Committee for Risk Assessment

RAC

Annex 1

Background document

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

1-methyl-2-pyrrolidone (NMP)

EC number: 212-828-1
CAS number: 872-50-4

CLH-O-0000004066-78-03/F

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

Adopted

6 June 2014

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: 1-methyl-2-pyrrolidone

EC Number: 212-828-1

CAS Number: 872-50-4

Index Number: 606-021-00-7

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Version number: 2 **Date: August 2013**

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	**1-methyl-2-pyrrolidone**
EC number:	**212-828-1**
CAS number:	**872-50-4**
Annex VI Index number:	**606-021-00-7**
Degree of purity:	**>99.0 % (w/w)**
Impurities:	**butyrolactone and methylamine (as indicated by 1 registrant, this may not be representative for all registrants)**

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	Skin Irrit. 2; H315 Eye Irrit. 2; H319 Repr. 1B; H360D***; C≥ 5% STOT SE 3; H335; C≥ 10%
Current proposal for consideration by RAC	Repr. 1B; H360D (removal of the SCL)
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Skin Irrit. 2; H315 Eye Irrit. 2; H319 Repr. 1B; H360D STOT SE 3; H335; C≥ 10%

1.3 Proposed harmonised classification and labelling based on CLP Regulation

The scope of this proposal is limited to a removal of the SCL for Repr. 1B; H360D but does not include the classification for Repr. 1B; H360D itself.

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives		None	Not classified	Out of the scope of this proposal
2.2.	Flammable gases		None	Not classified	Out of the scope of this proposal
2.3.	Flammable aerosols		None	Not classified	Out of the scope of this proposal
2.4.	Oxidising gases		None	Not classified	Out of the scope of this proposal
2.5.	Gases under pressure		None	Not classified	Out of the scope of this proposal
2.6.	Flammable liquids		None	Not classified	Out of the scope of this proposal
2.7.	Flammable solids		None	Not classified	Out of the scope of this proposal
2.8.	Self-reactive substances and mixtures		None	Not classified	Out of the scope of this proposal
2.9.	Pyrophoric liquids		None	Not classified	Out of the scope of this proposal
2.10.	Pyrophoric solids		None	Not classified	Out of the scope of this proposal
2.11.	Self-heating substances and mixtures		None	Not classified	Out of the scope of this proposal
2.12.	Substances and mixtures which in contact with water emit flammable gases		None	Not classified	Out of the scope of this proposal
2.13.	Oxidising liquids		None	Not classified	Out of the scope of this proposal
2.14.	Oxidising solids		None	Not classified	Out of the scope of this proposal
2.15.	Organic peroxides		None	Not classified	Out of the scope of this proposal
2.16.	Substance and mixtures corrosive to metals		None	Not classified	Out of the scope of this proposal
3.1.	Acute toxicity - oral		None	Not classified	Out of the scope of this proposal
	Acute toxicity - dermal		None	Not classified	Out of the scope of this proposal
	Acute toxicity - inhalation		None	Not classified	Out of the scope

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
					of this proposal
3.2.	Skin corrosion / irritation		None	Skin Irrit. 2; H315	
3.3.	Serious eye damage / eye irritation		None	Eye Irrit. 2; H319	
3.4.	Respiratory sensitisation		None	Not classified	Out of the scope of this proposal
3.4.	Skin sensitisation		None	Not classified	Out of the scope of this proposal
3.5.	Germ cell mutagenicity		None	Not classified	Out of the scope of this proposal
3.6.	Carcinogenicity		None	Not classified	Out of the scope of this proposal
3.7.	Reproductive toxicity	Repr. 1B; H360D	None	Repr. 1B; H360D; SCL: ≥ 5.0%	
3.8.	Specific target organ toxicity –single exposure		≥10.0%	STOT SE 3; H335; SCL: ≥ 10.0%	
3.9.	Specific target organ toxicity – repeated exposure		None	Not classified	Out of the scope of this proposal
3.10.	Aspiration hazard		None	Not classified	Out of the scope of this proposal
4.1.	Hazardous to the aquatic environment		None	Not classified	Out of the scope of this proposal
5.1.	Hazardous to the ozone layer		None	Not classified	Out of the scope of this proposal

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling:

Signal word: Danger

GHS07: exclamation mark
GHS08: health hazard

Hazard statements:
H315: Causes skin irritation.
H319: Causes serious eye irritation.
H360D: May damage the unborn child
H335: May cause respiratory irritation

Precautionary statements: Not relevant as precautionary statements are not included in Annex VI of CLP.

Proposed notes assigned to an entry: None

:

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

1-Methyl-2-pyrrolidone is currently classified for developmental toxicity as Repr. 1B; H360D with a SCL of 5%. The justification of this classification for developmental toxicity and setting of the current SCL for 1-methyl-2-pyrrolidone can be found in the Annex 1 (A-D) of this report.

2.2 Short summary of the scientific justification for the CLH proposal

According to the criteria in the 'Guidance on the Application of the CLP Criteria' (as described in tables 3.7.2.5.4 and 3.7.2.5.5 of this guidance) the current SCL of 5% for developmental toxicity of 1-methyl-2-pyrrolidone should be removed. The GCL of 0.3% for Repro 1B is then applicable. Analysis of the oral reproductive studies showed multiple ED_{10} levels for effects fulfilling the classification criteria for developmental toxicity with values between 4 and 400 mg/kg bw/day. These values correspond to a medium potency group (i.e. boundaries: 4 mg/kg bw/day < ED_{10} value < 400 mg/kg bw/day) for 1-methyl-2-pyrrolidone (no modifying factors affecting the preliminary potency). In combination with the already established category 1 classification for reproductive toxicity (Repr. 1B; H360D), the GCL of 0.3% can be assigned to 1-methyl-2-pyrrolidone, resulting in the removal of the current SCL of 5%.

2.3 Current harmonised classification and labelling

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

1-methyl-2-pyrrolidone is currently classified as:

Hazard class: Skin Irrit. 2

Eye Irrit. 2

Repr. 1B; C≥ 5%

STOT SE 3; C≥ 10%

Hazard Statement: H315

H319

H360D***

H335

2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015.

2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

The table below provides an overview of the self-classification of the registrants concerning reproductive toxicity (ECHA C&L inventory as accessed November 6th, 2012).

Table 4: Overview of the self-classification for reproductive toxicity of NMP by the registrants.

Classification category for reproductive toxicity	SCL	Total number of notifiers	% of notifiers
Repr. 1A	-	2	0.1
Repr. 1A	C ≥5%*	1	0.05
Repr. 1B	-	405	19.4
Repr. 1B	C ≥5%	1635	78.3
Repr. 1B	5% ≤ C < 100%	9	0.4
Repr. 2	-	3	0.1
No Repr.	-	32	1.5

* According to the C&L inventory of ECHA, this SCL of this self-classification concerns classification for Repr. 1B.

2.4.2 Current self-classification and labelling based on DSD criteria

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

NMP is a reproductive substance included in the candidate list for substances of very high concern. A risk management options analyses was performed by the Netherlands to determine the best way forward to reduce the possible risks associated with this substance. It was concluded that a combination of a reduction of the SCL for reproductive toxicity, resulting ultimately in a lower concentration in consumer mixtures, and a restriction was the best way forward. Further, the Scientific Committee on Consumer Safety (SCCS, 2011) concluded that: “With the information available at the time of assessment, the SCCS is of the opinion that the presence of NMP with a maximum use concentration of 5% in cosmetic products is not safe for the consumer.”.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

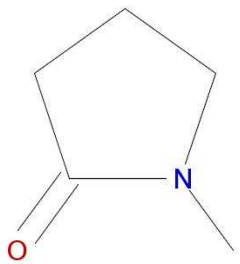
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	212-828-1
EC name:	1-methyl-2-pyrrolidone
CAS number (EC inventory):	872-50-4
CAS number:	872-50-4
CAS name:	2-Pyrrolidinone, 1-methyl-
IUPAC name:	1-methylpyrrolidin-2-one
CLP Annex VI Index number:	606-021-00-7
Molecular formula:	C_5H_9NO
Molecular weight range:	99.1311

Structural formula:



1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
1-methyl-2-pyrrolidone	usually > 99.0% (w/w)		The concentration included is the value made public by one of the REACH registrants but may not be representative for all registrants

Current Annex VI entry: Not applicable

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Non-specified	confidential		The substance does not contain impurities relevant for harmonised classification and labelling
y-butyrolactone EC 202-509-5 CAS 96-48-0	confidential		The impurity made public by one of the REACH registrants but may not be representative for all registrants
methylamine EC 200-820-0 CAS 74-89-5	confidential		The impurity made public by one of the REACH registrants but may not be representative for all registrants

Current Annex VI entry:

y-butyrolactone: none

methylamine: Press. Gas, Flam. Gas 1; H220, Skin Irrit. 2: H315, Eye Dam. 1; H318, Acute Tox 4*: H332 and STOT SE 3: H335

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
-				The substance does not contain additives relevant for harmonised classification and labelling

Current Annex VI entry: Not applicable

1.2.1 Composition of test material

The test item used in the developmental toxicity studies was 1-methyl-2-pyrrolidone without further specification unless stated in the relevant study.

1.3 Physico-chemical properties

Table 9: Summary of physico - chemical properties

Property	Value	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	Clear, colorless liquid	
Melting/freezing point	-24.2 °C	
Boiling point	204 °C at 1013 hPa	
Relative density	1.03 g/cm3 at 25 °C	
Vapour pressure	0.32 hPa at 20 °C	
Surface tension	not surface active	Based on chemical structure, no surface activity is predicted.
Water solubility	miscible	
Partition coefficient n-octanol/water	-0.46 at 25 °C	
Flash point	91 °C (cc)	
Flammability	Combustible liquid The substance has no pyrophoric properties and does not liberate flammable gases on contact with water.	Flammability derived from flash point. Based on chemical structure pyrophoric properties and flammability in contact with water are not to be expected.
Explosive properties	non explosive	There are no chemical groups associated with explosive properties present in the molecule.
Self-ignition temperature	245 °C	
Oxidising properties	no oxidising properties	The Substance is incapable of reacting exothermically with combustible materials on the basis of the chemical structure.
Granulometry	not relevant	Substance is marketed or used in a non solid or granular form.
Stability in organic solvents and identity of relevant degradation products	not applicable	The stability of the substance is not considered as critical.
Dissociation constant	not applicable	The substance does not contain any ionic structure.
Viscosity	1.661 mPa_s at 25 °C	

The information in this table is based on information from the registrant's dossier and ECHA's public registration information as accessed 06-11-2012.

2 MANUFACTURE AND USES

Quantities

The total tonnage band is 10,000 – 100,000 tonnes per annum (ECHA public registration information as accessed 30 October 2012)

2.1 Manufacture

Not relevant for this dossier.

2.2 Identified uses

Table 10: Uses by workers in industrial settings.

Confidential	IU number	Identified Use (IU) name	Substance supplied to that use	Use descriptors
	1	Manufacture of NMP		**Process category (PROC):** PROC 1: Use in closed process, no likelihood of exposure PROC 2: Use in closed, continuous process with occasional controlled exposure PROC 3: Use in closed batch process (synthesis or formulation) PROC 4: Use in batch and other process (synthesis) where opportunity for exposure arises PROC 8a: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at non-dedicated facilities PROC 8b: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at dedicated facilities PROC 15: Use as laboratory reagent **Environmental release category (ERC):** ERC 1: Manufacture of substances ERC 4: Industrial use of processing aids in processes and products, not becoming part of articles ERC 6a: Industrial use resulting in manufacture of another substance (use of intermediates) **Sector of end use (SU):** SU 8: Manufacture of bulk, large scale chemicals (including petroleum products) SU 9: Manufacture of fine chemicals SU 0: Other: SU 3: Industrial uses: uses of substances as such or in preparations at industrial sites **Subsequent service life relevant for that use?:** yes
	2	Distribution of NMP	as such (substance itself)	**Process category (PROC):** PROC 1: Use in closed process, no likelihood of exposure PROC 2: Use in closed, continuous process with occasional controlled exposure PROC 3: Use in closed batch process (synthesis or formulation) PROC 4: Use in batch and other process (synthesis) where opportunity for exposure arises PROC 8a: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at non-dedicated facilities PROC 8b: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at dedicated facilities

Confidential	IU number	Identified Use (IU) name	Substance supplied to that use	Use descriptors
				PROC 9: Transfer of substance or preparation into small containers (dedicated filling line, including weighing) PROC 15: Use as laboratory reagent **Environmental release category (ERC):** ERC 1: Manufacture of substances ERC 2: Formulation of preparations **Sector of end use (SU):** SU 8: Manufacture of bulk, large scale chemicals (including petroleum products) SU 9: Manufacture of fine chemicals SU 0: Other: SU 3: Industrial uses: uses of substances as such or in preparations at industrial sites **Subsequent service life relevant for that use?:** yes
	3	Formulation & (Re)Packing of Substances and Mixtures	as such (substance itself)	**Process category (PROC):** PROC 1: Use in closed process, no likelihood of exposure PROC 2: Use in closed, continuous process with occasional controlled exposure PROC 3: Use in closed batch process (synthesis or formulation) PROC 4: Use in batch and other process (synthesis) where opportunity for exposure arises PROC 5: Mixing or blending in batch processes for formulation of preparations and articles (multistage and/or significant contact) PROC 8a: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at non-dedicated facilities PROC 8b: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at dedicated facilities PROC 9: Transfer of substance or preparation into small containers (dedicated filling line, including weighing) PROC 14: Production of preparations or articles by tabletting, compression, extrusion, pelletisation PROC 15: Use as laboratory reagent **Environmental release category (ERC):** ERC 2: Formulation of preparations **Sector of end use (SU):**

Confidential	IU number	Identified Use (IU) name	Substance supplied to that use	Use descriptors
				SU 10: Formulation [mixing] of preparations and/or re-packaging (excluding alloys) SU 0: Other: SU 3: Industrial uses: uses of substances as such or in preparations at industrial sites **Subsequent service life relevant for that use?:** yes
	4	Use of NMP in coatings (industrial)	in a mixture	**Process category (PROC):** PROC 1: Use in closed process, no likelihood of exposure PROC 2: Use in closed, continuous process with occasional controlled exposure PROC 3: Use in closed batch process (synthesis or formulation) PROC 4: Use in batch and other process (synthesis) where opportunity for exposure arises PROC 5: Mixing or blending in batch processes for formulation of preparations and articles (multistage and/or significant contact) PROC 7: Spraying in industrial settings and applications PROC 8a: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at non-dedicated facilities PROC 8b: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at dedicated facilities PROC 9: Transfer of substance or preparation into small containers (dedicated filling line, including weighing) PROC 10: Roller application or brushing PROC 13: Treatment of articles by dipping and pouring PROC 14: Production of preparations or articles by tabletting, compression, extrusion, pelletisation PROC 15: Use as laboratory reagent **Environmental release category (ERC):** ERC 4: Industrial use of processing aids in processes and products, not becoming part of articles **Sector of end use (SU):** SU 0: Other: SU 3: Industrial uses: uses of substances as such or in preparations at industrial sites **Subsequent service life relevant for that use?:** no
	5	Use of NMP in cleaning agents (industrial)	in a mixture	**Process category (PROC):** PROC 1: Use in closed process, no likelihood of exposure PROC 2: Use in closed, continuous process with occasional controlled exposure

Confidential	IU number	Identified Use (IU) name	Substance supplied to that use	Use descriptors
				PROC 3: Use in closed batch process (synthesis or formulation) PROC 4: Use in batch and other process (synthesis) where opportunity for exposure arises PROC 7: Spraying in industrial settings and applications PROC 8a: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at non-dedicated facilities PROC 8b: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at dedicated facilities PROC 10: Roller application or brushing PROC 13: Treatment of articles by dipping and pouring **Environmental release category (ERC):** ERC 4: Industrial use of processing aids in processes and products, not becoming part of articles **Sector of end use (SU):** SU 0: Other: SU 3: Industrial uses: uses of substances as such or in preparations at industrial sites **Subsequent service life relevant for that use?:** no
	6	Use of NMP in functional fluids (industrial)	in a mixture	**Process category (PROC):** PROC 1: Use in closed process, no likelihood of exposure PROC 2: Use in closed, continuous process with occasional controlled exposure PROC 3: Use in closed batch process (synthesis or formulation) PROC 4: Use in batch and other process (synthesis) where opportunity for exposure arises PROC 8a: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at non-dedicated facilities PROC 8b: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at dedicated facilities PROC 9: Transfer of substance or preparation into small containers (dedicated filling line, including weighing) **Environmental release category (ERC):** ERC 7: Industrial use of substances in closed systems **Sector of end use (SU):** SU 0: Other: SU 3: Industrial uses: uses of substances as such or in preparations at industrial sites

Confidential	IU number	Identified Use (IU) name	Substance supplied to that use	Use descriptors
				Subsequent service life relevant for that use?: no
	7	Use of NMP in laboratories (industrial)	as such (substance itself) in a mixture	**Process category (PROC):** PROC 10: Roller application or brushing PROC 15: Use as laboratory reagent **Environmental release category (ERC):** ERC 4: Industrial use of processing aids in processes and products, not becoming part of articles **Sector of end use (SU):** SU 8: Manufacture of bulk, large scale chemicals (including petroleum products) SU 0: Other: SU 3: Industrial uses: uses of substances as such or in preparations at industrial sites **Subsequent service life relevant for that use?:** no

The information in this table is copied from the registrants dossier (d.d. 05-04-2011) and based on information from ECHA's public registration information as accessed 06-11-2012.

