gains of males given 600 or 1000 mg/kg bw/day and females given 300, 600, or 1000 mg/kg bw/day were decreased by greater than 12 % of control values and were attributed to lower feed consumption due to unpalability of DBNPA-treated diets. Slight but statistically significantly increases in relative liver and spleen weights and decreases in absolute brain and testes weights were also noted in males given 1000 mg/kg bw/day and were considered secondary to the lower final body weight of these mice. No treatment-related gross pathologic findings or histopathologic effects were observed in these organs at any dose level .

Minor decrements in red blood cell count, hemoglobin concentration and hematocrit were noted in males given 1000 mg/kg bw/day. These effects were interpreted to be likely secondary to the decrement in body weight and feed consumption

There were no clear treatment-related gross pathologic or histopathological finding in any organ of either males or females given DBNPA.

The NOAEL was 100 mg/kg bw/day (equal to 45 mg/kg bw/day) for females and 300 mg/kg bw/day (equal to 133 mg/kg bw/day) for males based on decreased body weight and body weight gains at greater concentrations of DBNPA.

Chronic studies

[redacted] A6.4.2/01 performed a chronic toxicity/carcinogenicity combined chronic neurotoxicity study. Groups of 60 male and 60 female F344/DuCrl rats were fed diets formulated to provide 0 (control), 3, 20, or 150 mg DBNPA/kg body weight/day for up to two years. Ten rats/sex/dose level were necropsied after one year of treatment (chronic toxicity group), five rats/sex/dose level were necropsied at this time for chronic neuropathology assessment, and the remaining 50 rats/sex/dose level were fed the respective diets for up to two years. The chronic neurotoxicity study has been reported separately (A6.5[redacted]), with a no-observed-effect level (NOEL) of 20 mg/kg/day (actual dose of approximately 9.6 mg/kg/day).

Over the course of the study, male rats from the low-, middle-, and high-dose groups received time-weighted average doses of 1.431, 9.546, and 71.29 mg/kg/day, respectively; female rats from the low-, middle-, and high-dose groups received acceptable time-weighted average doses of 1.434, 9.551, and 71.32 mg/kg/day, respectively. These time-weighted average doses were lower than the targeted doses of 3, 20, or 150 mg/kg/day due to the rapid degradation of DBNPA over the seven-day mixing interval used throughout the study. A degradation factor of 0.47 was applied to the percent of test material in the diet to calculate the test material intake.

Males and females given 150 mg/kg/day had treatment-related, statistically identified lower mean body weights and feed consumption during the latter portion of the study. At study termination, the body weight gains of males and females given 150 mg/kg/day were 15.1% and 12.8% lower than controls, respectively. There were no treatment-related effects on body weights or feed consumption of males or females given 3 or 20 mg/kg/day.

The only treatment-related clinical observation was an increased incidence of perineal urine soiling in males given 150 mg/kg/day. The urine soiling may have been caused by decreased grooming of the perineal region in response to the presence of excretory by-products of DBNPA in the urine.

Hematologic alterations in males given 150 mg/kg/day consisted of slightly lower reticulocyte counts at 3, 6, 12, 18, and 24 months. This decrease was only statistically signficantly lower than concurrent controls at 3 and 6 months. Higher red blood cell counts, hemoglobin concentrations, and hematocrits were observed at 18 and 24 months. These alterations were interpreted to be of minimal toxicological significance since there was no associated anemia or adverse clinical signs such as dehydration. There were no hematologic effects in males given 3 or 20 mg/kg/day, or in females at any dose level.
Males and females given 150 mg/kg/day had treatment-related higher serum chloride concentrations throughout the study, whereas males and females given 20 mg/kg/day had slightly higher chloride concentrations only at 3 months. The higher chloride concentrations were attributed to interference of serum bromine (associated with test material administration) with the specificity of the chloride electrode of the clinical chemistry analyzer to detect chlorine as well as bromine. As such, increased chlorine concentrations were interpreted to be a non-adverse marker of exposure to DBNPA.

Table 78: Red blood cell Count, hemoglobin concentration and hematocrit (%) in two year chronic rat study (A6.4.2/01[redacted])

RBC Count ($E^6/\mu l$)	Males Dose (mg/kg/day)				
	0	Historical Controls	3	20	150
18 months	8.97	8.11 – 9.74 [a]	9.13	9.27	9.72*
24 months	8.49	6.65 – 7.83 [a]	7.75	8.08	10.48*
Hemoglobin (HGB) Concentration (g/dl)	Males Dose (mg/kg/day)				
	0	Historical Controls	3	20	150
18 months	15.4	14.3 – 16.3 [a]	15.6	16.1	16.6$
24 months	14.9	12.4 – 14.0 [a]	14.0	14.5	18.2*
Hematocrit (%)	Males Dose (mg/kg/day)				
	0	Historical Controls	3	20	150
18 months	47.4	40.2 – 50.8 [a]	47.9	49.3	51.1$
24 months	44.9	37.7 – 43.5 [a]	42.3	42.7	53.9*

* Statistically different from control mean by Dunnett's Test, alpha = 0.05.
$ Statistically different from control mean by Wilcoxon's Test, alpha = 0.05.
[a] Range from three studies conducted between 2004 and 2007.

Table 79: Triglyceride and chloride concentrations in two year chronic study (A6.4.2/01[redacted]

Triglycerides (mg/dl)	Males Dose (mg/kg/day)				
	0	Historical Controls	3	20	150
3 months	111	78 – 143 [a]	112	114	77*
6 months	170	198 [b]	110*	104*	81*
12 months	94	66 [b]	96	67	93
18 months	160	134 [b]	139	126	135
24 months	81	76 [b]	98	166	80
Triglycerides (mg/dl)	Females Dose (mg/kg/day)				
	0	Historical Controls	3	20	150
3 months	40	46 – 54 [a]	36	41	38
6 months	64	59 [b]	63	58	49*
12 months	68	71 [b]	78	61	61
18 months	122	120 [b]	136	140	115
24 months	105	103 [b]	108	127	118
Chloride (mmol/l)	Males Dose (mg/kg/day)				
	0	Historical Controls	3	20	150
3 months	102	95 – 101 [c]	103	**106***	**119***
6 months	103	95 – 101 [d]	104	104	**112***
12 months	99	95 – 103 [d]	99	100	**103***
18 months	103	99 – 105 [d]	103	103	**109***
24 months	102	102 [d]	103	101	**106**
Chloride (mmol/l)	Females Dose (mg/kg/day)				
	0	Historical Controls	3	20	150
3 months	103	97 – 103 [c]	104	**105***	**116***
6 months	103	95 – 102 [d]	103	104	**110***
12 months	103	96 – 103 [d]	101	102	103
18 months	104	99 – 104 [d]	103	104	**108***
24 months	100	99 – 100 [d]	101	99	**105**

* Statistically different from control mean by Dunnett's Test, alpha = 0.05.
[a] Range from four studies conducted between 2004 and 2007.
[b] Data from one study conducted in 2007.
[c] Range from eight studies conducted between 2004 and 2007.
[d] Range from three studies conducted between 2004 and 2007.
Bold type indicates effects considered treatment related.

There was a general tendency for males given 150 mg/kg/day to have slightly higher urine pH than controls at 3, 6, 12, and 18 months. This alteration was interpreted to be treatment related, likely due to the presence of cyanoacetamide, the major excretory product in the urine of rats given DBNPA. Males and females given 150 mg/kg/day had a general tendency to have higher urinary ketone levels than controls at 3, 6, 12, 18, and 24 months. The higher ketone levels were associated with significantly lower body weights in males and females given 150 mg/kg/day, and may be the result of a secondary effect on lipid catabolism to meet energy needs.

Table 80: Urine ketones (A6.4.2/01[redacted])

Urine Ketones	Males Dose (mg/kg/day)			
	0	3	20	150
3 months	+ (10)	+ (10)	+ (7) ++ (3)	+ (7) ++ (3)
6 months	Trace (1) + (9)	+ (10)	+ (9) ++ (1)	+ (10)
12 months	Negative (1) Trace (1) + (8)	Trace (1) + (9)	Trace (1) + (9)	+ (9) ++ (1)
18 months	Negative (2) Trace (6) + (2)	Trace (5) + (5)	Negative (3) Trace (5) + (2)	Trace (1) + (9)
24 months	Negative (7) Trace (3)	Negative (8) Trace (2)	Negative (5) Trace (5)	Negative (1) Trace (5) + (4)
Urine Ketones	Females Dose (mg/kg/day)			
	0	3	20	150
3 months	Negative (4) Trace (6)	Negative (3) Trace (7)	Negative (4) Trace (6)	Negative (1) Trace (5) + (4)
6 months	Negative (7) Trace (3)	Negative (6) Trace (3)	Negative (5) Trace (5)	Trace (8) + (2)
12 months	Negative (6) Trace (4)	Negative (8) Trace (1)	Negative (5) Trace (5)	Trace (6) + (4)
18 months	Negative (9) Trace (1)	Negative (5) Trace (5)	Negative (9) Trace (1)	Negative (1) Trace (9)
24 months	Negative (10)	Negative (9) Trace (1)	Negative (8) Trace (2)	Negative (1) Trace (8) + (1)

Data tabulated as number of animals (N) with the stated value.
+ = slight, ++ = moderate.

The primary treatment-related histopathologic effect in animals from the 24-month sacrifice consisted of statistically-identified increases in the incidence of very slight or slight diffuse follicular cell hyperplasia in the thyroid glands of males and females given 150 mg/kg/day, and in males given 20 mg/kg/day. Treatment-related thyroid follicular hyperplasia was also present at the 12-month sacrifice in males and females given 150 mg/kg/day. The hyperplasia did not appreciably worsen during the final 12 months of the study. This effect was interpreted to be caused by the bromine component of DBNPA as thyroid follicular cell hyperplasia is a commonly recognized effect of exposure to exogenous compounds containing bromine. The increase seen in incidence of follicular cell hyperplasia of the follicular epithelium in rats after a chronic exposure during 2 years was very slight. During the two years the change was not associated with increased cancer risk and the genotoxicity studies were negative. According to a Commission group of specialized experts[2] the rat is a more

[2] Summary record – Commission group of specialised experts in the fields of Carcinogenicity, mutagenicity and reprotoxicity, ECBI/49/99, 1999, excerpt of agenda item 3.1

sensitive species with regards to changes of the thyroid and development of epithelial thyroid tumors after long term exposure to non-genotoxic agents and the changes should not be considered representative for humans.

Females given 150 mg/kg/day had a treatment-related statistically-identified increase in the incidence of slight vacuolization (consistent with fatty change) of individual hepatocytes. The fatty change of the liver may have been secondary to lower feed consumption and body weights of this dose group during the final months of the study or could be an effect of the metabolite DBAA. This slight increase in hepatocyte fat vacuoles was not accompanied by pathological increases in AST or ALT. Male rats did not have a similar effect on liver vacuolization at any dose level.

Males given 150 mg/kg/day had statistically-identified decreases in the incidence of large granular lymphocyte (LGL) leukemia, adenomas of the pars distalis of the pituitary gland, and moderate or severe chronic progressive glomerulonephropathy of the kidneys compared to controls. The decreased incidence of these findings was interpreted to be related to the lower feed consumption of rats from this dose group for the majority of the two-year dosing period.

No treatment-related increase in neoplasms was observed in either male or female rats at any dose level, indicating that DBNPA did not have an oncogenic potential under the conditions of this study.

Based on treatment-related hyperplasia of the thyroid follicular cells in males given 20 or 150 mg/kg/day, the no-observed-effect level (NOEL) for males was 3 mg/kg/day (actual dose of 1.431 mg/kg/day). Females given 150 mg/kg/day had treatment-related lower body weights and feed consumption, and histopathologic thyroid and liver effects. The only effect of treatment in females given 20 mg/kg/day was slightly higher serum bromide concentration (recorded as chloride) at the 3-month time point. This observation was interpreted to be a non-adverse marker of exposure to DBNPA. Therefore, females had a no-observed-adverse effect level (NOAEL) of 20 mg/kg/day and a NOEL of 3 mg/kg/day (actual doses of 9.551 and 1.434 mg/kg/day, respectively). No chronic mouse study was submitted by the applicant. It is however assessed that it is not likely that a second study in another rodent species will provide additional information.

No effects were observed in the genotoxicity studies. Effects concerning follicular cell hyperplasia in the thyroid glands were seen in the 90 day dog feeding study and the 2 year chronic rat study in both males and females. However the effects were not seen in the 90 day rat feeding study, the 90 day mouse feeding study or the multi-generation rabbit study. The hyperplasia did not appreciably worsen during the final 12 months of the study and no proliferative development in the hyperplasia leading to tumors was observed in the 2 year rat study.

Table 81: Histopathologic Thyroid Effects – 12 Months (A6.4.2/01[redacted])

Sex	**Males**				**Females**			
Dose (mg/kg/day)	0	3	20	150	0	3	20	150
Thyroid (number examined)	10	10	10	10	10	10	10	10
Hyperplasia, follicular cell, diffuse								
-very slight	0	0	0	3	0	0	0	2
-slight	0	0	0	5	0	0	0	0

Bold type indicates the effects were interpreted to be treatment related.

Table 82: Histopathologic Thyroid Effects – 24 Months (A6.4.2/01[redacted])

Sex	**Males**				**Females**			
Dose (mg/kg/day)	0	3	20	150	0	3	20	150
Thyroid (number examined)	49	50	50	50	49	50	50	50

Hyperplasia, follicular cell, diffuse								
-very slight	1	2	8*	14*	0	0	0	17*
-slight	0	0	0	22*	0	0	0	4

Bold type indicates the effects were interpreted to be treatment related.

*Statistically identified by Yate's Chi-square test, alpha = 0.05, two-sided.

Table 83: Histopathologic Liver Effects – 24 Months (A6.4.2/01[redacted])

Sex	**Males**				**Females**			
Dose (mg/kg/day)	0	3	20	150	0	3	20	150
Liver (number examined)	50	50	50	50	50	50	50	50
Vacuolization; consistent with fatty change, individual hepatocytes								
-very slight	5	4	11	8	26	24	23	11*
-slight	16	6	13	20	7	9	8	32*
-moderate	0	0	0	0	0	0	1	0

Bold type indicates the effects interpreted to be treatment related.

