Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

1,5-naphthylene diisocyanate

EC Number: 221-641-4

CAS Number: 3173-72-6

CLH-O-0000006855-63-01/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 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

17 September 2020

CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

1,5-Naphthylene diisocyanate;

[NDI]

EC Number: **221-641-4**

CAS Number: **3173-72-6**

Index Number: **615-007-00-X**

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Version number: **2.0**

Date: **June 2019**

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON FOR 1,5-NAPHTHYLENE DIISOCYANATE

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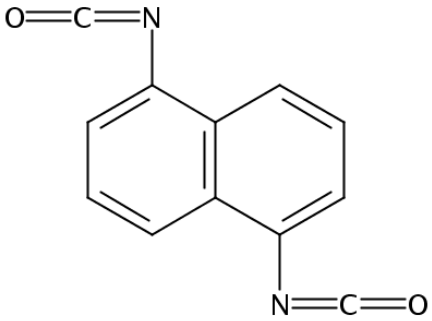
1 IDENTITY OF THE SUBSTANCE

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)	1,5-Diisocyanatonaphthalene
Other names (usual name, trade name, abbreviation)	NDI
ISO common name (if available and appropriate)	-
EC number (if available and appropriate)	221-641-4
EC name (if available and appropriate)	1,5-Naphthylene diisocyanate
CAS number (if available)	3173-72-6
Other identity code (if available)	-
Molecular formula	$C_{12}H_6N_2O_2$
Structural formula	
SMILES notation (if available)	O=C=Nc1cccc2c(cccc12)N=C=O
Molecular weight or molecular weight range	210.19 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	-
Description of the manufacturing process and identity of the source (for UVCB substances only)	-
Degree of purity (%) (if relevant for the entry in Annex VI)	

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)**
1,5-diisocyanatonaphthalene EC No. 221-641-4 CAS No. 3173-72-6	-	cf. Table 3	In addition to CLH: Skin Sens. 1 (H317)

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 3: Current, proposed, and resulting harmonised classification and labelling for NDI

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	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	615-007-00-X	1,5-naphthylene diisocyanate	221-641-4	3173-72-6	Acute Tox. 4* Skin Irrit. 2 Eye Irrit. 2 Resp. Sens. 1 STOT SE 3 Aquatic Chronic 3	H332 H315 H319 H334 H335 H412	GHS08 GHS07 Dgr	H332 H315 H319 H334 H335 H412			
Dossier submitter's proposal	TBD [split-entry 1]	1,5-naphthylene diisocyanate [containing < 0.1 % (w/w) of particles with an aerodynamic diameter of below 50 µm]	221-641-4	3173-72-6	**Retain** Skin Irrit. 2 Eye Irrit. 2 Resp. Sens. 1 STOT SE 3 Aquatic Chronic 3 **Add** Skin Sens. 1A **Delete** Acute Tox. 4*	**Retain** H315 H319 H334 H335 H412 **Add** H317 **Delete** H332	**Retain** GHS08 GHS07 Dgr	**Retain** H315 H319 H334 H335 H412 **Add** H317 **Delete** H332			
	TBD [split-entry 2]	1,5-naphthylene diisocyanate [containing ≥ 0.1 % (w/w) of particles with an aerodynamic diameter of below 50 µm]	221-641-4	3173-72-6	**Retain** Skin Irrit. 2 Eye Irrit. 2 Resp. Sens. 1 STOT SE 3 Aquatic Chronic 3 **Add** Skin Sens. 1A **Modify** Acute Tox. 2	**Retain** H315 H319 H334 H335 H412 **Add** H317 **Modify** H330	**Retain** GHS08 Dgr **Add** GHS06 **Remove** GHS07	**Retain** H315 H319 H334 H335 H412 **Add** H317 **Modify** H330		**Add** Inhalation: ATE = 0.27 mg/L (dusts or mists)	

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON FOR 1,5-NAPHTHYLENE DIISOCYANATE

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	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)		
Resulting entry in Annex VI if adopted by RAC and agreed by Commission	TBD [split-entry 1]	1,5-naphthylene diisocyanate [containing < 0.1 % (w/w) of particles with an aerodynamic diameter of below 50 µm]	221-641-4	3173-72-6	STOT SE 3 Skin Irrit. 2 Eye Irrit. 2 Resp. Sens. 1 Skin Sens. 1A Aquatic Chronic 3	H335 H315 H319 H334 H317 H412	GHS07 GHS08 Dgr	H335 H315 H319 H334 H317 H412			
	TBD [split-entry 2]	1,5-naphthylene diisocyanate [containing ≥ 0.1 % (w/w) of particles with an aerodynamic diameter of below 50 µm]	221-641-4	3173-72-6	Acute Tox. 2 STOT SE 3 Skin Irrit. 2 Eye Irrit. 2 Resp. Sens. 1 Skin Sens. 1A Aquatic Chronic 3	H330 H335 H315 H319 H334 H317 H412	GHS06 GHS08 Dgr	H330 H335 H315 H319 H334 H317 H412		Inhalation: ATE = 0.27 mg/L (dusts or mists)	

Table 4: Reason for not proposing harmonised classification and status under public consultation

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

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

The current CLH has been taken over into Annex VI to the CLP regulation from the previous legislation, i.e. the Dangerous Substances Directive (Dir. 67/548/EEC). Further details are not known to the Dossier Submitter (DS).

RAC general comment

The current harmonised classification for 1,5-naphthylene diisocyanate (NDI), which is used in the plastic industry as a curing agent, was transposed from the previous legislation (the Dangerous Substances Directive, Dir. 67/548/EEC) to Annex VI of the CLP Regulation, but further details are not available.

The CLH report has been created based on data submitted by the lead registrant in the REACH registration dossier, and further relevant data were retrieved as part of a general literature search in the context of the restriction proposal for diisocyanates recently submitted to ECHA by the Dossier Submitter (DS). Also, SCOPUS and PubMed databases were searched for relevant literature.

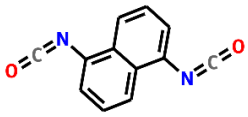


Figure: NDI structure

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Skin sensitisation

Diisocyanates are known for their potential to cause respiratory and skin sensitisation. A restriction dossier submitted recently by the German MSCA (German CA, 2016) shows that allergic respiratory symptoms may occur after sensitisation via the inhalation or the dermal route. Also the opposite, i.e. dermal contact allergy in individuals sensitised to diisocyanates via inhalation has been observed.

Skin sensitisation is not an endpoint with obligatory CLH, but because of its link to respiratory sensitisation (for which CLH is mandatory), the DS considers it essential that individuals handling diisocyanates are sufficiently protected against the risks arising from dermal exposure to diisocyanates.

Moreover, only 11/36 (as of 25.02.2019) notifiers have self-classified NDI for skin sensitisation which together with the huge annual tonnage and wide-spread use of the substance further highlights the need to install sufficient protection against the risks of dermal exposure to NDI on an EU-wide scale.

Acute Toxicity

There is agreement among the EU MSCAs that whenever an entry in Annex VI is amended, any minimum classifications (such as the Acute Tox. 4* for NDI) need to be clarified.

5 IDENTIFIED USES

A summary of the information available on ECHA's public website (accessed 2017-12-14) is given below[1].

5.1 General

This substance is manufactured and/or imported in the European Economic Area in 1 000 – 10 000 tonnes per year. This substance is used in formulation or re-packing, at industrial sites and in manufacturing.

5.2 Consumer Uses

ECHA has no public registered data indicating whether or in which chemical products the substance might be used. ECHA has no public registered data on the routes by which this substance is most likely to be released to the environment.

5.3 Article service life

ECHA has no public registered data on the use of this substance in activities or processes at the workplace. ECHA has no public registered data on the routes by which this substance is most likely to be released to the environment. ECHA has no public registered data indicating whether or into which articles the substance might have been processed.

5.4 Widespread use by professional workers

ECHA has no public registered data indicating whether or in which chemical products the substance might be used. ECHA has no public registered data on the types of manufacture using this substance. ECHA has no public registered data on the use of this substance in activities or processes at the workplace. ECHA has no public registered data on the routes by which this substance is most likely to be released to the environment.

5.5 Formulation or re-packing

ECHA has no public registered data indicating whether or in which chemical products the substance might be used.

This substance is used in the following activities or processes at workplace: transfer of chemicals, closed processes with no likelihood of exposure, closed batch processing in synthesis or formulation, transfer of substance into small containers, laboratory work, closed, continuous processes with occasional controlled exposure, batch processing in synthesis or formulation with opportunity for exposure and mixing in open batch processes.

Release to the environment of this substance can occur from industrial use: formulation of mixtures, formulation in materials, as an intermediate step in further manufacturing of another substance (use of intermediates) and for thermoplastic manufacture.

5.6 Uses at industrial sites

ECHA has no public registered data indicating whether or in which chemical products the substance might be used.

This substance is used for the manufacture of: chemicals.

This substance is used in the following activities or processes at workplace: Transfer of chemicals, closed processes with no likelihood of exposure, closed batch processing in synthesis or formulation, transfer of substance into small containers, laboratory work, closed, continuous processes with occasional controlled

[1] The text is a mixture of excerpts from ECHA's public website and of text prepared by the DS. Direct use of original text is not specifically marked.

exposure, batch processing in synthesis or formulation with opportunity for exposure and mixing in open batch processes.

Release to the environment of this substance can occur from industrial use: formulation of mixtures, formulation in materials, as an intermediate step in further manufacturing of another substance (use of intermediates) and for thermoplastic manufacture.

5.7 Manufacture

This substance is used in the following activities or processes at workplace: Closed processes with no likelihood of exposure, closed, continuous processes with occasional controlled exposure, closed batch processing in synthesis or formulation, transfer of chemicals at dedicated facilities, transfer of substance into small containers and laboratory work.

Release to the environment of this substance can occur from industrial use: manufacturing of the substance.

6 DATA SOURCES

This report has been created based on the data submitted by the lead registrant in the REACH registration dossier for NDI. In addition, further relevant data were retrieved as part of a general literature search in the context of the restriction proposal for diisocyanates recently submitted to ECHA by the DS.

A supplementary literature search was performed in the SCOPUS database on 2017-06-30 for all references in the areas of medicine, pharmacology, toxicology, or environment published in 2015-2017 and containing the keyword „isocyanate". Also the PubMed database was searched for that keyword and time range.

7 PHYSICOCHEMICAL PROPERTIES

Table 5: Summary of physicochemical properties (all data taken from REACH registration dossier)

Property	Value	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Solid	Experimental
Melting/freezing point	127 °C	Measured
Boiling point	167 °C (at 7 hPa)	Measured
Relative density	1.41 g ml^{-1} (20 °C)	Measured: According to OECD 109 and EU A.3 using a pycnometer.
Vapour pressure	8.0 10^{-4} Pa (25.0 °C) 1.1 10^{-1} Pa (60.4 °C) 9.5 10^{-1} Pa (79.6 °C)	Measured: According to OECD 104 and EU A.4 using the gas saturation method.
Surface tension	n.a. (substance reacts with water, hydrolysis)	Data waiver
Water solubility	Measured n.a. (substance reacts with water, hydrolysis, half-life < 1 hour at pH 4, 7 and 9) Calculated: 36.8 mg/L	Calculated using general solubility equation (GSE, p. 60 ECHA Guidance R.7a v.5.0)
	Calculated value of the hydrolysis product 1,5-diaminonaphthalene 195.8 mg/L	Calculated using general solubility equation (GSE, p. 60 ECHA Guidance R.7a v.5.0)
Partition coefficient n-octanol/water	Measured n.a. (substance reacts with water, hydrolysis) Calculated $logK_{OW}$ = 4.37	Calculated by KOWWIN v.141 using a "fragment constant" methodology
	Measured value of the hydrolysis product 1,5-diaminonaphthalene $logK_{OW}$ = 0.89	Measured
Granulometry	Particle size: > 875 µm — Amount: 82.0 % > 100 - — 16.7 %	Measured by dry dispersion technique by combined manual sieving at 875 µm and laser diffraction

Property	Value	Comment (e.g. measured or estimated)
	< 875.0 µm > 50 - < 100 µm: 1.0 % < 50 µm: 0.3 %	It was observed that significant amounts of the substance stick to the vibrational feeding system, which was used for laser diffraction analysis. Additionally, agglomeration of fine particles in vibrational feeder was likely to occur due to the cohesive nature of the substance.
Stability in organic solvents and identity of relevant degradation products	N.a. (stability in organic solvents is not a critical property of the substance)	Data waiver
Dissociation constant	N.a. (substance reacts with water, hydrolysis, half-life < 1 hour at pH 4, 7 and 9)	Data waiver
Viscosity	N.a. (substance is solid)	Data waiver

8 EVALUATION OF PHYSICAL HAZARDS

Not assessed in this dossier

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

9.1 Hydrolysis

According to the IUCLID summary in the lead registration dossier, NDI hydrolyses by 50 % within less than 1 hour at pH 4, 7 and 9 in water. 1,5-Diaminonaphthalene and carbon dioxide are found to be the main degradation products, followed by an urea-dimer in minor amounts (Bayer, 2006b).