Table 11: Uses by professional workers

Confidential	IU number	Identified Use (IU) name	Substance supplied to that use	Use descriptors
	1	Use of NMP in coatings (professional)	in a mixture	**Process category (PROC):** PROC 1: Use in closed process, no likelihood of exposure PROC 2: Use in closed, continuous process with occasional controlled exposure PROC 3: Use in closed batch process (synthesis or formulation) PROC 4: Use in batch and other process (synthesis) where opportunity for exposure arises PROC 5: Mixing or blending in batch processes for formulation of preparations and articles (multistage and/or significant contact) PROC 8a: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at non-dedicated facilities PROC 8b: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at dedicated facilities PROC 10: Roller application or brushing

Confidential	IU number	Identified Use (IU) name	Substance supplied to that use	Use descriptors
				PROC 11: Non industrial spraying PROC 13: Treatment of articles by dipping and pouring PROC 15: Use as laboratory reagent PROC 19: Hand-mixing with intimate contact and only PPE available. **Environmental release category (ERC):** ERC 8a: Wide dispersive indoor use of processing aids in open systems ERC 8c: Wide dispersive indoor use resulting in inclusion into or onto a matrix ERC 8d: Wide dispersive outdoor use of processing aids in open systems ERC 8f: Wide dispersive outdoor use resulting in inclusion into or onto a matrix **Sector of end use (SU): 22** **Subsequent service life relevant for that use?:** no
	2	Use of NMP in cleaning agents (professional)	in a mixture	**Process category (PROC):** PROC 1: Use in closed process, no likelihood of exposure PROC 2: Use in closed, continuous process with occasional controlled exposure PROC 3: Use in closed batch process (synthesis or formulation) PROC 4: Use in batch and other process (synthesis) where opportunity for exposure arises PROC 8a: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at non-dedicated facilities PROC 8b: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at dedicated facilities PROC 10: Roller application or brushing PROC 11: Non industrial spraying PROC 13: Treatment of articles by dipping and pouring **Environmental release category (ERC):** ERC 8a: Wide dispersive indoor use of processing aids in open systems ERC 8d: Wide dispersive outdoor use of processing aids in open systems **Sector of end use (SU): 22** **Subsequent service life relevant for that use?:** no

Confidential	IU number	Identified Use (IU) name	Substance supplied to that use	Use descriptors
	3	Use of NMP in agrochemicals (professional)	in a mixture	**Process category (PROC):** PROC 1: Use in closed process, no likelihood of exposure PROC 2: Use in closed, continuous process with occasional controlled exposure PROC 4: Use in batch and other process (synthesis) where opportunity for exposure arises PROC 8a: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at non-dedicated facilities PROC 8b: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at dedicated facilities PROC 11: Non industrial spraying PROC 13: Treatment of articles by dipping and pouring **Environmental release category (ERC):** ERC 8a: Wide dispersive indoor use of processing aids in open systems ERC 8d: Wide dispersive outdoor use of processing aids in open systems **Sector of end use (SU): 22** **Subsequent service life relevant for that use?:** no
	4	Use of NMP in functional fluids (professional)	in a mixture	**Process category (PROC):** PROC 1: Use in closed process, no likelihood of exposure PROC 2: Use in closed, continuous process with occasional controlled exposure PROC 3: Use in closed batch process (synthesis or formulation) PROC 8a: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at non-dedicated facilities PROC 9: Transfer of substance or preparation into small containers (dedicated filling line, including weighing) PROC 20: Heat and pressure transfer fluids in dispersive, professional use but closed systems **Environmental release category (ERC):** ERC 9a: Wide dispersive indoor use of substances in closed systems ERC 9b: Wide dispersive outdoor use of substances in closed systems **Sector of end use (SU): 22**

Confidential	IU number	Identified Use (IU) name	Substance supplied to that use	Use descriptors
				Subsequent service life relevant for that use?: no
	5	Use of NMP in road and construction applications (professional)	in a mixture	**Process category (PROC):** PROC 8a: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at non-dedicated facilities PROC 8b: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at dedicated facilities PROC 9: Transfer of substance or preparation into small containers (dedicated filling line, including weighing) PROC 10: Roller application or brushing PROC 11: Non industrial spraying PROC 13: Treatment of articles by dipping and pouring **Environmental release category (ERC):** ERC 8f: Wide dispersive outdoor use resulting in inclusion into or onto a matrix **Sector of end use (SU): 22** **Subsequent service life relevant for that use?:** no

The information in this table is copied from the registrants dossier (d.d. 05-04-2011) and based on information from ECHA's public registration information as accessed 06-11-2012.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Not applicable

3.1 *[Insert hazard class when relevant and repeat section if needed]*

3.1.1 Summary and discussion of

3.1.2 Comparison with criteria

3.1.3 Conclusions on classification and labelling

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

The absorption, distribution, metabolism, and excretion of [2-14C] 1-methyl-2-pyrrolidone (NMP) was studied in the rat (Haskell, 1995). Radioactive NMP was administered either intravenously (50 mg/kg), orally (single doses of 5 mg/kg and 50 mg/kg, or multiple doses of 50 mg/kg/day for 8 consecutive days), dermally (10 mg/kg for 6 h) or by inhalation (single 6h exposure to 10 ppm or 100 ppm) to Crl:CD BR ® rats. Blood, urine and faeces were collected until 120h postdose. The maximum concentration (C_{max}), the time to reach C_{max}. (T_{max}), and area under the plasma concentration versus time curve (AUC) were determined for total radioactivity (NMP and metabolites) and for intact NMP. No NMP was detected in the plasma after the low oral dose, low inhalation exposure, or dermal application. Elimination half-life ($t_{1/2}$) was measured for both total radioactivity and NMP (when possible). Urine and feces were collected for approximately 5 days post-exposure and were analyzed for radioactivity and the presence of metabolites. Tissues, organs, cage wash, and feces residues were collected at sacrifice and analyzed for radioactivity.

The rank order of concentration of total radioactivity in plasma (C_{max}) was intravenous > multiple high oral dose > high inhalation exposure > high oral dose > low oral dose > low inhalation exposure > dermal exposure. The rank order of time to reach C_{max} (T_{max}) was intravenous > inhalation > oral > dermal. The rank order for elimination half-life of total radioactivity was dermal > intravenous > oral and inhalation.

The concentration of NMP in the plasma (C_{max}) was the highest after intravenous administration, followed by both high oral and high inhalation exposures. The concentration of NMP in plasma was too low to be detected by the method used for analysis after low oral, low inhalation, or dermal exposures. The time to reach C_{max} (T_{max}) ranged between instantaneous after the intravenous dose to 2 h after the multiple oral high dose. Elimination half-life of NMP was similar between groups and ranged between 1 and 3.3 h. The half-life of NMP could not be determined after low oral, low inhalation, or dermal exposures.

The oral bioavailability of NMP was 48% for male rats and 101% for female rats. The determination of bioavailability in male rats was more accurate because earlier blood time points were sampled than in females. The calculated intravenous AUC for male rats was higher, resulting in a lower bioavailability value. Thus, the actual bioavailability of female rats is probably lower than 101%. The volume of distribution was 0 .7 L/kg of male rats and 1 .8 L/kg for female rats.

The rapid decline of NMP compared to total radioactivity suggests rapid and extensive first pass metabolism.

Approximately 44 and 43% of the topically applied dose was absorbed by male and female rats, respectively. Absorption of [2-14C] NMP after dermal application was estimated from total radioactivity excreted in urine, feces, and cage wash and from radioactivity retained in tissues, organs, and skin at the application site. The steady state absorption rate was 4 .65 $\mu g/h/cm^2$ for male rats and 4 .0 $\mu g/h/cm^2$ for female rats. The permeability constant (ICP) was 1 .94 x 10^{-4} cm/h for male rats and 1 .91 x 10^{-4} cm/h for female rats. Half of the absorbed dose in the female rats was retained at the skin application site (54% of absorbed dose) which created a depot for NMP. In male rats, only 26% of the applied dose was recovered at the skin application site, indicating that a significant portion of the applied dose (as compared to females) was absorbed into the systemic circulation.

During inhalation exposure, approximately 7% of 10 ppm [2-14C] NMP vapor was absorbed and 9% of 100 ppm [2-14C] NMP was absorbed. The total absorption of [2-14C] NMP by each animal was estimated from the total radioactivity excreted in urine, feces, cage wash, and radioactivity retained in tissues and organs.

Recovery of radioactivity after all routes of administration ranged between 87 and 102% of the administered dose *(absorbed* dose for dermal and inhalation exposures). Approximately 69 to 87% of the absorbed dose was excreted in the urine after 96 to 120 h postdose, except after dermal exposure where only 36 to 58% of the absorbed dose was excreted in the urine. The majority of the radioactivity excreted in the urine was eliminated within 24 h after the end of the exposure.

Typically, 2 to 9% of the absorbed dose was recovered in the feces and 0 .3 to 2% was recovered in tissues and organs (after 96-120 h), mostly in the carcass. Additional radioactivity was recovered in the cage wash and feed residue.

Tissue residue levels of radioactivity 4-5 days after dosing were very low. At sacrifice, the liver and kidneys contained the highest concentration of radioactivity. The next highest radioactivity concentration was in the G.I. tract contents and tissue (especially after oral administration), lungs, skin, and carcass (especially after inhalation exposure). However, concentrations of radioactivity in most tissues and organs was <0.1% of the absorbed dose.

Three radioactive components were separated from the urine of male and female rats after all routes of administration. The major urinary NMP metabolite was identified as 1-methyl-5-hydroxy-2-pyrrolidone (5-hydroxy-NMP), based on HPLC and mass spectral comparisons with an authentic standard. The two other metabolites were not identified.

In conclusion, NMP was readily absorbed after all four routes of administration. Once absorbed, NMP was distributed, metabolized, and eliminated in the urine with negligible tissue residues remaining after 4-5 days postdose (Haskell, 1995).

Sitarek and Kilanowicz (2006) studied the tissue distribution and excretion of NMP after oral exposure in rats. ^{14}C-NMP was administered to male and female rats (Imp:WIST inbred) intraperitoneally in a dose of 250 mg/kg bw (350 kBq/rat). Blood and tissues (liver, kidney, lungs, brain, spleen, testicles, epididymis, and seminal vesicles) were sampled for NMP analysis by measuring ^{14}C-radioactivity. Maximum radioactivity in serum was obtained between 45 min and 4h after exposure. A decrease in radioactivity was detected after 6h, and 24h after administration it was very low. Highest tissue levels of radioactivity (calculated for 1 g of tissue) in male animals were found 4h after exposure in adrenals, kidney, seminal vesicles, testes, muscle, liver, brain, lungs. In female rats, highest activity was detected in muscles, kidneys, lungs, ovaries, brain, sciatic nerve, adrenals and liver also 4h after administration. During 72h following administration, approximately 80% of the dose was excreted in urine. Elimination of the compound via the faeces was far less significant (only about 5% of the dose was excreted at once). The authors concluded that no clear differences in tissue distribution are present between male and female animals. Absorption from the peritoneal cavity is rapid, disappearance from the blood is monophase, and kidneys are the main route of excretion of NMP and/or its metabolites (Sitarek and Kilanowicz, 2006).

4.1.2 Human information

Oral

In a human volunteer study, the metabolic pathway of NMP was elucidated (Åkesson and Jönsson 1997). Three healthy male volunteers were administered a single dose of 100 mg NMP orally. All urine was collected during nine consecutive days. Identification and quantification of the metabolites were performed by gas chromatography/mass spectrometry (GC/MS). NMP, 5-hydroxy-*N*-methyl-2-pyrrolidone (5-HNMP), *N*-methylsuccinimide (MSI), and 2-hydroxy-*N*-methylsuccinimide (2-HMSI) were found in urine. The mean excreted fractions for NMP, 5-HNMP, MSI, and 2-HMSI were 0.8%, 44%, 0.4%, and 20%, respectively. There was no conjugation with glucuronic acid or sulfate or either 5-HNMP or 2-HMSI. One-third of the orally dosed NMP was not recovered in urine as either NMP, 5-HNMP, MSI, or 2-HMSI. The half-lives for 5-HNMP, MSI, and 2-HMSI in urine were approximately 4, 8, and 17 h, respectively.

Dermal

A human volunteer study was performed by Keener et al (2007). Four healthy male subjects were topically exposed to NMP in six different experimental designs involving the variation of exposure time and solvent concentration. A pad was spiked with NMP and attached to the back of tone hand of every participant. The six designs comprised application of 100% NMP for 2h (D1), 50% NMP for 2h (D2), 10% NMP for 2h (D3), 100% NMP for 30 min (D4), 50% NMP for 30 min (D5) and 10% NMP for 30 min (D6). The total volume of urine was collected during the exposure up to 72 h thereafter. NMP and its metabolites were analysed using GC/MS.

The urinary concentration of the metabolites upon exposure to undiluted NMP for 2 h increased rapidly with 5-HNMP reaching a maximum at 4–5 h and 2-HMSI after 26–29 h. The application of aqueous NMP solutions resulted in a delay of the peak time for 5-HNMP of approximately 6 h as compared with the undiluted solvent. An average dermal absorption of 5.4±1.5 mg NMP cm^{-2} h^{-1} was calculated for a 2 h exposure to undiluted NMP (6.5±2.0 mg NMP cm^{-2} h^{-1} for a 30 min exposure). Aqueous dilution of NMP to 50% was followed by a decrease of the absorption to 0.9±0.5 mg NMP cm^{-2} h^{-1}. NMP metabolite concentrations in the range of the detection limits were

found only in isolated urine samples after exposure to 10% NMP in aqueous dilution (Keener et al., 2007).

Inhalation

In a human volunteer study, six healthy male subjects (age: 28-41 year) were exposed by inhalation to NMP in a 5.6 m^3 inhalation chamber for 8 hours (Jönsson and Åkesson, 2003). Two experiments were performed in this study. Four different exposures at the intended NMP concentrations of 0, 10, 25 and 50 mg/m^3 were performed in experiment 1. The obtained time-weighted average (TWA) air concentrations of NMP were 0, 10 (range 8-13), 24 (range 22-26), and 53 (range 44-60) mg/m^3. Five-minute breaks were taken after 2, 4, and 6h for biological sample collection. Due to incomplete sample collection in experiment 1, three of the subjects (subjects 3, 4 and 6) were exposed a second time in the chamber for 8h at an intended concentration of 50 mg/m^3 (experiment 2). The obtained TWA air levels were 50, 52 and 47 mg/m^3 respectively.

In experiment 1, blood was collected before, and at 4, 8, 9, 10, 12, 16, 24, 32, and 48 h after the start of exposure. In experiment 2, blood was collected before the start of exposure, at the end of exposure and then every morning for the next 9 days. Plasma was obtained and used for analysis of NMP and its metabolites. In experiment 1, urine was collected at 2-h intervals up to 16 h after the start of exposure, at 4-h intervals up to 28 h and, finally, at 8-h intervals up to 52 h. In experiment 2, urine samples were collected before, and at 4, 8, 12, 16, 20, 24, and 32 h after the start of exposure and then every morning for the next 8 days. Urine was used for analysis of creatinine, and NMP and its metabolites. Toxicokinetic analysis was performed based on the obtained NMP (and metabolite) concentrations.

The concentration of 2-hydroxy-*N*-methylsuccinimide (2-HMSI) in plasma and urine rose during exposure and reached a peak approximately 15 h after the end of exposure. It then decayed according to a one-compartment model with a half-time of about 18 h. There were very close correlations between the NMP air levels, on the one hand, and concentrations of 2-HMSI in plasma (r=0.98) and creatinine-adjusted urinary 2-HMSI levels (r=0.96), on the other. The renal clearances were 0.13, 1.4, 0.12 and 1.2 l/h for NMP, 5-hydroxy-*N*-methyl-2-pyrrolidone (5-HNMP), *N*-methylsuccinimide (MSI) and 2-HMSI, respectively. The total clearances were 11.4, 3.2, 8.5 and 1.1 l/h for NMP, 5-HNMP, MSI and 2-HMSI, respectively. The apparent volumes of distribution were 41, 28, 120 and 28 l for NMP, 5-HNMP, MSI and 2-HMSI, respectively (Jönsson and Åkesson, 2003).

Bader et al (2007) studied the inhalation absorption of NMP in human volunteers. Sixteen male volunteers (average age: 26.5 ± 2.4 years) were exposed by inhalation to NMP in a 29 m^3 exposure chamber. The exposure conditions included three different NMP concentrations (10, 40, 80 mg/m3) that were kept constant throughout the experiments and one variable exposure scenario with a baseline concentration of 25 mg/m^3 NMP and four 15 min periods with an increased concentration of 160 mg/m^3 and inter-peak intervals of 2 h. Effective concentrations for resting/workload conditions were 10.7/10.4, 40.9/40.4, 71.9/72.3, 79.9/79.4 mg/m^3. The influence of physical activity on the uptake and elimination of NMP was studied under otherwise identical exposure conditions but involving moderate workload on a bicycle ergometer (75 W for 6 x 10 min). Every urine sample voided immediately before, during and up to 40 h after the exposure sessions was collected

separately (total sampling interval: 48 h). NMP and metabolites in urine were analysed using GC/MS.

Urinary NMP increased rapidly after the onset of exposure and an elimination peak within the first hour post-exposure was generally observed. The elimination of NMP in urine was completed after 24 h post-exposure. All analytes showed a close correlation between their post-shift peak concentrations and airborne NMP. An exposure to 80 mg/m^3 under resting conditions resulted in urinary peak concentrations of 2,400 µg/L NMP, 117 mg/g creatinine 5-HNMP and 32 mg/g creatinine 2-HMSI (workload conditions: 3,400 µg/L NMP, 150 mg/g creatinine 5-HNMP, 44 mg/g creatinine 2-HMSI). Moderate workload enhanced the total uptake of NMP by approximately one third. The authors conclude that differences between the estimated and the observed total amount of urinary metabolites point to a significant contribution of dermal absorption on the uptake of NMP. This aspect, together with the influence of physical workload, should, according to the authors, be considered for the evaluation of a biological limit value for NMP (Bader et al., 2007).

Bader et al. (2008) studied the inhalation and dermal absorption of NMP from the vapour phase. Human male volunteers (20-30 year) were exposed in a 29 m^3 exposure chamber to 80 mg/m^3 NMP for 8h under either whole-body, i.e. inhalational plus dermal, or dermal only conditions. For dermal-only exposure, the volunteers were equipped with a Scott Proflow2 face shield masks that covered the whole face and supplied activated carbon filtered air. The influence of physical activity on the uptake and elimination of NMP was studied under otherwise identical exposure conditions but involving moderate workload on a bicycle ergometer (75 W for 6 x 10 min). Every urine sample voided immediately before, during and up to 40 h after the exposure sessions was collected separately (total sampling interval: 48 h). NMP and metabolites in urine were analysed using GC/MS.

Percutaneous uptake delayed the elimination peak times and the apparent biological halflives of NMP and 5-HNMP. Under resting conditions, dermal-only exposure resulted in the elimination of 71 ± 8 mg NMP equivalents as compared to 169 ± 15 mg for wholebody exposure. Moderate workload yielded 79 ± 8 mg NMP (dermal-only) and 238 ± 18 mg (whole-body). Thus, dermal absorption from the vapour phase may contribute significantly to the total uptake of NMP, e.g. from workplace atmospheres (Bader et al., 2008).

Poet et al. (2010) developed a physiologically based pharmacokinetics based pharmacokinetic (PBPK) model, which was used in combination with benchmark dose (BMD) modeling to derive a human equivalent concentration that could be safely used at the workplace. The PBPK model described the kinetics of NMP in human and rat. The rat PBPK model was used to determine the relationship between NMP concentrations in maternal blood and decrements in fetal/pup body weight. Body weight decrements seen after inhalation exposures occurred at lower NMP blood levels than those observed after oral and dermal exposures. In addition, benchmark dose (BMD) modeling was used to better define a point of departure (POD) for fetal/pup body weight changes by using dose-response information from two key inhalation studies in rats. These PODs and the human PBPK model were then used to estimate the human equivalent concentrations (HEC) that could be safely used in the workplace. The geometric mean of the PODs derived from the key studies was estimated to be 350 mg*hr/L (expressed in terms of internal dose), a value which corresponds to a HEC of 480 ppm (occupational exposure of 8 hours/day, 5 days/week). The BMC human equivalent values that were calculated by means of the rat and human PBPK models based

on internal dose (area under the curve for parent NMP) were considerably larger (approximately 4.6 -fold, 105 ppm (rat) as compared to 480 ppm (human)) than would be obtained using rat external concentration (ppm) as the dose measure. The authors conclude that the HEC of 480 ppm is much higher than recently developed internationally-recognized OELs for NMP (Poet et al., 2010).

4.1.3 Summary and discussion on toxicokinetics

Non-human data are available for the oral, dermal, inhalation, intravenous and intraperitoneal route. For the oral route, no data are available for rabbit. NMP is well absorbed after oral, dermal and inhalation exposure. The available data show that NMP is rapidly excreted upon oral, dermal and inhalation exposure. NMP does not have a bioaccumulation potential. Once absorbed, NMP was distributed, metabolized and eliminated in the urine with negligible tissue residues remaining after 4-5 day postdose. The primary metabolite was 1-methyl-5-OH-2-pyrrolidone >50% of the applied dose.