***Statistically identified by Yate's Chi-square test, alpha = 0.05, two-sided.Table 84: Cumulative mortality – Males – 24 months (A6.4.2/01[redacted]**

DOSE (MG/KG/DAY)

TEST DAYS	0		3		20		150	
001-028	0	0/50	0	0/50	0	0/50	2	1/50
029-056	0	0/50	0	0/50	0	0/50	2	1/50
057-084	0	0/50	0	0/50	0	0/50	2	1/50
085-112	0	0/50	0	0/50	0	0/50	2	1/50
113-140	0	0/50	0	0/50	0	0/50	2	1/50
141-168	0	0/50	0	0/50	0	0/50	2	1/50
169-196	0	0/50	0	0/50	0	0/50	2	1/50
197-224	0	0/50	0	0/50	0	0/50	2	1/50
225-252	0	0/50	0	0/50	0	0/50	2	1/50
253-280	0	0/50	0	0/50	0	0/50	2	1/50
281-308	0	0/50	0	0/50	0	0/50	4	2/50
309-336	0	0/50	0	0/50	0	0/50	4	2/50
337-364	0	0/50	0	0/50	0	0/50	4	2/50
365-392	0	0/50	0	0/50	0	0/50	4	2/50
393-420	0	0/50	0	0/50	0	0/50	4	2/50
421-448	0	0/50	0	0/50	2	1/50	4	2/50
449-476	2	1/50	6	3/50	2	1/50	4	2/50
477-504	4	2/50	6	3/50	2	1/50	6	3/50
505-532	4	2/50	8	4/50	6	3/50	6	3/50
533-560	6	3/50	8	4/50	6	3/50	6	3/50
561-588	6	3/50	16	8/50	10	5/50	10	5/50
589-616	14	7/50	16	8/50	14	7/50	12	6/50
617-644	16	8/50	24	12/50	20	10/50	12	6/50
645-672	24	12/50	34	17/50	26	13/50	20	10/50
673-700	48	24/50	40	20/50	40	20/50	28	14/50
701-733	48	24/50	42	21/50	40	20/50	28	14/50
734-738	48	24/50	42	21/50	40	20/50	28	14/50

DATA PRESENTED FIRST AS PERCENT MORTALITY AND FOLLOWED BY NUMBER DEAD OVER TOTAL NUMBER OF ANIMALS IN GROUP SCHEDULED FOR THE CHRONIC ONCOGENICITY EVALUATION.
THERE WERE NO STATISTICAL DIFFERENCES FROM CONTROLS BY THE GEHAN-WILCOXON PROCEDURE, ALPHA=0.05.

Table 85: Cumulative Mortality – Females- 24 months (A6.4.2/01[redacted])

	DOSE (MG/KG/DAY)							
TEST DAYS	0		3		20		150	
001-028	0	0/50	0	0/50	0	0/50	0	0/50
029-056	0	0/50	0	0/50	0	0/50	0	0/50
057-084	0	0/50	0	0/50	0	0/50	0	0/50
085-112	0	0/50	0	0/50	0	0/50	0	0/50
113-140	0	0/50	0	0/50	0	0/50	0	0/50
141-168	0	0/50	0	0/50	0	0/50	0	0/50
169-196	0	0/50	0	0/50	0	0/50	2	1/50
197-224	0	0/50	0	0/50	0	0/50	2	1/50
225-252	0	0/50	0	0/50	0	0/50	2	1/50
253-280	0	0/50	0	0/50	0	0/50	2	1/50
281-308	0	0/50	0	0/50	0	0/50	2	1/50
309-336	0	0/50	0	0/50	0	0/50	2	1/50
337-364	0	0/50	0	0/50	0	0/50	2	1/50
365-392	2	1/50	0	0/50	0	0/50	2	1/50
393-420	2	1/50	0	0/50	0	0/50	2	1/50
421-448	2	1/50	0	0/50	0	0/50	2	1/50
449-476	2	1/50	2	1/50	0	0/50	2	1/50
477-504	4	2/50	2	1/50	0	0/50	2	1/50
505-532	4	2/50	4	2/50	0	0/50	2	1/50
533-560	4	2/50	4	2/50	2	1/50	4	2/50
561-588	4	2/50	4	2/50	2	1/50	8	4/50
589-616	4	2/50	6	3/50	4	2/50	8	4/50
617-644	4	2/50	6	3/50	10	5/50	12	6/50
645-672	4	2/50	12	6/50	16	8/50	16	8/50
673-700	14	7/50	12	6/50	24	12/50	22	11/50
701-733	16	8/50	12	6/50	24	12/50	22	11/50
734-738	16	8/50	12	6/50	24	12/50	22	11/50

DATA PRESENTED FIRST AS PERCENT MORTALITY AND FOLLOWED BY NUMBER DEAD OVER TOTAL NUMBER OF ANIMALS IN GROUP SCHEDULED FOR THE CHRONIC ONCOGENICITY EVALUATION.
THERE WERE NO STATISTICAL DIFFERENCES FROM CONTROLS BY THE GEHAN-WILCOXON PROCEDURE, ALPHA=0.05.

Table 86: Organ and organ/body weights summary – Males – 24 months (A6.4.2/01[redacted])

Sex	Males				
Dose (mg/kg/day)	Historical Control[a]	0	3	20	150
Final Body Weight (g)	410.9-416.4	396.2	403.3	410.0	**354.5***
Brain, absolute (g)	2.097-2.120	2.090	2.097	2.076	*1.999**
Brain, relative (g/100g bw)	0.511-0.519	0.534	0.526	0.510	*0.567**
Heart, absolute (g)	1.197-1.252	1.179	1.187	1.184	*1.109*$*
Kidney, absolute (g)	3.026-3.157	2.987	2.981	2.978	*2.732*$*
Kidney, relative (g/100g bw)	0.737-0.761	0.762	0.747	0.732	*0.774*$*
Liver, absolute (g)	11.494-11.717	12.130	12.095	12.151	*10.337*$*
Spleen, absolute (g)	1.467-2.362	1.539	3.340	1.594	*1.032*$*
Testes, relative (g/100g bw)	0.972-1.179	1.254	1.116	1.493	*1.673**

* Statistically different from control mean by Dunnett's Test, alpha = 0.05.
$ Statistically different from control mean by Wilcoxon's Test, Alpha = 0.05.
Bold type indicates the effects were interpreted to be treatment-related.
Italic type indicates effects are secondary to decreased final body weight.
[a] Historical control data were taken from three dietary studies conducted between 2004 and 2007.

Table 87: Organ and organ/body weights summary – Females – 24 months (A6.4.2/01 [redacted])

Sex	Females				
Dose (mg/kg/day)	Historical Control[a]	0	3	20	150
Final Body Weight (g)	272.0-278.9	279.5	282.2	282.6	**261.9$**
Adrenals, absolute (g)	0.064-0.081	0.065	0.064	0.125	*0.058$*
Brain, absolute (g)	1.886-1.905	1.910	1.917	1.899	*1.821**
Heart, absolute (g)	0.803-0.892	0.910	0.884	0.926	*0.861**
Kidney, relative (g/100g bw)	0.715-0.723	0.704	0.699	0.717	*0.754**
Liver, relative (g/100g bw)	2.656-2.754	2.768	2.726	2.812	*2.872$*
Uterus, absolute (g)	1.332-2.474	1.728	1.817	1.578	*1.110$*
Spleen, absolute (g)	0.674-1.060	1.312	0.752$	1.092	0.769
Spleen, relative (g/100g bw)	0.248-0.387	0.474	0.278$	0.400	0.294

* Statistically different from control mean by Dunnett's Test, alpha = 0.05.
$ Statistically different from control mean by Wilcoxon's Test, Alpha = 0.05.
Bold type indicates the effects were interpreted to be treatment related.
Italic type indicates effects are secondary to decreased final body weight.
[a] Historical control data were taken from three dietary studies conducted between 2004 and 2007.

Dermal route

In a 28 d study A6.3.2/01 [redacted] male and female Fischer 344 rats (5/sex/dose) were exposed to daily dermal applications of 0 (distilled water control), 0V (TEG-vehicle control), 2.5, 5, 10 and 20% of DBNPA at a rate of 2 mL/kg bw for 6 h/day, 5 days/week for 10 applications during the first two weeks of the study. The doses are equivalent to 57, 115, 241 and 515 mg/kg bw/day. Due to lack of dermal irritation and toxicity the study was continued for another 9 applications at a rate of 4 mL/kg bw for another two weeks. This corresponds to 115, 233, 482 and 1030 mg/kg bw/day. The test substance was applied to an area of approximately 30 cm^2 that was clipped free of hair prior to first treatment and as needed thereafter. During the first two weeks of the study the test solutions were uniformly spread over the application site with a syringe. The application site was then covered with an absorbent gauze patch that was fixed by an elastic bandaging tape. During the second half of the study the test material was directly applied to the absorbent gauze due to doubling of the dose volume. After six hours the bandage tape was removed and the dosing area was cleaned with a water-dampened towel.

The skin reaction was evaluated according to a modified DRAIZE scheme. Clinical signs were observed daily. After 4 weeks, animals were sacrificed and examined by necropsy. Weights of heart, brain, liver, kidneys, adrenals and testes were recorded. All animals were examined for gross pathological alterations. Statistical analysis for variance was conducted.

All animals survived the 4-week treating period with no signs of systemic toxicity, except one female rat (20% dosing group, sacrificed on day 12). The hemorrhagic lesion in the urethral area of this one female rat was considered to be spontaneous and not related to treatment.

Body weight gain was affected by treatment at the four dose levels used. Male mean body weights were reduced 6, 5 and 4% at the 5, 10 and 20% DBNPA dose levels, respectively. Although the body weights at these dose levels were identified as being statistically different from control values, the lack of a dose-response relationship indicated that the observed difference in body weights were of questionable biological significance. In the control, vehicle control and low dose group (2.5 %) no skin reactions were observed. Some rats in the 5, 10 and 20% dosing groups showed a slight and inconsistent dermal irritation at the application

site consisting of very slight to well-defined erythema, very slight to moderate edema, scabbing and scarring for males, and only very slight erythema for females. In general males were more affected at the application site than females At the 20% DBNPA dose level, one male and three females were completely unaffected by the treatment.

There were no signs of systemic toxicity at necropsy. Organ weights were comparable to controls. Gross treatment-related pathologic alterations in rats at termination of the study were limited to the skin at the site of test material application.

The doses applied in this study ranged between 57 and 1030 mg/kg bw/d (based on an increase of application volume from 2 to 4 mL/kg bw/d) and caused no biologically relevant systemic toxicity (clinical signs, body weight, organ weight).

[redacted] A6.7 evaluated DBNPA for local and systemic toxicity after repeated dermal application for six hours/day, five days/week. Groups of 10 Fischer 344 rats/sex/dose received a 20% solution of DBNPA in tetraethylene glycol (TEG) vehicle at dose levels of 103, 309 or 1031 mg/kg bw/day. A vehicle control group of male and female rats received TEG at a dose level of approximately 3360 mg TEG/kg bw/day. A sham control group was wrapped only. The study was conducted according to OECD Guideline No. 411 (adopted 1981) and EPA guideline 82-3 (adopted 1984).

Parameters evaluated included general appearance and demeanor, topical response of skin at the application site, body weights, feed consumption, hematology, clinical chemistry, urinalysis, selected organ weights, gross pathologic and histopathologic observations. A functional observational battery was conducted after 12 weeks of exposure.

Mortalities did not occur during the study. A dose-related topical response of the skin was noted and limited to the application site. The dermal irritation was characterised by erythema, edema and scabs/crust at the test site of most high-dose (1031 mg DBNPA/kg bw/day) male and female rats. Dermal abnormalities observed in the low (103 mg/kg bw/day) and intermediate (309 mg/kg bw/day) dose groups at any time during the study occurred sporadically and were considered incidental and not related to the test material. At study termination gross and histopathological examination of rats given 1031 mg/kg/day showed a localized response at the dermal test site which consisted of epidermal hyperkeratosis, inflammation and erosion/ulceration plus dermal inflammation and necrosis. This localized response did not occur in the rats given 103 or 309 mg/kg bw/day or the controls. The test site of the sham control and vehicle control groups was within limits throughout the observation period.

No signs of systemic toxicity were observed during the study at any dose group. Body weight gain revealed no findings related to treatment with test material. No differences were observed in the functional observational battery among the control and treated groups of rats. There were no treatment-related changes in feed consumption, haematology, clinical chemistry, urinalysis parameters or organ weights. In addition, there were no systemic gross or histopathological lesions attributed to treatment with DBNPA or the vehicle control. Based upon the absence of any adverse systemic effects, the NOEL for systemic toxicity of DBNPA administered by the dermal route was 1031 mg/g bw/day for male and female Fischer 344 rats. The NOAEL for local effects was 309 mg/kg bw/day based on gross pathology and histopathological findings at dermal test site.

Inhalation route

There are no repeat dose inhalation studies. Inhalative exposure to DBNPA is not significant due to the technical characteristics, the physical chemical properties and the use patterns. The repeated dose and subchronic inhalation toxicity is covered by oral toxicity studies.

Neurotoxicity

A one year chronic neurotoxicity study was conducted as part of a two-year chronic toxicity/oncogenicity study to assess the effects of dietary exposure to DBNPA (A6.5[redacted]). DBPNA exposure occurred via the diet at targeted dose levels of 0, 3, 20, and 150 mg/kg/day in male and female F344/DuCrl rats (actual doses were approximately 0, 1.4, 9.6 and 72.1 mg/kg/day). The neurotoxicity subgroup contained ten rats/sex/dose, and was evaluated pre-exposure, and at 1, 3, 6, 9 and 12 months of exposure using an automated test of motor activity and a functional observational battery (FOB) including determinations of grip performance, rectal temperature, and landing foot splay. Following 12 months of exposure five rats/sex/dose were perfused, and tissues from the central and peripheral nervous system of the control and high-dose groups were submitted for neuropathological examination.

There were no ranked FOB observations that could be attributed to treatment. Treatment-related categorical FOB observations consisted of increased urine soiling of males and females at 150 mg/kg/day DBNPA at 12 months of age. There was a statistically significant, treatment-related decrease in body weight of males given 150 mg/kg/day, such that after 12 months of exposure to DBNPA the mean body weights of these rats were 10% less than controls. There was also a statistically significant decrease in hindlimb grip performance of males and females given 150 mg/kg/day when compared to controls. Males given 150 mg/kg/day had a 20% decrease in hindlimb grip performance on month 9 when compared to controls, but this effect was attenuated on month 12. Females given 150 mg/kg/day

had a fairly consistent decrease (~13%) in hindlimb grip performance from months 1-12 when compared to controls. No treatment-related effects were seen on forelimb grip performance, landing foot splay, rectal temperature, or motor activity at any time. There were not treatment-related gross or histopathologic findings in either the central or peripheral nervous system following 12 months of dietary exposure.