In the view of the DS (and as proven by experimental data), this provides a sufficient time window for protein-hapten complex formation which is regarded as the Molecular Initiating Event (MIE) in the Adverse Outcome Pathway (AOP) for skin sensitisation (OECD, 2012).

9.2 ADME

In the registration dossier, the lead registrant has provided the following statement with relevance to available toxicokinetic information for NDI regarding the dermal and inhalation routes:

"Experimental toxicokinetic studies were not performed. NDI is a white to yellowish organic solid with a very low vapour pressure under normal ambient conditions (8 x 10-6 hPa at 25 °C), therefore inhalation exposure to the vapour is expected to be negligible. Currently available data on particle size during worst-case end-use of NDI indicate a thoracic percentage of 0.02 % that can be inhaled by humans and may reach the thoracic region. […] NDI proved as skin sensitiser in a local lymph node assay (LLNA) in mice, therefore at least some dermal bioavailability after dermal contact is expected" (Bayer, 2010).

10 EVALUATION OF HEALTH HAZARDS

10.1 Acute toxicity - oral route

Not assessed in this dossier

10.2 Acute toxicity - dermal route

Not assessed in this dossier

10.3 Acute toxicity - inhalation route

Table 6: Summary table of animal studies for acute inhalation LC_{50} determination

<table>
<tr><th>Method, guideline, deviations</th><th>Species, strain, sex, no/group</th><th>Test substance, duration of exposure, form, dose levels, and particle size, results</th><th>Reference</th></tr>
<tr><td>OECD 403/EU B.2

GLP claimed

Reliability 2 (reliable with restrictions): Only summary available

Key study</td><td>Rat, Wistar, 5M+ 5F per group</td><td>NDI, aerosol (dust), 1 x 4 h, nose-only

<table>
<tr><td>Dose level (mg/m³)</td><td>0</td><td>96</td><td>189</td><td>238</td><td>314</td><td>384</td><td>541</td></tr>
<tr><td>MMAD (µm)</td><td>NA</td><td>3.1</td><td>3.2</td><td>4.0</td><td>3.6</td><td>3.8</td><td>3.1</td></tr>
<tr><td>GSD (µm)</td><td>NA</td><td>1.6</td><td>1.7</td><td>2.1</td><td>1.5</td><td>1.5</td><td>1.6</td></tr>
<tr><td>Mortality
(no. dead/no. exposed)
M
F</td><td>

0/5
0/5</td><td>

0/5
0/5</td><td>

0/5
0/5</td><td>

3/5
3/5</td><td>

3/5
3/5</td><td>

4/5
4/5</td><td>

4/5
4/5</td></tr>
<tr><td>Mortality
(%, M+F combined)</td><td>0</td><td>0</td><td>0</td><td>60</td><td>60</td><td>80</td><td>80</td></tr>
</table>

LC_{50} (4h) = 0.27 mg/L

Cf. text for further details (including clinical signs)</td><td>(Bayer, 1995a)</td></tr>
<tr><td>Non-guideline

GLP claimed

Reliability 2 (reliable with restrictions): Only summary available, observation time post-exposure: 7 d (instead of 14 d as recom-mended by OECD TG 403), MMAD outside the range recom-mended by OECD TG 403

Supporting study</td><td>Rat, Wistar, 18 M/group</td><td>NDI, aerosol (dust), 1 x 4 h, nose-only

<table>
<tr><td>Dose level (mg/m³)</td><td>0</td><td>56</td><td>140</td><td>148</td><td>240</td><td>245</td><td>1 050</td></tr>
<tr><td>MMAD (µm)</td><td>NA</td><td>3.1</td><td>4.1§</td><td>6.9§</td><td>5.4§</td><td>9.0§</td><td>10.1§</td></tr>
<tr><td>GSD (µm)</td><td>NA</td><td>1.9</td><td>1.9</td><td>2.4</td><td>2.1</td><td>2.5</td><td>2.8</td></tr>
<tr><td>Mortality
(no. dead/no. exposed)</td><td>0/18</td><td>0/18</td><td>2/18</td><td>0/18</td><td>7/18</td><td>0/18</td><td>18/18</td></tr>
<tr><td>Mortality
(%)</td><td>0</td><td>0</td><td>11</td><td>0</td><td>39</td><td>0</td><td>100</td></tr>
</table>

LC_{50} (4 h) not calculated

Cf. text for further details (including clinical signs)</td><td>(Bayer, 2003)</td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations</th><th>Species, strain, sex, no/group</th><th>Test substance, duration of exposure, form, dose levels, and particle size, results</th><th>Reference</th></tr>
<tr><td>Similar to OECD 403/EU B.2

GLP claimed

Reliability 2 (reliable with restrictions): Only summary available, MMAD outside the range recommended by OECD TG 403

Supporting study</td><td>Rat, Wistar, 5M+5F per group</td><td>NDI, aerosol (dust), 1 x 1 h, nose-only

<table>
<tr><td>Dose level (mg/m³)</td><td>0</td><td>1 285</td><td>2 075</td></tr>
<tr><td>MMAD (µm)</td><td>NA</td><td>4.6§</td><td>8.1§</td></tr>
<tr><td>GSD (µm)</td><td>NA</td><td>1.6</td><td>1.7</td></tr>
<tr><td>Mortality
(no. dead/no. exposed)
M
F</td><td>

0/5
0/5</td><td>

1/5
0/5</td><td>

0/5
0/5</td></tr>
<tr><td>Mortality
(%, M+F combined)</td><td>0</td><td>10</td><td>0</td></tr>
</table>

For details regarding clinical signs, cf. text.</td><td>(Bayer, 1995b)</td></tr>
</table>

§ Outside the range recommended by OECD TG 403 (1-4 µm).

Three studies in animals are available in the registration dossier which are summarised in more detail below, using excerpts from the study summaries provided by the lead registrant for NDI under REACH. The design of a fourth study dated 1946, which is mentioned on ECHA's public dissemination site ("2 rats, 2 mice, 1 rabbit and 1 guinea pig were exposed to NDI (4000 mg heated to 150 °C in a 400 litre cage)") was not considered relevant for this dossier.

10.3.1 4 h acute inhalation study in rats (Bayer, 1995a)

In an OECD TG 403/EU B.2-conform acute inhalation toxicity study, 5 male and 5 female Wistar rats per group (source: Harlan-Winkelmann, Borchen, Germany; age at study initiation: 2-3 months; housing: individual; diet and water: ad libitum; acclimation period: ≥ 5 d) were exposed to 0, 96, 189, 238, 314, 384, or 541 mg NDI aerosol/m³ for 4 h via nose-only exposure.

According to a particle size analysis, in the 96, 189, 238, 314, 384, and 541 mg/m^3 exposure groups the MMAD (mass median aerodynamic diameter) was 3.1, 3.2, 4.0, 3.6, 3.8 and 3.1 µm, respectively, with a geometric standard deviation (GSD) of 1.6, 1.7, 2.1, 1.5, 1.5 and 1.6, respectively.

Body weights were recorded immediately prior to exposure, on days 3, 7, and weekly thereafter. Animals were observed for clinical signs several times on the day of dosing and ≥ 1/d during the 4 wk observation period. A Functional Observational Battery (FOB) as well as a gross pathological examination was performed.

Results regarding mortality and clinical signs are shown in Table 7.

Table 7: Overview of the results of an acute inhalation toxicity study with NDI, (Bayer, 1995a) reproduced from the summary in the registration dossier with slight editorial modifications

Sex	Gravimetric concentration (mg/m^3)	Toxicological results (no. dead/no. with clinical signs after cessation of exposure/number exposed)	Onset and duration of clinical signs	Onset of mortality
Males	0	0/0/5	-	-
	96	0/5/5	4 h – 8 d	-
	189	0/5/5	4 h – 7 d	-
	238	3/5/5	4 h – 7 d	1 d
	314	3/5/5	4 h – 7 d	1 d – 2 d
	384	4/5/5	4 h – 11 d	1 d
	541	4/5/5	4 h – 11 d	1 d
Females	0	0/0/5	-	-
	96	0/5/5	4 h – 8 d	-
	189	0/5/5	4 h – 7 d	-
	238	3/5/5	4 h – 7 d	1 d – 2 d
	314	3/5/5	4 h – 7 d	1 d – 2 d
	384	4/5/5	4 h – 6 d	1 d
	541	4/5/5	4 h – 6 d	1 d

Below the detailed report of the findings in this study is reproduced verbatim from the IUCLID summary submitted by the lead registrant for NDI:

*"**Mortality**: Aerosol (dust) concentrations to 238 mg/m^3 and above induced test substance-related mortality within the first two post-exposure days. Exposure to concentrations equal or less than 189 mg/m^3 test compound were tolerated without mortality.*

***Clinical signs**: All animals exposed to the test compound showed bradypnoe, laboured breathing pattern, nose/snout area with red encrustations, reduced motility, flaccid appearance, ungroomed hair-coat and piloerection starting at 96 mg/m^3. In addition, rales, salivation, serous discharge from nose, cyanosis and apathy was seen at 189 mg/m^3 and above (see also table 7 for onset and duration of signs).*

***Body weight**: Decreased body weights were observed in all groups exposed to the test compound (at 96 mg/m^3 and above).*

***Gross pathology**: White foamy discharge from snout, red encrustation in the muzzle area, lungs with dark-red colourations and spongy (oedematous) appearance, foam in trachea, distended hydrothorax, lobulation of liver, and pale parenchymatous organs were observed in animals sacrificed during the observation period. In rats sacrificed at the end of the observation period an increased incidence of macroscopical findings was observed on lungs. However, the findings appeared not to be induced in a clear concentration-dependent manner.*

***Other findings**: All animals showed normal reflexes. At 96 mg/m^3 and above a concentration-dependent decrease of body temperature was recorded."*

The LC_{50} (aerosol, 4 h) in this study as calculated by the author was 270 mg/m^3 air for male and female rats combined. Regarding the calculation of the LC_{50}, the summary provides the following information*: "If calculation of a median lethal concentration (LC50) is possible, it is performed by computer (HP 3000) according to the method of AP. Rosiello, I.M. Essigmann, and G.N. Wogan (1977[2]) as modified by Pauluhn (1983). This method is based on the maximum-likelihood method of C.I. Bliss (1938). If only 2 pairs of values with greater than 0 % lethality and less than 100 % are available then the first linear approximation is based on these values and a homogeneity test is not performed. The interpolated concentration at 50 % lethality in this case was designated at approximate LC50".*

FOB results are not reported in the summary available in the registration dossier, but are also not considered by the DS to be relevant for acute inhalation toxicity classification.