Human data are available for the oral, dermal and inhalation route. NMP is well absorbed. The data further point towards metabolism of NMP to 5-hydroxy-*N*-methyl-2-pyrrolidone (5-HNMP), *N*-methylsuccinimide (MSI), and 2-hydroxy-*N*-methylsuccinimide (2-HMSI).

Overall, both in human as well as animals (i.e. rat), NMP is well absorbed via the various exposure routes. Further, NMP is highly metabolized and NMP itself and its metabolites are excreted mainly via the urine. The major metabolite of NMP is 1-methyl-5-OH-2-pyrrolidone in rat whereas in humans the major metabolite is 5-hydroxy-*N*-methyl-2-pyrrolidone (5-HNMP).

As the lowest ED10 (to be used to set an SCL in general, or in this case remove the SCL) is based on the oral rabbit developmental toxicity study (See also paragraph 4.11.4 of this CLH report), an evaluation of the toxicokinetic differences between rabbits and humans to oral exposure should be taken into account when determining the potency group of a substance. For humans some information is available on the kinetics of NMP after oral exposure, however this is limited to a study focused on the metabolic pathway of NMP. For rabbits, information on the kinetic profile of NMP after oral exposure was not found. A comparison between kinetics in humans and rabbits after oral exposure is therefore not possible.

4.2 Acute toxicity

Not applicable

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

4.2.1.2 Acute toxicity: inhalation

4.2.1.3 Acute toxicity: dermal

4.2.1.4 Acute toxicity: other routes

4.2.2 Human information

4.2.3 Summary and discussion of acute toxicity

4.2.4 Comparison with criteria

4.2.5 Conclusions on classification and labelling

4.3 Specific target organ toxicity – single exposure (STOT SE)

Not applicable

4.3.1 Summary and discussion of Specific target organ toxicity – single exposure

4.3.2 Comparison with criteria

4.3.3 Conclusions on classification and labelling

4.4 Irritation

Not applicable

4.4.1 Skin irritation

4.4.1.1 Non-human information

4.4.1.2 Human information

4.4.1.3 Summary and discussion of skin irritation

4.4.1.4 Comparison with criteria

4.4.1.5 Conclusions on classification and labelling

4.4.2 Eye irritation

4.4.2.1 Non-human information

4.4.2.2 Human information

4.4.2.3 Summary and discussion of eye irritation

4.4.2.4 Comparison with criteria

4.4.2.5 Conclusions on classification and labelling

4.4.3 Respiratory tract irritation

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015.

4.4.3.1 Non-human information

4.4.3.2 Human information

4.4.3.3 Summary and discussion of respiratory tract irritation

4.4.3.4 Comparison with criteria

4.4.3.5 Conclusions on classification and labelling

4.5 Corrosivity

Not applicable

4.5.1 Non-human information

4.5.2 Human information

4.5.3 Summary and discussion of corrosivity

4.5.4 Comparison with criteria

4.5.5 Conclusions on classification and labelling

4.6 Sensitisation

Not applicable

4.6.1 Skin sensititsation

4.6.1.1 Non-human information

4.6.1.2 Human information

4.6.1.3 Summary and discussion of skin sensitisation

4.6.1.4 Comparison with criteria

4.6.1.5 Conclusions on classification and labelling

4.6.2 Respiratory sensitisation

4.6.2.1 Non-human information

4.6.2.2 Human information

4.6.2.3 Summary and discussion of respiratory sensitisation

4.6.2.4 Comparison with criteria

4.6.2.5 Conclusions on classification and labelling

4.7 Repeated dose toxicity

Not applicable

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

4.7.1.2 Repeated dose toxicity: inhalation

4.7.1.3 Repeated dose toxicity: dermal

4.7.1.4 Repeated dose toxicity: other routes

4.7.1.5 Human information

4.7.1.6 Other relevant information

4.7.1.7 Summary and discussion of repeated dose toxicity

4.7.1.8 Summary and discussion of repeated dose toxicity findings relevant for classification according to DSD

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015.

4.7.1.9 Comparison with criteria of repeated dose toxicity findings relevant for classification according to DSD

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015.

4.7.1.10 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification according to DSD

This paragraph is considered irrelevant seen the repeal of Directive 67/548/EEC with effect from 1 June 2015.

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

Not applicable

4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

4.8.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

4.8.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

4.9 Germ cell mutagenicity (Mutagenicity)

Not applicable

4.9.1 Non-human information

4.9.1.1 In vitro data

4.9.1.2 In vivo data

4.9.2 Human information

4.9.3 Other relevant information

4.9.4 Summary and discussion of mutagenicity

4.9.5 Comparison with criteria

4.9.6 Conclusions on classification and labelling

4.10 Carcinogenicity

Not applicable

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

4.10.1.2 Carcinogenicity: inhalation

4.10.1.3 Carcinogenicity: dermal

4.10.2 Human information

4.10.3 Other relevant information

4.10.4 Summary and discussion of carcinogenicity

4.10.5 Comparison with criteria

4.10.6 Conclusions on classification and labelling

4.11 Toxicity for reproduction

Table 12 presents an overview of the most relevant studies taken into consideration for developmental toxicity. As this proposal only aims at removing the current SCL, the focus is on studies for the oral route. The studies for the dermal and inhalation route are added for completeness. In addition, as this proposal only aims at removing the current SCL, based on developmental effects, effects on fertility are not evaluated.

Table 12: Summary table of reproductive toxicity studies relevant for developmental toxicity

Species	Route	*dose mg/kg/day ppm **Conc. mg/m^3	Exposure time (hr/day)	Exposure period : number of days during pregnancy	Observations and remarks	Reference
Oral route:						
rat (Sprague - Dawley)	oral: gavage	0, 125, 250, 500, 750 mg/kg bw/day 1-methyl-2-pyrrolidone	Daily	Exposure: day 6 through day 20 of gestation (1x/day)	500 + 750 mg/kg: Reduced maternal body weight + maternal food consumption 250 mg/kg: reduced maternal body weigth gain 500 + 750 mg/kg: increased incidence of (litters with) malformed fetuses ≥ 250 mg/kg: reduced fetal body weight <u>NOAEL</u> - maternal toxicity: 125 mg/kg bw/day - developmental toxicity: 125 mg/kg bw/day	Saillenfait *et al*. (2001, 2002) OECD Guideline 414 (Prenatal Develop-mental Toxicity Study)
rabbit (New Zealand White)	oral: gavage	0, 55, 175, 540 mg/kg bw/day (nominal conc.)	daily	Exposure: day 6 through 18 of gestation (1x/day)	- Reduced body weight gain: 175 mg/kg bw/day (GD 6-12) and 540 mg/kg bw/day (GD 6-19) - Reduced food consumption: 540 mg/kg bw/day - One abortion at 540 mg/kg bw/day - Increased post-implantation loss, reduced live litter size and reduced mean uterine weight at 540 mg/kg bw/day - cardiovascular + skeletal malformations at 540 mg/kg bw/day <u>NOAEL</u> -maternal toxicity: 55 mg/kg bw/day - developmental toxicity: 175 mg/kg bw/day	International Research and Development Corporation (IRDC) (1991)
rat (Wistar) male/female	oral: feed	0; 50; 160; 500/350 mg/kg bw/day (nominal in	Daily	two-generation study Exposure period: - F0: 10 weeks premating, mating,	- high dose level reduced from 500 to 350 mg/kg bw/day due to severe pup mortality in the first litter (F1a pups) - No adverse effects on fertility and reproduction in all groups - no substance-related adverse effects at 50 and 160 mg/kg bw/day (F0, F1a/b, F2, F2a/b males/females)	BASF AG, Department of Toxicology (1999) OECD Guideline 416 (Two-Generation

Species	**Route**	***dose mg/kg/day ppm **Conc. mg/m^3**	**Exposure time (hr/day)**	**Exposure period : number of days during pregnancy**	**Observations and remarks**	**Reference**
		diet)		gestation/lactation and rest period of F1a and F1b offspring, - F1: after weaning during 10 weeks premating, mating, gestation/lactation and rest period F2a/F2b offspring - F2: until weaning Premating exposure period (males/females): 10 weeks Duration of test: approx. 54 weeks (continuous)	- reduced body weight gain and food intake at 500/350 mg/kg bw/day (P0, F1) - renal toxicity (organ weights and histopathology) at 500/350 mg/kg bw/day - increased pup mortality, reduced body weight gain in pups treated at 500/350 mg/kg bw/day <u>NOAEL:</u> - Fertility/reproduction: 350 mg/kg bw/day (male/female, P- and F1-generation) - developmental toxicity: 160 mg/kg bw/day (male/female, F1 and F2 generation pups)	Reproduction Toxicity Study) EPA OPPTS 870.3800 (Reproduction and Fertility Effects) EU Method B.35 (Two-Generation Reproduction Toxicity Test)
rat (CD) male/female	oral: feed	0, 50, 160, 500/350 mg/kg bw/day (nominal in diet)	daily	two-generation study Exposure period: - F0: 10 weeks premating, mating, gestation/lactation and rest period of F1a and F1b offspring - F1: after weaning during 10 weeks premating, mating, gestation/lactation and rest period F2a/F2b offspring	- high dose level reduced from 500 to 350 mg/kg bw/day due to severe pup mortality in the first litter (F1a pups) - no adverse effects on reproductive performance or fertility in all groups - no signs of maternal toxicity noted after high dose level reduction to 350 mg/kg bw/day. - decrease in the number of F2b pups surviving lactation and a decrease in pup body weights at 350 mg/kg bw/day - no adverse effect of NMP treatment was observed in the P- and F-generation male rats, including mortality, body weights, feed consumption and clinical observations. <u>NOAEL:</u>	Huntingdon Life Science (1999) OECD Guideline 416 (Two-Generation Reproduction Toxicity Study) EPA OPPTS 870.3800 (Reproduction and Fertility Effects) EU Method B.35 (Two-Generation Reproduction Toxicity

Species	**Route**	***dose mg/kg/day ppm **Conc. mg/m^3**	**Exposure time (hr/day)**	**Exposure period : number of days during pregnancy**	**Observations and remarks**	**Reference**
				- F2: until weaning Premating exposure period (females/males): 10 weeks Duration of test: approx. 58 weeks, from receipt of F0 to the sacrifice of the F1 parental generation and F2b pups (continuous)	- Fertility/reproduction: 350 mg/kg bw/day (male/female, P- and F1-generation) - developmental toxicity: 160 mg/kg bw/day (male/female, F1 and F2 generation pups)	Test)
Rats (Imp:WIST)	gavage	100, 300, 1000 mg/kg bw/day (males only) With an additional control	5 days/week, during a total period of 10 weeks before mating and 1 week during mating	N/A (only male animals were exposed)	1000 mg/kg bw/day: reduced male fertility 300 mg/kg bw/day: reduction in postnatal survival until day 4 1000 mg/kg bw/day: only 2 out of 44 females delivered; total number of pups was 6	Sitarek and Stetkiewicz, 2008
Rats Sprague-Dawley	gavage	332 and 997 mg/kg With an additional control	Daily	GD 6 to 15	At 332 mg/kg : Maternal body weights not reported. Placental and foetal weight lower than control (14-20% and 10% respectively). No difference in implantation rate, litter size or resorptions. At 997 mg/kg : Marked reductions in maternal body weight and placental weight were observed. There was a large number of resorptions (24/29 dams showed complete resorption) and only 15	EPA 1987 (based on the French classification proposal, 2002)

Species	**Route**	***dose mg/kg/day ppm **Conc. mg/m^3**	**Exposure time (hr/day)**	**Exposure period : number of days during pregnancy**	**Observations and remarks**	**Reference**
					live and 1 dead foetus were present at term. Observations in the live fetuses included reduction in fetal weight (37%), malformations considered as indicative of foetal retardation in 8 out of 15 foetuses), and 14 runts. No other information is available.	
Rats (Crl:CD)	Gavage (100 % pure)	40, 125 and 400 mg/kg/day with an additional vehicle control (water)	Daily 5ml/kg	GD 6 to 15	- Maternal toxicity : no treatment-related clinical observations. Body weight gain was depressed during treatment at 400 mg/kg (GD 6 9, GD 9-12, GD 6-15) No changes in food consumption. - Developmental toxicity : At 400 mg/kg : Reduced fetal body weight (10-11 %) and an increased incidence of stunted fetuses No teratogenic effects. <u>NOAEL</u> -maternal toxicity: 125 mg/kg bw/day - developmental toxicity : 125 mg/kg/day	Exxon 1992

Species	**Route**	***dose mg/kg/day ppm **Conc. mg/m^3**	**Exposure time (hr/day)**	**Exposure period : number of days during pregnancy**	**Observations and remarks**	**Reference**
Inhalation route:						
Rat (Sprague - Dawley)	inhalation: vapour (whole body)	0, 30, 60, 120 ppm (0, 123, 247, 494 mg/m^3) (nominal conc.)	(6 hours/day)	Exposure: day 6 through day 20 of gestation	60 + 120 ppm: reduced maternal body weight 120 ppm: reduced food consumption No change in the mean number of implantations sites, live fetuses and the incidences of non-live implants and resorptions between treatment groups. Concentration-related decrease in fetal body weight (sign at 120 ppm) No change in the incidence and types of malformations between groups. NOAEC: - maternal toxicity: 30 ppm - developmental toxicity: 60 ppm	Saillenfait *et al*. (2001, 2003) OECD Guideline 414 (Prenatal Developmental Toxicity Study)
Rabbit (Himalayan)	inhalation (nose/head only)	0, 200, 500, 1000 mg/m^3 (0, 49, 122, 243 ppm) (nominal conc.)	(1x/day)	Exposure: day 7 through day 19 of gestation	No signs of maternal toxicity (clinical findings, body weight, body weight gain, corrected body weight, gross pathology) at any concentration. Increased occurrence of one skeletal variation (i.e. supernumerary 13th ribs) at 1.0 mg/L NOAEC: - maternal toxicity: 1 mg/mL - developmental toxicity: 0.5 mg/L	BASF AG, Department of Toxicology (1993) OECD Guideline 414 (Prenatal Developmental Toxicity Study) EPA OTS 798.4900 (Prenatal Developmental Toxicity Study)
Rats (25/dose	Whole body inhalati	0, 100 and 360 mg/m^3 (0, 24.3 and	6hr/day	GD 6 to 15	Sporadic lethargy and irregular respiration was found in several dams, at both levels, only during the 3 first days of exposure.	Lee *et al*., 1987

Species	**Route**	***dose mg/kg/day ppm **Conc. mg/m^3**	**Exposure time (hr/day)**	**Exposure period : number of days during pregnancy**	**Observations and remarks**	**Reference**
) (Crl:CD)	on (100 % pure)	87.3 ppm) Aerosol			No adverse effects on maternal and fetal body weight, nor increases in the incidences of resorptions and of malformations and variations (external, soft tissue and skeletal).	
Rats (14-16 litters/dose) (Mol :WIST)	Whole body inhalation (≥99.5 % pure)	0 and 150 ppm (i.e. 0 and 620 mg/m^3)	6 hr/day	GD 7 to 20 Behavioural developmental toxicity study	No effects on maternal weight gain during gestation, gestation length, the number of pups and neonatal death. Reduced body weight of litters from birth throughout weaning. Slight delay in some pre-weaning development milestones and reflexes (i.e. ear unfolding, surface righting reflex, incisor eruption, eye opening). Post-weaning behavioural tests : There was no effect on learning of low grade tasks, motor function (rotorod), and activity level (open field). Some changes were found in more difficult tasks, including the reversal procedure in Morris water maze and in operant delayed special alternation.	Hass *et al*., 1994
Rats (20-23 pregnant females) (Mol :WIST)	Whole body inhalation (≥99.5 % pure)	0 and 165 ppm (i.e. 0 and 680 mg/m^3) (highest technically possible concentration, 40-50 % relative humidity in the inhalation	6 hr/day	GD 4 to 20 (vaginal plug = GD 1)	No maternal toxicity reported Increased number of dams with pre-implantation loss (11/20 and 20/23 at 0 and 165 ppm, respectively) . However, no significant differences in the incidence of pre-implantation loss/litter (13.4 and 20.5 % at 0 and 165 ppm) and in the number of implantations. No effect on corpora lutea, live fetuses and resorptions. Slight decrease in fetal body weight (significant difference only when adjusted for litter size). The incidence of bones showing delayed ossification tended to increase. It was significantly higher for digits and cervical vertebrae. No treatment-related malformations.	Hass *et al*., 1995

Species	**Route**	***dose mg/kg/day ppm **Conc. mg/m^3**	**Exposure time (hr/day)**	**Exposure period : number of days during pregnancy**	**Observations and remarks**	**Reference**
		chambers) with an additional control (air). Primarily vapour phase				
Rats (10 males and 20 females/ dose) (Crl:CD)	Whole body inhalation (vapours)	0, 10, 51 and 116 ppm (i.e. 0, 41, 210, 478 mg/m^3) The authors indicated that 116 ppm was the highest concentration possible without formation of aerosols under their experimental conditions	6 hr/day, 7 days/week Males: pre-mating and mating periods (Total > 100 days) Females: pre-mating, mating gestation,	2 generations exposed	No adverse effects on the indices of reproductive performance of males and females An exposure-related reduction in response to sound was noted at 116 ppm in the first generation. No other signs of NMP-related toxicity were observed among parental rats (e.g. body weight, weight of testes and ovaries, and histological examination of the reproductive organs). At 116 ppm, a slight decrease in the body weight of the F1 offspring was seen at birth that persisted till weaning.	Solomon *et al*., 1995

Species	Route	*dose mg/kg/day ppm **Conc. mg/m^3	Exposure time (hr/day)	Exposure period : number of days during pregnancy	Observations and remarks	Reference
			lactation (Total > 106 days). (Interruption from GD 20 to Day 4 post-partum).			

Species	**Route**	***dose mg/kg/day ppm **Conc. mg/m^3**	**Exposure time (hr/day)**	**Exposure period : number of days during pregnancy**	**Observations and remarks**	**Reference**
Dermal route:						
rat (Sprague - Dawley)	dermal	0, 75, 237, 750 mg/kg bw/day (nominal conc.)	(8 hours/day, 1x/day)	Exposure: day 6 through day 15 of gestation	- reduced maternal body weight (gain) (750 mg/kg bw/day) - topical signs of irritation (dose-dependent) - colored urine (indication of systemic test substance availability) - reduced number of live fetuses, increased resorption rate (750 mg/kg bw/day) - reduced fetal weight, indications of retarded skeletal development, increased appearance of skeletal malformations (e.g., fused, surplus or cleft ribs, fusion of skull bones) → 750 mg/kg bw/day <u>NOAEL:</u> - maternal toxicity: 237 mg/kg bw/day - developmental toxicity: 237 mg/kg bw/day	Food and Drug Research Laboratories (FDRL) (1979) equivalent or similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study)
rabbit (Himala yan)	dermal	0, 100, 300, 1000 mg/kg bw/day (nominal in water)	(1x/day)	Exposure: day 7 through 19 of gestation	- No adverse maternotoxic effects (body weight, food intake, clinical signs) - reddish-brown discolouration of the urine - increased occurrence of accessory 13th rib(s) in the fetuses at the high dose group <u>NOAEL:</u> - maternal toxicity: 1000 mg/kg bw/day - developmental toxicity: 300 mg/kg bw/day	BASF AG, Department of Toxicology (1993) OECD Guideline 414 (Prenatal Developmental Toxicity Study) EPA OTS 798.4900 (Prenatal Developmental Toxicity Study)
Sprague-Dawley rats (22-24 pregnant females/	Dermal (99.9 %	75, 237, and 750 mg/kg/day, with an additional	8hr/day	GD 6 to 15	Dose range finding study (3-5 pregnant females/dose; 500, 1100, and 2500 mg/kg) At 2500 mg/kg : all dams died or aborted prior to caesarean. At 1100 mg/kg : Depressed maternal weight gain during gestation, 4/5 litters completely resorbed.	Becci *et al*., 1992

Species	Route	*dose mg/kg/day ppm **Conc. mg/m^3	Exposure time (hr/day)	Exposure period : number of days during pregnancy	Observations and remarks	Reference
dose)	pure)	negative control group (water) and two positive control groups (one by gavage and one by dermal application) Not occlusive (25 cm^2)			At 500 mg/kg : No evidence of adverse effects on the mother and the conceptus. Main Study (75, 237, and 750 mg/kg/day) Maternal toxicity : - Patches of dry skin at the application site, the severity of which increased with the dose. - At the high dose, decrease in the body weight gain during gestation. - No maternal effects at 75 and 237 mg/kg. Developmental toxicity : - At 750 mg/kg: Increase in the incidence of resorptions, decreases in the number of viable fetuses and in the fetal body weight (20 %). Delayed ossification of several bones (i.e. skull, hyoid, sternebrae, vertebrae) and increase in the incidence of extra ribs. Skeletal malformations including fused/split ribs (8 fetuses from 5 litters), and fusion of the exoccipital and atlas bones (4 fetuses from 4 litters). No increase in the incidence of soft tissue variations or malformations. - No treatment-related effects at 75 and 237 mg/kg. <u>NOAEL</u> - maternal toxicity : 237 mg/kg bw/day - Developmental toxicity : 237 mg/kg/day.	