Based on the significant decrease in male body weights and the slight decrease in hindlimb grip performance in males and females of the high-dose group, the chronic dieteary no-observed-effect level (NOEL) for DBNPA neurotoxicity in F344/DuCrl rats was 20 mg/kg/day (actual dose of approximately 9.6 mg/kg/day). It is unclear whether the effect on hindlimb grip performance represented a specific effect on the nervous system due to the small maginitude of change in grip performance (<20%), the non-specific nature of this endpoint (i.e., influenced by neuromuscular function, general systemic well being etc.), and the lack of correlating findings in other neurobehavioral and neuropathological endpoints. The decrease in hindlimb grip performance represents a minimal change (i.e. the coefficient of variation for hindlimb grip performance in control rats from 4 historical studies conducted in the laboratory was approximately 15%). No treatment-related findings for other behavioral or functional correlates were found at any time throughout the study. Specifically, forelimb grip strength, considered a better indicator of neurobehavioral effects than hindlimb grip performance, was unaffected by treatment throughout the study. No gross or histopathological changes were found in central or peripheral nervous system tissues or the associated muscle tissue, indicating that there was no selective effect of DBNPA exposure on the hindlimb neuromuscular structure. Lastly, it is important to consider that grip strength is an apical measure that is influenced by a number of factors including strength, motivation, aversiveness to the procedure, body weight, and experimental history.

Due to the small maginitude of change in grip performance (<20%), the non-specific nature of this endpoint (i.e., influenced by neuromuscular function, general systemic well being etc.), and the lack of correlating findings in other neurobehavioral and neuropathological endpoints it is unclear whether the effect on hindlimb grip performance represented a specific effect on the nervous system and it is not assessed that classification is justified on basis of the data.

Confidential Table 88: Male body Weights (g) (A6.5 [redacted])

DOSE MKD		DAYS ON TEST 1	29	85	176	261	365
0	MEAN	129.7	239.7	325.5	385.8	423.4	452.8
	S.D.	17.5	16.6	21.2	23.0	26.5	30.1
	N=	10	10	10	10	10	10
3	MEAN	134.9	242.7	333.4	396.6	436.8	459.1
	S.D.	15.7	17.0	21.3	24.8	28.5	30.6
	N=	10	10	10	10	10	10
20	MEAN	132.7	237.3	325.3	386.2	424.1	447.6
	S.D.	18.5	17.1	15.9	20.6	22.8	21.4
	N=	10	10	10	10	10	10
150	MEAN	133.6	240.5	315.9	362.4	391.5	407.6
	S.D.	18.1	15.5	20.4	23.5	29.2	28.9
	N=	10	10	10	10	10	10

Table 89: Categorical FOB Observations: Urine soiling (A6.5 [redacted])

FOB 9 MONTH

SEX:	MALES				FEMALES			
DOSE (MKD):	0	3	20	150	0	3	20	150
NUMBER OF ANIMALS OBSERVED:	10	10	10	10	10	9	10	10
Soiling								
Perineal, Urine	0	0	1	3	0	0	0	1

FOB 12 MONTH

SEX:	MALES				FEMALES			
DOSE (MKD):	0	3	20	150	0	3	20	150
NUMBER OF ANIMALS OBSERVED:	10	10	10	10	9	9	10	10
Soiling								
Perineal, Urine	1	0	0	3	0	0	0	3

Table 90: Hindlimb Grip Performance (g) – Males (A6.5 [redacted])

DOSE MKD		SESSION (MONTHS AFTER DOSE INITIATION) Baseline	1	3	6	9	12
0	MEAN	440.3	771.5	887.7	892.7	867.3	756.5
	S.D.	72.9	151.3	78.3	164.8	136.0	132.7
	N=	10	10	10	10	10	10
3	MEAN	482.8	777.5	927.8	891.4	957.2	766.3
	S.D.	76.5	98.7	125.3	105.6	158.1	150.9
	N=	10	10	10	10	10	10
20	MEAN	468.6	735.7	921.2	926.0	794.1	734.6
	S.D.	66.3	65.9	147.1	96.9	123.6	118.3
	N=	10	10	10	10	10	10
150	MEAN	481.4	701.7	899.8	813.3	694.7	715.9
	S.D.	97.0	127.3	93.9	164.5	140.4	107.3
	N=	10	10	10	10	10	10

Table 91: Hindlimb Grip Performance (g) – Females (A6.5 [redacted])

DOSE MKD		SESSION (MONTHS AFTER DOSE INITIATION) Baseline	1	3	6	9	12
0	MEAN	366.4	578.0	629.9	647.7	606.7	524.4
	S.D.	73.7	91.6	65.6	100.9	78.9	42.5
	N=	10	10	10	10	10	9
3	MEAN	399.9	593.0	652.4	626.4	602.4	556.4
	S.D.	76.5	102.0	119.1	127.0	98.1	99.4
	N=	10	10	10	9	9	9
20	MEAN	399.1	540.4	604.1	630.5	647.4	517.2
	S.D.	57.3	58.1	80.5	113.1	95.9	82.9
	N=	10	10	10	10	10	10
150	MEAN	377.3	504.3	582.5	561.0	523.7	454.9
	S.D.	48.6	66.4	80.9	103.1	115.4	59.2
	N=	10	10	10	10	10	10

For further details on the studies, please refer to annex II to this report.

Table 92: Extrapolation of equivalent effective dose for toxicity studies of greater or lesser duration than 90 days

Study reference	Effective dose (mg/kg/d)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
No data available				

10.12.2 Comparison with the CLP criteria

The data from the subchronic toxicity studies conducted in multiple species with multiple routes of exposure support the position that treatment with DBNPA does not produce toxicological/histopathological effects that could be considered to target a specific organ with repeated exposure.

A 90 day dermal study in F344 rats showed no signs of toxicity up to the highest applied dose of 1000 mg/kg bw/day indicating that there is no potential for target organ toxicity via the dermal route of exposure.

Two 28 day oral studies, one exposing dogs to DBNPA via the diet and the other exposing Sprague Dawley rats to DBNPA via gavage, showed no evidence of adverse effects related to treatment. Dyspnoea occurred in rats exposed to 50 mg/kg bw/day but this was related to gavage application of the test material and was not seen in dietary studies of longer duration in any animals tested.

A 90 day gavage study in Sprague Dawley rats showed orophayngeal irritation and dyspnoea at high doses (35/30 mg/kg bw/day), again related to gavage administration of test material. An increase in adrenal weight was reported in high dose animals but was not found in other 90 day studies, indicating that this is not a target organ of DBNPA toxicity. Furthermore, the bolus dosing in this study is not relevant to the manner in which humans would be exposed, indicating that this study does not adequately address human risk. No other treatment-related effects were found in the study.

One 90 day drinking water study was performed in Sprague-Dawley rats. Increased relative kidney weights in males (20 and 100 ppm at pH 4) and females (500 ppm at pH 8) and increased incidence of minimal cytoplasmic swelling and vacuolization of rental tubular epithelial cells in females (500 ppm at pH 8) were observed. This more typical age-related kidney change is of little significance with respect to human hazard potential, and was not observed in any other (sub)chronic study. Increased relative liver weights were observed in males (100 and 500 ppm at pH 8) and in females (500 ppm at pH 8). In the absence of histological and physiological findings these findings were considered unrelated to treatment and incidental. NOAEL is established at 100 ppm (equal to 10.8-17.3 mg/kg bw/d).

Three 90 day dietary studies were conducted, in F344 rats, CD-1 mice, and Beagle dogs. In rats, an increase in extramedullary hematopoiesis and urinary ketones was observed at high doses. It is not until dosages of 133 and 130 mg/kg/day are administered that potentially significant (but not severe) responses are observed. In mice, toxicity was secondary to reductions in body weight. The NOAEL was established at 133 mg/kg bw/d for males and 45 mg/kg bw/d for females on the basis of reduced body weight and body weight gains. Therefore, no target organ was identified. In Beagle dogs, an increase in follicular hypertrophy in the thyroid gland was observed. The changes to the thyroid weight were within or close to the historical control ranges and there was no histopathological evidence of injury (inflammation, necrosis, proliferation) indicating that no organ dysfunction is evident.

Finally, a 2 year cancer bioassay was performed in F344 rats. A very slight to slight diffuse thyroid hyperplasia was present at 12 months in males and females at the administered dose of 150 mg/kg bw/day. Progression between 12 and 24 months was minimal. By 24 months, 20 mg/kg bw/day males exhibited a small increase in the incidence of a very slight thyroid gland hyperplasia. The follicular cell hyperplasia was characterized by a diffuse increase in the cellularity of the follicular epithelium. No increase in thyroid nodules or follicular cell adenomas or carcinomas were observed. No effects were observed in the genotoxicity studies. Effects

concerning follicular cell hyperplasia in the thyroid glands were seen in the 90 day dog feeding study and the 2 year chronic rat study in both males and females. However the effects were not seen in the 90 day rat feeding study, the 90 day mouse feeding study or the multi-generation rabbit study. The hyperplasia did not appreciably worsen during the final 12 months of the study and no proliferative development in the hyperplasia leading to tumors was observed in the 2 year rat study.

10.12.3 **The available studies demonstrate that exposure to DBNPA does not target any specific organ on repeated exposure that would be relevant with regards to exposure pathway and/or unique species sensitivities not present in humans. Observations were inconsistent across studies and non-adverse at tolerable doses. While thyroid changes were observed in both the 90 day dog study and 2 year cancer study in rats, the increased thyroid weights and follicle dilation observed in dogs did not result in histopathological evidence of injury indicating that no organ dysfunction is evident. In rats after a chronic exposure during 2 years the increase in incidence of follicular cell hyperplasia of the follicular epithelium was very slight. During the two years the change was not associated with increased cancer risk and the genotoxicity studies were negative. According to a Commission group of specialized experts[3] the rat is a more sensitive species with regards to changes of the thyroid and development of epithelial thyroid tumors after long term exposure to non-genotoxic agents and the changes should not be considered representative for humans. It is therefore concluded that the effects seen in the 2 year rat study and in the 90 dog study is not sufficient to trigger classification as STOT RE.ConfidentialConclusion on classification and labelling for STOT RE**

Classification for specific target organ toxicity is not required under the terms of Regulation (EC) No 1272/2008 and subsequent amendments to that legislation.

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

[3] Summary record – Commission group of specialised experts in the fields of Carcinogenicity, mutagenicity and reprotoxicity, ECBI/49/99, 1999, excerpt of agenda item 3.1

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.

Additional key elements

The 2-week inhalation study in rats submitted during the public consultation is summarised in the following table.

Summary of the 2-week inhalation study in rats		
Type of study; Year	**Method**	**Observations**
Rat 2-week inhalation 2013	Non-guideline GLP Substance: DBNPA, purity 99.87% Rat, strain F344/DuCrl Nose-only Exposure 6h per day, 5 days per week (10 exposures in total) 5/sex/concentration Concentrations: 0, 0.51, 5.4, 50/25 mg/m^3 (days 1-3 50 mg/m^3; day 4 no exposure; from day 5 on 25 mg/m^3; average 31 mg/m^3) MMAD 2.2±1.5, 1.7±2.4 and 1.8±2.0 µm at 0.51, 5.4 and 50/25 mg/m^3 respectively Examinations: haematology, clinical chemistry, bronchoalveolar lavage (BAL), organ weights, gross pathology; histopathology of the respiratory tract, liver, and thyroid	50/25 mg/m^3 (31 mg/m^3): • Clinical signs: from day 3 slow, noisy, laboured respiration, ungroomed appearance, perinasal soiling; these clinical signs started improving on day 5 and reversed by the end of the study • Bw loss and reduced food consumption days 1-6; m ↓ terminal bw by 13% • ↑ eosinophils, monocytes (only m) and reticulocytes in the blood • ↑ number of BAL cells (macrophages 3.2/3.9-fold m/f, also eosinophils and neutrophils); ↑ BAL total protein • ↑ lung weight (absolute by 20%/33% m/f) • Histopathological findings in the lung, larynx and nasal cavity (presented in a separate table below) 5.4 mg/m^3: • ↑ eosinophils in the blood • ↑ BAL total protein • ↑ lung weight (absolute by 15%) • Histopathological findings in the lung, larynx and nasal cavity 0.51 mg/m^3: • Histopathological findings in the larynx

Incidence and severity of selected histopathological findings in the respiratory tract in this study are summarised below (severity grades: + very slight or slight, ++ moderate, +++ severe).

Incidence and severity of selected histopathological findings in the respiratory tract of rats from the 2-week inhalation study				
Concentration (mg/m³)	**0**	**0.51**	**5.4**	**50/25**
Number of animals examined	10	10	10	10
Nasal cavity				
Inflammation of the epithelium and lamina propria, multifocal			6 (+)	10 (+, ++)
Hyperplasia of the respiratory and/or transitional epithelium, multifocal				10 (+)
Hyperplasia of the squamous epithelium, multifocal			10 (+)	10 (+)
Squamous metaplasia of the epithelium, multifocal				9 (+, ++)
Degeneration of the olfactory epithelium, multifocal				7 (+)
Degeneration of the olfactory nerve, multifocal				3 (+)
Larynx				
Inflammation of the lamina propria, multifocal		7 (+)	10 (+, ++)	10 (+, ++)
Hyperplasia of the respiratory epithelium, multifocal or diffuse		1 (+)	10 (+)	10 (+)
Squamous metaplasia of the respiratory epithelium, multifocal		1 (+)	10 (+)	10 (+)
Necrosis of the respiratory epithelium, individual cells, multifocal			7 (+)	9 (+)
Necrosis of the lamina propria, multifocal			1 (+)	2 (++)
Fibrosis of the lamina propria, multifocal		1 (+)	10 (+)	9 (+, ++)
Necrosis of the cartilage, multifocal				3 (+)
Lung				
Peribronchial and perivascular inflammation, multifocal			10 (+)	10 (+)
Hyperplasia of the bronchiolar epithelium, multifocal			10 (+)	10 (+)
Mucous cell metaplasia of the bronchial and bronchiolar epithelium, multifocal			10 (+)	10 (+)
Necrosis of the bronchiolar epithelium, multifocal			10 (+)	10 (+)
Alveolar histiocytosis			10 (+)	10 (+)

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**.

10.13 Aspiration hazard

Table 93: Summary table of evidence for aspiration hazard

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Not applicable, the substance is a solid.				

10.13.1 Short summary and overall relevance of the provided information on aspiration hazard

Not applicable, the substance is a solid.

10.13.2 Comparison with the CLP criteria

Not applicable, the substance is a solid. Although the definition of aspiration in section 3.10.1.2 of Regulation (EC) No 1272/2008 includes the entry of solids into the respiratory system, classification

according to point (b) in Table 3.10.1 for Category 1 is intended to apply to liquid substances and mixtures only.

10.13.3 Conclusion on classification and labelling for aspiration hazard

Classification for an aspiration hazard is not required under the terms of Regulation (EC) No 1272/2008 and subsequent amendments to that legislation.