[2] Rosiello, A. P.

10.3.2 4 h acute inhalation study in rats (Bayer, 2003)

In a non-guideline 4 h acute toxcicity study which focused on an analysis of bronchoalveolar lavage (BAL) parameters rather than lethality, 18 male Wistar rats per group (source: Harlan-Winkelmann, Borchen, Germany; age at study initiation: ca. 2 months; housing: individual; diet and water: ad libitum; acclimation period: ≥ 5 d) were exposed to 0, 56, 140, 148, 240, 245, and 1 050 mg NDI/m^3 for four hours. Six males per group were serially sacrificed on post-exposure days 1, 3, and 7.

In the 56, 140, 148, 240, 245 and 1050 mg/m^3 exposure groups the MMAD (GSD) were 3.1 (1.9), 4.1 (1.9), 6.9 (2.4), 5.4 (2.1), 9.0 (2.5) and 10.1 (2.8) µm, respectively. Except for the 56 mg/m^3 group, all of these distributions were outside the MMAD range recommended in OECD TG 403.

Body weights were recorded on days 1, 3, and 7 (prior to sacrifice), animals were observed for clinical signs several times on the day of exposure and at least 1/d thereafter, and their rectal temperature was measured ca. half an hour after the end of exposure. BAL fluid was collected on post-exposure days 1, 3, and 7 and analysed for indicators of inflammatory response and lower respiratory tract damage as well as interactions with pulmonary phospholipids: Lactate dehydrogenase (LDH), total protein, ß-N-Acetyl-glucosamidase (ß-NAG), phospholipids (phosphatidylcholine), phospholipids (phosphatidylcholine)/cell, and total number of lavaged cells (including the volume and diameter).

Results regarding mortality, clinical signs, and rectal temperature are shown in Table 7.

Table 8: Overview of the results of an acute inhalation toxicity study with NDI (Bayer, 2003), reproduced from the summary in the registration dossier with slight editorial modifications

Group no.	Gravimetric concentration (mg/m^3)	Toxicological results (no. dead/no. with clinical signs after cessation of exposure/number exposed)	Onset and duration of clinical signs	Onset of mortality	Rectal temperature (°C)
1	0	0/0/18	-	-	38.0
2	56	0/18/18	0 d – 7 d	-	32.6
3	140	2/18/18	0 d – 7 d	1 d, 2 d	33.1
4	148	0/18/18	0 d – 7 d	-	31.4
5	240	7/18/18	0 d – 3 d	1 d, 2 d	30.4
6	245	0/18/18	0 d – 7 d	-	31.6
7	1 050	18/18/18	0 d – 1 d	0 d, 1 d	30.4

Below the detailed report of the findings in this study is reproduced verbatim from the IUCLID summary submitted by the lead registrant for NDI:

"*Clinical signs:*

Control: All rats tolerated the exposure without specific signs

56 mg/m^3 (MMAD: 3.1 µm): Bradypnoe, laboured breathing pattern, breathing sounds, piloerection, haircoat ungroomed, nasal discharge (serous), nostrils: reddened, nostrils: red encrustations, stridor, motility reduced, limp, high-Iegged gait, muzzle: red encrustations

140 mg/m^3 (MMAD: 4.1 µm): Bradypnoe, laboured breathing pattern, breathing sounds, piloerection, haircoat ungroomed, nasal discharge (serous), nostrils: reddened, nostrils: red encrustations, stridor, motility reduced, limp, high-legged gait.

148 mg/m^3 (MMAD: 6.9 µm): Bradypnoe, laboured breathing pattern, breathing sounds, piloerection, haircoat ungroomed, nasal discharge (serous), nostrils: red encrustations, stridor, motility reduced, limp, high-legged gait, tremor, muzzle: red encrustations, nares: red encrustations

240 mg/m^3 (MMAD: 5.4 µm): Bradypnoe, laboured breathing pattern, breathing sounds, piloerection, haircoat ungroomed, nasal discharge (serous), nostrils: red encrustations, motility reduced, limp, high-Iegged gait, giddiness, tremor, muzzle: red encrustations, nares: red encrustations, blepharospasm, cyanosis, chromodakryorrhea.

245 mg/m^3 (MMAD: 9.0 µm): Bradypnoe, laboured breathing pattern, breathing sounds, piloerection, haircoat ungroomed, nasal discharge (serous), nostrils: reddened, nostrils: red encrustations, stridor,

motility reduced, limp, high-legged gait, giddiness, muzzle: red encrustations, nares: red encrustations, blepharospasm, cyanosis, chromodakryorrhea, dyspnea

1 050 mg/m^3 (MMAD: 10.1 µm): Bradypnoe, laboured breathing pattern, breathing sounds, piloerection, nasal discharge (serous), nostrils: red encrustations, stridor, motility reduced, limp, high-legged gait, giddiness, tremor, muzzle: red encrustations, blepharospasm, dyspnea

Body weight:

Mean body weights of rats in all exposure groups were markedly different from the control group.

Gross pathology:

In all groups exposed to the test substance concentration-dependent macroscopic alterations of the respiratory tract were observed.

Other findings:

Rectal temperature: Results are presented in Table […][3].

Bronchoalveolar Lavage and Lung Weights: At all time points the average recovery of the lavage fluid instilled into the lung was high (approximately 80 % of the instilled volume was recovered). Nevertheless, BALF-parameters were recalculated according to the recovered total volume (adjustment factor = volume instilled/volume recovered). BALC-parameters were adjusted to the total cell number in BAL. Absolute lung weights were significantly increased in all […] exposure groups. Despite the increase observed lung weights of the exposure groups were indistinguishable from the control group on day 7. From all endpoints the increase in protein was most prominent. Taking into account the relative extent of protein changes, which maximum was approximately 50-times the control value on post-exposure day 1. Accordingly, this endpoint is considered to be the most sensitive one to assess early changes. Despite the magnitude of effect, the increased extravasation was rapidly reversible and reached the level of the control on day 7 at the latest. Phospholipids in BALF and especially BALC as well as LDH and TCC were increased in all exposure groups at the day 3 time point. The increase of TCC and LDH appears to be associated and may correspond with the removal of cellular debris and surfactant. Accordingly, elevated LDH is conceived to be associated with increased phagolysosomal activities of alveolar macrophages which is substantiated, at least in part, by increased levels of ß-NAG. The influx of protein into the alveoli and the elevated lung weights were contingent upon the actually respirable mass (total mass concentration x fraction penetrating the alveolar region; which approximate cut-off for rats is 5 µm) rather than total concentration." (Bayer, 2003).

While not suitable as a basis for classifications as such, the study demonstrates that acute toxicity of NDI is a function not only of the total air concentration, but in particular of the particle size distribution: An external concentration of 140 mg NDI (MMAD: 4.1 µm)/m^3 resulted in 11 % mortality, while a concentration of 148 mg NDI (MMAD: 6.9 µm)/m^3 did not cause any mortality at all. Likewise 240 mg NDI (MMAD: 5.4 µm)/m^3 were lethal for 39 %, while 245 mg NDI (MMAD: 9.0 µm)/m^3 were survived by all of the animals in the respective test group.

With respect to the BAL findings Pauluhn has claimed that:

"The comparison of actual exposure concentrations (total mass collected by filter analyses) and alveolar exposure intensities suggests that the magnitude of BAL protein is governed by the respirable fraction of particles rather than the total airborne concentration. […].*The dependence of BAL protein on the alveolar exposure intensity rather than total exposure concentration suggests that the mass of particles capable penetrating the alveolar region is decisive for the outcome of study* […]. *Thus, for* […] *NDI it could be shown that the critical mode of action of acute inhalation toxicity is restricted to the respirable fraction rather than the total exposure concentration."* (Pauluhn, 2004)

On the other hand, it cannot be fully excluded that also effects on parts of the respiratory tract other than the alveolar region may have contributed to the overall toxicity and hence to the observed mortality. For example, with respect to the effect on rectal temperature, Pauluhn and Mohr observed that:

[3] Table 8 in this dossier

"Inhalation exposure to upper respiratory tract sensory irritants, for example, is known to evoke in rodents a remarkable decrease in body temperature, possibly via reflex stimulation of local receptors in this region of the tract. This response is concentration dependent, rapid in onset, and reversible within hours of cessation of exposure […]. *Despite the apparent temporary nature of the response of rats and mice to irritant exposure, these secondary effects are important for a number of reasons. First, the induced decreases appear to be a primary component of a more general response by the rodent to toxic insult. Second, the magnitude of changes in thermoregulatory function may be potentiated or attenuated by a number of experimental conditions or stresses that may differ from one laboratory to another. Thus, initial experimental conditions may play an important role in the final toxic outcome, thereby compromising the ability to compare results across species and studies in which these experimental factors are neither monitored nor controlled. Third, it is as yet unclear whether this physiological response to xenobiotic agents is unique to rodents or if it also occurs in larger mammals and humans. However, it is quite possible that humans have a greater thermal inertia due to the larger body mass and therefore do not exhibit this response to any measurable degree* [...]. *Hence, in addition to the direct effects of such irritants on the function and the structure of the pulmonary system, there may be substantial indirect effects related to changes in extrapulmonary parameters that in turn significantly modify the final toxic outcome"* (Pauluhn and Mohr, 2000).

Overall, the DS agrees that the results in (Bayer, 2003) suggest a strong dependency of NDI-associated lethality on the particle size distribution of the test material. (Pauluhn, 2004) has demonstrated the correlation of BAL fluid parameters with particle MMAD.

10.3.3 1 h acute inhalation study in rats (Bayer, 1995b)

In a study similar to OECD TG 403/EU B.2, but with an exposure duration of 1 h only, 5 male and 5 female Wistar rats per group (source: Harlan-Winkelmann, Borchen, Germany; age at study initiation: 2-3 months; housing: individual; diet and water: ad libitum; acclimation period: ≥ 5 d) were exposed to 0, 1 285, or 2 075 mg NDI aerosol/m³ for 1 h via nose-only exposure.

In the 1 285 and 2 075 mg/m^3 exposure groups, the MMAD (GSD) were 4.6 (1.6) and 8.1 (1.7) µm which is outside the MMAD range recommended by OECD TG 403 (1-4 µm).

Body weights were recorded immediately prior to exposure, on days 3, 7, and weekly thereafter. Animals were observed for clinical signs several times on the day of dosing and ≥ 1/d during the 4 wk observation period. A Functional Observational Battery (FOB) as well as a gross pathological examination was performed.

The results are summarised by the registrant as follows: *"Aerosol (dust) concentrations up to 1 285 mg/m^3 did induce test substance related mortality (males: 1 out of 5 rats died; females: no mortality). Exposure to the limit concentration of 2 075 mg/m^3 test compound was tolerated without mortality. Mortality occurred on post-exposure day ten. Necropsy findings support the conclusion that a causal relationship between lethality and lung damage existed. Exposure to concentrations of 1 285 mg/m^3 and higher were followed by concentration-dependent signs suggestive of irritation of the respiratory tract (e.g. bradypnoe, dyspnoea, laboured breathing pattern, rales, nose/snout area with red encrustations, serous discharge from nose, cyanosis) and non-specific signs such as reduced motility and flaccid muscle tone. The duration of signs (maximum duration up to day 9) was dependent on respiratory signs."* (Bayer, 1995b)

FOB results are not reported in the summary available in the registration dossier, but are also not considered by the DS to be relevant for acute inhalation toxicity classification.

Given that MMADs of the test materials used in this study were clearly outside the range recommended by OECD TG 403, this study is not used for classification.