4.11.1 Effects on fertility

Not applicable

4.11.1.1 Non-human information

4.11.1.2 Human information

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

Developmental effects included for the analysis of ED_{10} values are highlighted in grey in tables 13-17.

Oral

In a prenatal developmental toxicity study, pregnant Sprague-Dawley rats were treated by oral gavage with aqueous NMP solutions in doses of 0, 125, 250, 500 or 750 mg/kg bw/day during gestational days (GD) 6 through 20 (Saillenfait *et al*., 2002).
Maternal body weight was significantly decreased from GD 15–21 at 500 mg/kg, and from GD 12–21 at 750 mg/kg. Significant decreases in maternal body weight gain were observed throughout the treatment period at 500 and 750 mg/kg (except for GD 12-15 for the 500 mg/kg bw group). In contrast to the authors of this study, the registrant considers the non-significant 9% reduction in maternal body weight gain at 250 mg/kg bw/day as biologically relevant and bases a maternal LOAEL on this effect. Also seen the clear dose effect relation, the dossier submitter agrees that this is a biological relevant effect. Food consumption was reduced on GD 9–12 and 18–21 at 500 mg/kg, and during all the intervals measured at 750 mg/kg.
No significant effect of NMP was noted on the pregnancy rate and the number of corpora lutea and implantations sites. Post-implantation loss and resorptions were increased in the 500 mg/kg and 750 mg/kg groups, showing a steep dose-response relationship. At 750 mg/kg, the number of live foetuses was greatly reduced due to a marked increase in the number of resorptions. Only eight out of 25 dams in this treatment group had live foetuses. The incidence of foetal deaths was low in all NMP-treated groups, but tended to increase with the dose. Doses of 250 to 750 mg/kg produced significant dose-related decreases in foetal body weights (males, females, total).
Gestational parameters from the pregnant rats are shown in the Table 13:

Table 13: Gestational parameters from pregnant Sprague-Dawley rats given NMP by gavage on GD 6-20

	Dose (mg/kg bw/day)				
	0	**125**	**250**	**500**	**750**
All litters[A]	21	22	24	25	25
No. of corpora lutea per dam	14.6 ± 2.4 [B]	14.6 ± 1.6	14.3 ± 1.9	14.5 ± 1.7	14.8 ± 1.7
Mean no. of implantation sites per litter	13.3 ± 3.2	13.6 ± 3.0	13.3 ± 3.2	14.0 ± 2.0	13.8 ± 3.0
Mean % post-implantation loss per litter [C]	4.1 ± 6.1	9.3 ± 21.3	4.5 ± 6.6	10.6 ± 10.5 *	94.2 ± 11.2**
Mean %dead foetuses per litter	0.0 ± 0.0	0.4 ± 1.6	0.0 ± 0.0	1.2 ± 3.4	3.2 ± 7.1
Mean % resorption sites per litter	4.1 ± 6.1	8.9 ± 21.2	4.5 ± 6.6	9.4 ± 8.9 *	91.0 ± 16.0**
Live litters[D]	21	21	24	25	8
Mean no. of live foetuses per litter	12.7 ± 3.1	13.1 ± 2.6	12.7 ± 3.0	12.4 ± 2.1	2.4 ± 2.3 **
Mean % male foetuses per litter	44.2 ± 17.5	46.1 ± 11.9	53.6 ± 14.7*	50.4 ± 17.5	91.7 ± 17.8 **

Foetal body weight (g)					
-All foetuses	5.73 ± 0.5	5.59 ± 0.22	5.18 ± 0.35**	4.02 ± 0.21**	3.01 ± 0.39 **
-Male foetuses	5.79 ± 0.42	5.74 ± 0.25	5.32 ± 0.45**	4.18 ± 0.22**	3.03 ± 0.40
-Female foetuses	5.62 ± 0.50	5.47 ± 0.20	5.02 ± 0.29**	3.88 ± 0.28**	3.09 ± 0.47 **

*, ** Significant differences from the vehicle control $P< 0.05$ and $P< 0.01$, respectively
[A] Includes all animals pregnant at euthanization.
[B] Values are expressed as means±SD.
[C] Resorptions plus dead foetuses.
[D] Includes all animals with live foetuses at euthanization.

The overall incidence of malformed foetuses per litter and the percentage of litters containing at least one malformed foetus were significantly increased at 500 and 750 mg/kg. A number of external (anasarca, anal atresia), visceral (persistent truncus arteriosus) and skeletal (fusion or absence of cervical arches were most prominent) malformations occurred only in NMP-treated groups, and a consistent dose-dependent trend was found in the incidence of these defects. Incidences of malformations and variations in foetuses are shown in table 14.

Table 14: Incidences of malformations and variations in foetuses of Sprague-Dawley rats given NMP by gavage on GD 6-20

	Dose (mg/kg bw/day)				
	0	**125**	**250**	**500**	**750**
Total no. of fetuses (litters) examined [A]:					
External	267 (21	276 (21)	304 (24)	311 (25)	19 (8)
Visceral	134 (21)	138 (21)	152 (24)	156 (25)	10 (6)
Skeletal	133 (20)	138 (21)	152 (24)	155 (25)	9 (5)
A. Fœtal malformations :					
External malformations [B]:					
Anasarca	0	0	0	6 (5)	1 (1)
Proboscis	0	0	0	0	1 (1)
Cleft palate	0	0	0	0	1 (1)
Anal atresia and tail, absent or vestigial	0	0	1 (1)	7 (5)	0
Omphalocele	0	1 (1)	0	0	0
No. (%) of foetuses with external malformations	0	1 (0.4)	1 (0.3)	11 (3.5)**	3 (15.8)**
No. (%) of litters with external malformations	0	1 (4.8)	1 (4.8)	9 (36.0)**	3 (37.5)*
Mean % of foetuses with external malformations per litter (mean ± SD)	0	0.4 ± 1.7 [C]	0.3 ± 1.7	3.3 ± 5.0	20.8 ± 36.5
Visceral malformations:					
Anophthalmia	1 (1)	0	0	0	0
Cardiovascular malformations [between square brackets: as % of total no. of fetuses with visceral malformations]	0 [0%]	0 [0%]	0 [0%]	10# (9) [6.4%]	6 # (4) [60%]
- Dextrocardia	0	0	0	1 (1)	0
- Truncus arteriosus, persistent [between square brackets: as % of total no. of fetuses with visceral malformations]	0 [0%]	0 [0%]	0 [0%]	5 (4) [3.2%]	2 (2) [20%]
- Aorta, transposed	0	0	0	2 (2)	2 (2)
- Aorta, overriding and/or enlarged and pulmonary artery, narrow	0	0	0	3 (3)	1 (1)
- Interventicular septum defect, solitary	0	0	0	1 (1)	1 (1)
No. (%) of foetuses with visceral malformations	1 (0.7)	0	0	10 (6.4)*	6 (60.0)**
No. (%) of litters with visceral malformations	1 (4.8)	0	0	9 (36.0)*	4 (66.7)**
Mean % of foetuses with visceral malformations per litter (mean ± SD)	0.6 ± 2.7	0	0	6.1 ± 8.7	66.7 ± 51.6#

	Dose (mg/kg bw/day)				
	0	**125**	**250**	**500**	**750**
Skeletal malformations:					
Facial bones, abnormal	0	0	0	0	1 (1)
Atlas and exoccipital, fused	0	0	0	1 (1)	2 (2)
Atlas, axis and/or cervical archs, fused	0	0	0	7 (5)	3 (2)
Cervical archs, absent [D]	0	0	0	2 (2)	1 (1)
Thoracic archs, fused	0	0	0	0	2 (2)
Thoracic centra second and/or fourth absent	0	0	0	2 (2)	0
Vertebrae, thoracic, lumbar, and/or sacral, absent	0	0	0	2 (2)	0
Sacral archs, fused	0	0	0	0	1 (1)
Ribs, absent	0	0	0	1 (1)	0
Ribs, fused	0	0	0	0	2 (2)
Cleft sternum	0	0	0	0	2 (2)
No. (%) foetuses with skeletal malformations	0	0	0	14 (9.0)**	5 (55.6)**
No. (%) litters with skeletal malformations	0	0	0	12 (48.0)**	3 (60.0)**
Mean % foetuses with skeletal malformations per litter (mean ± SD)	0	0	0	9.6 ± 11.7##	46.7 ± 44.7#
No. (%) foetuses with any malformations	1 (0.4)	1 (0.4)	1 (0.33)	30 (9.6)**	11 (57.9)**
No. (%) litters with any malformations	1 (4.8)	1 (4.8)	1 (4.2)	18 (72.0)**	6 (75.0)**
Mean % foetuses with any malformations per litter (mean ± SD)	0.3 ± 1.4	0.4 ± 1.7	0.3 ± 1.7	9.6 ± 8.3##	58.3 ± 43.6##
B. Foetal variations:					
External variations [B]					
Nostril, misshapen	0	0	0	1 (1)	0
Club foot	0	1 (1)	1 (1)	1 (1)	0
No. (%) of foetuses with external variations	0	1 (0.4)	1 (0.3)	2 (0.6)	0
No. (%) of litters with external variations	0	1 (4.8)	1 (4.2)	2 (8.0)	0
Mean % of foetuses with external variations per litter	0	0.3 ± 1.4 [C]	0.3 ± 1.3	0.5 ± 1.9	0
Visceral variations					
Palate rugae, misshapen in the center of palate	0	0	0	1 (1)	0
Uterine horn, small and oviduct, misshapen	0	0	1 (1)	0	0
Ovaries, displaced	0	0	0	1 (1)	0
Testis, displaced	0	0	0	1 (1)	0
Kidney, small	0	0	0	0	1 (1)
Dilated renal pelvis	0	0	0	2 (2)	0
Distended ureter	4 (4)	0	1 (1)	1 (1)	2 (1)
No. (%) of foetuses with visceral variations	4 (3.0)	0	2 (1.3)	5 (3.2)	3 (30.0)**
No. (%) of litters with visceral variations	4 (19.0)	0	2 (8.3)	5 (20.0)	2 (33.3)
Mean % of foetuses with visceral variations per litter	2.7 ± 5.8	0	1.3 ± 4.4	3.3 ± 7.0	16.7 ± 27.9
Skeletal variations					
Skull, incomplete ossifications [D]:					
- frontals and parietal	1 (1)	0	0	55## (17)	8## (5)
- supraoccipital	1 (1)	0	0	13 (6)	8## (5)
- interparietal	1 (1)	0	0	0	0
Hyoid, absent	1 (1)	0	0	0	0
Sternebrae:					
- first and second, fused	0	0	1 (1)	1 (1)	0
- incomplete ossification or absent, no. 5 and/or 6	0	1 (1)	7 (7)	43## (21)	6## (5)
- incomplete ossification or absent, other than no.	0	0	0	6 (5)	3 (3)

	Dose (mg/kg bw/day)				
	0	**125**	**250**	**500**	**750**
5 and/or 6					
Ribs:					
- cervical, rudimentary	2 (2)	1 (1)	6 (6)	19 (10	1 (1)
- 14th, supernumerary	18 (8)	26 (13)	29 (13)	38 (18)	6 (3)
- 13th, short (uni or bilateral)	2 (1)	0	0	0	0
Thoracic vertebral centra:					
- first absent	0	0	0	2 (2)	2 (2)
- Incomplete ossification (one or two)	13 (8)	7 (4)	3 (3)	15 (11)	5# (4)
No. (%) of foetuses with skeletal variations	33 (24.8)	33 (23.9)	41 (27.0)	115 (74.2)**	9 (100.0)**
No. (%) of litters with skeletal variations	14 (70.0)	15 (71.4)	19 (79.2)	25 (100.0)*	5 (100.0)
Mean (%) of foetuses with skeletal variations per litter	24.7 ± 20.3	22.6 ± 22.1	26.2 ± 25.8	74.2 ± 24.9##	100.0 ± 0.0##

[A] Only live foetuses were examined
[B] The incidence of individual malformation or defect is presented as number of foetuses (number of litters). A single foetus may be represented more than once in listing of the individual malformations/variations.
[C] Mean ± SD
[D] Absent = alizarine red S negative
*, ** Significant differences from the vehicle control $P< 0.05$ and $P< 0.01$, respectively, Fischer's test
#, ## Significant differences from the vehicle control $P< 0.05$ and $P< 0.01$, respectively, Mann-Whitney test

Based on the observed data, the NOAEL for maternal and developmental toxicity can both be considered as 125 mg/kg bw/day.

In a developmental toxicity study with rabbits, groups of 20 inseminated New Zealand White rabbits were administered by oral gavage dose levels of 0, 55, 175 and 540 mg/kg bw/day of an aqueous NMP solution on gestation day 6 through 18 (International Research and Development Corporation (IRDC) (1991)). A dose-dependent reduced body weight gain and food consumption was observed which was significant at 540 mg/kg bw/day. At a dose of 175 mg/kg bw/day, body weight gain was significantly reduced only during GD 6-12. Reduced food intake was however not significant at the 175 mg/kg bw/day dose level. Furthermore, one abortion was observed at a dose level of 540 mg/kg bw/day. Increased post-implantation loss, reduced live litter size and reduced mean uterine weight were observed at 540 mg/kg bw/day as well. Gestational parameters from the pregnant rabbits are shown in the table 15.

Table 15: Gestational parameters of pregnant rabbits given NMP by gavage on GD 6-18

	Dose (mg/kg bw/day)			
	0	**55**	**175**	**540**
All litters	20	18	18	17
No. of corpora lutea per dam	13.6 ± 3.08	14.1 ± 3.54	13.5 ± 3.33	13.5 ± 2.17
Mean no. of implantation sites per litter	9.1 ± 3.08	7.6 ± 4.06	6.7 ± 3.33	7.0 ± 3.35
Mean post-implantation loss per litter	0.4 ± 0.68	0.8 ± 1.82	0.7 ± 0.91	1.8 ± 1.94 *
Preimplantation loss %	35.5	44.4	50.6	45.3
Postimplantation loss %	5.5	10.9	10.0	25.9
Resorptions (total)	0.3 ± 0.58	0.5 ± 0.99	0.7 ± 0.91	1.8 ± 1.94
Mean % dead foetuses per litter	0.03	0.28	0	0
Live litters	20	17	18	15
Mean no. of live foetuses per litter	8.6 ± 2.96	6.8 ± 4.32	6.0 ± 2.95	5.2 ± 3.10
Mean % of male foetuses per litter	51.2	55.9	50.0	55.8

Foetal body weight (g):				
-All foetuses	41.6 ± 8.14	46.3 ± 7.33	47.3 ± 8.32	42.9 ± 5.27
-Male foetuses	42.0 ± 8.44	46.8 ± 6.73	47.5 ± 7.43	43.3 ± 5.95
-Female foetuses	41.4 ± 7.84	43.9 ± 7.37	45.3 ± 8.38	41.0 ± 4.93
Mean uterine weight	471 ± 123	461 ± 210	402 ± 173	357 ± 135

* Significantly different from control group (P≤ 0.05)

Both cardiovascular (i.e. bulbous aortic arch, pulmonary trunk stenosis, ductus arteriosis stenosis and intraventricular septal defect) as well as skeletal (skull bones) malformations were observed at the high dose level. Incidences of malformations and variations in foetuses are shown in the table 16.

Table 16: Incidences of malformations and variations in foetuses of rabbits dosed with NMP by gavage on GD 6-18

	Dose (mg/kg bw/day)			
	0	**55**	**175**	**540**
Total no. of fetuses (litters) examined:	19	17	18	15
External	161	128	108	83
Visceral	161	128	108	83
Skeletal	161	128	108	83
Malformations:				
Omphalocele	0	0	0	1 (1)
Aortic arch stenosis	1 (1)	0	0	0
Bulbous aortic arch [between square brackets: as % of total no. of fetuses with visceral malformations]	1 (1) [0.6%]	0 [0%]	1 (1) [0.9%]	20 (8) [24%]
Pulmonary trunk stenosis [between square brackets: as % of total no. of fetuses with visceral malformations]	1 (1) [0.6%]	0 [0%]	1 (1) [0.9%]	17 (6) [20%]
Bulbous pulmonary trunk	0	0	0	1 (1)
Ductus arteriosus stenosis	2 (2)	0	1 (1)	14 (6)
Right subclavian stenosis	0	0	0	1 (1)
Interventricular septal defect [between square brackets: as % of total no. of fetuses with visceral malformations]	2 (2) [1.2%]	0 [0%]	2 (2) [1.9%]	24 (8) [28.9%]
Pulmonary hypoplasia	0	1 (1)	0	0
Gallbladder agenesis	0	1 (1)	1 (1)	0
Malformed ovaries	0	0	1 (1)	0
Malformed skull bone(s)	0	0	0	6 (4)
Fused skull bones	0	1 (1)	0	0
Vertebral malformation with or without an associated rib malformation	1 (1)	2 (2)	0	0
Rib malformation	0	0	0	1 (1)
Interrupted ossification of a rib	1 (1)	1 (1)	0	0
Fused sternebrae	3 (2)	2 (2)	5 (4)	7 (6)
Forked scapula	0	0	2 (1)	1 (1)
Total foetuses (litters) with malformations	9 (7)	5 (5)	10 (7)	36 (12) *
Variations:				
Hemorrhagic iris	0	0	1 (1)	0
Retroesophageal aortic arch	0	0	0	1 (1)
Retroesophageal right subclavian	0	0	0	1 (1)
Accessory left subclavian	1 (1)	0	0	0
Left carotid arises from innominate	20 (11)	36 (13)	4 (4)	0

Azygous lobe of lung absent	1 (1)	0	1 (1)	4 (3)
Gallbladder hypoplasia	9 (6)	7 (5)	0	6 (5)
Renal papillae not developed	1 (1)	0	0	0
Skull reduced in ossification	0	0	0	1 (1)
Misshapen skull bone	0	1 (1)	1 (1)	25 (9)
Hyoid arches(s) bent	11 (6)	7 (5)	9 (6)	13 (8)
Accessory skull bone	1 (1)	1 (1)	0	2 (2)
27 presacral vertebrae	21 (13)	15 (8)	32 (10)	70 (13)
Extra vertebral ossification site	0	0	1 (1)	2 (1)
Greater than 12 pairs of full ribs	82 (19)	54 (15)	88 (16)	74 (14)
13th rudimentary rib(s)	32 (14)	24 (10)	9 (8)	4 (3)
7th cervical rib(s)	3 (3)	3 (2)	4 (3)	0
Sternebra(e) #5 and/or #6 unossified	10 (5)	8 (3)	9 (6)	7 (6)
Misaligned sternebra	1 (1)	1 (1)	0	0
Extra sternebra	0	5 (3)	1 (1)	0
Extra sternal ossification site(s)	3 (2)	4 (2)	1 (1)	1 (1)
Total foetuses (litters) with variations	126 (19)	101 (17)	102 (17)	82 (15)

* significantly different from the control group; P≤0.05 as per Chi Square test

The NOAEL for maternal toxicity and developmental toxicity were 55 mg/kg bw/day and 175 mg/kg bw/day respectively.