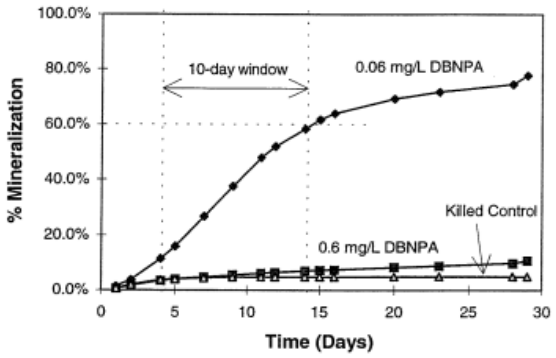
11 EVALUATION OF ENVIRONMENTAL HAZARDS (ADDITIONAL DETAILS RELATING TO ANY PARTICULAR ENVIRONMENTAL STUDY CAN BE FOUND IN ANNEX 1)

11.1 Rapid degradability of organic sub stances

11.1.1 Ready biodegradability

[redacted]) A7.1 .1.2.1/01 and [redacted] A7.1.1.2.1/04:

Two valid studies according to OECD guideline 301B was submitted. In the first study the guideline was followed with the exception that the concentration of test material (DBNPA) was reduced to 0.06 or 0.6 mg/L. It should be noted that the lower concentrations of DBNPA were necessary to avoid potential inhibitory effects of DBNPA. After 28 days, mineralisation of 0.06 mg/L reached 78%. With approximately 58% mineralisation within the 10-day window, the pass level for ready biodegradability of 60% was missed by 2%. It should be noted that a little deviation from the 60% mineralisation could be due to uncertainty in measurement. The DT_{50} value for mineralisation can be read from the degradation curve to be about 12 days (see the graph below). In contrast, mineralisation of 0.6 mg DBNPA/L reached only 11% after 28 days suggesting that the microbiological activity is partially inhibited at this level. The main conclusions are regarded as acceptable, and the study is given a **Reliability Indicator of 1**. However, the strict criteria of ready biodegradation (i.e., 10-day window) were not quite met, and not all validity criteria were addressed (Inorganic content/total carbon content: OECD 301B, Point 31, page 22) ;however, this validity criterion does not apply for ^{14}C labeled study because all calculation is based on 14C radioactivity. There is no ^{14}C in organic carbon (i.e. $14CO_2$) in the test system.



In the second study the OECD guideline 301B was also followed with the exception that the concentration of test material (DBNPA) was reduced to 0.05 mg/L. The lower concentration of DBNPA was necessary to avoid potential inhibitory effects of DBNPA. After 28 days, mineralisation of 0.05 mg /L reached only 35%. Intermediates/degradation products has not been identified. Thuse the DBNPA did not meet the criterion tob e classified as ready biodegradable. The main conclusions are regarded as acceptable, and the study is given a ***Reliability indicator of 1*** by RMS. The reason for the different results oft he two valdi studies is not known, but was likely due to variations in the microbial inoculum.

Based on the results of the two ready biodegradability tests of DBNPA according to OECD guideline 301B it hast o be concluded that DBNPA is not readily biodegradable even though a rapid degradation of DBNPA is observed in the additional biodegradation studies in activated sludge and in water and sediment studies

11.1.2 Hydrolysis

[redacted] A7.1.1.1.1/01

A hydrolysis study was conducted according to FIFRA Guideline 161-1. DBNPA was dissolved in buffer solutions of pH 5, 7 and 9 at 25.3 ± 0.2°C. For analysis of degradation products and for material balancing radio labelled DBNPA was used, as well.

Under aseptic conditions in darkness with a solution temperature of 25.3 ± 0.2°C, the following half-lives of DBNPA were determined: 67.3 days at pH 5, 63.2 hours at pH 7 and 73.6 minutes at pH 9. From the results it can be concluded that the hydrolysis rate of DBNPA is strongly pH-dependent.

At the end of the test, the material balance of DBNPA for three replicates ranged from 92.3 - 112% for pH 5, 7 and 9. Volatiles were not formed. The sampling was at day 50 for pH 5, at 4.1 days for pH 7 and at 85 minutes for pH 9. Analysis of the degradation products by isocratic elution HPLC with radiometric detection revealed the presence of: bromoacetamide and dibromoacetic acid as well as four unknown metabolites. The identification of degradation products by gradient elution HPLC with radiometric detection indicated the presence of dibromoacetic acid, bromoacetic acid, dibromoacetonitrile and three unknown metabolites.

Mass balances are only available at the end of the test. The identity of the following hydrolysis products representing 10% or more of the applied dose are unknown: A (up to 66.9%) , C (up to 18.5%), and 3 (up to 18.5%). **Reliability Indicator = 3**

[redacted] A7.1.1.1.1/07

In another hydrolysis study at 25.3 °C, the following half-lives of DBNPA were determined: 200-202 hours at pH 5, 45.8-62.8 hours at pH 7 and 0.76 – 1.00 hour at pH 9. The degradation products were dibromoacetic acid, dibromoacetamide and oxalic acid. **Reliability Indicator = 3**

[redacted] A7.1.1.1.1/06

hydrolysis as a function of pH and temperature was investigated. DBNPA was dissolved in phosphate buffer over the pH range 7.3-8.6 at 25, 37 and 47.7° C. The results revealed that the decomposition is more rapid at higher pH. **Reliability Indicator = 3.**

[redacted] A7.1.1.1.1/03

In this study, hydrolysis rates for DBNPA were determined following OECD 111 guidelines 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. The levels of DBNPA and brominated hydrolysis products were determined using reverse-phase high performance liquid chromatography (HPLC) with ultraviolet detection (UV) detection.

Hydrolysis of DPNBA occurred in all of the reaction mixtures with the rate increasing with an increase in either the solution pH or temperature. At pH 4, hydrolysis half-lives ranged from 578 hours at 50°C to 69 hours at 70°C. At pH 7, half-lives ranged from 65 hours at 25°C to 3 hours at 50°C. At pH 9, half-lives ranged from 5.2 hours at 13°C to 0.31 hours at 35°C (see Table 94).

Table 94: Half-Life and Pseudo-first order rate constants for DBNPA Hydrolysis in Buffered Solutions at pH 4, 7, and 9 at Three Temperatures.

Guideline / Test method	pH	Temperature (°C)	K_h[a](hours-1)	Half-Life[b] (hours)	Coefficient of Determination (r^2)	Estimated Half-Life at 12°C (hours)	**Reference and RI**
	4	50	0.0012	578	0.9591	12079	
		60	0.0044	158	0.9612	7348	[redacted] A7.1.1.1.1./03

OECD 111 guidelines		70	0.0101	69	0.9438	7141	**RI = 1**
	7	25	0.0107	65	0.9928	183	
		37.5	0.0422	16	0.9778	123	
		50	0.2289	3.0	0.9964	62	
	9	13	0.1328	5.2	0.9936	5.6	
		25	0.5797	1.2	0.9577	3.4	
		35	2.2124	0.31	0.9913	1.9	

[a] pseudo-first-order rate constant determined at indicated pH;

[b] Half Life = (ln 2)/Kh

Overall 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 are as follows: Dibromoacetonitrile − 54 to 79%; Dibromoacetamide – not detectable (nd) to 35%; Dibromoacetic acid – 6 to 11%; and DBMAL – 6 to 11%. **Reliability Indicator = 1**

Considering the hydrolytic stability of DBNPA determined under environmental pH and temperature conditions, it can be concluded that the substance is not hydrolytically stable at environmentally relevant pH and temperature.

The following Half-Lifes are determined for 12 °C, based on the Arrhenius reported by [redacted] A7.1.1.1.1./03

DBNPA:

At pH 4and 12 ºC = 12079 hours.

At pH 7and 12 ºC = 183 hours.

At pH 9and12 ºC = 5.6 hours

11.1.2.1 Inherent and enhanced ready biodegradability tests

[redacted] A7.1.2.1.1/01

Die away test of DBNPA in activated sludge (STP simulation): The test was performed according to [redacted], which is recommended in the TNsG on data requirements (page 110) to assess the aerobic biodegradation of active substances in activated sludge under real life conditions (simulation test). The test consisted of 1.5 L activated sludge sampled from the oxidation ditch of a municipal waste water treatment plant. The test material was added at a concentration of 0.038 mg/L. The closed test system was aerated with moistured CO_2-free air and connected to a gas trapping system to collect volatile degradation products and the evolved CO_2. Samples were collected after 0, 1, 5, 15, 30, 45, 60, and 90 minutes and after 2, 4, 6, 8, 12, 24, 48, 96, and 120 hours.

The results of the activated sludge die away test show a fast biodegradability of DBNPA under a real life situation. DBNPA degraded immediately as no DBNPA was found at the first sample collection at 1 minute. Due to general measurement uncertainties in laboratories, a conservative assumption was made by RMS that the DT_{50} for primary degradation of DBNPA is 10 minutes. For an unknown metabolite of DBNPA, the DT_{50} was calculated to be 9.9 hours. After 96 h, 53% of the total radioactivity evolved as CO_2 in the die away test (plateau was reached) opposed to 5 % in the abiotic control. The sum of dissolved and extractable radioactivity (related to parent compound and degradation products) decreased from 85% to 7% within the same period (weaker decrease from 80 to 49% in the abiotic control). Altogether these data shows that microbially mediated mineralisation of DBNPA plays an important role. The radioactivity which remained associated with the solid fraction (incorporated into biomass or irreversibly bound to it) increased from 25% to 33% (abiotic control: increase from 11 to 57%). Recovery of the total radioactivity in all probes sampled during the incubation was approximately 100% indicating reliability of the analytical work.

The half-life for mineralisation (in this case the time in which 50% of the total evolved $^{14}CO_2$ was observed) was calculated via a fit of the CO_2 production to a first-order production model to be 8.1 h. This is in line with the parameters for the metabolite decay, indicating that the microbiologically-mediated mineralisation rate is governed by the rate of formationand degradation of the metabolite. If hydrolysis of DBNPA would have been solely responsible for the primary degradation, a much higher initial rate and lower total yield of $^{14}CO_2$ production should have been observed at the beginning of the test, as the hydrolysis of DBNPA is immediately followed by a decarboxylation step which could give only ~33% of the theoretical $^{14}CO_2$ yield. Hence, the fast primary degradation of DBNPA is most likely not due to hydrolysis, but due to a chemical degradation in which cyanoacetamide is formed by the loss of the two bromines from DBNPA.

After 4 days 53% of the total radioactivity evolved as CO_2 in the die away test opposed to 5% in the abiotic control. The sum of dissolved and extractable radioactivity (related to parent compound and degradation products) decreased from 85% to 7% within the same period (weaker decrease from 80 to 49% in the abiotic control). Altogether these data can be considered as clear hint for an extensive microbially mediated mineralization of DBNPA. The radioactivity which remained associated with the solid fraction (incorporated into biomass or irreversebly bound to it) increased from 25% to 33%. **Reliability Indicator = 2**

[redacted] A7.1.2.1.1/02

"Evaluation of the effect of 2,2-Dibromo-3-Nitrilo-Propionamide (DBNPA) on a semi-continuous activated sludge treatment system" confirms the rapid primary degradation of DBNPA. The addition of DBNPA to raw, untreated sewage resulted in a rapid transformation, especially at the lowest concentration of DBNPA (detection limit approximately 0.5 mg/L). These results are consistent with the known reactivity of DBNPA with dissolved organic carbon (DOC) in aqueous systems. The study was not conducted according to guidelines but was conducted according to GLP**. Reliability Indicator = 3**

[redacted] A7.1.1.2.1/01

The biodegradation of DBNPA in seawater was assessed using a modification of the shake flask method in OECD 306. The modifications of the test procedure involved lowering the initial concentrations of the test compound and measuring $^{14}CO_2$ production as an indicator of biodegradation. The [^{14}C] DBNPA was added to reaction mixtures at two concentrations (0.02 and 0.10 mg/L) prepared with authentic seawater amended with inorganic nutrients.

Rapid primary degradation of [^{14}C]DBNPA was observed at both initial concentrations. At the high dose, the half-life was about one day, while at the low dose already on day one no DBNPA remained. As in the abiotic control on day three still 40% of DBNPA remained, it can be concluded that the primary degradation of DBNPA is to a considerable extent biologically-mediated. After 28 days, 17 to 22% of the [^{14}C]DBNPA was mineralised to $^{14}CO_2$ in viable test solutions, compared to 7% mineralisation of [^{14}C]DBNPA in the abiotic control prepared with $HgCl_2$ sterilant. After 60 days, $^{14}CO_2$ levels reached 26 to 36% in viable test solutions, while levels in the abiotic control remained at approximately 7%. Limited production of $^{14}CO_2$ in the abiotic control indicated that the mineralisation observed was predominated by the biological process. It can be concluded that DBNPA exhibits inherent primary biodegradability in the marine environment. **Relability Indicator = 2**

The results of the activated sludge die away test show a fast biodegradability of DBNPA under a real life situation. DBNPA degraded immediately as no DBNPA was found at the first sample collection at 1 minute. Due to general measurement uncertainties in laboratories, a conservative assumption was made that the DT_{50} for primary degradation of DBNPA is 10 minutes.

DBNPA exhibits inherent primary biodegradability in the marine environment.

11.1.2.2 Water, water-sediment and soil degradation data (including simulation studies)

11.1.2.2.1 Water and Water-sediment degradation

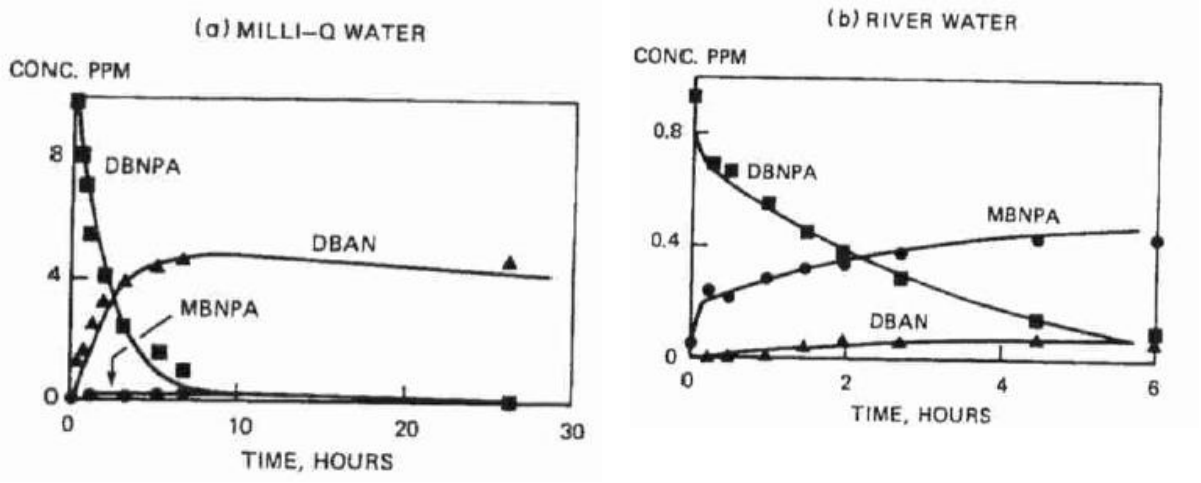
[redacted] Aerobic aquatic degradation of DBNPA in purified and natural waters.