Furthermore, studies with 1 h-exposure may be used for classification in principle by applying an extra assessment factor, but this would be associated with a high degree of uncertainty: *"Based on the 4-h LC50 values, 1-h LC50s of NDI and mMDI* […] *solid respirable aerosols* […] *were markedly higher than predicted because of the larger particle size commonly associated with higher test concentrations* […]. *This demonstrates that especially for irritant aerosols the extrapolation from a 4-h to a 1-h LC_{50} utilizing*

commonly applied conventions, i.e., a time-adjustment factor of 4 […], *may lead to over-conservative estimates."*

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

In a guideline-conform 4 h acute inhalation toxicity test in rats, the LC_{50} for NDI was determined at 270 mg/m^3, or 0.27 mg/L (Bayer, 1995a), which will be taken as the basis for classification.

Supporting studies demonstrated a dependency of NDI-associated mortality on the particle size distribution. These studies are not used for classification directly because they applied 1 h exposure only and/or used test materials with MMADs outside the range recommended in OECD TG 403. They are, however, relevant for the evaluation whether the split-entry concept for acute toxic substances via the inhalation route is applicable to NDI.

10.3.5 Comparison with the CLP criteria

10.3.5.1 The "split-entry concept" and its applicability to NDI

In section 3.1.2.3.2 (p. 242), the ECHA "Guidance on the Application of the CLP Criteria" notes:

"The use of highly respirable dusts and mists is ideal to fully investigate the potential inhalation hazard of the substance. However, it is acknowledged that these exposures may not necessarily reflect realistic conditions. For instance, solid materials are often micronised to a highly respirable form for testing, but in practice exposures will be to a dust of much lower respirability. Similarly, pastes or highly viscous materials with low vapour pressure need strong measures to be taken to generate airborne particulates of sufficiently high respirability, whereas for other materials this may occur spontaneously. In such situations, specific problems may arise with respect to classification and labelling, as these substances are tested in a form (i.e. specific particle size distribution) that is different from all the forms in which these substances are placed on the market and in which they can reasonably be expected to be used.

A scientific concept has been developed as a basis for relating the conditions of acute inhalation tests to those occurring in real-life, in order to derive an adequate hazard classification. This concept is applicable only to substances or mixtures which are proven to cause acute toxicity through local effects and do not cause systemic toxicity (Pauluhn, 2008)." (ECHA, 2017)

In (Pauluhn, 2008), further guidance on the applicability of the EU split–entry concept is provided. In this context, criteria are defined which are supportive or prohibitive for its use (Table 9).

Table 9: Criteria supportive of or prohibitive for the use of the split-entry concept (by default all criteria refer to findings from an acute 4 h inhalation study using the OECD (2007) protocol), from (Pauluhn, 2008)

<table>
<tr><th></th><th>Mandatory endpoint</th><th>RT (ET-TB)</th><th>Pulmonary (alveolar)</th><th>Supportive of the use of split-entry</th><th>Prohibitive for the use of split-entry</th></tr>
<tr><td>Non-inhalation route (acute)</td><td rowspan="6">Yes</td><td rowspan="2">-</td><td rowspan="2">-</td><td>Low toxicity</td><td>High toxicity</td></tr>
<tr><td>MMAD</td><td>< approx. 4 µm</td><td>>> 4 µm</td></tr>
<tr><td>Irritation/inflammation</td><td>Minimal</td><td>Yes</td><td rowspan="2">Yes</td><td>-</td></tr>
<tr><td>Lethality dependent on particle size</td><td rowspan="2">-</td><td rowspan="2">-</td><td>No</td></tr>
<tr><td>Onset of lethality</td><td>Immediate (up to day 7)</td><td rowspan="2">If delayed in onset (≥ 8d)</td></tr>
<tr><td>Respiratory distress</td><td>Minimal</td><td>Yes</td><td>Yes</td></tr>
</table>

Evidence on severe non-respiratory tract toxicity	-	-	-	No	Yes, if not secondary
Necropsy findings in succumbed rats	Yes			Hepatisation, lung enlarged, oedema	No findings in lungs
Increase in BAL protein	Supportive		Yes	Yes	-
Histopathology				Major lesions restricted to lower RT	Major lesions distributed throughout RT
Severe extrapulmonary organ damage	-		-	No	Yes

MMAD: mass median aerodynamic diameter of particulate atmosphere in the vicinity of the breathing zone of animals and measured by cascade impactor, post-exposure days are relative to day 0 (exposure day); RT: respiratory tract; ET: extrathoracic region (pharynx, nasal passages); TB: tracheobronchial region; - : not applicable.

In Table 10, relevant findings for NDI from 4 h acute toxicity tests via the inhalation route are summarised and compared with the above mentioned criteria. The table also shows the DS's conclusions on whether an endpoint is considered supportive or prohibitive for the use of the split-entry concept.

Table 10: Comparison between the criteria from Pauluhn (2008) and relevant findings for NDI

Criterion	**Data for NDI**			**DS's conclusion for NDI**
	Bayer, 2003	**Bayer, 1995a**	**Other study**	
Non-inhalation route (acute)	Not applicable	Not applicable	Oral, OECD 423 (Schuengel, 2006): LD_{50} > 2 000 mg/kg bw/d	Supportive
MMAD	MMAD: 3.1-10.1 µm	MMAD: 3.1-4.0 µm; Key study for LC_{50}	Not applicable	
Irritation/ inflammation	Absolute lung weights significantly increased; increase in BALF protein; increase in BALF phospholipids and especially BALC, LDH, and TCC	Distended hydrothorax; lungs with dark-red colourations and spongy (oedematous) appearance		
Lethality dependent on particle size	56 mg/m³ (3.1 µm MMAD): no mortality 140 mg/m³ (4.1 µM MMAD): 2/18 dead 148 mg/m³ (6.9 µm MMAD): no mortality 240 mg/m³ (5.4 µm MMAD): 7/18 dead 245 mg/m³ (9.0 µm MMAD): no mortality 1050 mg/m³ (10.1 µm MMAD): 18/18 dead	96 mg/m³ (3.1 µm MMAD): no mortality 189 mg/m³ (3.2 µm MMAD): no mortality 238 mg/m³ (4.0 µm MMAD): 6/10 dead 314 mg/m³ (3.6 µm MMAD): 6/10 dead 384 mg/m³ (3,8 µm MMAD): 8/10 dead 541 mg/m³ (3,1 µm MMAD): 8/10 dead	Not applicable	Supportive
Onset of lethality	Onset of lethality: days 0-2	Onset of lethality: days 1-2		
Respiratory distress	Onset of clinical signs: Day 0 Signs: bradypnoe, laboured breathing pattern, breathing sounds, stridor, nasal discharge (serous), nostrils: reddened, red encrustations, dyspnoe	Onset of clinical signs: 4 h Signs: bradypnoe, laboured breathing pattern, rales, nose/snout area with red encrustations, serous discharge from nose		
Evidence on severe non-respiratory tract toxicity	No effects reported (decrease of rectal body temperature not considered severe)	No effects reported (decrease of rectal body temperature not considered severe)		
Necropsy findings in succumbed rats	"Absolute lung weights were significantly increased in all exposure groups."	Lungs with dark-red colourations and spongy (oedematours) appearance		

Criterion	Data for NDI			DS's conclusion for NDI
	Bayer, 2003	Bayer, 1995a	Other study	
Increase in BAL protein	"From all endpoints the increase in protein was most prominent. […] this endpoint is considered to be the most sensitive one to assess early changes."	BALF not examined		
Histopathology	Data not available	Data not available		Criterion cannot be evaluated.
Severe extrapulmonary organ damage	Severe extrapulmonary organ damage not reported	Severe extrapulmonary organ damage not reported (lobulation of liver/pale parenchymatous organs not considered severe)		Supportive

In the view of the DS, there is sufficient supportive evidence from the toxicological data that the split-entry concept is applicable to NDI.

10.3.5.2 Comparison with the CLP criteria

According to Annex I Table 3.1.1 of the CLP regulation, NDI falls into category 2 for acute toxicity via the inhalation route (Table 11).

Table 11: Comparison of the LC_{50} value for NDI with the classification criteria for dusts and mists according to Table 3.1.1 of the CLP regulation

CLP Acute Toxicity Category	Relevant ATE for dusts/mists (mg/L)	LC_{50}-value calculated for NDI (mg/L)	Resulting classification	Reference
Category 1	≤ 0.05	0.27	**Acute Tox. 2**	(Bayer, 1995a)
Category 2	> 0.05 - ≤ 0.5			
Category 3	> 0.5 - ≤ 1.0			
Category 4	> 1.0 - ≤ 5.0			

With regard to the split-entry concept, the DS proposes to establish a split entry for NDI in analogy to tolylfluanid (index numbers 613-116-00-7/613-116-01-4) and several per(oxo)borates (index numbers 005-017-00-7/005-017-01-4, 005-018-00-2/005-018-01-X, and 005-019-00-8/005-019-01-5):

- If NDI contains < 0.1 % (w/w) of particles with an aerodynamic diameter of below 50 µm, no classification for acute toxicity via the inhalation route is warranted.
- If NDI contains ≥ 0.1 % (w/w) of particles with an aerodynamic diameter of below 50 µm, it should be classified as acutely toxic via inhalation in category 2 (Acute Tox. 2, H330: Fatal if inhaled).

10.3.6 Conclusion on classification and labelling for acute inhalation toxicity

- If NDI contains < 0.1 % (w/w) of particles with an aerodynamic diameter of below 50 µm, no classification for acute toxicity via the inhalation route is warranted.
- If NDI contains ≥ 0.1 % (w/w) of particles with an aerodynamic diameter of below 50 µm, it should be classified as acutely toxic via inhalation in category 2 (Acute Tox. 2, H330: Fatal if inhaled).

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

NDI's current Annex VI entry for acute toxicity is Acute Tox. 4*; H332. The DS proposed to modify this classification into a **split entry** as follows:

- 1,5-naphthylene diisocyanate [containing **< 0.1 % (w/w) of particles with an aerodynamic diameter of below 50 µm]: no classification**
- 1,5-naphthylene diisocyanate [containing **≥ 0.1 % (w/w) of particles with an aerodynamic diameter of below 50 µm]: Acute Tox. 2; H330**. Inhalation: ATE = 0,27 mg/L (dusts or mists).

According to the REACH registration dossier, the substance is solid at 20°C and has a very low vapour pressure (8.0 x 10^{-4} Pa at 25.0°C). The particle size distribution of the registered substance is:

Particle size	Amount
> 875 µm	82.0 %
> 100 - < 875.0 µm	16.7 %
> 50 - < 100 µm	1.0 %
< 50 µm	0.3 %

This was measured by dry dispersion technique by combined manual sieving at 875 µm and laser diffraction. It was observed that significant amounts of the substance stick to the vibrational feeding system, which was used for laser diffraction analysis. Additionally, agglomeration of fine particles in the vibrational feeder was likely to occur due to the cohesive nature of the substance.

In the REACH registration dossier, the lead registrant provided the following statement with relevance to available toxicokinetic information for NDI regarding the inhalation route: "*Experimental toxicokinetic studies were not performed. NDI is a white to yellowish organic solid with a very low vapour pressure under normal ambient conditions (8 x 10^{-6} hPa at 25°C), therefore inhalation exposure to the vapour is expected to be negligible. Currently available data on particle size during worst-case end-use of NDI indicate a thoracic percentage of 0.02% that can be inhaled by humans and may reach the thoracic region.*" (Bayer, 2010).

For evaluation of acute inhalation toxicity, three studies in Wistar rats were available, summarised in the table below. The DS only had access to the study summaries in the registration dossier. Therefore, the excerpts reported below are from the study summaries provided by the lead registrant for NDI under REACH or reproduced by the DS from the summary in the REACH registration dossier with slight editorial modifications.