In a two-generation reproduction toxicity study, groups of Wistar rats (n = 25 per sex) were given 1-methyl-2-pyrrolidone (NMP) daily via the diet at initial dose levels of 0, 50, 160 or 500 mg/kg bw/day over a 10-week premating period and throughout the mating, gestation, lactation and a rest period between pregnancies (BASF AG, Department of Toxicology, 1999). The concentrations in the diet were adjusted regularly in respect to the actual body weight gain. Due to severe pup mortality in the first litter (F1a), the highest dose level was reduced to 350 mg/kg bw/day for the further course of the study. Each generation gave birth to two litters (F1a+F1b, F2a+F2b). The parental animals for the second generation were selected from pups of the second litter (F1b).

NMP had no adverse effects on reproductive performance or fertility of the F0 or F1 parental animals of all substance-treated groups (examinations included estrous cycle data, mating behavior, conception, gestation, parturition, lactation and weaning as well as sperm parameters, sexual organ weights, gross and histopathological findings of these organs (including differential ovarian follicle counts)). No substance-related adverse effects were observed in the F0 males and females, F1a and F1b pups, F2 males and females, F2a and F2b pups at the dose levels of 50 and 160 mg/kg bw/day. However, signs of systemic toxicity were observed in the high dose group, both in the parental animals as well as in the pups. Parental toxicity consisted of reduced body weight gain and food intake as well as kidney findings in form of impaired organ weight and histopathological findings. Developmental toxicity was evidenced by increased pup mortality and reduced body weight gain, including corresponding effects in the investigated organs, in pups treated at 500/350 mg/kg bw/day. Table 17 presents the observed foetal effects. The NOAEL for reproductive performance/fertility was 350 mg/kg bw/day. The NOAEL for developmental toxicity was 160 mg/kg bw/day.

Table 17: Summary of observed effects in foetuses of Wistar rats dosed NMP by oral gavage in a 2-generation study

	Dose (mg/kg bw/day)			
	0	**50**	**160**	**500/350 *****

Total no. of dams (F0) with F1a pups	25	25	24	24
Numbers of dams (F0) with complete litters lost (F1a) at the end of the lactation period [between square brackets: as % of total no. dams]	0 [0%]	0 [0%]	0 [0%]	18 [75%]
Total no. of pups (F1a)	327	330	326	296
Number of liveborn pups (F1a)	320	319	318	272 **
Number of stillborn pups (F1a) [between square brackets: as % of total no. of pups]	7 [2.1%]	11 [3.3%]	8 [2.5%]	24 ** [8.1%]
Pup (F1a) mortality % (died)	2.8	7.6	2.8	54**
Pup (F1a) mortality % (cannibalization)	1.2	1.5	0.9	23**
Foetal body weight F1a pups (g): males + females				
Day 1	6.4±0.42	6.1±0.56	6.1±0.48	5.4±0.92**
Day 4 (pre-culling)	9.6±1.06	8.7±1.31	9.0±1.15	8.0±2.07
Day 4 (post-culling)	9.5±1.01	8.7±1.30	9.0±1.17	8.0±2.07*
Day 7	14.7±1.22	13.5±1.68	14.1±1.57	11.9±4.25**
Day 14	30.0±1.48	28.7±2.61	29.6±2.49	26.9±2.59*
Day 21	49.3±2.62	47.6±4.34	49.0±3.99	44.2±2.92**
Foetal body weight gain F1a pups (g): males + females				
Days 1-4	3.1±0.73	2.6±0.82	2.9±0.73	2.0±1.19**
Days 4-7	5.2±0.47	4.7±0.61	5.1±0.70	3.8±.2.28**
Days 7-14	15.3±0.74	15.2±1.24	15.5±1.29	13.8±1.80*
Days 14-21	19.3±1.47	19.0±1.86	19.4±1.75	17.3±0.80*
Days 4-21	39.8±1.98	38.9±3.28	40.0±3.19	35.6±2.23**
Foetal organ weight F1a pups: males + females				
Brain – in grams	1.433±0.0419	1.402±0.0496*	1.420±0.0437	1.359±0.0773*
Brain – to body weight ratio	2.913±0.1553	2.989±0.2287	2.940±0.2186	3.126±0.2049
Thymus – in grams	0.163±0.0119	0.156±0.0256	0.167±0.0215	0.153±0.0168
Thymus – to body weight ratio	0.330±0.0254	0.330±0.0412	0.341±0.0330	0.349±0.0249
Spleen – in grams	0.197±0.0275	0.189±0.0355	0.213±0.0296	0.162±0.0161*
Spleen – to body weight ratio	0.397±0.0460	0.397±0.0565	0.437±0.0562**	0.371±0.0394
Total no. of dams (F0) with F1b pups	24	25	25	25
Numbers of dams (F0) with complete litters lost (F1b) at the end of the lactation period [between square brackets: as % of total no. dams]	1 [4.2%]	0 [0%]	0 [0%]	0 [0%]
Total no. of pups (F1b)	326	339	327	275
Number of liveborn pups (F1b)	336	329	321	270 **
Number of stillborn pups (F1b) [between square brackets: as % of total no. of pups]	0 [0%]	10 ** [2.9%]	6 * [1.8%]	5 * [1.8%]
Pup (F1b) mortality % (died)	3.3	5.0	3.1	5.5
Pup (F1b) mortality % (cannibalization)	5.4	2.4	0.9	7.3
Foetal body weight F1b pups (g): males + females				

Day 1	6.2±0.56	6.1±0.40	6.2±0.58	6.0±0.59
Day 4 (pre-culling)	8.9±1.21	8.5±1.05	8.8±1.33	7.9±1.72*
Day 4 (post-culling)	8.9±1.20	8.6±1.05	8.8±1.30	7.9±1.71*
Day 7	13.7±2.23	13.2±1.80	13.7±1.99	11.7±3.01**
Day 14	29.5±2.32	28.1±2.91	29.1±3.13	25.9±5.24**
Day 21	48.4±3.62	47.2±5.20	48.5±4.93	43.6±7.90*
Foetal body weight gain F1b pups (g): males + females				
Days 1-4	2.7±0.85	2.4±0.80	2.5±0.83	1.9±1.25*
Days 4-7	4.9±1.24	4.6±1.03	4.9±1.04	3.8±1.41**
Days 7-14	15.4±1.49	15.0±1.61	15.4±1.67	14.2±2.58
Days 14-21	18.9±1.89	19.1±2.47	19.4±2.31	17.7±2.95
Days 4-21	39.3±3.22	38.7±4.55	39.7±4.22	35.7±6.45*
Foetal organ weight F1b pups: males + females				
Brain – in grams	1.425±0.0375	1.410±0.0501	1.422±0.0577	1.356±0.0966**
Brain – to body weight ratio	2.981±0.2131	3.010±0.3065	2.978±0.2132	3.308±0.6364*
Thymus – in grams	0.167±0.0172	0.166±0.0172	0.168±0.0241	0.157±0.0354
Thymus – to body weight ratio	0.347±0.0275	0.352±0.0404	0.350±0.0437	0.365±0.0437
Spleen – in grams	0.205±0.0345	0.203±0.04533	0.213±0.0388	0.174±0.0520*
Spleen – to body weight ratio	0.421±0.0521	0.425±0.0574	0.438±0.0512	0.395±0.0678
Numbers of dams (F1) with complete litters lost (F2a) at the end of the lactation period	0	0	0	1
Number of liveborn pups (F2a)	333	309	309	244
Number of stillborn pups (F2a)	8	2	3	4
Pup (F2a) mortality % (died)	2.6	3.9	2.6	6.9*
Pup (F2a) mortality % (cannibalization)	1.2	1.9	1.3	2.0
Foetal body weight F2a pups (g): males + females				
Day 1	6.2±0.48	6.0±0.63	6.2±0.66	5.9±0.68
Day 4 (pre-culling)	9.0±1.23	8.8±1.36	9.0±1.38	8.2±1.59
Day 4 (post-culling)	9.1±1.22	8.8±1.39	9.0±1.38	8.2±1.61
Day 7	14.1±1.82	13.6±1.87	14.1±1.82	12.5±2.51*
Day 14	28.7±2.68	27.07±3.04	28.7±2.71	25.09±4.05*
Day 21	46.0±4.03	45.3±5.17	46.7±4.49	42.9±5.95
Foetal body weight gain F2a pups (g): males + females				
Days 1-4	2.8±0.83	2.7±0.79	2.8±0.80	2.3±1.03
Days 4-7	5.1±0.74	4.8±0.69	5.1±0.66	4.3±1.01**
Days 7-14	14.6±1.16	14.1±1.63	14.6±1.41	13.4±1.71*
Days 14-21	17.3±1.88	17.6±2.48	18.1±2.20	17.0±2.08
Days 4-21	37.0±3.16	36.5±4.18	37.7±3.47	34.7±4.53
Foetal organ weight F2a pups: males + females				
Brain – in grams	1.425±0.0394	1.414±0.0393	1.430±0.0518	1.394±0.0667
Brain – to body weight ratio	3.126±0.2738	3.148±0.2885	3.084±0.2446	3.290±0.3302*
Thymus – in grams	0.159±0.0145	0.152±0.0143	0.158±0.0180	0.155±0.0260
Thymus – to body weight ratio	0.346±0.0272	0.337±0.0390	0.339±0.0285	0.361±0.0490
Spleen – in grams	0.186±0.0356	0.184±0.0366	0.192±0.0339	0.174±0.0380
Spleen – to body weight ratio	0.402±0.0593	0.402±0.0495	0.408±0.0513	0.400±0.0461

Numbers of dams (F1) with complete litters lost (F2b) at the end of the lactation period	0	0	0	0
Number of liveborn pups (F2b)	336	325	336	315
Number of stillborn pups (F2b)	12	8	4	5
Pup (F2b) mortality % (died)	2.9	2.7	1.5	7.8**
Pup (F2b) mortality % (cannibalization)	0.9	2.1	1.5	7.2**
Foetal body weight F2b pups (g): males + females				
Day 1	6.3±0.57	6.3±0.58	6.3±0.46	6.0±0.55
Day 4 (pre-culling)	8.8±1.50	8.7±1.34	9.1 ±0.97	7.9±1.45*
Day 4 (post-culling)	8.9±1.52	8.7±1.34	9.1±0.96	7.9±1.47*
Day 7	13.5±2.15	13.2±1.93	14.1±1.30	11.6±2.45**
Day 14	28.1±3.05	27.5±3.23	29.1±2.26	25.2±3.76**
Day 21	46.4±4.51	45.8±4.89	48.6±3.74	43.3±5.77
Foetal body weight gain F2b pups (g): males + females				
Days 1-4	2.6±1.02	2.4±0.84	2.8±0.64	1.9±0.96*
Days 4-7	4.7±0.80	4.5±0.89	5.0±0.53	3.8±1.05**
Days 7-14	14.6±1.34	14.4±1.67	14.9 ±1.30	13.6±1.66
Days 14-21	18.3±2.06	18.3±2.07	19.5±1.99	18.2±2.39
Days 4-21	37.6±3.34	37.1±3.83	39.5±3.11	35.5±4.62
Foetal organ weight F2b pups: males + females				
Brain – in grams	1.401±0.0482	1.394±0.0484	1.410±0.0445	1.370±0.0620*
Brain – to body weight ratio	3.039±0.2288	3.081±0.2742	2.923±0.1896	3.204±0.3488
Thymus – in grams	0.157±0.0201	0.150±0.0165	0.164±0.0202	0.153±0.0191
Thymus – to body weight ratio	0.339±0.0325	0.329±0.0308	0.338±0.0355	0.354±0.0427
Spleen – in grams	0.190±0.0376	0.203±0.0495	0.212±0.0310*	0.177±0.0326
Spleen – to body weight ratio	0.406±0.0528	0.439±0.0729	0.436±0.0417	0.406±0.0385

* and ** : Significantly different from the control group; P≤0.01 and P≤0.05 respectively
*** F1a are pups of dams exposed to 500 mg/kg bw/day as highest dose
F1b are pups of dams exposed to 350 mg/kg bw/day as highest dose

In a second two-generation reproduction toxicity study, groups of CD rats (n = 30 per sex) were given NMP via the diet at initial dose levels of 0, 50, 160 or 500 mg/kg bw/day over a 10 -week premating period and throughout the mating, gestation, lactation and a rest period between pregnancies (Huntingdon Life Science, 1999). The concentrations in the diet were adjusted regularly in respect to the actual body weight gain. Due to severe pup mortality in the first litter (F1a), the highest dose level was reduced to 350 mg/kg bw/day for the further course of the study. Each generation gave birth to two litters (F1a+F1b, F2a+F2b). The parental animals for the second generation were selected from pups of the second litter (F1b).

NMP had no adverse effects on reproductive performance or fertility of the F0 or F1 parental animals of all substance-treated groups (examinations included gonadal function, the estrous cycle, mating behavior, conception, gestation, parturition, lactation and weaning, and the growth and development of the offspring). NMP treatment-related effects in the P-generation were confined to the female rats where there was a decrease in body weights at the end of gestation and the beginning of lactation as well as decreased feed consumption during lactation when treated with the 500

mg/kg bw/day dose of NMP. The F1-pups whose dams were exposed to 500 mg/kg bw/day NMP had a decrease in mean litter size, pup survival, and pup body weights during lactation. Because of pup toxicity at the 500 mg/kg bw/day NMP dose level, the dose level was decreased to 350 mg/kg bw/day for the remainder of the study. There were no signs of maternal toxicity noted after high dose level reduction to 350 mg/kg bw/day. However, F2b pups only at 350 mg/kg bw/day had a decrease in the number of pups surviving lactation and a decrease in pup body weights. No adverse effect of NMP treatment was observed in the P- and F- generation male rats, including mortality, body weights, feed consumption and clinical observations. At necropsy, parental animals revealed significant organ weight changes, however, they were considered not treatment-related due to the absence of changes in the other sex and the absence of corresponding histopathological findings. Table 18 presents the observed foetal effects. The NOAEL for reproductive performance/fertility was 350 mg/kg bw/day. The NOAEL for developmental toxicity was 160 mg/kg bw/day.

Table 18: Summary of observed effects in foetuses of CD rats dosed NMP by oral gavage in a 2-generation study

	Dose (mg/kg bw/day)			
	0	**50**	**160**	**500/350 *****
Numbers of dams (F0) with complete litters lost (F1a) at the end of the lactation period	0	0	0	13**
Number of liveborn pups (F1a)	342	335	339	349*
Number of stillborn pups (F1a)	6	5	2	20*
Pup (F1a) mortality % (dying,missing, cannibalized)	5.0	2.4	1.5	57.9**
Foetal body weight F1a pups (g): males + females				
Day 1	6.7±0.65	6.9±0.39	6.8±0.77	5.3±0.78**
Day 4 (pre-culling)	9.6±1.08	10.1±0.85	9.8±1.29	7.4±1.64**
Day 4 (post-culling)	9.6±1.09	10.1±0.82	9.9±1.29	7.4±1.61**
Day 7	14.9±1.23	15.3±1.26	15.0±1.97	10.8±2.53**
Day 14	29.9±2.41	29.4±2.24	30.2±3.50	24.3±477**
Day 21	44.9±5.41	45.2±4.83	44.4±5.89	37.7±6.15**
Foetal organ weight F1a pups: males + females				
Brain – in grams	1.453±0.0765	1.460±0.0812	1.456±0.0689	1.335±0.0683**
Brain – to body weight ratio	3.264±0.3644	3.266±0.2719	3.324±0.4882	3.560±0.4390
Thymus – in grams	0.2014±0.0401	0.1959±0.0376	0.2030±0.0401	0.1859±0.0494
Thymus – to body weight ratio	0.4442±0.0518	0.4316±0.0550	0.4530±0.0620	0.4795±0.0641
Spleen – in grams	0.1836±0.0620	0.1789±0.0430	0.1865±0.0477	0.1654±0.0408
Spleen – to body weight ratio	0.3983±0.0993	0.3915±0.0609	0.4145±0.8390	0.4281±0.0665
Numbers of dams (F0) with complete litters lost (F1b) at the end of the lactation period	0	0	0	0
Number of liveborn pups (F1b)	350	333	357	373
Number of stillborn pups (F1b)	6	5	4	4
Pup (F1b) mortality % (dying,missing, cannibalized)	13	4	5	11
Foetal body weight F1b pups (g):				

males + females				
Day 1	6.6±0.7	7.0±0.43*	6.6±0.43	6.5±0.58
Day 4 (pre-culling)	9.6±1.09	10.1±0.86	9.4±0.84	9.5±1.12
Day 4 (post-culling)	9.6±1.05	10.1±0.87	9.4±0.87	9.5±1.14
Day 7	15.4±1.52	15.7±1.22	14.9±1.72	15.2±1.88
Day 14	31.2±2.69	31.5±1.99	30.7±3.12	30.2±3.71
Day 21	51.5±5.12	50.9±3.44	49.3±4.48	49.4±5.90
Foetal organ weight F1b pups: males + females				
Brain – in grams	1.5331±0.0736	1.5738±0.0927	1.5467±0.0816	1.5320±0.0820
Brain – to body weight ratio	3.0216±0.2739	3.0911±0.2600	3.1625±0.2687	3.1806±0.4555
Thymus – in grams	0.2571±0.0539	0.2774±0.0571	0.2421±0.0428	0.2541±0.0376
Thymus – to body weight ratio	0.4979±0.0803	0.5447±0.1237	0.4911±0.0731	0.5196±0.0645
Spleen – in grams	0.2606±0.0591	0.2629±0.0698	0.2493±0.0510	0.2551±0.0449
Spleen – to body weight ratio	0.5041±0.0934	0.5140±0.1445	0.5036±0.0745	0.5169±0.0579
Numbers of dams (F1) with complete litters lost (F2a) at the end of the lactation period	0	0	0	0
Number of liveborn pups (F2a)	400	404	413	352
Number of stillborn pups (F2a)	3	2	3	3
Pup (F2a) mortality % (dying,missing, cannibalized)	7.8	2.5**	2.4**	4.3
Foetal body weight F2a pups (g): males + females				
Day 1	7.0±0.65	6.9±0.57	6.9±0.58	6.5±0.65**
Day 4 (pre-culling)	10.1±1.21	10.1±1.10	10.0±0.96	9.7±1.13
Day 4 (post-culling)	10.1±1.17	10.1±1.14	10.0±0.98	9.7±1.13
Day 7	16.4±1.91	16.3±1.97	16.5±1.21	15.8±1.94
Day 14	32.9±2.34	31.8±3.73	32.8±2.19	32.1±2.83
Day 21	53.4±3.94	51.6±5.63	52.7±3.31	50.6±5.25
Foetal organ weight F2a pups: males + females				
Brain – in grams	1.545±0.0844	1.542±0.0804	1.533±0.0695	1.491±0.0604*
Brain – to body weight ratio	2.914±0.1714	3.019±0.2622	2.927±0.2090	2.953±0.3418
Thymus – in grams	0.253±0.0345	0.239±0.0279	0.251±0.0298	0.258±0.0357
Thymus – to body weight ratio	0.476±0.0621	0.468±0.0599	0.479±0.0660	0.504±0.0458
Spleen – in grams	0.258±0.0426	0.229±0.0437*	0.251±0.0304	0.258±0.0478
Spleen – to body weight ratio	0.482±0.0549	0.443±0.0607*	0.476±0.0480	0.502±0.0587
Numbers of dams (F1) with complete litters lost (F2b) at the end of the lactation period	0	0	0	0
Number of liveborn pups (F2b)	376	387	346	331**
Number of stillborn pups (F2b)	14	9	4	0**
Pup (F2b) mortality % (dying,missing, cannibalized)	3.5	5.7	3.8	16.6**
Foetal body weight F2b pups (g): males + females				
Day 1	7.1±0.65	6.7±1.01	6.7±0.72	6.4±0.86*
Day 4 (pre-culling)	10.3±1.27	9.7±1.54	9.3±1.14	8.7±2.11**
Day 4 (post-culling)	10.2±1.26	9.6±1.59	9.3±1.18	8.7±2.10**