The study : Doc. 792-002; A7.1.2.2.1/01 described below is a scientific publication on the hydrolytic degradation of DBNPA and the degradation of DBNPA in the presence of organic material occurring in natural waters. The main purpose of this study was to investigate the influence of organic material present in natural waters on the degradation rates of DBNPA. Organic material was measured as total organic carbon (TOC). Eight separate experiments were conducted over a dosage of 1-10 mg DBNPA in unfiltered waters with increasing measured TOC levels (Mili-Q purified water, water from lake Huron at 3 TOC levels and water from 3 rivers). Experiments were conducted under normal laboratory lighting. The pH of the waters was adjusted to pH 8.0-8.1 with 0.1N NaOH prior to addition of DBNPA. All tests were performed at 24-25ºC, except for one study, which was performed at 17ºC. Milli-Q water with a very low total organic carbon content served as comparison control.

A computer simulation model based on the chemistry presented [redacted] (refer to Doc III-A, Section A7.1.1.1.1/02), as extended by the inclusion of an MBNPA step, was used in simulating the results of a series of laboratory degradation studies.

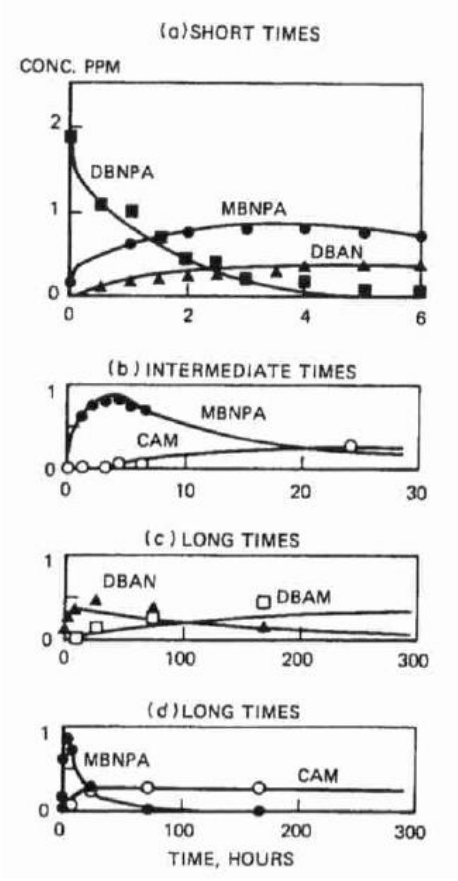
The study shows that organic material present in natural waters contributes to the degradation of DBNPA. In addition, it confirms two reaction pathways: (1) DBNPA -> DBAN -> DBAM and (2) DBNPA -> MBNPA-> CAM. The two pathways are in line with those found by Exner, J.H. et al. (see chapter 4.1.1.2.4).

The degradation in the presence of organic material yields the one-bromine species monobromonitrilopropionamide (MBNPA), which is further degraded by loosing the second bromine to yield cyanoacetamide (CAM). It was observed that only little MBNPA or CAM was formed in the absence of measurable total organic carbon (TOC) and proportionally higher amounts of MBNPA and CAM were formed at higher TOC concentrations. The figures of the publication show measured values (dots) along with regression lines according to a simulated concentration/time relationship model. Two typical degradation profiles are shown in the figures below. The effects of the higher TOC/DBNPA ratio in driving the degradation towards MBNPA and CAM can be seen in the figures (note shorter time range in the right figure); the ratio of TOC to DBNPA, rather than the absolute level of either, controls the distribution of degradation between the two pathways.



Comparison of high and low TOC waters: Data and simulations. Concentrations in ppm (mg/L). (a) Milli-Q Water. Initial DBNPA 10 ppm, pH 8, 24°C, TOC 1 ppm; (b) River water. Initial DBNPA 1 ppm, pH 8, 17.5°C, TOC 6.2 ppm.

Development through time is shown in the figure below for one of the experiments. The rapid degradation of DBNPA is evident. It is accompanied by the formation, then degradation of MBNPA. The degradation of MBNPA is accompanied by the formation of CAM. The plots also show that dibromoacetonitrile (DBAN) is formed at the beginning and then degraded. The degradation of DBAN coincides with the formation of dibromoacetamide (DBAM).



Data and simulations for Chippewa River water. Concentrations in ppm. Initial DBNPA 2.06 ppm; pH 8, 25 °C, TOC 12 ppm. (a) Short reaction times showing DBNPA, MBNPA, DBAN; (b) Intermediate reaction times showing MBNPA, CAM; (c) Long reaction times showing DBAN, DBAM; (d) Long reaction times showing MBNPA, CAM.

From the study, it can be deduced that organic material present in natural waters directs the degradation route of DBNPA towards the formation of MBNPA and CAM, i.e. the degradation pathway 2 becomes predominant. Where both water and organic matter are present in the environment, it is expected that DBNPA will degrade via both pathways in parallel. The study is a none-guideline study that does not follow GLP. However, the test conditions are well-described and the quality of the results are of an acceptable level, so the publication is given a ***Reliability Indicator of 2*** by RMS.

By visual analysis of the different graphs presented in the figures shown, it is possible to estimate three DT_{50} values for different conditions (DBNPA initial concentration and TOC content):

Initial DBNPA concentration: 2 ppm; pH 8; 12 ppm TOC; 25 °C (river water): DT_{50} = ca. 1 hour

Initial DBNPA concentration: 10 ppm; pH 8; 1 ppm TOC; 24 °C (Milli-Q water): DT_{50} = ca. 2 hours

Initial DBNPA concentration: 1 ppm; pH 8; 6.2 ppm TOC; 25 °C (river water): DT_{50} = ca. 2 hours*

*rates were determined at 17.5 °C and were extrapolated to 25 °C.

Hence, it is considered reasonable to use a DT_{50} value of 2 hours for the degradation of DBNPA in surface waters at 25ºC., corresponding to 5.7 hours at 12ºC.

[redacted]; aerobic and anaerobic

In 2007, two studies of [redacted]: Doc. 714-002 (A7.1.2.2.2/01) and Doc. 715-001 (A7.1.2.2.2/02) were submitted as key studies for degradation of DBNPA in water-sediment systems. As reported in Doc. 714-002, DBNPA degraded rapidly in a water-sediment system under aerobic conditions with a half-life of less than 5 hours. The major degradation products (> 10% yield on basis of the applied dose) detected were cyanoacetamide (up to 59.8% in water, 17.6% in sediment) and dibromoacetic acid (up to 67.3% in water, not detected in sediment) from degradation Pathway 2 and degradation Pathway 1 of DBNPA, respectively. It should also be noted that we lack identification of one major metabolite, a so-called 'Unknown metabolite" (up to 10.9% in water, 0.2% in sediment). Mineralisation was at a maximum level of 10.7% of the initial dose at day 30 and the yield of bound residues increased throughout the course of the study to a maximum of 16.3% of the initial dose at day 30. These results show that a field accumulation study in sediment is not needed.

In the water-sediment study under anaerobic conditions (Doc. 715-001), DBNPA degraded with a half-life of less than 4 hours. The major degradation products detected were oxalic acid (up to 10.3% in water, not detected in sediment), cyanoacetamide (up to 59.6% in water, 17.5% in sediment) and dibromoacetic acid (up to 28.8% in water, not detected in sediment). At Ddy 48, approximately 9.5% and 3.3% of the initial dose of radioactivity was captured in the sodium hydroxide traps and the ethylene glycol traps, respectively. This level had increased steadily from day 14 of the study. The radioactivity represented volatile degradation products. Bound residue yields increased throughout the course of the study to a maximum of 7.9% of the initial dose at day 48.

RMS do not regard the two above-mentioned water-sediment studies as valid. According to OECD 308, "Recoveries should range from 90% to 110% for labelled chemicals". Unfortunately, the material balances are not satisfactory in the two studies. In addition, it is not clear how the material balances is calculated from the available data. The two studies do not meet the primary requirements of OECD 308, so they are given a ***Reliability Indicator of 3***

[redacted] A7.1.2.2.2/03

The study of [redacted] confirms the rapid degradation of DBNPA. The degradation of DBNPA was investigated in microcosms prepared with water and sediment collected from the Tittabawassee and Muskegon rivers and incubated at 20 ± 2°C for 56 days. Overall, DBNPA reached non-detectable levels (<1.5% of initial radioactivity) within one hour (mixing time prior to day 0 sampling) in both the water and sediment layers as the test compound was rapidly transformed. Cyanoacetamide and cyanoacetic acid were identified as degradation products formed at greater than 10% yield within one day in both viable and sterilized microcosms. During the remainder of the study the degradation products, in turn, were mineralized to $^{14}CO_2$, strongly bound to the sediment, or incorporated into biomass in the viable microcosms. The formation of the degradation products is consistent with the reported degradation pathway involving indirect photolysis as well as the reaction of DBNPA with nucleophilic substances.

During the 56 days study [redacted] A7.1.2.2.2/03, material balance was 71-105% of initial radioactivity applied. In addition, DBNPA reached non-detected levels in the water and sediment layers of both the viable and sterile control microcosms within one hour. There is one day between the first and the second samples in each test system, so we are concerned that the metabolites are not adequately addressed in the beginning of the study. It should be noted that Dibromoacetic aid constitutes up to 67.3% (Replicate 1) and 65.5% (Replicate 2) of the initial radioactivity applied in water at hour 0 in the study of [redacted] (A7.1.2.2.2/01), but this major transformation product ($\geq$ 10% on amount basis of the applied dose) is not identified in the study of [redacted] (A7.1.2.2.2/03). The validity criteria according to OECD 308 cannot be considered as fulfilled, why the study is given a ***Reliability Indicator of 3*** by RMS.

11.1.2.2.2 Soil

Only a supportive study on degradation in soil is available. The substance is readily biodegradable and abiotic degradation of the substance plays an important role in the environment. . The degradation of DBNPA in soils seems to be fast and the expected degradation half-life is estimated to be 2 days at 12° C.

11.1.2.3 Photochemical degradation

11.1.2.3.1 Water

[redacted] A7.1.1.1.2/01

In the study, the effect of natural sunlight on the degradation of aqueous solutions of DBNPA was tested at pH 5, 7, and 9. The test was performed under aseptic conditions at mean temperatures of 22.9 ± 3.8°C with a range of 16.0 - 28.0°C at 41°46´ latitude North (Wareham, Massachusetts, USA) in the month of September. As the UV-visible absorption spectra for DBNPA at pH 5, 7, and 9 showed no appreciable absorption from 290 to 700 nm, the quantum yield for DBNPA was not calculated. For the same reason, the observed degradation effects were attributed to hydrolysis, oxidation and indirect photolysis, but not to direct photolysis.

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 pH 5 dark controls did not degrade appreciably during the four days of exposure. The pH 7 and 9 controls had a hydrolytic half-life of 53.2 hours and 34.3 minutes, respectively. The observed degradation in the dark controls was attributed to hydrolysis, whereas in the exposed solutions hydrolysis, oxidation and indirect photolysis took place. The results indicate that indirect photolysis plays a role in degradation of DBNPA in water at pH 5 and pH 7, but this role is not further quantified or estimated.

Material balance results were in the range of 96.4 - 100.1% of initial radioactivity applied. It should be noted that mass balance data was only analysed at hour 0 and at study completion at each pH in triplicate. In addition, we are not able to follow the percentage of DBNPA and its degradation products during the course of the test. At the termination of the study, the following degradation products were identified:

- **Oxalic acid** (at pH 5, 7, 9); formed via the hydrolysis pathway.

- **Bromoacetamide** (at pH 9); no available information about the formation.

- **Dibromoacetic acid** (at pH 5, 7, 9); formed via the hydrolysis pathway.

RMS lacks identification of two major degradation products (≥ 10% on amount basis of the applied dose): Unknown A (61.4%, only at pH 9) and Unknown C (up to 21.8%, at pH 5, 7) included in Table 95. It should be noted that photolytic products were not distinguished from hydrolytic products in the study. The study is given a ***Reliability Indicator of 3*** However, this study provides useful and relevant information for exposure and risk assessment in the aquatic environment- esp. for pH 5 and 7 conditions, where hydrolysis is apparently slower than photolysis.

Table 95: Distribution of radioactivity across DBNPA and its observed degradates in % activity, determined by isocratic elution HPLC

	pH 5 (hour 38.08)		pH 7 (hour 14.67)		pH 9 (hour 1.00)	
	Exposed	Control	Exposed	Control	Exposed	Control
DBNPA	16.7	68.6	0	0.550	0.010	0
Oxalic acid	3.15	0	11.8	13.2	11.3	29.9
Bromoacetamide	0	0	0	0	14.6	16.7

Dibromoacetic acid	63.7	31.4	66.5	74.9	11.5	0
Bromoacetic acid	0	0	0	0	0	
Dibromoacetonitrile	0	0	0	0	0	0
Unknown A	0	0	0	0	61.4	51.7
Unknown B	0	0	0	0	1.22	1.78
Unknown C	16.5	0	21.8	11.3	0	0

Note:

Average recoveries at pH 3 were as follows: 91.6±12.9 for oxalic acid, 103±5.0 for DBNPA, 99.6±7.93 for bromoacetamide and 92.3±5 for dibromoacetic acid.

The limits of detection were indicated as: 7.93 mg/L for oxalic acid, 7.32 mg/L for DBNPA, 3.01 mg/L for bromoacetamide and 4.72 mg/L for dibromoacetic acid.

In the aquatic environment, especially at pH 5 and 7, hydrolysis is slower than photolysis.

[redacted] A7.1.1.1.2/03

The effect of natural sunlight was investigated on the degradation of aqueous solutions of DBNPA at pH 3, 5, 7 and 9. The test was performed in Israel (Temprature up to 43.0ºC) over a 19 day exposure period. The study was conducted according to FIFRA Guideline 161-1. The concentrations of DBNPA and identified degradation products were followed using HPLC with UV detection. The following transformation products were identified:

DBAc: dibromoacectic acid

CA: cyanoacetic acid

MBNPA: 2-bromo-2-nitropropionamide
MBA: dibromoacetamide

DBACN: dibromoacetonitrile.