***Table*:** *Summary by the DS of the animal studies for acute inhalation LC_{50} determination (originally Table 6 in the CLH proposal). The texts referred to are in the original CLH proposal*

<table>
<tr><th>Method, guideline, deviations</th><th>Species, strain, sex, no/group</th><th>Test substance, duration of exposure, form, dose levels, and particle size, results</th><th>Reference</th></tr>
<tr><td>OECD 403/EU B.2

GLP claimed

Reliability 2 (reliable with restrictions): Only summary available

Key study</td><td>Rat, Wistar, 5M+ 5F per group</td><td>NDI, aerosol (dust), 1 x 4 h, nose-only

<table>
<tr><th>Dose level (mg/m³)</th><td>0</td><td>96</td><td>189</td><td>238</td><td>314</td><td>384</td><td>541</td></tr>
<tr><th>MMAD (µm)</th><td>NA</td><td>3.1</td><td>3.2</td><td>4.0</td><td>3.6</td><td>3.8</td><td>3.1</td></tr>
<tr><th>GSD (µm)</th><td>NA</td><td>1.6</td><td>1.7</td><td>2.1</td><td>1.5</td><td>1.5</td><td>1.6</td></tr>
<tr><th>Mortality (no. dead/no. exposed)
M
F</th><td>
0/5
0/5</td><td>
0/5
0/5</td><td>
0/5
0/5</td><td>
3/5
3/5</td><td>
3/5
3/5</td><td>
4/5
4/5</td><td>
4/5
4/5</td></tr>
<tr><th>Mortality (%, M+F combined)</th><td>0</td><td>0</td><td>0</td><td>60</td><td>60</td><td>80</td><td>80</td></tr>
</table>
LC_{50} (4h) = 0.27 mg/L

Cf. text for further details (including clinical signs)</td><td>(Bayer, 1995a)</td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations</th><th>Species, strain, sex, no/group</th><th>Test substance, duration of exposure, form, dose levels, and particle size, results</th><th>Reference</th></tr>
<tr><td>Non-guideline

GLP claimed

Reliability 2 (reliable with restrictions): Only summary available, observation time post-exposure: 7 d (instead of 14 d as recom-mended by OECD TG 403), MMAD outside the range recom-mended by OECD TG 403

Supporting study</td><td>Rat, Wistar, 18 M/group</td><td>NDI, aerosol (dust), 1 x 4 h, nose-only

<table>
<tr><th>Dose level (mg/m³)</th><td>0</td><td>56</td><td>140</td><td>148</td><td>240</td><td>245</td><td>1 050</td></tr>
<tr><th>MMAD (µm)</th><td>NA</td><td>3.1</td><td>4.1§</td><td>6.9§</td><td>5.4§</td><td>9.0§</td><td>10.1§</td></tr>
<tr><th>GSD (µm)</th><td>NA</td><td>1.9</td><td>1.9</td><td>2.4</td><td>2.1</td><td>2.5</td><td>2.8</td></tr>
<tr><th>Mortality (no. dead/no. exposed)</th><td>0/18</td><td>0/18</td><td>2/18</td><td>0/18</td><td>7/18</td><td>0/18</td><td>18/18</td></tr>
<tr><th>Mortality (%)</th><td>0</td><td>0</td><td>11</td><td>0</td><td>39</td><td>0</td><td>100</td></tr>
</table>
LC_{50} (4 h) not calculated

Cf. text for further details (including clinical signs)</td><td>(Bayer, 2003)</td></tr>
</table>

<table>
<tr>
<td>Similar to OECD 403/EU B.2

GLP claimed

Reliability 2 (reliable with restrictions): Only summary available, MMAD outside the range recommended by OECD TG 403

Supporting study</td>
<td>Rat, Wistar, 5M+5F per group</td>
<td>NDI, aerosol (dust), 1 x 1 h, nose-only

<table>
<tr><td>Dose level (mg/m³)</td><td>0</td><td>1 285</td><td>2 075</td></tr>
<tr><td>MMAD (µm)</td><td>NA</td><td>4.6§</td><td>8.1§</td></tr>
<tr><td>GSD (µm)</td><td>NA</td><td>1.6</td><td>1.7</td></tr>
<tr><td>Mortality
(no. dead/no. exposed)
M
F</td><td>

0/5
0/5</td><td>

1/5
0/5</td><td>

0/5
0/5</td></tr>
<tr><td>Mortality
(%, M+F combined)</td><td>0</td><td>10</td><td>0</td></tr>
</table>

For details regarding clinical signs, cf. text.</td>
<td>(Bayer, 1995b)</td>
</tr>
</table>

§ Outside the range recommended by OECD TG 403 (1-4 µm).

Bayer (1995a) was identified as the **key study** by the DS and used as the basis for the classification proposal. In this study, using 1 x 4h nose-only exposure to aerosol (dust), mortality was seen at concentrations of 238 mg/m^3 and above (see table above). Decreased body weights were observed in all groups exposed to the test compound. None of the rats in the control group exhibited any clinical signs. In the test substance groups, clinical signs were observed in all rats at all tested dose levels. They included bradypnea, laboured breathing pattern, nose/snout area with red encrustations, reduced motility, flaccid appearance, ungroomed hair-coat and piloerection. In addition, rales, salivation, serous discharge from nose, cyanosis and apathy were seen at 189 mg/m^3 and above. At 96 mg/m^3 and above, also a concentration-dependent decrease of body temperature was recorded. The onset and duration of the clinical signs was 4h – 11 days in all dose groups and both sexes. The onset of mortality, where it occurred, was 1 – 2 days. Gross pathology findings in the animals sacrificed during the observation period of 4 weeks included white foamy discharge from snout, red encrustation in the muzzle area, lungs with dark-red colourations and spongy (oedematous) appearance, foam in trachea, distended hydrothorax, lobulation of liver, and pale parenchymatous organs. In rats sacrificed at the end of the observation period, an increased incidence of macroscopical findings was observed on lungs. However, the findings appeared not to be induced in a clear concentration-dependent manner. The LC_{50} value (aerosol, 4h) was determined as 270 mg/m^3 for male and female rats combined. In this study, the particle sizes varied between 3.1 – 4.0 µm (mass median aerodynamic diameter (MMAD), geometric standard deviations (GSD), between 1.5 – 2.1 µm).

The other two available studies were considered by the DS as supporting, but important in demonstrating a dependency of NDI associated mortality on the particle size distribution. These studies were not used for classification directly because they either applied only 1h exposure and/or used test materials with MMADs outside the range recommended in OECD TG 403. However, they were seen by the DS as relevant for the evaluation whether the split-entry concept for acute toxic substances via the inhalation route is applicable to NDI.

The non-guideline, 1 x 4h, nose-only study (Bayer, 2003) focused on an analysis of bronchoalveolar lavage (BAL) parameters rather than lethality. In the 56, 140, 148, 240, 245 and 1050 mg/m^3 exposure groups, the MMAD (GSD) were 3.1 (1.9), 4.1 (1.9), 6.9 (2.4), 5.4 (2.1), 9.0 (2.5) and 10.1 (2.8) µm, respectively. Except for the 56 mg/m^3 group, all of these distributions were outside the MMAD range recommended in OECD TG 403. All rats in the control group tolerated the exposure without clinical signs. In all exposure groups, mean body weights were markedly different from the control group. The clinical signs were similar to those reported in Bayer (1995a), again visible in all rats in each test compound group, and mostly comparable across all of the exposure groups. In this study, the onset and duration of the clinical signs was 0 – 7 days. The onset of mortality, when it occurred, was 0 – 2 days. In gross pathology, in all groups exposed to the test substance, concentration dependent macroscopic alterations of the respiratory tract were observed.

Moreover in Bayer (2003), BAL fluid was collected on post-exposure days 1, 3, and 7 and analysed for indicators of inflammatory response and lower respiratory tract damage as well as for interactions with pulmonary phospholipids. According to the registration dossier, *absolute lung weights were significantly increased in all […] exposure groups*. Despite the increase, observed lung weights of the exposure groups were indistinguishable from the control group on day 7. From all endpoints, increase in protein was most prominent. The conclusion by the lead registrant on the BAL results was that the influx of protein into the alveoli and the elevated lung weights were dependent upon the actually respirable mass (total mass concentration x fraction penetrating the alveolar region; approximate cut-off for rats is 5 µm) rather than total concentration.

The DS noted that while Bayer (2003) is not a suitable study as a basis for classification as such, it demonstrated that acute toxicity of NDI is a function of not only the total air concentration, but in particular of the particle size distribution. An external concentration of 140 mg/m^3 NDI (MMAD: 4.1 µm) resulted in 11% mortality, while a concentration of 148 mg/m^3 NDI (MMAD: 6.9 µm) did not cause any mortality (see table above). Likewise, 240 mg/m^3 NDI (MMAD: 5.4 µm) was lethal for 39%, while all of the animals survived exposure to 245 mg/m^3 NDI (MMAD: 9.0 µm). Overall, the DS agreed that these results suggest a strong dependency of NDI associated lethality on the particle size distribution of the test material, and that Pauluhn (2004) demonstrated the correlation of BAL fluid parameters with particle MMAD.

In the third study (Bayer, 1995b), the study design was similar to OECD TG 403, but with an exposure duration of 1 x 1h only, creating a high degree of uncertainty (see table above). In addition, the MMADs of the test materials used were clearly outside the range recommended by OECD TG 403 (1 – 4 µm): in the 1285 and 2075 mg/m^3 exposure groups, the MMAD (GSD) were 4.6 (1.6) and 8.1 (1.7) µm. The results were summarised by the registrant as follows: "*Aerosol (dust) concentrations up to 1 285 mg/m^3 did induce test substance related mortality (males: 1 out of 5 rats died; females: no mortality). Exposure to the limit concentration of 2075 mg/m^3 test compound was tolerated without mortality. Mortality occurred on post-exposure day ten. Necropsy findings support the conclusion that a causal relationship between lethality and lung damage existed. Exposure to concentrations of 1285 mg/m^3 and higher were followed by concentration-dependent signs suggestive of irritation of the respiratory tract (e.g. bradypnoea, dyspnoea, laboured breathing pattern, rales, nose/snout area with red encrustations, serous discharge from nose, cyanosis) and non-specific signs such as reduced motility and flaccid muscle tone. The duration of signs (maximum duration up to day 9) was*

dependent on respiratory signs" (Bayer, 1995b).

Split-entry concept

In section 3.1.2.3.2 (p. 242), the ECHA Guidance on the Application of the CLP Criteria (v5.0, 2017, hereafter the CLP guidance) notes: *"The use of highly respirable dusts and mists is ideal to fully investigate the potential inhalation hazard of the substance. However, it is acknowledged that these exposures may not necessarily reflect realistic conditions. For instance, solid materials are often micronised to a highly respirable form for testing, but in practice exposures will be to a dust of much lower respirability. Similarly, pastes or highly viscous materials with low vapour pressure need strong measures to be taken to generate airborne particulates of sufficiently high respirability, whereas for other materials this may occur spontaneously. In such situations, specific problems may arise with respect to classification and labelling, as these substances are tested in a form (i.e. specific particle size distribution) that is different from all the forms in which these substances are placed on the market and in which they can reasonably be expected to be used.*

A scientific concept has been developed as a basis for relating the conditions of acute inhalation tests to those occurring in real-life, in order to derive an adequate hazard classification. This concept is applicable only to substances or mixtures which are proven to cause acute toxicity through local effects and do not cause systemic toxicity (Pauluhn, 2008)" (ECHA, 2017).

In Pauluhn (2008), further guidance on the applicability of the EU split–entry concept is provided. In this context, criteria are defined which are supportive or prohibitive for its use (see table below). Relevant findings for NDI from the two 4h acute toxicity tests via the inhalation route are summarised by the DS in next table below (the DS's comparison between the criteria from Pauluhn (2008) and relevant findings for NDI…), and compared with the above mentioned criteria. This latter table also shows the DS's conclusions on whether each finding is considered supportive or prohibitive for the use of the split-entry concept.