Day 7	16.7±1.69	15.2±2.63	14.8±2.01*	14.0±3.31**
Day 14	32.0±3.31	31.2±3.86	29.0±5.38	29.6±4.88
Day 21	52.8±4.34	50.5±6.08	46.4±9.73**	47.7±6.69*
Foetal organ weight F2b pups: males + females				
Brain – in grams	1.5743±0.0745	1.5786±0.0823	1.5082±0.1321*	1.5238±0.0801
Brain – to body weight ratio	2.9860±0.2512	3.1552±0.2953	3.4992±1.2790	3.2431±0.3931
Thymus – in grams	0.2587±0.0283	0.2430±0.0405	0.2254±0.0603	0.2481±0.0490
Thymus – to body weight ratio	0.4885±0.0470	0.4789±0.0496	0.4803±0.0784	0.5172±0.0634
Spleen – in grams	0.2802±0.0416	0.2519±0.0634	0.2328±0.0686*	0.2714±0.0504
Spleen – to body weight ratio	0.5268±0.0535	0.4922±0.0868	0.4894±0.0820	0.5665±0.0674

* and ** : Significantly different from the control group; P≤0.01 and P≤0.05 respectively
*** F1a are pups of dams exposed to 500 mg/kg bw/day as highest dose
F1b are pups of dams exposed to 350 mg/kg bw/day as highest dose

Sitarek and Stetkiewicz (2008) assessed the reproductive toxicity and gonadotoxicity of NMP. Male rats were exposed to NMP via oral gavage in doses of 0, 100, 300 and 1000 mg/kg bw/day for 5 days/week during a total period of 10 weeks before mating and 1 week during mating. Body weight and food and water intake of male rats were studied during exposure. After the 10-week premating exposure period, the exposed males were mated with un-exposed females during one week. After the mating period, the male animals were autopsied and were studied for toxic effects. Analysis included body weight, organ weight, macrospcopic evaluation of organs, and histopathological analysis of testis and epididymis. Evaluation of the pregnant females included behaviour, body weight gain and daily food and water intake. Furthermore, assessment of early postnatal development of the offspring was done until the end of the lactation period (28 days). NMP at a dose of 1000 mg/kg bw/day was found to produce reduced male fertility and extensive damage to seminiferous epithelium in the seminal tubules of the testis. NMP at doses of 100 mg/kg bw/day did not influence the viability or the development of their offspring. Exposure of the males to 300 mg/kg bw/day was found to induce a reduction in postnatal survival until day 4. In the group of the 1000 mg/kg bw/day exposed males, only 2 out of 44 females delivered, and the total number of pups was 6.

In a developmental toxicity study, Sprague-Dawley rats were exposed by oral gavage to 0, 332 and 997 mg/kg bw/day NMP on gestation day 6 through 15 (EPA 1987; as summarised in the French classification proposal, 2002). At the dose of 332 mg/kg bw/day, placental and foetal weight was lower than control (14-20% and 10% respectively). There was no difference in implantation rate, litter size or resorptions. Maternal body weights were not reported. At the dose of 997 mg/kg bw/day, marked reductions in maternal body weight and placental weight were observed. There was a large number of resorptions (24/29 dams showed complete resorption) and only 15 live and 1 dead fetuses were present at term. Observations in the live fetuses included reduction in fetal bodyweight (37%), malformations considered as indicative of foetal retardation in 8 out of 15 foetuses, and 14 runts.

In a developmental toxicity study, Crl:CD rats were exposed by oral gavage to 0, 40, 125 and 400 mg/kg bw/day NMP on gestation day 6 through 15 (Exxon 1992). Maternal body weight gain was depressed during treatment at 400 mg/kg at GD 6-9, GD 9-12, GD 6-15 (14, 18, and 53 g, respectively at 0 mg/kg compared to 7, 15, and 42 g, respectively at 400 mg/kg). However, there

was no statistical difference in weight gain during the overall gestation period (GD 0-21) and after correction for gravid uterine weight. Furthermore, food consumption was unchanged. At 400 mg/kg, reduced fetal body weight (10-11%) was observed. There were no statistically significant differences between treated and control for any uterine implantation parameter. Foetal variations and malformations were observed in all groups, including controls, although the types and incidences were similar between treated and control groups. An increased incidence of stunted fetuses was observed (fetuses: 1/340, 1/393, 2/395, and 12/397; litters: 1/21, 1/25, 2/24, and 6/25; at 0, 40, 125 and 400 mg/kg, respectively. The NOAEL for maternal and developmental toxicity were considered as 125 mg/kg/day.

Inhalation

The developmental toxicity of inhaled NMP was studied in Sprague–Dawley rats (Saillenfait *et al*. (2001, 2003)). Pregnant rats were exposed whole body to NMP vapours at concentrations of 0, 30, 60 and 120 ppm, equivalent to 0, 123, 247 and 494 mg/m^3. Rats were exposed 6 h/day, on GD 6 through 20. Maternal body weight gain was significantly decreased at 247 and 494 mg/m^3 on GD 6–13 and maternal food consumption was reduced at 494 mg/m^3 on GD 13–21. No statistically significant difference in the gestational weight change corrected for the weight of the gravid uterus was observed, at any of the NMP concentrations. Fetal toxicity indicated by reduced fetal weight was observed at 494 mg/m^3. There were no adverse effects on embryo/fetal viability or evidence of teratogenicity at any concentration tested. Only two malformations were observed (1 in control group, 1 in low dose group) and the incidences of external, visceral and skeletal variations did not indicate any adverse effects related to NMP exposure. The NOAECs for maternal and developmental toxicity were 123 and 247 mg/m^3, respectively.

In the second inhalation developmental toxicity study (BASF AG, Department of Toxicology (1993)) inseminated Himalayan rabbits (15/dose) were exposed (nose-head) to 0, 200, 500, 1000 mg/m^3 NMP for 6 h/day during GD 7 through 19. No signs of maternal toxicity were observed: no clinical signs or mortality were seen and body weights were comparable between all dose groups. However, it was mentioned that maternal toxicity expressed as prolonged clotting time, decreased plasma protein content and slightly increased liver weight occurred in a pretest at concentrations of 1000 and 2000 mg/m^3. Food consumption was not measured in this study. Developmental toxicity was observed at 1000 mg/m^3 as indicated by an increased occurrence of accessory 13th rib(s) (skeletal variation). Other incidences of variations were within the historical control range, lacked a dose-response relationship or did not show significant statistical or biological changes. No effects on the incidence of malformations were found. Based on the result of this study, the NOAEC for maternal toxicity was 1000 mg/m^3 and the NOAEC for developmental toxicity was 500 mg/m^3.

In a developmental toxicity study, Crl:CD rats were inhalation exposed (whole body inhalation) to 0, 100 and 360 mg/m^3 NMP (0, 24.3 and 87.3 ppm) for 6 h/day on gestation day 6 to 15 (Lee *et al*., 1987). Sporadic lethargy and irregular respiration was observed in several dams at both 100 mg/m^3 (24.3 ppm) and 360 mg/m^3 (87.3 ppm) during the 3 first days of exposure. No adverse effects on maternal and fetal body weight, nor increases in the incidences of resorptions and of malformations and variations (external, soft tissue and skeletal) were observed.

In an inhalation developmental toxicity study, Mol:WIST rats were whole body exposed to 0 and 151 ppm NMP (0 and 620 mg/m^3) for 6 hr/day on gestation day 7 to 20 (Hass *et al*., 1994). No effects on maternal weight gain during gestation were observed. Furthermore, no changes in gestation length, the number of pups and neonatal death were observed. A reduced body weight of litters was observed from birth throughout weaning until the age of 5 weeks. Some pre-weaning development milestones and reflexes (i.e. i.e. ear unfolding, surface righting reflex, incisor eruption, eye opening) showed a slight delay. Post-weaning behavioural tests showed that there was no effect on learning of low grade tasks, motor function (rotorod), and activity level (open field). Some changes were found in more difficult tasks, including the reversal procedure in Morris water maze and in operant delayed special alternation.

In a subsequent inhalation developmental toxicity study, Mol:WIST rats were whole body exposed to 0 and 165 ppm NMP (0 and 680 mg/m^3) for 6 hr/day on gestation day 4 to 20 (Hass *et al*., 1996; based on the French classification proposal, 2002). No maternal toxicity was reported (mortality, clinical signs, no reduction in food consumption and in body weight changes, including weight gain corrected from uterus weight). There were significantly more dams with pre-implantation loss (11/20 and 20/23 at 0 and 165 ppm, respectively). However, there were no significant differences in the incidence of pre-implantation loss/litter (13.4 and 20.5 % at 0 and 165 ppm) and in the number of implantations. No effect on corpora lutea, live fetuses and resorptions. A slight decrease in fetal body weight (significant difference only when adjusted for litter size) was observed. The incidence of bones showing delayed ossification tended to increase, which was significantly higher for digits and cervical vertebrae. There were no treatment-related malformations observed.

In a 2-generation inhalation study, Crl:CD rats were inhalation (whole body) exposed to 0 and 116 ppm NMP (0 and 478 mg/m^3) for 6h/day, 7 days/week (Solomon *et al*., 1995). No adverse effects on the indices of reproductive performance of males and females were detected (i.e. mating performance, fertility, gestation length, and number of offspring delivered and carried through the lactation period). An exposure related reduction in response to sound was noted at 116 ppm in the first generation. No other signs of NMP-related toxicity were observed among parental rats (e.g. body weight, weight of testes and ovaries, and histological examination of the reproductive organs). At 116 ppm, a slight decrease in the body weight of the F1 offspring was seen at birth that persisted till weaning.

Dermal

In the first dermal prenatal developmental toxicity study (Food and Drug Research Laboratories (FDRL), 1979), NMP was administered dermally to Sprague-Dawley rats during gestation day 6 through 15 during 8 hours per day. NMP was dosed at 0, 75, 237 and 750 mg/kg bw/day to 25 females per dose group. The test compound was applied unchanged under open conditions to a shaven skin area of 25 cm² at the back of each animal for eight hours daily from gestation day 6 through day 15. The dams were fitted with collars to prevent ingestion of the test compound. Maternal toxicity, presented as a reduction in body weight, was observed at 750 mg/kg bw/day. Food consumption was not measured. The average number of live fetuses was statistically significantly decreased in the high dose group and linked to that the number of resorptions was increased. Further, the fetal weights in the high dose group were significantly reduced. Examination of skeletal and sof tissue abnormalities revealed an increased incidence of missing sternebrae, extra

ribs, incomplete ossification of vertebrae and incomplete closure and fusions in the skull at 750 mg/kg bw/day. The NOAEL for maternal and developmental toxicity was 237 mg/kg bw/day.

In the second dermal prenatal developmental study (BASF AG, Department of Toxicology, 1993) Himalayan rabbits (15/dose group) were dermally exposed on the intact shaven skin (using a semiocclusive dressing) to 0, 100, 300 and 1000 mg/kg bw/day NMP for 6 hours daily during gestation day 7 through 19. No treatment-related clinical signs were observed in the pregnant dams and maternal body weights and food consumption were not affected. No substance-related differences in conception rate, implantation sites or in the number of postimplantation losses, resorptions and viable fetuses were observed. The occurrence of accessory 13^{th} rib(s) clearly increased in the 1000 mg/kg bw.day group. In addition, the incidence of incomplete ossification of sacral vertebral arches and the talus was increased. The NOAEL for maternal and developmental toxicity was 1000 and 300 mg/kg bw/day respectively.

In a developmental toxicity study, Sprague-Dawley rat (22-24 pregnant females/dose) were dermally exposed to 0, 75, 237 and 750 mg/kg bw/day NMP on gestation day 6 to 15 (Becci *et al*., 1982). The test compound was applied to a shaven skin area of 25 cm² at the back of each animal for eight hours daily from gestation day 6 through day 15. The dams were fitted with collars to prevent ingestion of the test compound. A preceding dose-finding study (3-5 pregnant females/dose; 500, 1100, and 2500 mg/kg) showed that at 2500 mg/kg all dams died or aborted prior to caesarean. At 1100 mg/kg, maternal body weight gain was depressed during gestation, and 4 out of 5 litters completely resorbed. At the lowest dose of 500 mg/kg, no evidence of adverse effects on the mother and the conceptus were observed. In the main study, patches of dry skin at the application site were observed, with a severity increasing with the dose. At the dose of 750 mg/kg bw, a decrease in the maternal body weight gain was observed during gestation (no information available on maternal weight gain minus uterine weight on GD 21). No maternal effects were seen at 75 and 237 mg/kg. Developmental effects were observed at 750 mg/kg. Increase in the incidence of resorptions, decreases in the number of viable fetuses and in the fetal body weight (20%) were shown. Furthermore, delayed ossification of several bones (i.e. skull, hyoid, sternebrae, vertebrae) and increase in the incidence of extra ribs) were observed. Skeletal malformations including fused/split ribs (8 fetuses from 5 litters), and fusion of the exoccipital and atlas bones (4 fetuses from 4 litters). No increase in the incidence of soft tissue variations or malformations were observed. No treatment-related developmental effects were observed at doses of 75 and 237 mg/kg. The NOAELs for developmental toxicity and maternal toxicity were considered to be 237 mg/kg bw/day.

4.11.2.2 Human information

A case report is available in the public literature which describes a case of a late miscarriage in a woman who sustained both occupational dermal contact and repeated inhalational exposure to NMP throughout her first trimester of pregnancy (Solomon et al. 1996). The patient is a 23-year old pregnant woman, 9 weeks pregnant with her first child. The pregnancy so far had been uneventful. The patient did not smoke, drink alcohol, or use caffeine. She was on no medications except prenatal vitamins, which however did contain 4000 IU of vitamin A. Past medical history was completely negative. The patient was referred by her obstetrician to an occupational medicine physician for evaluation of concerns about possible chemical exposures at work. She worked as a laboratory technician at a company that develops, manufactures and sells custom chemicals. Her

work at the QC laboratory included operating two atomic spectrophotometers for analysing samples for production runs. Each solid sample to be analysed was dissolved in NMP. Approximately 1 L of NMP was used a day. There was local exhaust ventilation over the spectrophotometers, but there was no local ventilation over the countertop, on which the patient filtered NMP by pouring it from a 5-gallon container through an ion-exchange column. After evaluation of the workplace, her work assignment was not latered though she was given a half-face air-purifying respirator in addition to her other protective equipment (i.e. a laboratory coat, safety goggles, and latex gloves).

At around the 16th week of pregnancy, there was a spill of NMP at work, which the patient cleaned up. She noted that the latex glove she was wearing dissolved in the solvent and there was extensive direct skin contact to her hands and into a break in the skin. Over the next 4 days, the patient felt ill with malaise, headache, nausea, and vomiting. She saw her obstetrician 2 weeks later, and still had evidence of chemical stains on her hands at that time. Though it was recommended by the obstetrician that she be referred to an alternate job, she remained on the same job for 2 more weeks as the company responded there were no alternate jobs available. Altogether, she had daily exposure to NMP for an average of 42 hours each week until the 20th week of pregnancy.

Follow-up ultrasound examination 1 month later showed early intrauterine growth retardation (IUGR). Gestational age as determined by biparietal diameter was nearly 25 weeks, whereas humerus and femur length measurements, as well as abdominal circumference, corresponded with a 21-week gestational age. A follow-up ultrasound 3 weeks later confirmed the presence of IUGR. During this time, maternal weight gain was appropriate for gestational age. Thus the poor fetal growth cannot be attributed to insufficient maternal weight gain. On physical examination 2 weeks later, no fetal activity was detected, and no fetal heart sounds were identifiable by Doppler ultrasound. The patient was hospitalized for prostaglandin induction, and delivered a stillborn fetus. By the original ultrasound estimated date of confinement, this was the 31st week of gestation. Autopsy revealed a 430 g male fetus, with a crown-rump length of 25.0 cm, and which appeared clinically to be at 29 weeks of age. There were no identifiable abnormalities of the organs, although there was extensive autolysis. No chromosomal abnormalities could be idenitified. The placenta was small for the gestational age, weighing only 52 g. There were placental changes of villous fibrosis and focal acute chorionitis, which may have occurred ante- or post-mortem. The cord appeared normal with three vessels and no torsion.

An industrial hygiene evaluation (incl air sampling) of the QC laboratory was performed afterwards. Detectable concentrations of NMP were found in the technicians breathing zone and in an area sample. The authors stated that these results may not represent the same conditions that pertained when the patient had been working, but they do indicate a potential for inhalation exposure during usual tasks (Solomon et al., 1996).

4.11.3 Other relevant information

No other relevant information on developmental toxicity is available.

4.11.4 Summary and discussion of reproductive toxicity

1-Methyl-2-pyrrolidone is currently classified for developmental toxicity. This proposal only aims at removing the current SCL and not on changing the classification for reproductive toxicity. The GCL of 0.3% corresponding to a Repro 1B classification would then be applicable. Therefore, no summary and justification of the current classification is required. Only a justification of the removal of the SCL is required.

The criteria for setting SCLs for reproductive toxicity focus on the ED_{10} after oral exposure for effects fulfilling the classification criteria. Three studies were selected for analyses: oral rat developmental study (Saillenfait *et al.*, 2001), oral rabbit developmental study (International Research and Development Corp. (IRDC), 1991), and a 2-generation rat study (BASF AG, Department of Toxicology, 1999). A second 2-generation rat study (Huntingdon Life Science, 1999) was available, however, was not included for analyses as similar results were observed as in the first 2-generation rat study.

Developmental effects such as postimplantation losses, effects on the cardiovascular system and mortality fulfilled the classification criteria and were therefore included for the analysis of ED_{10} values (analyzed effects are highlighted in grey in tables 13-17). Effects such as reduced body weight of the pups and incomplete ossification were excluded.