Two sets of graphs were obtained;

1) Changes observed in the concentrations of CA, MBNPA and DBNPA, i.e. main pathway products
2) Changes observed in the concentrations of secondary degradation products.

There was a rapid fall-off of DBNPA wit concomitant increases of the other products. At pH 3, the rise and fall ofMBNPA is shown clearly.

As the pH increases DBNPA fall-off is more rapid and the two main pathway products become less important. The secondary degradation products increase in intensity as pH rises.

At pH 3, the most intense is DBAc, reaching an intensity after 3 weeks of less than one third that of the maximal intensity of MBNPA and less than one tenth that of DBNPA.

At pH 5 DBAc reaches an intensity of less than one tenth that of DBNPA after approximately 3 weeks. The absolute amount of MBAm is questionable because an acceptable standard was not available and the response factor was therefore estimated to be equivalent to that of DBAm.

At pH 7 DBACN peaks in three days and recedes. Its maximum is less than one tenth that of the starting amount of DBNPA.

At pH 9 only MBAm and DBACN are of any significance but fall-off after peaking in 2 – 3 hours.

The total recovered weight did not equal the initial weight of DBNPA An analysis of bromide ion was performed on Day 19. The overall material recovery was obtained by adding the bromide to the totals of the other measured components. The over-estimate, as compared to starting concentration of DBNPA, is probably due to the faulty estimate of the response factor of MBAm. It is probable that other products have been formed which were not detected, such as, monobromoacetic acid (MBAc), acetic acid, oxalic acid, glycolic acid and carbon dioxide

Due to lack of UV absorbance in the sunlight spectral region, DBNPA is not degradable by direct photodegradation in water; rather, indirect photolytic mechanisms appear to be responsible for the observed accelerated degradation (relative to hydrolysis alone) under sunlight-irradiated conditions. Furthermore, the DBNPA is not persistent to other degradation processes (e.g. biodegradation and hydrolysis) which indicates that the rate of indirect aqueous photolysis is of minor importance in the fate process for this substance.

11.1.2.3.2 Air

The photochemical and oxidative decomposition of DBNPA in air was evaluated based on theoretical grounds by a calculation according to Atkinson. The calculation was performed with the help of the programme AOPWIN, Atmospheric Oxidation Programme v1.92 for (© 2000 US Environmental Protection Agency):

SMILES : O=C(N)C(C(#N))(Br)Br

CHEM : Acetamide, 2,2-dibromo-2-cyano-

MOL FOR: C3 H2 Br2 N2 O1

MOL WT : 241.87

-----SUMMARY (AOP v1.92): HYDROXYL RADICALS (25 deg C)-----

Hydrogen Abstraction = 0.0000 E-12 cm3/molecule-sec

Reaction with N, S and -OH = 2.0000 E-12 cm3/molecule-sec

Addition to Triple Bonds = 0.0000 E-12 cm3/molecule-sec

Addition to Olefinic Bonds = 0.0000 E-12 cm3/molecule-sec

Addition to Aromatic Rings = 0.0000 E-12 cm3/molecule-sec

Addition to Fused Rings = 0.0000 E-12 cm3/molecule-sec

OVERALL OH Rate Constant = 2.0000 E-12 cm3/molecule-sec

HALF-LIFE = 8.022 Days (24-hr day; 0.5E6 OH/cm3)

HALF-LIFE = 192.528 Hrs

-----SUMMARY (AOP v1.91): OZONE REACTION (25 deg C)-----

****** NO OZONE REACTION ESTIMATION ******

(ONLY Olefins and Acetylenes are Estimated)

Experimental Database: NO Structure Matches

The outcome from the AOPWIN programme shows that DBNPA degrades in the atmosphere by reaction with OH radicals, having a DT_{50} value of 192.5 hours or a DT_{50} value of about 8 days. As DBNPA is only slightly volatile for both its pure form (vapour pressure = 1.19 x 10^{-3} Pa at 19.2 °C), and from aqueous solution (estimated Henry's Law Constant = 2.04 x 10^{-5} Pa m^3 mol^{-1}at pH 7 and 20 °C) release to air and long-range transport in the atmosphere does not seem likely.

11.1.3 Summary of degradation

According to its chemical properties, DBNPA degrades via two concurrent pathways:

Pathway 1: DBNPA, DBAN, DBAM, DBAA, Glyoxylic acid, Oxalic acid, Carbon dioxide.

Pathway 2: DBNPA, MBNPA, CAM, CAA, Malonic acid.

Pathway 1 is the hydrolytical pathway and pathway 2 becomes relevant when DBNPA comes in contact with sulphur containing reducing species ("nucleophiles"), and/or light, and/or organic material.

DBNPA undergoes several degradation processes (e.g. nucleophilic reaction, biodegradation, indirect photolysis and hydrolysis) , which are not mutually-exclusive in relevant surface water compartments. In such compartments, the combined action of nucleophilic reaction, hydrolysis, biodegradation, and indirect photolysis may result in residence times which are much shorter than indicated by rates or half-lives of any of these reactions alone.

The following processes are involved in the degradation of DBNPA in the environment:

- Hydrolysis
- Indirect photolysis (this pathway is of minor importance)
- Biodegradation
- Reaction with sulphur containing reducing species (nucleophiles)
- Reaction with organic material
- Decomposition in the presence of soil.

Table 96 summarises the degradation rates and DT_{50} values for DBNPA in different media and under different conditions.

Table 96: Degradation behaviour of DBNPA

Media and conditions	Degradation rates
Hydrolysis	pH 4, 12°C: DT_{50} = 12079 hours

DBNPA (A7.1.1.1.1/03)	pH 7, 12ºC: DT_{50} = 183 hours pH 9, 12ºC: DT_{50} = 5.6 hours
Biodegradation of DBNPA (ready test) (A7.1.1.2.1/01)	78% mineralisation after 28 days. 58% mineralisation within 10-day window. the 60% trigger value was only missed by 2%.
Biodegradation of DBNPA (ready test) (A7.1.1.2.1/04)	35% mineralisation after 28 days. DBNPA is not ready biodegradable
Biodegradation (activated sludge die away test) (A7.1.2.1.1/01)	Immediate rapid primary degradation of DBNPA at 20 ± 2°C. A DT_{50} of 10 min. After 4 days 53% of the total radioactivity evolved as CO_2 in the die away test opposed to 5% in the abiotic control. A DT_{50} for mineralization was determined to be 8.1 hours.
Biodegradation of DBNPA in seawater (A7.1.1.2.3/01)	17 - 22% mineralisation after 28 days 26 - 36% mineralisation after 60 days at 18.6 ± 0.7°C.
.Degradation of DBNPA in air; Indirect Photolysis	DT_{50} = 192.5 hours or ca. 8 days. Temperature assumed to be 25°C.
Degradation in soil (A7.1.1.1.1/02)*	DT_{50} = 4 - 25 hours (primary degradation). at pH 4.8 - 7.5 and room temperature. Temperature assumed to be 20°C. DT_{50} of 11 hours is calculated (geometric mean, n = 7) at 20ºC, corresponding to 20.9 hours at 12ºC.
Degradation of DBNPA in natural surface water (A7.1.2.2.2./03)	DT_{50} = 2 hours. pH 8 and temperature 24-25°C. Corresponding to 5.7 hours at 12ºC.

*) The results support the assumption that a fast degradation in soil is expected.

Note: In the table, the temperatures assumed to be 25°C are purely assumed but conservative reference values since they lead to higher DT_{50} values at 12°C compared to lower reference temperatures (for example, a reference temperature of 20°C).

For many studies in this section, primary degradation is described. However, it should be noted that the CLP regulation (4.1.2.9.3) states '*Many degradation data are available in the form of degradation half-lives and these can be used in defining rapid degradation provided that ultimate biodegradation of the substance, i.e. full mineralisation, is achieved. Primary biodegradation does not normally suffice in the assessment of rapid degradability unless it can be demonstrated that the degradation products do not fulfil the criteria for classification as hazardous to the aquatic environment*'. The Guidance on the Application of the CLP criteria (p. 563) further states that '*Data on primary degradation can only be used where it is demonstrated that the degradation products shall not be classified as hazardous to the aquatic environment, i.e. that they do not fulfil the classification criteria*'. *In all studies that demonstrate primary degradation within the 16 day window and the degradation products are already classified under CLP or have no data available, rapid degradability cannot be demonstrated and the conclusion must be 'not rapidly degradable'*. Degradation products of DBNPA is estimated to have either the same or lower toxicity compared to DBNPA and therefore it must be concluded that for all available studies that only demonstrate primary degradation only can be reported to as supporting evidence and cannot justify the conclusion that DBNPA is rapidly degrable according to CLP. However, the most important data for considering rapid degradability under CLP are data from ready biodegradability tests and simulation tests. For DBNPA we have two valid ready biodegradability tests. One test showing 78% mineralisation after 28 days, 58% mineralisation within 10-day window. With approximately 58% mineralisation within the 10-day window, the pass level for ready biodegradability of 60% was missed by 2%. It should be noted that a little deviation from the 60% mineralisation could be due to uncertainty in measurement. The DT_{50} value for mineralisation can be read from the degradation curve to be about 12 days. Based on this study DBNPA is considered be rapidely degradable; however, DBNPA does not fulfille the strict criteria of ready biodegradable. In the other valid readi biodegradability test DBNPA only have 35% miniralisation after 28 days and DBNPA is therefore not eady biodegradable. In addition we have a activated sludge die away test showing a DT_{50} value for mineralization of 8.1 hours, After 4 days 53% of the total radioactivity evolved as CO_2 in the die away test opposed to 5% in the abiotic control. The sum of dissolved and extractable radioactivity (related to parent compound and degradation products) decreased from 85% to 7% within the same period (weaker decrease from 80 to 49% in the abiotic control). Altogether these data can be considered as clear hint for an extensive microbially mediated mineralization of DBNPA. The radioactivity which remained associated with the solid fraction (incorporated into biomass or irreversebly bound to it) increased from 25% to 33%. Finally other biodegradation studies are available which show fast degradation of DBNPA in natural waters/sediments and soils.

Based on the above data DBNPA is not considered to be ready biodegradable.

11.2 Environmental transformation of metals or inorganic metals compounds

Table 97: Summary of relevant information on rapid environmental transformation

Method	Results	Remarks	Reference
No data available			

11.2.1 Summary of data/information on environmental transformation

DBNPA does not contain any metal ions and is not classified as an inorganic metal compound. Environmental transformation of metals and inorganic metal compounds is therefore not applicable to this substance or any of it's degradation products.

11.3 Environmental fate and other relevant information

Volatilisation from water:

The Henry's law constant of DBNPA, calculated on the basis of the measured vapour pressure and water solubility, is 2.04 x 10^{-5} Pa * m^3 * mol^{-1}at pH 7 and 20ºC. The Henry's law constant for water itself is about

0.03 Pa * m^3 * mol^{-1}at pH 7 and 20ºC, hence volatilisation of DBNPA from surface waters is expected to be negligible.

Adsorption onto/desorption from soils:

[redacted] A7.1.3/01

One adsorption /desorption screening test is available. The adsorption/desorption behaviour of DBNPA was determined on four different soils according to the EPA FIFRA Guideline 163-1, which is comparable with OECD Guideline 106 “Adsorption - Desorption Using a Batch Equilibrium Method”. A screening test on adsorption and desorption, an equilibrium phase test and an adsorption isotherm test were performed. The choice of measurement method was liquid scintillation counting, so it cannot be excluded that degradation products are not tested due to the instability of DBNPA in water.

Table 98: Adsorption onto / desorption from four soils

EPA FIFRA Guideline 163-1 GLP Reliability = 2	Adsorbed a.s. [%]	K_{ads} Confidential[L/kg]	$K_{ads\ F}$ [1] [L/kg]	$K_{ads\ OC\ F}$ [2] [L/kg]	K_{des} [3] Step 1 [L/kg]	K_{des} [3] Step 2 [L/kg]	K_{dOC} [4] Confidential[L/kg]	K_{ads} / K_{des} [5]	Degradation products Name	Degradation products [%] of a.s.	Reference
Kansas Silty Clay Loam	20.7	1.30	2.05	99.5	8.08	27.4	3.02	calculated on the basis of the mean K_{ads} and the overall mean K_{des}	Not investigated		[redacted] A7.1.3/01
Georgia Loamy Sand	5.74	0.306	0.367	39.0	0.109 *	-3.68*	n.c.				
Georgia Sandy Clay Loam	4.15	0.217	0.782	663	-6.16*	-10.8*	n.c.				
Kansas Loamy Sand	27.8	1.93	1.55	146	10.4	39.9	7.78				
Arithmetic mean values	**14.6**	**0.94**	**1.19**	**236.9**	**9.24** **	**33.65** **	**5.41**	**0.13**			
					mean overall K_{des} = 7.26						

[1] K_a = Freundlich adsorption coefficient

[2] K_{aOC} = Freundlich adsorption coefficient based on organic carbon content

[3] K_d = Desorption coefficient

[4] K_{dOC} = Desorption coefficient based on organic carbon content

[5] K_a / K_d = Adsorption / Desorption distribution coefficient

* *Since the percent adsorbed material was low (approximately 5%) the desorption phase cannot provide accurate desorption values*.

** *Values with * were not considered for calculating the means*

n.c. = not calculated

The mean adsorption coefficient was calculated as:

$K_{ads\ oc\ F}$ = 236.9 L/kg

The study is given a ***RI of 2*** by RMS as it is not performed strictly according to guideline.

The K_{oc} was also estimated by a QSAR for non-hydrophobic substances from the K_{ow} of DBNPA (log K_{ow} = 0.8). By using equation ($logK_{oc}$ = 0.52 logKow + 1.02), the K_{oc} was calculated to be 27.3 L/kg.

The mean adsorption coefficient was calculated as $K_{ads\ oc\ F}$ = 236.9 L/kg based on an adsorption /desorption screening test. Although using the experimentally determined K_{oc} rather than QSAR estimated value is preferable, it should be taken into account that this value does not correspond to DBNPA alone but also to the sum of its degradation products. No specific analytical determination of DBNPA was carried in the assay, but the concentration was determined via scintillation counting and combustion, therefore does not allow to differentiate between DBNPA and any degradation products formed.

11.4 Bioaccumulation

The log Pow of DBNPA is 0.8. Consequently, the substance has a low potential for bioaccumulation.

Table 99: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
EPA, Draft 5/1/72, p-v-37; Fish non-GLP Reliability =3	***Steady-state BCF*** Whole fish: 13.0 Muscle: 9.9 ***Uptake rate const:*** Whole fish: 2.7 Muscle: 2.2 ***Depuration rate constant:*** Whole fish: 0.21 Muscle: 0.21 ***Depuration time (DT_{50}):*** DT_{50} = 3.3 days	Initial test concentration: 5 ppb, 50 ppb Exposure: 30 days Log Pow: 0.8 Metabolites: Not investigated	[redacted] A7.4.2/01

Calculation of BCF from log P_{OW}: Calculation of the BCF_{fish} are not applicable for substances with a log P_{ow} below 2.