Table: *Criteria supportive of or prohibitive for the use of the split-entry concept (by default all criteria refer to findings from an acute 4h inhalation study using the OECD (2007) protocol), from Pauluhn (2008), originally Table 9 in the CLH proposal*

<table>
<tr><th></th><th>Mandatory endpoint</th><th>RT (ET-TB)</th><th>Pulmonary (alveolar)</th><th>Supportive of the use of split-entry</th><th>Prohibitive for the use of split-entry</th></tr>
<tr><td>Non-inhalation route (acute)</td><td rowspan="6">Yes</td><td rowspan="2">-</td><td rowspan="2">-</td><td>Low toxicity</td><td>High toxicity</td></tr>
<tr><td>MMAD</td><td>< approx. 4 µm</td><td>>> 4 µm</td></tr>
<tr><td>Irritation/inflammation</td><td>Minimal</td><td>Yes</td><td rowspan="2">Yes</td><td>-</td></tr>
<tr><td>Lethality dependent on particle size</td><td rowspan="2">-</td><td rowspan="2">-</td><td>No</td></tr>
<tr><td>Onset of lethality</td><td>Immediate (up to day 7)</td><td rowspan="2">If delayed in onset (≥ 8d)</td></tr>
<tr><td>Respiratory distress</td><td>Minimal</td><td>Yes</td><td>Yes</td></tr>
<tr><td>Evidence on severe non-respiratory tract toxicity</td><td>-</td><td rowspan="4">-</td><td rowspan="2">-</td><td>No</td><td>Yes, if not secondary</td></tr>
<tr><td>Necropsy findings in succumbed rats</td><td>Yes</td><td>Hepatisation, lung enlarged, oedema</td><td>No findings in lungs</td></tr>
<tr><td>Increase in BAL protein</td><td rowspan="2">Supportive</td><td rowspan="2">Yes</td><td>Yes</td><td>-</td></tr>
<tr><td>Histopathology</td><td>Major lesions restricted to lower RT</td><td>Major lesions distributed throughout RT</td></tr>
<tr><td>Severe extrapulmonary organ damage</td><td>-</td><td>-</td><td>No</td><td>Yes</td></tr>
</table>

MMAD: mass median aerodynamic diameter of particulate atmosphere in the vicinity of the breathing zone of animals and measured by cascade impactor, post-exposure days are relative to day 0 (exposure day); RT: respiratory tract; ET: extrathoracic region (pharynx, nasal passages); TB: tracheobronchial region; - : not applicable.

Table: *The DS's comparison between the criteria from Pauluhn (2008) and relevant findings for NDI (originally Table 10 in the CLH proposal*)

Criterion	Data for NDI			DS's conclusion for NDI
	Bayer, 2003	Bayer, 1995a	Other study	
Lethality dependent on particle size	56 mg/m³ (3.1 µm MMAD): no mortality 140 mg/m³ (4.1 µM MMAD): 2/18 dead 148 mg/m³ (6.9 µm MMAD): no mortality 240 mg/m³ (5.4 µm MMAD): 7/18 dead 245 mg/m³ (9.0 µm MMAD): no mortality 1050 mg/m³ (10.1 µm MMAD): 18/18 dead	96 mg/m³ (3.1 µm MMAD): no mortality 189 mg/m³ (3.2 µm MMAD): no mortality 238 mg/m³ (4.0 µm MMAD): 6/10 dead 314 mg/m³ (3.6 µm MMAD): 6/10 dead 384 mg/m³ (3,8 µm MMAD): 8/10 dead 541 mg/m³ (3,1 µm MMAD): 8/10 dead	Not applicable	Supportive
Onset of lethality	Onset of lethality: days 0-2	Onset of lethality: days 1-2		
Respiratory distress	Onset of clinical signs: Day 0 Signs: bradypnoe, laboured breathing pattern, breathing sounds, stridor, nasal discharge (serous), nostrils: reddened, red encrustations, dyspnoe	Onset of clinical signs: 4 h Signs: bradypnoe, laboured breathing pattern, rales, nose/snout area with red encrustations, serous discharge from nose		
Evidence on severe non-respiratory tract toxicity	No effects reported (decrease of rectal body temperature not considered severe)	No effects reported (decrease of rectal body temperature not considered severe)		
Necropsy findings in succumbed rats	"Absolute lung weights were significantly increased in all exposure groups."	Lungs with dark-red colourations and spongy (oedematours) appearance		
Increase in BAL protein	"From all endpoints the increase in protein was most prominent. […] this endpoint is considered to be the most sensitive one to assess early changes."	BALF not examined		
Histopathology	Data not available	Data not available		Criterion cannot be evaluated.
Severe extrapulmonary organ damage	Severe extrapulmonary organ damage not reported	Severe extrapulmonary organ damage not reported (lobulation of liver/pale parenchymatous organs not considered severe)		Supportive

The DS concluded that there is sufficient supportive evidence from the toxicological data that the split-entry concept is applicable to NDI. In addition, according to the CLP Regulation, Annex I, Table 3.1.1, NDI meets Category 2 criteria for acute toxicity via the inhalation route as the calculated LC_{50} was 0.27 mg/L. With regard to the split-entry concept, the DS proposed to establish a split entry for NDI in analogy to tolylfluanid (index numbers 613-116-00-7/613-116-01-4) and several per(oxo)borates (index numbers 005-017-00-7/005-017-01-4, 005-018-00-2/005-018-01-X, and 005-019-00-8/005-019-01-5):

- If NDI contains < 0.1% (w/w) of particles with an aerodynamic diameter of below 50 µm, no classification for acute toxicity via the inhalation route is warranted.
- If NDI contains ≥ 0.1% (w/w) of particles with an aerodynamic diameter of below 50 µm, it should be classified as Acute Tox. 2; H330: Fatal if inhaled, with an ATE = 0,27

mg/L (dusts or mists).

Comments received during consultation

One Member State Competent Authority (MSCA) agreed with the proposed classification for acute inhalation toxicity and the use of a split-entry as proposed by the DS. One Company-Manufacturer and two Company-Importers agreed with the use of a split entry, and the two entries being Acute Tox. 2; H330 and no classification. However, they disagreed with the cut-off limit proposed by the DS and proposed that NDI with a concentration of particles with aerodynamic diameter of below 50 µm should not be classified if their concentration is < 0.1% w/w while be classified as Acute Tox. 2 if ≥ 0.1% w/w.

In their comment, the lead registrant remarked that the thoracic fraction of the substance is the toxicity determining parameter when a split-entry concept applies to acute inhalation toxicity. They stated that the the-cut off limit proposed by the DS was only based on analogy to previous entries. They assumed that the cut-off of 50 µm proposed by the DS was based on the parameters laid down in the plant protection product (PPP) regulation, and stated that in their opinion, analogy to the PPP tolylfluanid is not applicable to an industrial chemical without known spray applications, such as NDI.

Furthermore, the lead registrant presented calculations with the purpose of transposing the available acute inhalation toxicity data on NDI to the typical particle-size of the substance as produced. In their calculation, they used a concentration of the thoracic fraction of 0.02% w/w which they claim correspond to the NDI as produced. In addition, the calculations aimed to recompute the thoracic fraction percentage which would not to trigger classification considering the respective ATE interval of the individual categories of acute inhalation toxicity. Based on these calculations, they proposed the following cut-off limits for classification:

- C_{th} < 5.4% no classification for acute toxicity via the inhalation route.
- C_{th} ≥ 5.4% classified as Acute Tox. 2; H330.

The DS replied by noting that the aim is to classify all possible NDI materials, not just one specific material. Furthermore, they mentioned that, while ECHA's CLP guidance refers to the split-entry concept, it does not provide a workable definition of the thoracic fraction. Moreover, the upper limit of 50 µm used was not derived from the Plant Protection Product Regulation, but from the table 1 in norm EN481. According to this table, 50 µm marks the lowest particle size without contribution to the thoracic fraction (vs. 0.1% of the particles at 40 µm, 1.0% at 30 µm, 3.0% at 25 µm etc.). The DS additionally noted that EN481 also describes the thoracic fraction as a cumulative (log)normal distribution with a median of 11.64 µm and a geometric standard deviation of 1.5. Consequently, the 50 µm limit chosen in previous cases where the split-entry concept was applied might be considered as quite conservative. The DS also stated that the use of 10 µm as the upper limit of the thoracic fraction is not acceptable to as 55.5% of the particles with a diameter of 11 µm, and still 9.1% of the particles with 20 µm diameter, contribute to the thoracic fraction (EN481, table 1). In the Currenta study (2019), submitted during the consultation, almost 74% of the test material had a particle size of 10-50 µm. The DS noted that the proposal from the manufacturer to define classification borders based on the percentage of the thoracic fraction rather than a specific particle size cut-off would bear a considerable risk of under classification if the 10 µm limit was used. Therefore, the DS would rather prefer the classification borders would be defined based on an upper limit particle size. If the percentage of thoracic fraction would be used, then a clear definition would be needed to

allow for a correct and unambiguous determination of that fraction.

A Company-Importer first expressed their support for the lead-registrant's comment and argumentation. In addition, they, along with a second Company-Importer, suggested to use already existing values for the definition of the diameter of inhalable dust, in order to have a harmonisation of different legal regulations. They presented as reference the ADR 2019 (chapter 2.2.61.1.3), which describes the principle requirement for the testing of a substance for acute toxicity by inhalation. This is defined by min 10% w/w of inhalable dust with an aerodynamic radius of < 10 µm. Therefore, they suggested to define the particle size accordingly by < 10 µm instead of < 50 µm as proposed by the DS.

The DS restated their opinion, in particular that an upper limit of 10 µm does not appear sufficiently conservative based on norm EN481.

Assessment and comparison with the classification criteria

There are three studies available to assess NDI's acute inhalation toxicity. Of these, only one can be used directly for classification. This key study was performed according to OECD TG 403 and under GLP in Wistar rats (5 M + 5 F), using 1 x 4h nose-only exposure (Bayer, 1995a). The DS assessed the reliability of this study as 2, due to only the summary being available in the REACH registration dossier. In this study, the LC_{50} value was 0.27 mg/L. As shown in the table below, the LC_{50} value meets the criteria for classification as Acute Tox. 2.

Table: *Comparison of the LC_{50} value for NDI with the classification criteria for dusts and mists according to Table 3.1.1 of the CLP regulation (originally Table 11 in the CLH proposal)*

CLP Acute Toxicity Category	Relevant ATE for dusts/mists (mg/L)	LC_{50}-value calculated for NDI (mg/L)	Resulting classification	Reference
Category 1	≤ 0.05	0.27	**Acute Tox. 2**	(Bayer, 1995a)
Category 2	> 0.05 - ≤ 0.5			
Category 3	> 0.5 - ≤ 1.0			
Category 4	> 1.0 - ≤ 5.0			

The two other GLP studies available cannot be used directly for classification. Bayer (2003) was reported by the lead registrant as a non-guideline study, and the MMAD of the test material was outside the range recommended by OECD TG 403. While the study design of the third study (Bayer, 1995b) was otherwise similar to OECD TG 403, it deviated on the exposure time, 1h instead of 4h, and in the MMAD of the test material which was outside the recommended range. LC_{50} values were not derived in these two studies. In Bayer (2003), where rats were exposed to NDI for 1 x 4h, larger particles (~7-9 µm, GSD ~2.5 µm) tested at dose levels of ~140-150 and ~240-245 mg/m^3 did not cause mortality while smaller particles (~4-5.5 µm, GSD ~2 µm) tested at corresponding dose levels did (see table "Summary by the DS of the animal studies for acute inhalation LC_{50} determination…" above). In Bayer (1995b), 1 x 1h exposure was not lethal at 2075 mg/m^3, when the MMAD was 8.1 µm (GSD 1.7 µm) and, caused 10% mortality at a substantially lower dose level of 1285 mg/m^3 when the particle MMAD was 4.6 µm (GSD 1.6 µm).