The ED_{10} values for effects fulfilling the classification criteria were determined using bench mark dose software (PROAST). For comparison, also linear interpolation was used to calculate the ED_{10} values. The ED_{10} values are provided in table 19.

In the benchmark approach a dose-response model is fitted to the data, and this model is used for estimating the dose at a certain level of response. The benchmark dose (BMD) is a dose level, derived from the estimated dose-response curve, associated with a specified change in response (BMR) (Slob, 2002; EFSA, 2009). According to Kaylock et al. (1996) and Krewski et al. (2002), a minimum of 3 dose groups (incl. control) is needed to fit a good dose-response curve, based on data from developmental studies. As the number of dose groups in the developmental studies are 3 (incl. control) or more, this is considered sufficient.
Effects expressed in numbers of litters were excluded for BMD analyses, as no distinction can be made between the numbers of pups affected per litter. The number of affected litters is therefore not representative for the size of the effect and not useable for analyses of dose-responses. The same accounts for effects expressed in number of females with stillborn pups. No distinction can be made between females with one stillborn pup and females with 10 stillborns, therefore these data were excluded from further assessments.
More information on the BMD-analysis (including the software output) can be found in Annex 2 of this CLH-report.

Calculation of the ED_{10} values by linear interpolation can be found in Annex 3 of this CLH report.

The ED_{10} values calculated with the linear interpolation method are in the same range, though some changes are observed as compared to the ED_{10} values calculated with the BMD-analysis. Only two ED_{10} values calculated with linear interpolation are outside the 90% confidence interval of the ED_{10} as calculated with the BMD-analysis.

Linear interpolation uses only 2 data-points to determine the ED_{10}. In contrast, BMD-analysis uses all the data-points to determine the ED_{10} (BMD_{10}). For this reason the accuracy of the ED_{10}, as calculated with the BMD-analysis, is much higher. Further, by calculating the ED_{10} using BMD-analysis also information on the uncertainty is provided (i.e. the lower and upper 95% confidence limit of the benchmark dose).

Table 19: ED_{10} levels for reproductive toxicity endpoints for effects fulfilling the classification criteria based on data of oral animal studies on developmental toxicity.

Study	Endpoint	ED_{10} (mg/kg bw/day) (as calculated by BMD-analysis)[1]	ED_{10} (mg/kg bw/day) (as calculated by linear interpolation)
Rat, oral Saillenfait AM *et al.* (2001, 2002)	postimplantation loss (see table 13)	520 (504-540)	511
	cardiovascular malformations (see table 14)	528 (499-561)	517
	truncus arteriosus (see table 14)	626 (558-865)	601
Rabbit, oral International Research and Development Corp. (IRDC) (1991)	postimplantation loss (see table 15)	**225** (171-319)	**301**
	Interventricular septal defect (see table 16)	**337** (269-475)	**301**
	Bulbous aortic arch (see table 16)	**379** (304-495)	**328**
	Pulmonary trunk stenosis (see table 16)	401 (321-521)	**360**
Rat, oral 2-generation study BASF AG, Department of Toxicology (1999)	Pup (F1a-generation [2]) mortality (see table 17)	**360** (347/346-374/375)[3]	**226**
	Complete litters (F1a-generation [2]) lost at the end of the lactation period (see table 17)	**263** (203-328)	**205**
	Stillborn pups (F1a-generation [2]) (see table 17)	511 (501-615)	743

Values in bold are values corresponding to a medium potency group (i.e. boundaries: 4 mg/kg bw/day < ED_{10} value < 400 mg/kg bw/day)

[1] Values between brackets are BMDL and BMDU (i.e. the lower and upper 95% confidence limit of the benchmark dose)

[2] The BMD analyses was performed using both F1a and F1b. However, F1a was determinative for the BMD_{10}
[3] dependent on the model used for BMD-analysis

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

The substance has an entry in Annex VI of the CLP Regulation including Repr. 1B H360D with a specific concentration level (SCL) of 5%. The proposal of The Netherlands is to remove this SCL. The basis for the proposal is that the current guidance on setting SCLs is not deemed to support an SCl greater than the general concentration limit (GCL).

Data is presented from 16 studies in rats and rabbits for oral, dermal and inhalation routes. The ED_{10} (bench mark dose) in these studies ranges from 225 to 626 mg/kg bw/day. As the cut-off for low potency reprotoxicants in the CLP guidance is 400 mg/kg bw/day, the DS argues that the criteria for setting a higher SCL are not fulfilled.

Comments received during public consultation

Comments were received from seven MS, all in support of the proposal. During public consultation a new study (Sitarek et al, 2012) was submitted The study was summarised and discussed by the dossier submitter in the RCOM document.

Assessment and comparison with the classification criteria

Comparison with the criteria

1-Methyl-2-pyrrolidone (NMP) has a harmonised classification for developmental toxicity as Repr. 1B; H360D with a SCL of 5%. According to the data on developmental effects following exposure to NMP included in the CLH report by the DS, and based on an analysis of this data according to the guidance for setting SCLs in the CLP Guidance (November 2013) the SCL which is currently set at 5.0% should be deleted.

Sixteen reproductive toxicity studies were included in the CLH report by the DS, thirteen in rats and three in rabbits and these involved oral, inhalation and dermal exposure. Three of these studies were selected for deriving ED_{10} values. The ED_{10} value was also derived by the DS (as reported in the RCOM) from a study submitted during the public consultation (Sitarek et al., 2012). The ED_{10} value, according to the CLP Guidance, is the lowest dose which induces reproductive toxic effects which fulfil the criteria for classification of reproductive toxicity with an incidence or magnitude of 10% after correction of the spontaneous incidence.

RAC agrees with the DS on the reproductive toxicity studies selected for analysis. The key studies selected were a rat and rabbit developmental toxicity study and a rat 2-generation study, all involving oral administration. A second 2-generation study was also evaluated by the DS, but this study was not included in the analysis since similar results were observed to those in the first 2-generation study. These study reports included sufficient information to derive ED_{10} values according to the requirements in the CLP Guidance for setting SCL (section 3.7.2.5). The developmental effects used to derive ED_{10} values and which fulfilled the criteria for classification for developmental toxicity were post-implantation losses, effects on the cardiovascular system and foetal mortality. There were two main reasons for not including the other developmental toxicity studies with oral, inhalation or dermal exposure to NMP in the ED_{10} analysis. (1) developmental effects were not shown in these studies that fulfil the criteria for classification, and (2) developmental effects occurred at higher doses than in the studies included for deriving ED_{10} values. According to the Guidance for setting SCL, section 3.7.2.5.3.1:

"For both developmental effects and on sexual function and fertility, the lowest ED_{10} for the effect(s) that fulfils the criteria for classification in the different studies, is then used as the ED_{10} that determine the potency of that substance".

RAC agreed with the DS in the selection of the methods use to derive the required ED_{10} values, i.e. the benchmark dose software (PROAST) and calculation by linear interpolation. Both methods are described in the Guidance for setting the SCL (section 3.7.2.5.3). In the benchmark approach, a dose-response model is fitted to the data, and this model is used for estimating the dose at a particular level of response. The use of the bench mark dose software is considered to result in a more precise estimate of the ED_{10} because all data from the dose-response curve are used. The estimated ED_{10} values from the three selected key studies both calculated by the bench mark dose software (PROAST) and by linear interpolation are given below.

The ED_{10} values for the most severe developmental effects from the key studies selected by the DS derived by bench mark dose software were 520 mg/kg bw/day (post-implantation loss, Saillenfait AM et al., 2001, 2002), 225 mg/kg bw/day (post-implantation loss, IRDC, 1991) and 263 mg/kg bw/day (complete litters lost at the end of the lactation period in rats, BASF AG, Department of Toxicology, 1999). For the same developmental effects from the three key studies the ED_{10} values calculated by linear interpolation were 511 mg/kg bw/day (post-implantation loss, LOAEL 500 mg/kg bw/day), 301 mg/kg bw/day (post-implantation loss, LOAEL 540 mg/kg bw/day) and 205 mg/kg bw/day (complete litters lost at the end of the lactation period, LOAEL 500 mg/kg bw/day), which showed that the ED_{10} values were in the same range for both methods used. According to the CLP Guidance, the lowest ED_{10} value of all the key studies for effects warranting classification determines the overall ED_{10} of the substance. RAC agreed that for NMP this was the ED_{10} values of 225 mg/kg bw/day derived by PROAST for post-implantation loss in the developmental toxicity study in rabbits (IRDC, 1991), and 205 mg/kg bw/day derived by linear interpolation for complete litters lost at the end of the lactation period in rats (BASF AG, Department of Toxicology, 1999).

The ED_{10} values from the Sitarek et al. (2012) study submitted during Public Consultation were calculated by the DS by linear intrapolation based on pup mortality. The calculated ED_{10} values were 199 and 84 mg/kg bw/day for indices of pup viability on pnd 4 and on pnd 21, respectively. The ED_{10} values from the Sitarek et al. (2012) study were shown to be lower than the ED_{10} values included in the CLH report. However, there were some uncertainties concerning whether the effect on pup mortality was a true developmental effect since it could also be related to an effect of NMP during lactation.

The ED_{10} values included by the DS and in Sitarek et al. (2012) corresponded to the medium potency group (i.e. within the range: 4 mg/kg bw/day < ED_{10} value < 400 mg/kg bw/day) for NMP. Furthermore, the oral rabbit study and the rat 2-generation study included additional ED_{10} values corresponding to the medium potency group (i.e. 337 mg/kg bw/day for an interventricular septal defect, 379 mg/kg bw/day for a bulbous aortic arch, 263 mg/kg bw/day for complete litters lost at the end of the lactation period and 360 mg/kg bw/day for pup mortality).

According to the CLP Guidance (section 3.7.2.5.5) for setting SCL, modifying factors should also be considered when deriving a SCL. The modifying factors include type and severity of the effect observed, data availability (e.g. limitations in the database), dose-response relationship, mode or mechanism of action, toxicokinetics and bioaccumulation of substances. These modifying factors are used to account for case-specific situations where the data indicate that the potency group for a substance as obtained by the preliminary assessment should be changed. The modifying factors were assessed for NMP

as follows:

Type and severity of the effect:

The type of effects observed in reproductive toxicity studies following exposure to NMP included post-implantation loss, malformation and foetal mortality and were considered to be severe. However, the ED_{10} was not close to the boundary of a higher potency group (ie not close to 4 mg/kg bw/day). Therefore, this did not change the potency group.

Data availability:

The data available for NMP were considered more than adequate considering the REACH requirements and did not justify adaptation of the potency group.

Dose-response relationship:

NMP showed a steep dose-response relationship and no adaptation of the potency group was considered necessary.

Mode or mechanism of action:

No information was available on the mode or mechanism of action of NMP for the induction of developmental effects. Therefore adaptation of the potency group was not necessary.

Toxicokinetics:

The lowest ED_{10} derived by the most precise method (PROAST) was from the rabbit oral developmental toxicity study (225 mg/kg bw/day) and a comparison of the kinetics of NMP after oral exposure in rabbit and human (if known) should be taken into account for the determination of the potency group for NMP. For humans, some information was available on the kinetics of NMP after oral exposure. However, this was limited to a study assessing the metabolic pathway of NMP. For rabbits, information on the kinetic profile of NMP after oral exposure was not found. A comparison between kinetics in humans and rabbits after oral exposure to NMP is therefore not considered possible. Therefore, no adaptation is needed.

Bio-accumulation of substance:

NMP was not considered to be a bio- accumulating substance from the data available in the CLH dossier and from the registration dossier.

Conclusion on modifying factors:

Based on the available data, RAC considered that no modifying factors were necessary which could affect the assessment of the potency of NMP. Therefore, NMP was considered a medium potency reproductive toxicant.

Conclusion

RAC agrees that the data for setting SCLs for developmental toxicity for NMP clearly shows that NMP corresponds to the medium potency group (i.e. boundaries: 4 mg/kg bw/day < ED_{10} value < 400 mg/kg bw/day, CLP Guidance table 3.7.2-d). According to CLP Guidance table 3.7.2-e, the GCL of 0.3% should be applied for NMP. RAC therefore considers that the current SCL of 5% should be removed and the GCL should be applied for NMP.

References
Sitarek K., Stetkiewicz J., Wasowicz W., 2012. Evaluation of Reproductive Disorders in Female Rats Exposed to N-Methyl-2-Pyrrolidone. Birth Defects Res (Part B) 95:195-201, 2012.

4.11.5 Comparison with criteria

Currently, 1-methyl-2-pyrrolidone is classified with a specific concentration limit (SCL) of 5% for Repr. 1B, H360. However, a removal of the specific concentration limit (SCL) for developmental toxicity (Repr. 1B; H360), which would then result in a GCL of 0.3%, according the 'Guidance on the Application of the CLP Criteria', is proposed as follows:

Based on the available data from the oral animal studies on developmental toxicity (as described in 4.11.2.1 and Table 12), the reproductive toxicity dose descriptor ED_{10} (effective dose with a 10% effect level above the background) was established for a number of effects warranting classification. This was done by calculating the bench mark dose (BMD) for a 10% change in response. Table 19 presents the calculated ED_{10} levels for effects fulfilling the classification criteria for the animal (oral) studies on reproductive toxicity.

For each of the analysed oral animal studies, i.e. oral rat study (Saillenfait *et al*., 2001), oral rabbit study (International Research and Development Corp. (IRDC), 1991), and the rat 2-generation study (BASF AG, Department of Toxicology, 1999), the most conservative ED_{10} values were 520 mg/kg bw/day (postimplantation loss), 225 mg/kg bw/day (postimplantation loss), 263 mg/kg bw/day (complete litters lost at the end of the lactation period), respectively. The lowest ED_{10} value of all the studies for effects warranting classification is determinative for the overall ED_{10} of the substance. For NMP this is the ED_{10} of 225 mg/kg bw/day for postimplantation loss in the developmental study in rabbits (IRDC, 1991). This ED_{10} value corresponds to the medium potency group (i.e. boundaries: 4 mg/kg bw/day < ED_{10} value < 400 mg/kg bw/day) for 1-methyl-2-pyrrolidone. Furthermore, the oral rabbit study and the rat 2-generation study included additional ED_{10} values corresponding to the medium potency group (i.e. 337 mg/kg bw/day for interventricular septal defect, 379 mg/kg bw/day for bulbous aortic arch, 263 mg/kg bw/day for complete litters lost at the end of the lactation period, 360 mg/kg bw/day for pup mortality). According to the 'Guidance on the Application of the CLP Criteria' (paragraph 3.7.2.5.5) modifying factors (i.e. for type of effect or severity, data availability, dose-response relationship, modes or mechanism of action, toxicokinetics, and bio-accumulation of substances) can be applied to account for case-specific data situations which indicate that the potency group for a substance as obtained by the preliminary assessment should be changed. The type of effect (postimplantation loss and malformation) could be considered as severe. However, the ED_{10} is not close to the border of a higher potency group (not close to 4 mg/kg bw/day). Therefore, this does not change the potency group. The available data for NMP is considered more than adequate compared to the REACH requirements and does not justify adaptation of the potency group. NMP shows a steep dose response relationship not warranting adaptation of the potency group. No information is available on the mode or mechanism of action of NMP for the induction of developmental effects. Therefore adaptation of the potency group is not necessary. As the lowest ED_{10} (225 mg/kg bw/day) is derived from the rabbit oral developmental toxicity study (IRDC, 1991), comparison of the kinetics of NMP after oral exposure in rabbit and human (if known) should be taken into account when determining the potency group of a substance. For humans some information is available on the kinetics of NMP after oral exposure, however this is limited to a study focused on the metabolic

pathway of NMP. For rabbits, information on the kinetic profile of NMP after oral exposure was not found. A comparison between kinetics in humans and rabbits after oral exposure is therefore not possible. Therefore, no adaptation is needed. NMP is not an accumulating substance as indicated by the available information in the registration dossier. Based on the available data, no modifying factors are considered necessary which can affect the preliminary potency. Therefore, NMP is considered a medium potency reproductive toxicant.

According to the criteria in the 'Guidance on the Application of the CLP Criteria' (as described in tables 3.7.2.5.4 and 3.7.2.5.5 of this guidance) a CL of 0.3% can thus be assigned. As Repro 1B classification has a GCL of 0.3%, this means that the current SCL of 5% should be removed.

4.11.6 Conclusions on classification and labelling

No conclusion on the classification is required as this proposal only concerns a change in SCLs for reproductive toxicity.

Based on the information available for NMP showing multiple ED_{10} levels for developmental effects between 4 and 400 mg/kg bw/day and no modifying factors affecting the preliminary potency, NMP is of medium potency and the current SCL of 5% for developmental toxicity of 1-methyl-2-pyrrolidone should be reduced to a level of 0.3%. However, as a Repro 1B classification has a GCL of 0.3%, this means that the SCL of 5% should be removed.

4.12 Other effects

Not applicable

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

4.12.1.2 Immunotoxicity

4.12.1.3 Specific investigations: other studies

4.12.1.4 Human information

4.12.2 Summary and discussion

4.12.3 Comparison with criteria

4.12.4 Conclusions on classification and labelling

5 ENVIRONMENTAL HAZARD ASSESSMENT

Not applicable

5.1 Degradation

5.1.1 Stability

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

5.1.2.2 Screening tests

5.1.2.3 Simulation tests

5.1.3 Summary and discussion of degradation

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

5.2.2 Volatilisation

5.2.3 Distribution modelling

5.3 Aquatic Bioaccumulation

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

5.3.1.2 Measured bioaccumulation data

5.3.2 Summary and discussion of aquatic bioaccumulation

5.4 Aquatic toxicity

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

5.4.1.2 Long-term toxicity to fish

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

5.4.2.2 Long-term toxicity to aquatic invertebrates

5.4.3 Algae and aquatic plants

5.4.4 Other aquatic organisms (including sediment)

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

6 OTHER INFORMATION

7 REFERENCES

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ANNEXES

Annex 1. Justification of current classification for developmental toxicity

A. Revision of the French proposal for harmonised classification, 2002.

B. Annex of Revision of the French proposal for harmonised classification, 2002

C. Minutes of the meeting of the Commission Working Group on the Classification and Labelling of Dangerous Substances, Ispra, 17-19 November 2003.

D. Minutes of the Meeting of the Technical Committee C&L on the Classification and Labelling of Dangerous Substances, Arona, 15-18 March 2005.

Annex 2. BMD-analysis of reproductive studies for NMP.

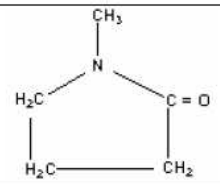
Annex 3. Calculation of ED10 values by linear interpolation

Annex 1. Justification of current classification for developmental toxicity

A. *Revision of the French proposal for harmonised classification, 2002.*

Relevant parts concerning 1-methyl-2-pyrrolidone were copied.