11.4.1 Estimated bioaccumulation

Calculation of the BCF_{fish} are not applicable for substances with a log P_{ow} below 2.

11.4.2 Measured partition coefficient and bioaccumulation test data

11.4.2.1 Aquatic bioaccumulation

[redacted] A7.4.2/01

A bioconcentration study of DBNPA in fishes was performed. The test species was Rainbow trout (*Oncorhynchus mykiss*) acclimatised to test conditions for 14 days. The water was taken from Lake Huron and passed over activated carbon. The temperature was 15±1 °C, the oxygen content ranged from 69-100% of air saturation and the pH value was 7.8 - 8.0. A total of 40 fishes were exposed to test vessels of 40 L volume with 1 replicate per concentration and control. During uptake, fishes were sampled after 6, 12, 24, 48 and 96 hours and 10, 20 and 30 days. During clearance, sampling was performed after 6, 12, 24, 72 and 144 hours. Per sampling, 3 fishes were dissected into muscle and remainder.

Our overall impression is that the test design and the test conditions are not thoroughly described, so it is very difficult to understand all the aspects of the study. It should be noted that the study does not follow GLP and the test principle in OECD 305. The depuration phase was already started after 4 days of exposure, when some of the fishes were transferred to fresh water to determine the rate of clearance. At that time, the plateau concentration was far from reached yet. In addition, we are not able to evaluate the validity of the test, because there is no information about the control group of fish during the study. Generally, mortalities and abnormalities are not addressed in the study. The BCF values for muscle and whole fish are low (see Table 4.1.2-1). Regarding the calculated BCFs, the study can be supportive despite a poor ***Reliability Indicator of 3***.

DBNPA is considered as having a low potential for bioaccumulation;

- there is no risk of secondary poisoning as indicated by the log Pow of 0.8, which is below the trigger value of 4, and by the rapid breakdown of DBNPA in the environment .
- the surface tension of DBNPA was determined to be 72.2 ± 0.6 mN · m^{-1} at 25.0 ± 0.5°C; therefore DBNPA cannot be considered to be surface active (as indicated by surface tension ≤ 50 mN · m^{-1}).
- there are no structural features indicating bioaccumulation.

DBNPA is considered to have a low potential for bioaccumulation in aquatic organisms as indicated by the log P_{OW} of 0.8, which is below the trigger value of 4, and supported by the results of the bioconcentration study in fish.

11.4.2.2 Terrestrial bioconcentration

Based on its physical chemical properties, bioconcentration is of no concern because of the low log Pow of 0.8, its ready biodegradability, its rapid biodegradation in soil, instability in contact with organic substances and its metabolism in mammals (DBNPA showed low potential for bioaccumulation in the rat).

11.5 Acute aquatic hazard

Table 100: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results[1]	Remarks	Reference
EPA-FIFRA 72-3; GLP **Reliability = 1**	Sheepshead minnow (*Cyprinodon variegatus)*	DBNPA (mean measured conc.)	LC_{50} = 3.4 mg/L (concentration range used: 0 – 10 mg/L)	Flow through Saline water (31-35‰), 96 h *at pH 7.9-8.1* 23 °C	[redacted] A7.4.1.1/01
No **Reliability = 3**	Sheepshead minnow (*Cyprinodon variegatus*)	DBNPA (nominal conc.)	LC_{50} = 1.4 mg/L (concentration range used: 0.6 – 6 mg/L)	Static 96h *at pH 7.9-8.1* 21-22°C No analytical verification of the test concentrations. Little information about the test material e.g. the purity	[redacted] A7.4.1.1/01a
US-EPA-662/3-75-009; GLP **Reliability = 3**	Bluegill sunfish (*Lepomis macrichirus*)	DBNPA (nominal conc.)	LC_{50} = 2.3 mg/L (concentration range used: 1.0 – 10 mg/L)	Static 96h *at pH 7.2-7.3* 22°C No analytical verification of the test concentrations. Little information about the test material e.g. the purity)	[redacted] A7.4.1.1/04
US-EPA-662/3-75-009; GLP **Reliability = 3**	Rainbow trout (*Oncorhynchus mykiss*)	DBNPA (nominal conc.)	LC_{50} = 2.3 mg/L (concentration range used: 0 – 10 mg/L)	Static 96 h *at pH 7.2-7.6* 12°C No analytical verification of the test concentrations. Little information about the test material e.g. the purity)	[redacted] A7.4.1.1/05

ASTM, 1980; GLP **Reliability = 3**	Fathead minnow (*Pimephales promelas*)	DBNPA (nominal conc.)	LC_{50} = 1.8 mg/L (concentration range used: 0.9 – 2.5 mg/L)	Static 96 h *at pH 7.8-8.1* 16.4-16.8°C The analytical verification of the test concentrations was only performed for the highest test concentration in a parallel test aquaria under superficially described test conditions. The measured concentrations are much lower than 80% of nominal concentrations (only 0.4% at termination of the test).	[redacted] A7.4.1.1/06
US EPA-FIFRA 72-3; **Reliability = 1** GLP	Mysid shrimp *(Mysidopsis bahia)**	DBNPA (mean measured conc.)	LC_{50} = 0.72 (concentration range used: 0.23 – 3 mg/L)	Flow through 96 h *at pH 7.9-8.1* 25 ± 1°C 31-32‰ The validity criterion was met.	[redacted] A.7.4.1.2/05
US EPA-FIFRA 72-3; **Reliability = 3** GLP	Eastern oyster *(Crassostrea virginica)**	DBNPA (extrapolated conc.)	EC_{50} = 0.17 (EC_{50} = 0.37; based on nominal conc.) (concentration range used: 0.13 – 1 mg/L)	Flow through 96 h *at pH 7.7-8.1* 20 ± 2°C Seawater used: 31‰ Analysis of the four lowest exposure solutions on days 0 and 4 resulted in measured concentrations which were below the detection limit for DBNPA.	[redacted] A.7.4.1.2/04
ASTM Standard, 1990; US-EPA-660/3-75-009, 1975; **Reliability = 3** GLP	*Daphnia magna*	DBNPA (nominal conc.)	EC_{50} = 0.60 EC_0 = 0.06 (concentration range used: 0.9 – 2.5 mg/L)	Semi-static 48 h *at pH 7.8 – 8.1* 20 ± 1°C Test solution was renewed after 24 hours; however as the half-life of DBNPA is about 2 hours under these conditions, RMS does not think the the exposure concentrations had remained within 80% on nominal values.	[redacted] A7.4.1.2/01
EPA-660/3-75-009(1975) **Reliability = 3**	*Daphnia magna*	DBNPA (nominal conc.)	EC_{50} = 0.9 (concentration range used: 0.75 – 1.8 mg/L)	Static 48 h *at pH 7.6* 25 °C	[redacted] A7.4.1.2/08

				Concentrations not measured	
EPA-660/3-75-009(1975) **Reliability = 3**	*Daphnia magna*	DBNPA (nominal conc.)	EC_{50} = 0.86 (concentration range used: 0.32 – 3.2 mg/L)	Static 48 h *at pH 7.8-8.3* 20 °C Concentrations not measured	[redacted]) [redacted] A7.4.1.2/09
OECD 201 GLP **Reliability = 3**	*Chlorella vulgaris*	DBNPA (nominal conc.)	E_bC_{50} = 0.38 mg/L NOEC = 0.1 mg/L (concentration range used: 0.9 – 2.5 mg/L)	Static 72 h *at pH 8* 24.1 ± 1°C Concentrations not measured	[redacted] A7.4.1.3/01
OECD 201 GLP **Reliability = 2**	*Scenedesmus subspicatus*	DBNPA (mean measured conc.)	E_bC_{50} = 0.9mg/L E_rC_{50} = 2.3 mg/L NOEC = 0.36 mg/L (initial concentration range used: 0.9 – 13.7 mg/L. After 72 hours: <LOQ – 3.59 mg/L)	Static 72 h *at pH 7.1-8.0* 24 ± 1°C As the analytical results was significantly below the acceptable 80% of nominal the effect concentrations were calculated based on the time-weighted mean measured test concentrations.	[redacted] A7.4.1.3/04

11.5.1 Acute (short-term) toxicity to fish

Five studies have tested the aquatic toxicity of DBNPA on fish, the study of DBNPA to Sheepshead minnow (*Cyprinodon variegatus*) has an acceptable Reliability Indicator and therefore its endpoint value (LC_{50} = 3.4 mg/L) is used to determine classification.

The available LC_{50} values for DBNPA for the different fish species are all in the same order of magnitude, ranging from 1.4 - 3.4 mg DBNPA /L.

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

The acute toxicity of DBNPA was tested to three species of invertebrates: *Daphnia magna*, *Crassostrea virginica* and *Mysidopsis bahia*.

The acute endpoint values for DBNPA for the different invertebrates species were 0.6- 0.9 mg/L (EC_{50} of *Daphnia magna*), 0.17 mg/L (EC_{50} of *Crassostrea virginica*) and 0.72 mg/L (LC_{50} of *Mysidopsis bahia*). Only the study of DBNPA to *Mysidopsis bahia* has an acceptable Reliability Indicator and therefore its endpoint value (LC_{50} = 0.72 mg/L) is used to determine classification.

11.5.3 Acute (short-term) toxicity to algae or other aquatic plants

A study was performed to assess the effect of DBNPA on the growth of the green alga *Scenedesmus subspicatus*. Following a preliminary range-finding test, *Scenedesmus s ubspicatus* was exposed to an aqueous solution of the test material at concentrations of 1.0, 2.0, 4.0, 8.0 and 16 mg/L (three replicate flasks per concentration) for 72 hours, under constant illumination and shaking at a temperature of 24 ± 1ºC. Due to the rapid degradation of DBNPA the substance tested was a mixture of DBNPA and its degradants.

For the test concentrations at 72 hours where the measured test concentration was less than the LOQ of the analytical method, a value of half the LOQ was used for the calculation of the time-weighted mean measured test concentration. Based on the time-weighted mean measured test concentrations the E_bC_{50} (72 h) was 0.90 mg/L; 95% confidence limits 0.73 - 1.1 mg/L and the E_rC_{50} (0 - 72 h) was 2.3 mg/L; 95% confidence limits 1.9 - 2.8 mg/L. NOEC was 0.36 mg/L.

The toxicity of DBNPA was originally tested to the freshwater alga *Chlorella vulgaris*. No analytical monitoring of the test material in the test solutions was performed even though DBNPA degrades very quickly. E_rC_{50} (0-24 hours) of 0.15 mg/L was determined, but E_rC_{50} (72 hours) was not given. E_bC_{50} (72 hours) was available, and was determined to be 0.28 mg/L. NOEC (72 hours) was 0.1 mg/L.

Conclusion

Based on the time-weighted mean measured test concentrations of DBNPA, the E_bC_{50} (72 h) was 0.90 mg/L; 95% confidence limits 0.73 - 1.1 mg/L and the E_rC_{50} (0 - 72 h) was 2.3 mg/L; 95% confidence limits 1.9 - 2.8 mg/L. NOEC was 0.36 mg/L.

11.5.4 Acute (short-term) toxicity to other aquatic organisms

Table 101: Summary of relevant information on acute aquatic toxicity – other organisms

Guideline / Test method	Endpoint / Type of test	Exposure		Results	Test material	Reference
		Design	Duration/ condition	EC_{50} (mg/L)		
OECD Respiration inhibition test (1981) GLP **Reliability = 3**	Respiration rate	Respiration inhibition test	3 h at pH 7.5 21°C	3.1 Norminal concentrations r ange from 0.5-30 mg/L Controls of DBNPA are not described in the study, so we are not able to check, if the two controls respiration rates are within 15 percent of each other.	DBNPA (no information about test concentrations)	[redacted] A7.4.1.4/01
OECD 209 (1984); EEC 92/69, Part C11, 1988 GLP **Reliability = 2**	Respiration rate	Respiration inhibition test	3 h at pH 7.8 20 ± 2°C	4.6 concentrations r ange from 1-500 mg/L Validity criteria is fulfilled	DBNPA (nominal conc.)	[redacted] A7.4.1.4/02

Two study reports on respiration inhibition of activated sludge are submitted. The study by [redacted] (A7.4.1.4/01) is a screening survey for IC_{50} values of test chemicals. The effects of a wide variety of inorganic and organic chemicals on activated sludge respiration rates were examined in the study. Statistical analysis of activated sludge data was conducted for 55 chemicals including Dibromonitrilopropionamide (= DBNPA), and the test with DBNPA was not individually reported.

The other study, performed by [redacted] (A7.4.1.4/02) fulfils the validity criteria required by OECD 209. The test organisms were activated sludge from a waste water treatment plant treating predominantly domestic wastewater. After incubation of sewage sludge over 3 hours treated with DBNPA, the EC_{50} was determined to be 4.6 mg/L. The study was rated with a **Reliability Indicator** of 2.

The two tests on the respiration inhibition of activated sludge resulted in comparable EC_{50} values for DBNPA; however the EC_{50} of 4.6 mg DBNPA/L was considered as the only valid value.

11.6 Long-term aquatic hazard

Table 102: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results			Remarks	Reference
			NOEC (mg/L)	**LOEC (**mg/L)	**MATC (**mg/L)		
EPA FIFRA 72-4; Embryo viability, survival of embryos at hatch and survival and growth (weight and length) of larvae after 60 days post-hatch exposure GLP **Reliability = 2**	Rainbow trout (*Oncorhynchus mykiss*)	DBNPA (mean measured conc.) Initial concentration range from 0 – 4.1 mg/L	0.47 mg/L	0.98	< 0.98 - > 0.47 Geometric mean = 0.68	Flow-through 85 days at pH 6.5-7.6 12 ± 1°C	[redacted] A7.4.3.2/01
EPA FIFRA 72-4 Effects on survival and reproduction GLP **Reliability = 3**	*Daphnia magna*	DBNPA (mean measured conc.) Initial concentration range from 0 – 1 mg/L	0.05 survival < 0.02 reproduction	0.08 survival 0.02 reproduction	< 0.08 - ≥ 0.05 survival Geometric mean = 0.06	Flow-through 28 days at pH 7.8-8.3 20 ± 1°C It was not reported, if abnormal appearance andor behaviour were observed during the study. The mortality of control daphnids exceed 20%. The mean number of offspring produced per parent animal surviving at the end of the test is below 60. The study does not met the validity criteria.	[redacted] A7.4.3.4/01
OECD 211 and USEPA OPPTS 850.1300 Mortality and sublethal effects that included reproduction and growth	*Daphnia magna*	DBNPA (mean measured conc.) Mean measured concentrations	**0.060** survival 0.060 reproduction	0.170 survival >0.060 reproduction*	0.100 survival	Flow-through 21 days at pH 7.8-8.3 19 - 22°C	[redacted] A7.4.3.4/02

GLP **Reliability = 2**		were <LOQ, 0.0055, 0.020, 0.060 and 0.17 mg/L	0.060 Growth (total body length) 0.060 Growth (dry weight)	>0.060 Growth (total body length)* >0.060 Growth (dry weight)*Confidential			
EPA FIFRA 72-4; GLP **Reliability = 2**	*Daphnia magna*	DBNPA Initial concentrations range from 0.007 to 0.9 mg/L	**0.07** 0.07 reproduction 0.54 Growth	0.12 reproduction	0.092 for reproduction	Flow-through 28 days at pH 7.2-9 20 ± 2°C	[redacted] A7.4.3.4/03

*Note: 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.