RAC agrees with the DS that both studies (Bayer, 2003 and 1995b) show that the particle size of NDI has an impact on its acute toxicity via the inhalation route.

NDI is a solid substance with a very low vapour pressure. Considering the results of the three inhalation toxicity studies, and section 3.1.2.3.2 (p. 242) of the CLP guidance cited under the sub-heading *"Split-entry concept"*, RAC agrees with the DS that a split-entry is applicable.

However, the available data on NDI do not allow determination of a "safe" NDI particle size warranting no classification, which could be used as the cut-off limit for the split entry. The largest particle size tested was 10.1 µm MMAD (GSD 2.8 µm) at one dose level of 1050 mg/m^3 (1.05 mg/L). This was the highest dose tested in the non-guideline study using a 1 x 4h nose-only exposure (Bayer, 2003), and it was 100% lethal. Although not directly applicable to classification purposes, this result would indicate at least a category 3 classification for the acute inhalation toxicity of this particle size.

Therefore, RAC agrees with the DS that 10 µm, as proposed in the consultation, is clearly not an acceptable cut-off limit for the split entry. On the other hand, the cut-off particle size of 50 µm proposed by the DS is a conservative value, aimed at ensuring that all of the particles are above the thoracic fraction.

There is no clear definition available for the particle size of the thoracic fraction, which is a spectrum below 50 µm. As mentioned also by the DS in their response to a consultation comment, the norm EN481 describes the thoracic fraction as a cumulative (log)normal distribution with a median of 11.64 µm and a GSD of 1.5. Furthermore, in the norm EN481, 50 µm marks the lowest particle size without contribution (whereas, 0.1% of the particles at 40 µm, 1.0% at 30 µm, 3.0% at 25 µm etc. contribute to the thoracic fraction). Similarly, according to the Particle Size-Selective Sampling Criteria for Airborne Particulate Matter by the American Conference of Governmental Industrial Hygienists (ACGIH®), thoracic particulate matter is composed by 50% of particles with 10 µm aerodynamic diameter and 2% of particles with 25 µm aerodynamic diameter.

As a practical solution, RAC agrees with the DS to set the cut-off limit to particles just above the thoracic fraction and that it is preferable to clearly define a specific particle size cut-off, rather than a percentage of the thoracic fraction. Especially, as there is no clear-cut definition for the thoracic fraction available. The same cut-off of particle size 50 µm has previously been used for the split entries of tolylfluanid (index numbers 613-116-00-7/613-116-01-4) and several per(oxo)borates (index numbers 005-017-00-7/005-017-01-4, 005-018-00-2/005-018-01-X, and 005-019-00-8/005-019-01-5). Consistency between the split-entries is considered by RAC as appropriate, when there is no specific data or other reason to justify deviating from the previous entries.

Concerning the proposed ATE of 0.27 mg/L (dusts or mists), RAC notes that there is a discrepancy between the LC_{50} value of 0.27 mg/L calculated in (Bayer, 1995a) and the acute inhalation toxicity data. Already at the dose level of 0.238 mg/L 60% of the animals died suggesting an actual LC_{50} < 0.24 mg/L. According to the available information, the LC_{50} was calculated according to the method of Rosiello *et al.* (1972) as modified by Pauluhn (1983), based on the maximum-likelihood method of Bliss (1938). It was stated that "*The interpolated concentration at 50% lethality in this case was designated at approximate* LC_{50}". RAC notes that considering the data (0-0-0-60-60-80-80% mortality, at 0, 0.096, 0.189, 0.238, 0.314, 0.384 or 0.541 mg/L), different curve-fitting equations might yield different LC_{50} value. However, considering that both the calculated 0.27 mg/L and data-based < 0.24 mg/L would result to the same category, RAC supports the ATE value proposed by the DS.

In conclusion, RAC agrees with the DS's proposal that the following split-entry classification is warranted for NDI:

- 1,5-naphthylene diisocyanate [containing < 0.1 % (w/w) of particles with an aerodynamic diameter of below 50 µm]**: no classification**
- 1,5-naphthylene diisocyanate [containing ⩾ 0.1 % (w/w) of particles with an aerodynamic diameter of below 50 µm]: **Acute Tox. 2; H330**. Inhalation: ATE = 0.27 mg/L (dusts or mists).

10.4 Skin corrosion/irritation

Not assessed in this dossier

10.5 Serious eye damage/eye irritation

Not assessed in this dossier

10.6 Skin corrosion/irritation

Not assessed in this dossier

10.7 Serious eye damage/eye irritation

Not assessed in this dossier

10.8 Respiratory sensitisation

Not assessed in this dossier. NDI already has a harmonised classification as Resp. Sens. 1.

10.9 Skin sensitisation

To the knowledge of the DS, no studies of the skin sensitising potential of NDI in humans are available. However, skin sensitisation test data in animals summarised in Table 12 below are available for NDI, which are sufficient for classification and labelling. It is stressed that all other diisocyanates currently classified as respiratory sensitisers in Annex VI of the CLP regulation also are classified as skin sensitisers.

Table 12: Summary table of the available animal studies on skin sensitisation for NDI

Method, guideline, deviations	Species, strain, sex, no/group	Test substance, vehicle	Study protocol	Results	Reference
Modified LLNA (LLNA/IMDS, Integrated Model for the Differentiation of Skin Reactions) Similar to OECD TG 429/EU B.42	Mouse, NMRI, 6 F/group	NDI (purity 99.8 %), acetone/olive oil (AOO), 4:1	25 µL 0, 2, 10 or 50 % NDI in AOO were applied to the dorsum of both ears in three consecutive days. Positive control: Hexylcinnamic aldehyde (CAS No 101-86-0) at 3, 10, and 30 % in AOO (4:1)	SI (weight of draining lymph nodes): 2 %: 3.51** 10 %: 3.79** 50 %: 3.47** SI (cell count in draining lymph nodes): 2 %: 4.06**	(Bayer, 2006)

Method, guideline, deviations	Species, strain, sex, no/group	Test substance, vehicle	Study protocol	Results	Reference
(LLNA) GLP claimed Reliability 2 (reliable with restrictions): Only summary available (with deficiencies in reporting, cf. text)				10 %: 4.15** 50 %: 4.42** $EC_{1.4}$ << 2 % Skin Sens. 1A	

** Statistically significant ($p \leq 0.05$)

In a modified LLNA test in mice, NDI concentrations of 2 % and above in acetone/olive oil (4:1) resulted in Stimulation Indices (SI) of > 4. An EC3 value was not calculated and individual or group DPM values were not provided, therefore the available information does not allow to determine whether NDI is a strong or even an extreme sensitiser. Only a IUCLID summary by the lead registrant for NDI with deficiencies in reporting was available to the DS which is reproduced here (as is, with slight editorial amendments by the DS):

Test type

LLNA, OECD TG 429/EU B.42. Modification: In addition, measurements of ear swelling and ear weight were done to discriminate the irritating potential from the sensitising potential of the test substance ("Integrated Model for the Differentiation of Skin reactions (IMDS)"). Incomplete reporting: No pre-screen test for irritancy and systemic toxicity, no experimental details regarding the measurement of proliferation. GLP claimed.

Test material

NDI, purity 99.8 %, Lot/batch no. P3YE591000. Vehicle: Acetone/olive oil 4:1.

Test animals

Species: Mouse. Strain: NMRI. Sex: Female. Source: Harlan-Winkelmann GmbH, Borchen, Germany. Age at study initiation: 9 weeks. Weight at study initiation: 26-36 g. Housing: Individual. Diet and water: Ad libitum. Acclimation period: at least seven days. Six animals per group.

Methods

Application: The test item in the formulation and the vehicle were applied epicutaneously onto the dorsal part of both ears of the animals. This treatment was repeated on three consecutive days (days 1, 2, and 3). The volume administered was 25µL/ear. The concentrations used were based on the experiences with the test system and the toxic properties of the test substance.

Positive control substance(s): Hexylcinnamic aldehyde (CAS No 101-86-0) formulated in acetone/olive oil (4:1) at concentrations of 3, 10, and 30 %.

Statistics: When it was statistically reasonable, the values from treated groups were compared with those from the control group by a one-way analysis of variance (ANOVA) when the variances are considered homogeneous according to a homogeneity testing like Cochran's test. Alternatively, if the variances are considered to be heterogeneous ($p \leq 0.05$), a non-parametric Kruskal-Wallis test has been used (Kruskal-Wallis ANOVA) at significance levels of 5 %. Two-sided multiple test procedures were done according to Dunnett or Bonferroni-Holm, respectively. Outlying values in the LN weights were eliminated at a probability level of 99 % by Nalimov's method. In addition, for the LLNA/IMDS the smallest significant differences in the means were calculated by Scheffels method, which according to Sachs can be used for both equal and unequal sample sizes.

Results

Positive control: The LLNA with hexylcinnamic aldehyde showed a clear sensitising potential.

Test group: The results show that the test item has a sensitising potential in mice after dermal application. Compared to vehicle treated animals there was a clear increase in weights of the draining lymph nodes (indices of 3.51, 3.79, and 3.47, resp.) and in the cell counts (indices of 4.06, 4.15, and 4.42, resp.) at dose groups of 2, 10, and 50 %. These changes are of statistical significance. The "positive level" of index 1.4 was exceeded for the cell counts in all dose groups.

Table 13: Summary of the LLNA/IMDS results (means of 6 animals per group)

Parameter investigated	Vehicle control	Dose groups		
		2 %	10 %	50 %
Stimulation index (weight of draining lymph nodes)	1.00	3.51*	3.79*	3.47*
Stimulation index (cell count in draining lymph nodes)	1.00	4.06*	4.15*	4.42*
Ear swelling in 0.01 mm on day 4 (index)	17.50 (1.00)	20.58* (1.18)	23.42* (1.34)	23.17 (1.32)
Ear weight in mg/8 mm diameter punch on day 4 (index)	11.03 (1.00)	14.29* (1.30)	16.41* (1.49)	17.97* (1.63)

* Statistically significant increase ($p \leq 0.05$)

The "positive level" of ear swelling which was a 2 x 10^{-2} mm increase, i.e. about 10 % of the control values, has been exceeded in all dose groups. A significant increase compared to vehicle treated animals regarding ear swelling and ear weights was detected in all dose groups. An increase in this parameter would point to an acute irritating (inflammatory) response. However, such an irritating property is also combined with a strong skin sensitising potential of a test compound.

The body weights of the animals were not affected by any treatment.

Registrant's summary and conclusion

1,5-Naphthylene diisocyanate (NDI) was investigated in the modified local lymph node assay (LLNA-IMDS) on female mice according to OECD TG 429. Concentrations of 0 (vehicle control), 2, 10, and 50 % formulated in acetone/olive oil (4:1) were tested. The results show that NDI has a sensitising potential in mice after dermal application. Compared to vehicle treated animals there was a significant increase regarding the weights of the draining lymph nodes and the cell counts in all dose groups. The corresponding cell count indices were 4.06, 4.15, and 4.42 exceeding the "positive level" of index 1.4. A significant increase compared to vehicle treated animals regarding ear swelling and ear weights was detected in all dose groups. An increase in this parameter would point to an acute irritant (inflammatory) response. However, such an irritant property is also combined with a strong skin sensitizing potential of a test compound (Bayer, 2006).