11.6.1 Chronic toxicity to fish

A single study by [redacted] (A7.4.3.2/01) reported the long-term toxicity of DBNPA to fish. Rainbow trout (Oncorhynchus mykiss) embryos and larvae were continuously exposed to DBNPA for 85 days under flow-through conditions at concentrations ranged from 0.096 to 4.1 mg/L. Observations were made on embryo viability, survival of embryos at hatch and survival and growth of larvae after 60 days of post-hatch.

The long-term exposure of fish to DBNPA resulted in an NOEC of 0.47 mg DBNPA /L, based on mean measured concentrations.

11.6.2 Chronic toxicity to aquatic invertebrates

[redacted] A7.4.3.4/01

Juvenile *Daphnia magna*, less than 24 hours old at test initiation, were continuously exposed to DBNPA for 28 days under flow-through conditions at concentrations ranged from 0.02 to 0.93 mg/L. Observations were made on survival of daphnids and number of offspring produced per female during the course of the test. The NOEC was determined to be 0.05 mg/L for survival and < 0.02 mg/L for reproduction. RMS cannot accept the lowest NOEC value: < 0.02 mg/L for reproduction. According to OECD 211, "the lowest test concentration must be low enough so that the fecundity at that concentration is not significantly lower than that in the control. If this is not the case, the test will have to be repeated with a reduced lowest concentration". Therefore, the study is given a ***Reliability Indicator of 3***.

[redacted] A7.4.3.4/03

Juvenile *Daphnia magna*, less than 24 hours old at test initiation, were continuously exposed to DBNPA for 21 days under flow-through conditions at concentrations ranging from 0.07 to 0.9 mg/L. Observations were made on survival and growth of daphnids and number of offspring produced per female during the course of the test.

During this study the pH ranged from 7.2 to 8.0; the dissolved oxygen concentration averaged 8.7 mg/L (6.0 – 9.8 mg/L); and the temperature averaged 20.2°C (19.9 – 20.7°C0. The light intensity ranged from 1062 to 1372 lux.

The five-day LC50 was calculated to be 0.732 mg/L and the 21-day LC50 was calculated to be 0.256 mg/L. There was 100% mortality at the 0.90 mg/L treatment level.

Statistical analysis of the number of progeny/female produced a 21-day EC50 of 0.13 mg/L and a NOEC of 0.07 mg/L. The lowest observed effect concentration (LOEC) was 0.12 mg/L. Taking the geometric mean of the LOEC and the NOEC, the maximum acceptable toxicant concentration (MATC) is calculated to be 0.092 mg/L. Analysis of the number of broods per female provided an EC50 of 0.16 mg/L and a NOEC of 0.07 mg/L. The LOEC was 0.12 mg/L ; consequently the MATC for this reproduction parameter is 0.092mg/L. Length varied non-systematically with a significant reduction in size (alpha 0.05) at 0.32 mg/L but not at other concentrations evaluated. The NOEC for length was judged to be 0.54 mg/L. the study is given a ***Reliability indicator of 2***.

[redacted] A7.4.3.4/02

The exposure to DBNPA was performed under flow-through conditions for a period of 21 days with *Daphnia magna*, less than 24 hours old at test initiation. Observations were made on survival of parental daphnids, number of offspring produced per female and growth of parental daphnids (total body length, body weight). The study is a combined reproduction and mortality test as the range of test concentrations include concentrations that have a statistical effect on adult survival. Nominal concentrations were 0.031, 0.063, 0.13,

0.25 and 0.50 mg/L. Mean measured concentrations were <LOQ, 0.0055, 0.020, 0.060 and 0.17 mg/L, representing NA, 8.8, 15, 24 and 34% of nominal concentrations.

The most sensitive indicator of toxicity is percent survival of parental daphnids. The survival is significantly reduced at 0.17 mg/L (mean measured concentration) compared to the pooled control based on Dunett's Multiple Comparison Test, and the NOEC is determined to be 0.060 mg/L mean measured concentration). The NOEC values for reproduction and growth (total body length, dry weight) are 0.060 mg/L (mean measured concentration). ***Reliability indicator of 2***.

In conclusion, the 21-day NOEC for DBNPA is 0.060 mg/L (mean measured concentration) based on percent survival.

11.6.3 Chronic toxicity to algae or other aquatic plants

Long term toxicity studies in fish and invertebrates are available for DBNPA. In accordance with the guidance, NOEC values from a short term toxicity study on algae (e.g. OECD 201) can be considered to be a chronic endpoint when used in conjunction with NOEC values from long-term fish and invertebrate studies.

Based on the time-weighted mean measured test concentrations of DBNPA, the NOEC was 0.36 mg/L.

11.6.4 Chronic toxicity to other aquatic organisms

There are no other chronic toxicity studies available for DBNPA.

11.7 Comparison with the CLP criteria

11.7.1 Acute aquatic hazard

The toxicity of DBNPA to aquatic organisms is well-documented by acute and long-term studies.

Acute toxicity EC_{50} values for DBNPA are available for three trophic levels represented by adequate data from fish, *Daphnia* and algae):

- 96-h LC_{50} Fish = 3.4 mg/L
- 48-hr EC_{50} Aquatic invertebrate = 0.72 mg/L.
- 72-hr E_rC_{50} Algae = 0.9 mg/L

Conclusion

Classification of a substance as Acute Category 1 is applicable if the LC/EC_{50} for any of the three trophic levels tested is less than or equal to 1 mg/L. For DBNPA the EC_{50} values for invertebrates (0.72 mg/L) is below the classification threshold and therefore classification as Acute 1; H400 with a M factor = 1 is required.

11.7.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

The following processes are involved in the degradation of DBNPA in the environment:

- Hydrolysis
- Indirect photolysis (this pathway is of minor importance)
- Biodegradation
- Reaction with sulphur containing reducing species (nucleophiles)
- Reaction with organic material
- Decomposition in the presence of soil.

Table 103 summarises the degradation rates for DBNPA in different media and under different conditions.

Table 103: Degradation behaviour of DBNPA

Media and conditions	Degradation rates
Hydrolysis DBNPA (A7.1.1.1.1/03)	pH 4, 12ºC: DT_{50} = 12079 hours pH 7, 12ºC: DT_{50} = 183 hours pH 9, 12ºC: DT_{50} = 5.6 hours
Biodegradation of DBNPA (ready test) (A7.1.1.2.1/01)	78% mineralisation after 28 days. 58% mineralisation within 10-day window. DT_{50} = ca. 12 days (mineralisation) at 20 ± 1°C.
Biodegradation of DBNPA (ready test) (A7.1.1.2.1/04)	35% mineralisation after 28 days.
Biodegradation (activated sludge die away test) (A7.1.2.1.1/01)	Immediate rapid primary degradation of DBNPA at 20 ± 2°C. A DT_{50} = 10 min. A DT_{50} for mineralization was determined to be 8.1 hours.
Biodegradation of DBNPA in seawater (A7.1.1.2.3/01)	17 - 22% mineralisation after 28 days. 26 - 36% mineralisation after 60 days at 18.6 ± 0.7°C.
Degradation of DBNPA in air; Indirect Photolysis	DT_{50} = 192.5 hours or ca. 8 days. Temperature assumed to be 25°C.
Degradation in soil (A7.1.1.1.1/02)*	DT_{50} = 4 - 25 hours (primary degradation). at pH 4.8 - 7.5 and room temperature. Temperature assumed to be 20°C. DT_{50} of 11 hours is calculated (geometric mean, n = 7) at 20ºC, corresponding to 20.9 hours at 12ºC.
Degradation of DBNPA in natural surface water (A7.1.2.2.2./03)	DT_{50} = 2 hours. pH 8 and temperature 24-25°C. Corresponding to 5.7 hours at 12ºC.

*) The results support the assumption that a fast degradation in soil is expected.

Note: In the table, the temperatures assumed to be 25°C are purely assumed but conservative reference values since they leads to higher DT_{50} values at 12°C compared to lower reference temperatures (for example, a reference temperature of 20°C).

The most important data for considering rapid degradability under CLP are data from ready biodegradability tests and simulation tests. For DBNPA we have two valid ready biodegradability tests. One showing 78% mineralisation after 28 days, 58% mineralisation within 10-day window. With approximately 58% mineralisation within the 10-day window, the pass level for ready biodegradability of 60% was missed by 2%. In the other valid test only 35% miniralisation was observed after 28 days. The DT_{50} value for mineralisation can be read from the degradation curve to be about 12 days. In addition we have an activated sludge die away test showing a DT_{50} value for mineralization of 8.1 hours, After 4 days 53% of the total radioactivity evolved as CO_2 in the die away test opposed to 5% in the abiotic control. The sum of dissolved and extractable radioactivity (related to parent compound and degradation products) decreased from 85% to 7% within the same period (weaker decrease from 80 to 49% in the abiotic control). Altogether these data can be considered as clear hint for an extensive microbially mediated mineralization of DBNPA. The radioactivity which remained associated with the solid fraction (incorporated into biomass or irreversebly bound to it) increased from 25% to 33%. Finally other biodegradation studies are available which show fast degradation of DBNPA in natural waters/sediments and soils.

Based on the above data DBNPA is not considered to be ready biodegrable.

DBNPA is considered to have a low potential for bioaccumulation in aquatic organisms as indicated by the log P_{OW} of 0.8, which is below the trigger value of 4, and supported by the results of the bioconcentration study in fish.

Chronic toxicity

Long-term toxicity NOECs for DBNPA are available for three trophic levels represented by adequate data from fish, Daphnia and algae:

- Fish (Rainbow trout, *Oncorhynchus mykiss*) NOEC (85 d) = 0.47 mg/L
- Aquatic invertebrate (*Daphnia magna*) NOEC (21 d) = 0.06 mg/L
- Algae (*Scenedesmus subspicatus*) NOEC (72 h) = 0.36 mg /L.

As the NOEC value of *Daphnia magna* is 0.06 mg/L, and DBNPA is rapidly degradable, classification as Chronic Category 2; H411 is required.

Application of a M-factor for chronic toxicity is not applicable, based on this chronic toxicity classification and rapid degradability of DBNPA.

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Acute

Classification of a substance as Acute Category 1 is applicable as the EC_{50} for DBNPA for invertebrates is 0.72 mg/L, hence below the classification threshold and therefore classification as Acute 1; H400 is required.

A M-factor of 1 is applicable, based on this Acute toxicity classification.

Chronic

Long-term toxicity NOEC for DBNPA is for Aquatic invertebrate (*Daphnia magna*) NOEC (21 d) = 0.06 mg/L.

As one long term NOEC value is < 0.1 mg/L, and DBNPA is readily biodegradable, classification as Chronic Category 2; H411 is required.

Application of a M-factor for chronic toxicity is not applicable, based on this Chronic toxicity classification and rapid degradability of DBNPA.

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.[4] After 28 days, mineralisation of 0.06 mg/L 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°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

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

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)	Static 0 hour: 0.9 – 13.7 mg/L (1	A7.4.1.3/04

			72h E_rC_{50} = 2.3 mg/L (time-weighted mm)	72 hours: <LOQ – 3.59 mg/L)	
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)**.

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

Table 104: Summary table of data concerning hazardous properties of the substance for the ozone layer

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data available				

12.1.1 Short summary and overall relevance of the provided information on ozone layer hazard

No atmospheric effect studies were available.

It should be noted that exposure of the atmospheric compartment to DBNPA is considered to be of no concern, as DBNPA has a very low vapour pressure (1.19 x 10-3 Pa at 19.2°C) and is not used in a manner which leads to direct release to the atmosphere.

The Henry's law constant of DBNPA, calculated on the basis of the vapour pressure and the water solubility, is 2.04 x 10^{-5} Pa m^3 mol^{-1} at pH 7 and 20ºC. Hence volatilisation of dissolved DBNPA from surface waters is expected to be negligible.

DBNPA is not an ozone-depleting substance. 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. These include chlorofluorocarbons-CFC, hydrochlorofluorocarbons-HCFC, and other halogenated chemicals such as halons. DBNPA is applied to water and its low Henry's Law constant means it preferentially partitions to water rather than air. In water it quickly degrades due to hydrolysis. In addition, DBNPA reacts rapidly with nucleophiles and organic matter. That small fraction of DBNPA that partitions from water to air degrades in the atmosphere by reaction with OH radicals, having an estimated DT_{50} value of 192.5 hours or about 8 days. As the release of DBNPA to the atmosphere is expected to be negligible, it does not seem likely that DBNPA will contribute to long-range transport of pollutants and to global warming.

DBNPA does not degrade in the atmosphere to produce sulfur dioxide or NOx the primary species involved in acidification.

12.1.2 Comparison with the CLP criteria

DBNPA is not an ozone-depleting substance and does not contain any chemical groups that are expected to be ozone depleting substance in the atmosphere. DBNPA does not degrade in the atmosphere to produce sulfur dioxide or NOx the primary species involved in acidification. As the release of DBNPA to the atmosphere is expected to be negligible, it does not seem likely that DBNPA will contribute to long-range transport of pollutants and to global warming. DBNPA does not fulfill the criteria for classification for effects to the ozone layer.

12.1.3 Conclusion on classification and labelling for hazardous to the ozone layer

Classification and labelling of DBNPA for hazardous effects to the ozone layer is not required.

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**.

13 ADDITIONAL LABELLING

No additional labelling required.

14 REFERENCES

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

15 ANNEXES

Annex I Extract from Competent Authority Report – Biocidal Products Regulation (EU) No 528/2012. Document III-A, study summaries – active substance, section A7: environment (non-confidential).

Anne II Extract from Competent Authority Report – Biocidal Products Regulation (EU) No 528/2012. Document III-A, study summaries – active substance, section A6: human health (non-confidential)