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

The LLNA/IMDS is a variant of the standard LLA test which uses cell counts in draining lymph nodes in order to avoid the use of radiolabel. In addition, lymph node weight is used as a parameter to assess irritancy. Basic validation information for the test is available from two publications which demonstrated good inter-laboratory comparability of results. In addition they showed that for BALB/c mice a cut-off Stimulation Index (SI) of 1.55 ($EC_{1.5}$) corresponded to the same statistical significance level as the EC_3 in the conventional LLNA (Ehling et al., 2005a; Ehling et al., 2005b). For NMRI mice (the strain used in the study with NDI), a slightly lower equivalent cut-off of 1.4 ($EC_{1.4}$) was noted.

OECD TG 429 defines performance standards which have to be met by alternative LLNA designs in order to meet test guideline requirements. Basketter and co-workers (Basketter et al., 2011) evaluated the performance of the LLNA/IMDS (termed "LNCC" in their study) vs. the standard LLNA for all of the reference substances listed in Annex 1 of OECD TG 429, albeit in CBA mice. Both tests agreed for 21 of the 22 reference substances, i.e. with an excellent concordance of > 95 %.

Remarkably, for four out of the 18 core reference substances and five out of the complete set of 22 reference substances, both tests (i.e. LNCC and the concurrent standard LLNA) failed to reproduce the LLNA results

published in Annex I to OECD TG 429 (of the five "malpredictions", one was categorised as a „false negative" and four were „false positives"). The authors discussed two important factors that might have been responsible for this finding:

- The "false" positive results were mostly obtained using higher concentrations than used in OECD TG 429, often in conjunction with evidence for skin irritation.
- As with any other biological test, the reproducibility of LLNA results is subject to variability and multiple measurements were not performed for all of the reference substances in OECD TG 429

They summarise their discussion by stating that *"[…] performance standards for the evaluation of any toxicological endpoint, not solely skin sensitization, as would be expected, can only to be as good as the data on which they are based. Additionally, it has to be borne in mind that biological variation can have similar impact on the PS standard data as well as on the assay being evaluated. Accordingly, there is true value in both ensuring good use of control data and maintaining a degree of flexibility when it comes to the overall validation interpretation"* (Basketter et al., 2011).

In line with these considerations, the DS concludes that by and large the LLNA/IMDS has been shown to reproduce the results from the standard LLNA very well.

In the study with NDI concentrations of 2 % NDI and greater consistently caused SI values of > 4, i.e. lymphocyte counts of more than four times that of the vehicle controls (cf. Table 13 above). Although not specified in the study summary, it is evident that the $EC_{1.4}$, i.e. the effective concentration causing a 1.4-fold increase in lymphocyte count must be << 2 %. Even considering that there is some uncertainty about the equivalence of the $EC_{1.4}$ in the LLNA/IMDS and the EC_3 in the standard test, the DS concludes that the findings from the former indicate that NDI is a strong skin sensitiser (Bayer, 2006).

10.9.2 Comparison with the CLP criteria

According to the criteria given in Table 3.4.3 of the CLP regulation, skin sensitisers fall into Skin Sens. Sub-category 1A based on the results from a standard LLNA test, if an EC_3 < 2 % is determined. In the available LLNA/IMDS for NDI, the applied concentration of 2 % already caused an SI of > 4, i.e. the $EC_{1.4}$ (equivalent to the EC_3 in the standard LLNA) was << 2 %. The DS concludes that NDI is a strong sensitiser and fulfils the criteria for classification as Skin Sens. 1A.

Table 14: Comparison of experimental results (mouse LLNA/IMDS) confirming the skin sensitisation potential of NDI in animals with the respective criteria of the CLP regulation and CLP guidance

Criteria acc. to Table 3.4.3 and Table 3.4.4 of the CLP regulation and CLP Guidance (ECHA, 2017)		Reference	EC_3	Resulting Classification
Skin Sens. 1A, Extreme	$EC_3 \leq 0.2$ %	(Bayer, 2006)	$EC_{1.4}$* << 2 %	**Skin Sens. 1A Strong sensitiser**
Skin Sens. 1A, Strong	0.2% < $EC_3 \leq 2$ %			
Skin Sens. 1B, Moderate	EC_3 > 2 %			

* Equivalent to the EC_3 in the standard LLNA.

Since 2 % was the lowest concentration tested in (Bayer, 2006), the available data do not allow for a decision on whether NDI should even be considered an extreme sensitiser (with the consequence of setting an SCL of 0.001 % acc. to Table 3.9 of the CLP guidance).

10.9.3 Conclusion on classification and labelling for skin sensitisation

Based on the results of the LLNA/IMDS, NDI should be classified as Skin Sens. 1A (hazard statement H317: May cause an allergic skin reaction).

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

No information on the skin sensitising potential of NDI in humans is available.
One modified LLNA (Integrated Model for the Differentiation of Skin reaction, LLNA/IMDS; similar to OECD TG 429) is presented in the CLH report (Bayer, 2006). The DS considered it reliable with restrictions (reliability 2) since only the study summary was available and, there are deficiencies in reporting, e.g. no information on pre-screen testing for irritancy and systemic toxicity, no experimental details regarding the measurement of proliferation. In this GLP study, NDI (purity 99.8%) was applied at concentrations of 2%, 10% or 50% in acetone/olive oil to female NMRI mice, 6 per group, to the dorsum of both ears for three consecutive days. Appropriate positive control was used (hexylcinnamic aldehyde), which showed a clear sensitising potential. Stimulation indices (SI) of both the cell count in draining lymph nodes and draining lymph nodes weights were significantly higher than in the vehicle control group, and well above 1.4 (a cut-off value for positive response for NMRI mouse strain; Ehling *et al.*, 2005b, see table below).
The "positive level" of ear swelling, defined at about 10% of the control values, was exceeded in all dose groups, which indicates an acute irritating response. The DS, however, pointed out that this irritating property was combined with a strong skin sensitising potential of the test compound. The body weights of the animals were not affected by any treatment.

Table: *Summary of the LLNA/IMDS results (means of 6 animals per group) (Table 10 from the CLH Report)*

Parameter investigated	Vehicle control	Dose groups		
		2%	10%	50%
Stimulation index (weight of draining lymph nodes)	1.00	3.51*	3.79*	3.47*
Stimulation index (cell count in draining lymph nodes)	1.00	4.06*	4.15*	4.42*
Ear swelling in 0.01 mm on day 4 (index)	17.50 (1.00)	20.58* (1.18)	23.42* (1.34)	23.17 (1.32)
Ear weight in mg/8 mm diameter punch on day 4 (index)	11.03 (1.00)	14.29* (1.30)	16.41* (1,49)	17.97* (1.63)

* Statistically significant increase ($p \leq 0.05$)

The DS provided justification for the validity of the assay performed (primarily good inter-laboratory comparability of results described by Ehling *et al.,* 2005a and 2005b; very good agreement with standard LLNA found by Basketter *et al.*, 2011), and concluded that the LLNA/IMDS has been shown to reproduce the results from the standard LLNA very well.

According to ECHA Guidance (Table 3.6), EC3 values in the range > 0.2 - ≤ 2 indicate strong skin sensitising potential. Since in the Bayer's study (2006), NDI concentration of 2% already caused SI values > 4, the DS considered that the EC1.4 (i.e. the effective concentration causing a 1.4-fold increase in lymphocyte count) must be well below 2%. This indicates that that NDI is a strong skin sensitiser, even taking into account some uncertainty about the equivalence of the EC1.4 in the LLNA/IMDS and the EC3 in the standard test. The DS therefore concluded that the criteria for classification as **Skin Sens. 1A** are fulfilled, according to the Table 3.4.3 of the CLP Regulation.

A specific concentration limit (SCL) has not been proposed because 2% was the lowest NDI concentration tested, and for an extreme sensitiser (which would warrant an SCL of 0.001%), EC3 ≤ 0.2% should be ascertained.

Comments received during consultation

Two MSCAs and one from Industry representative agreed with the DS's proposal.

Assessment and comparison with the classification criteria

RAC agrees with the DS that the only available assay (Bayer, 2006), a GLP study performed as a modified LLNA/IMDS assay, is reliable enough to provide a basis for classification on skin sensitisation.

This study is a LLNA/IMDS assay modified in a way to measure cell count and weights of draining lymph nodes in order to avoid radioactive labelling. Aim of IMDS assay is to discriminate sensitising from irritative potential of a test item by comparing the specific immune reaction in the draining lymph nodes (lymph nodes cell counts and lymph nodes weights) with the unspecific acute inflammatory skin reaction (ear swelling and weight of circular biopsies of the ears, Ehling *et al.*, 2005a). Validity of the LLNA/IMDS has been assessed, and a good inter-laboratory comparability was shown by Ehling *et al.* (2005a, 2005b), as well as very good agreement with standard LLNA (Basketter *et al.*, 2011). WHO also recognised this modification as "evaluated thoroughly in the context of interlaboratory trials" (WHO, 2008).

RAC acknowledges the study's limitations stated above but agrees with the DS that they do not have a major impact on the study's reliability. Namely, the study summary provides enough information for hazard assessment. Further, although there is no information on methodological details regarding cell proliferation measurement, the performing laboratory (Bayer HealthCare AG, Department of Toxicology, Wuppertal, Germany) is an experienced facility and has been involved in above mentioned inter-laboratory validation shortly before performing this assay (Bayer, 2006). Regarding the lack of pre-screen test for irritancy and systemic toxicity, an assessment of irritative potential of NDI was incorporated into IMDS assay, and the animals' body weights were not affected by the treatment.

The study results showed a marked increase in SIs with a dose response for cell count in draining lymph nodes (see table above). In agreement with the DS, RAC considers that these results cannot be explained only by irritative reaction. A clear increase in cell count SI (> 4) was observed already at 2% NDI concentration at which 18% increase in ear thickness was noted. According to ECHA Guidance, an excessive local skin irritation is indicated by an increase in ear thickness of ≥ 25%.

The 2nd ATP[4] and ECHA Guidance indicate that Skin Sens. 1A is applicable when EC3 value is ≤ 2. This value applies to standard LLNA. In case of modified LLNA, a value of 1.4 has been proposed as a cut-off for NMRI mouse strain used in the assay (Ehling *et al.*, 2005b). RAC agrees with the DS that, since in the Bayer study (2006) 2% concentration of NDI already

[4] Commission Regulation (EU) No 286/2011 of 10 March 2011 amending, for the purposes of its adaptation to technical and scientific progress, Regulation (EC) No 1272/2008 of the European Parliament and of the Council on classification, labelling and packaging of substances and mixtures

caused SI values of > 4, it could be assumed that the EC1.4 must be well below 2%. RAC therefore supports the DS's proposal to classify NDI as **Skin Sens. 1A; H334**, with no SCL.

10.10 Germ cell mutagenicity

Not relevant for this dossier

10.11 Carcinogenicity

Not relevant for this dossier

10.12 Reproductive toxicity

Not relevant for this dossier

10.13 Specific target organ toxicity-single exposure

Not relevant for this dossier

10.14 Specific target organ toxicity-repeated exposure

Not relevant for this dossier

10.15 Aspiration hazard

Not relevant for this dossier

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not relevant for this dossier

12 EVALUATION OF ADDITIONAL HAZARDS

Not relevant for this dossier

13 ADDITIONAL LABELLING

According to the CLP regulation, Annex II, section 2.4, the following special rule for supplemental label elements shall apply for mixtures containing NDI:

"Unless already identified on the label of the packaging, mixtures containing isocyanates (as monomers, oligomers, prepolymers, etc., or as mixtures thereof) shall bear the following statement:

EUH204 — 'Contains isocyanates. May produce an allergic reaction."

Additional labelling
According to the CLP regulation, Annex II, section 2.4, the following special rule for supplemental label elements shall apply for mixtures containing NDI: *"Unless already identified on the label of the packaging, mixtures containing isocyanates (as monomers, oligomers, prepolymers, etc., or as mixtures thereof) shall bear the following statement:* **EUH204 — Contains isocyanates. May produce an allergic reaction***".*

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