1. IDENTIFICATION OF THE SUBSTANCE

INDEX N° 606-021-00-7	EC. N°212-828-1	CAS n° 872-50-4	ID N°
1.1. *EINECS Name* If not in EINECS IUPAC Name	1-methyl-2-pyrrolidone		
1.2. SYNONYMS (state ISO name if available)	N-methyl-2-pyrrolidone; N-methylpyrrolidinone; N-methylpyrrolidone		
1.3. MOLECULAR FORMULA	C_5H_9NO		
1.4. STRUCTURAL FORMULA			
1.5. *PURITY (wt/wt)*	> 99 %		
1.6. *SIGNIFICANT IMPURITIES OR ADDITIVES, THEIR CONCENTRATIONS (wt/st)*	*Dimethylpyrolidones (mixtures of isomers) < 0.4 %* *Methylamine < 0.005 %* *γ-Butyrolactone < 0.05 %* *Water < 0.05 %*		
1.7. KNOWN USES for polymers.	INDUSTRIAL : Petrochemical industry. Formulating agent for dyes, insecticides and herbicides. Aqueous paints. Stripping and cleaning appplications. Solvent GENERAL PUBLIC : Varnishes stripper; graffiti removal; penetration enhancer for drugs; cosmetics. Belongs to HPVC's		
1.8. CURRENT CLASSIFICATION 19th ATP	Xi ; R 36/38 C ≥ 10 % Xi; R36/38		
1.9. PROPOSED CLASSIFICATION	[Xn; R48/20] or [R37] Xi; R36/38 [Repr. Cat. 3; R62] Repr. Cat.2; R61		
1.10 PROPOSED LABEL SYMBOL(S) :	R-PHRASE(S) : T R 61-62-36/38-48/20		

	S 53-45

4 TOXICOLOGICAL DATA (indicate conclusions and bibliographical references)

4.5. FERTILITY

Species	Route	Dose	Exposure time	Number of generations exposed	Observations and Remarks	Ref.
Rats (10 males and 20 females/dose) (Crl:CD)	Whole body inhalation (vapours)	10, 51 and 116 ppm (i.e. 41, 210, 478 mg/m³) with an additional control (air) The authors indicated that 116 ppm was the highest concentration possible without formation of aerosols under their experimental conditions	6 hr/day, 7 days/week Males: pre-mating and mating periods (Total > 100 days) Females : pre-mating, mating gestation, lactation (Total > 106 days). (Interruption from GD 20 to Day 4 post-partum).	2 generations	No adverse effects on the indices of reproductive performance of males and females were detected (i.e. mating performance, fertility, gestation length, and number of offspring delivered and carried through the lactation period). An exposure related reduction in response to sound was noted at 116 ppm in the first generation. No other signs of NMP-related toxicity were observed among parental rats (e.g. body weight, weight of testes and ovaries, and histological examination of the reproductive organs). At 116 ppm, a slight decrease in the body weight of the F1 offspring was seen at birth that persisted till weaning.	Solomon et al. 1995

Rats Wistar 25 males and 25 females	Oral diet	0-50-160-500 (originally)/ 350 (later) mg/kg/day the dose level was reduced to 350 mg/kg BW/day from the 126 th day because of pup toxicity at the 500 mg/kg/day	18 weeks	2 generations	At 500mg/kg/day, in the P1 generation: - Decreased in body weights and feed consumption at the end of gestation (day 20) and the beginning of lactation (day 1). (It cannot be excluded that the reduction in the offspring weight may have contributed to the decrease in maternal weight at the end of gestation). - No change in body weight for male. - Statistically significantly increased number of stillborn F1a pups and kidney weights in P1 males. - Statistically significantly decreased in body weight and in number of liveborn. - Significant decreased in mean litter size at day 4. At 500 mg/kg/day, in the F1 generation: Decrease in mean litter size, pup survival and pup body weights during lactation. At 350 mg/kg/day: no maternal toxicity or reduced pup survival, no adverse effects in the P1 and F1 (only F1b is exposed at 350 mg/kg) generation including mortality, body weights, feed consumption, clinical observations, reproductive performance or fertility. No effects on histological male and female in the P1. At 350mg/kg/day, in the F2 generation: decreased in the number of pups surviving lactation, and in pup body weights. No changes in microscopic evaluations of reproductive tissues as well in sperm assessments (P1-F1). Post weaning developmental landmarks (day of preputial separation and vaginal opening) determined in F1 generation was not affected. NOAEL for reproductive performance and fertility is 350 mg/kg/day for the F0 and F1 parental rats. NOAEL for developmental toxicity could be fixed at 160 mg/kg/day for F1 and F2 progeny.	BASF, 1999

Rats (30/sex/group)	Oral, diet	0, 50, 160 and 500 mg/kg	10 days prior to mating and continuing throughout mating, gestation and lactation for both generations	2 generations	Maternal toxicity was reported as reduced food intake, body weight, and/or body weight gain in the F0 and F1 generations at 500 mg/kg. There was evidence of developmental toxicity in both generations at 500 mg/kg, as evidenced by reduced litter size, reduced postnatal survival and pup weight. Significant reductions in the male fertility index and the female fecundity index of the F1 generation were also reported, without a clear NOAEL. Clear adverse effects were found at 500 mg/kg). *(Fertility index of males mated twice : 93-83, 72-69, 72-60, and 47-35 in the control, low-, mid- and high-dose groups, respectively. Fecundity index of females mated twice : 96-93, 82-74, 75-64, 61-50 in the control, low-, mid-, and high-dose groups, respectively)* There was also an increased incidence of F1 females with decreased corpora lutea at the high-dose. Histological changes were noted at the high dose. There was a reduced incidence of females with pigmented macrophages, which usually occur at the implantation sites (24, 18, 19, and 11 dams at 0, 50, 160, and 500 mg/kg, respectively). This correlates well with the findings that 1, 6, 5, and 13 animals at 0, 50, 160, and 500 mg/kg, respectively, never became pregnant. There was also an increased incidence of females with reduced corpora lutea in the treated groups (1, 3, 5, and 17 dams at 0, 50, 160, and 500 mg/kg, respectively). Hypospermia was observed in 3 males at 500 mg/kg. Diffuse bilateral testicular atrophy was observed in 1 male at the mid-dose and in 3 males at the high-dose (0 in control). Diffuse unilateral atrophy occurred in 1 male at 50 mg/kg and in 1 male at 160 mg/kg. No other information is available.	Cited by OEHHA 1999

Rats (30/sex/group)	Oral, diet	0, 50, 160 and 500 mg/kg	10 days prior to mating and continuing throughout mating, gestation and lactation for both generations	2 generations	Maternal toxicity was reported as reduced food intake, body weight, and/or body weight gain in the F0 and F1 generations at 500 mg/kg. There was evidence of developmental toxicity in both generations at 500 mg/kg, as evidenced by reduced litter size, reduced postnatal survival and pup weight. Significant reductions in the male fertility index and the female fecundity index of the F1 generation were also reported, without a clear NOAEL. Clear adverse effects were found at 500 mg/kg). *(Fertility index of males mated twice : 93-83, 72-69, 72-60, and 47-35 in the control, low-, mid- and high-dose groups, respectively. Fecundity index of females mated twice : 96-93, 82-74, 75-64, 61-50 in the control, low-, mid-, and high-dose groups, respectively)* There was also an increased incidence of F1 females with decreased corpora lutea at the high-dose. Histological changes were noted at the high dose. There was a reduced incidence of females with pigmented macrophages, which usually occur at the implantation sites (24, 18, 19, and 11 dams at 0, 50, 160, and 500 mg/kg, respectively). This correlates well with the findings that 1, 6, 5, and 13 animals at 0, 50, 160, and 500 mg/kg, respectively, never became pregnant. There was also an increased incidence of females with reduced corpora lutea in the treated groups (1, 3, 5, and 17 dams at 0, 50, 160, and 500 mg/kg, respectively). Hypospermia was observed in 3 males at 500 mg/kg. Diffuse bilateral testicular atrophy was observed in 1 male at the mid-dose and in 3 males at the high-dose (0 in control). Diffuse unilateral atrophy occurred in 1 male at 50 mg/kg and in 1 male at 160 mg/kg. No other information is available.	Cited by OEHHA 1999

By oral route in rat, there was evidence of reproductive toxicity at 500 mg/kg in one study (male and female fertility was affected) (OEHHA, 1999). Histological changes were observed at very high dose in subacute and subchronic toxicity studies

(higher than 1000 mg/kg). In a 2-years study in rat lesions in reproductive tract and testicular effects were reported at 678 mg/kg.
In other recent studies conducted on rats, no effect on fertility parameters, either no change in histological reproductive organs was observed at 350 mg/kg (BASF 1999, Huntington Life Science 1999).
Thus a classification **Repr. Cat. 3; R 62** could be discussed.

4.6. DEVELOPMENTAL TOXICOLOGY

Species	Route	*dose mg/kg/day ppm **Conc. mg/m³	Exposure time (hr/day)	Exposure period : number of days during pregnancy	Observations and remarks	Ref.

Sprague-Dawley rats (22-24 pregnant females/dose)	Dermal (99.9 % pure)	75, 237, and 750 mg/kg/day, with an additional negative control group (water) and two positive control groups (one by gavage and one by dermal application) Not occlusive (25 cm^2)	8hr/day	GD 6 to 15	<u>Dose range finding study</u> (3-5 pregnant females/dose; 500, 1100, and 2500 mg/kg) At 2500 mg/kg : all dams died or aborted prior to caesarean. At 1100 mg/kg : Depressed maternal weight gain during gestation, 4/5 litters completely resorbed. At 500 mg/kg : No evidence of adverse effects on the mother and the conceptus. <u>Main Study</u> (75, 237, and 750 mg/kg/day) Maternal toxicity : - Patches of dry skin at the application site, the severity of which increased with the dose. - At the high dose, decrease in the body weight gain during gestation. No information available on maternal weight gain minus uterine weight on GD 21. - No maternal effects at 75 and 237 mg/kg. Developmental toxicity : - At 750 mg/kg: Increase in the incidence of resorptions, decreases in the number of viable fetuses and in the fetal body weight (20 %). Delayed ossification of several bones (i.e. skull, hyoid, sternebrae, vertebrae) and increase in the incidence of extra ribs. Skeletal malformations including fused/split ribs (8 fetuses from 5 litters), and fusion of the exoccipital and atlas bones (4 fetuses from 4 litters). No increase in the incidence of soft tissue variations or malformations. - No treatment-related effects at 75 and 237 mg/kg. NOAEL for developmental toxicity : 237 mg/kg/day. NOAEL for maternal toxicity : 237 mg/kg ? The lower maternal weight may be due, at least partly, to the increased resorption rate and the lower fetal body weight.	Becci et al. 1982
Rabbits (Himalayan) 15/dose	Dermal	100, 300, and 1000 mg/kg (40% aqueous NMP) with an additional control (vehicle)	Semi-occlusive dressing 6hr/day	GD 7 to 19	There were no signs of maternal toxicity (death, food consumption, body weight, uterus weight), nor local effects at the application site. There was a significant increase in the incidence of foetuses with skeletal alterations, due to the occurrence of accessory 13th ribs. At 1000 mg/m^3,	BASF 1993, cited in HSE 1997 and IUCLID

					their fetal and litter incidences were 15% and 60%, respectively (historical value 8.4 and 40 %, respectively). There was no effect on fetal body weight, nor on the incidence of external, soft tissue and skeletal malformations. No effects were observed at 300 mg/kg.	
Rats (25/dose) (Crl:CD)	Whole body inhalation (100 % pure)	100 and 360 mg/m³ with an additional control (air). Aerosol	6hr/day	GD 6 to 15	Sporadic lethargy and irregular respiration was found in several dams, at both levels, only during the 3 first days of exposure. No adverse effects on maternal and fetal body weight, nor increases in the incidences of resorptions and of malformations and variations (external, soft tissue and skeletal).	Lee at al. 1987
Rabbits	Inhalation head only	200, 500 and 1000 mg/m³ (aerosol) (50% relative humidity)	6hr/day	GD 7 to 19	Preliminary study (5 animals/dose) (external fetal examination only) 300, 1000 and 2000 mg/m³ : No signs of toxicity in dams. Slight but not significant changes in maternal liver weights. Increase in maternal clotting times at 1000 mg/m³. Small but dose-related decrease in gravid uterine weight (99, 90, 82 and 71 g at 0, 300, 1000, and 2000 mg/m³. Concomitant findings included a dose-related decrease in the number of fetuses (which attained statistical significance at the high dose), and a statistical increase in post-implantation loss at 2000 mg/m³. Main study (15 animals/dose) 200, 500 and 1000 mg/m³ : (Vapour/aerosol : Mass Media Aerodynamic Diameter 2.7-3.5 µm) No effects on maternal body weight (corrected and uncorrected for uterus weight), food consumption, uterus weight. No effect on implantations, number of resorptions and live fetuses, and fetal body weight. No increase in the incidence of external, visceral or skeletal malformations. Clear increase in the incidence of accessory ribs at 1000 mg/m³. Smaller non-statistically significant increase in this parameter at 500 mg/m³. This was considered a common finding in rabbits, although the incidence was much higher than that of the concurrent value (80% at 1000 mg/m³ /32 % in control).	BASF 1991, 1993 Cited in HSE 1997 and IUCLID
Rats (14-16	Whole body	151 ppm (i.e. 620 mg/m³)	6 hr/day	GD 7 to 20	No effects on maternal weight gain during gestation, on the gestation	Hass et al. 1994

litters/dose) (Mol :WIST)	inhalation (≥99.5 % pure)	with an additional control (clean air)		Behavioural developmental toxicity study	length, on the number of pups and on neonatal death. Reduced body weight of litters from birth throughout weaning. This decrease was no longer present after the age of 5 weeks. Slight delay in some pre-weaning development milestones and reflexes (i.e. ear unfolding, surface righting reflex, incisor eruption, eye opening). Post-weaning behavioural tests : There was no effect on learning of low grade tasks, motor function (rotorod), and activity level (open field). Some changes were found in more difficult tasks, including the reversal procedure in Morris water maze and in operant delayed special alternation. The investigators questioned about a possible relationship between the partly transient decrease in body weight and delay in physical development.	
Rats (20-23 pregnant females) (Mol :WIST)	Whole body inhalation (≥99.5% pure)	165 ppm (i.e. 680 mg/m³) (highest technically possible concentration, 40-50 % relative humidity in the inhalation chambers) with an additional control (air). Primarily vapour phase	6 hr/day	GD 4 to 20 (vaginal plug = GD 1)	No maternal toxicity reported (mortality, clinical signs, no reduction in food consumption and in body weight changes, including weight gain corrected from uterus weight). There were significantly more dams with pre-implantation loss (11/20 and 20/23 at 0 and 165 ppm, respectively) . However, there were no significant differences in the incidence of pre-implantation loss/litter (13.4 and 20.5 % at 0 and 165 ppm) and in the number of implantations. No effect on corpora lutea, live fetuses and resorptions. Slight decrease in fetal body weight (significant difference only when adjusted for litter size). The incidence of bones showing delayed ossification tended to increase. It was significantly higher for digits and cervical vertebrae. No treatment-related malformations.	Hass et al. 1995
Rats (10 males and 20 females) (Crl:CD)	Whole body inhalation (vapours)	116 ppm (i.e. 478 mg/m³) with an additional control (air) The authors indicated that 116	6 hr/day, 7 days /week Part of a two-generation study	Males: pre-mating and mating periods (total > 100 days) Females : pre-mating,	The maternal body weight was not affected. The fetal body weight was significantly lower than control (7%). Pup body weight at birth was also reduced in the two-generation study.	Solomon et al. 1995

		ppm was the highest concentration possible without formation of aerosols under their experimental conditions		mating, and GD 0 to 20	There were no differences in the incidences of malformations and variations (external, visceral and skeletal).	
Sprague-Dawley rats (20-26 pregnant females/dose)	Whole body inhalation	0-30-60-120 ppm, (i.e. 124, 247, and 494 mg/m³) with an additional control (air). Vapours (40% relative humidity in the inhalation chambers)	6h/day	GD 6-20	All the animals survived the exposure. <u>Body weight change</u>: - Concentration-related reductions in maternal BW gain. - Significantly reduced during the first half of exposure at 60 and 120 ppm. No significant difference in corrected weight gain. <u>Food consumption:</u> - Concentration-related reductions in food intake. - Significant decrease in food consumption on GD 13-21 at 120 ppm. <u>Fetal BW:</u> - Concentration related decrease. - Statistically significantly at 120 ppm (5-6 % lower than control). No treatment related malformations. NOAEL for maternal toxicity is 30 ppm. NOAEL for developmental toxicity is 60 ppm. Inhalation exposure of pregnant rats to NMP (vapours) during the entire postimplantation phase of gestation is neither teratogenic nor embryolethal. Evidence of developmental toxicity was limited to intrauterine growth retardation that occurred in the presence of maternal toxicity.	Saillenfait, Gallissot, Morel, 2002
Rats Sprague-Dawley	gavage	332 and 997 mg/kg With an additional control	Daily	GD 6 to 15	At 332 mg/kg : Maternal body weights were not reported. Placental and foetal weight was lower than control (14-20% and 10% respectively). There was no difference in implantation rate, litter size or resorptions. At 997 mg/kg : Marked reductions in maternal body weight and placental weight were observed. There was a large number of resorptions (24/29 dams showed complete resorption) and only 15 live and 1 dead foetus were present at term. Observations in the live fetuses included reduction in fetal weight (37%), malformations considered as indicative of foetal retardation in 8 out of 15 foetuses), and 14 runts. No other information is available.	EPA 1987, cited in HSE 1997 and IUCLID

Rabbits (15-20/dose)	Gavage	55, 175, and 540 mg/kg/day	Daily	GD 6 to 18	- Maternal toxicity : Decreased food intake and weight gain during dosing at 175 and 540 mg/kg. - Developmental toxicity : At 540 mg/kg: Increased incidences of resorptions. Cardiovascular malformations and malformed skull bones. Increased incidence of misshapen skull bones and of 27 presacral vertebrae. NOAEL for maternal toxicity: 55 mg/kg/day. NOAEL for developmental toxicity: 175 mg/kg/day. No other information is available.	Cited by OEHHA 1999
Rats (Crl:CD)	Gavage (100 % pure)	40, 125 and 400 mg/kg/day with an additional vehicle control (water)	Daily 5ml/kg	GD 6 to 15	- Maternal toxicity : There was no treatment-related clinical observations. Body weight gain was depressed during treatment at 400 mg/kg (GD 6-9, GD 9-12, GD 6-15) (14, 18, and 53 g, respectively at 0 mg/kg compared to 7, 15, and 42 g, respectively at 400 mg/kg). However, there was no statistical difference in weight gain during the overall gestation period (GD 0-21) and after correction for gravid uterine weight. No changes in food consumption. - Developmental toxicity : At 400 mg/kg : Reduced fetal body weight (10-11 %) and an increased incidence of stunted fetuses (fetuses: 1/340, 1/393, 2/395, and 12/397; litters : 1/21, 1/25, 2/24, and 6/25; at 0, 40, 125 and 400 mg/kg, respectively). No teratogenic effects. NOAEL for maternal and developmental toxicity : 125 mg/kg/day	Exxon 1992
Rats Sprague-Dawley	Gavage (5 ml/kg) (≥ 99.5% pure)	125, 250, 500 and 750 mg/kg/day with an additional vehicle control group (water)	Daily (5ml/kg)	GD 6 to 20	Two dose-range finding studies were conducted (10-16 pregnant rats/dose, fetal external examination only) - First study : 500, 1000, and 1500 mg/kg. No test dams died. Maternal body weight gains were depressed at all doses. Administration of 1000 and 1500 mg/kg resulted in complete early resorptions in all litters. Significantly increased embryolethality (13.8 % resorptions versus 4.7 % in the control group) and decreased foetal body weight were observed at 500 mg/kg. Four foetuses exhibited external malformations including imperforate anus and absence of tail (four cases), proboscis and cyclopia (one case).	Saillenfait Gallissot, Langonné, Sabaté . 2002

					- Second study : 500, 625 and 750 mg/kg. Decreases in maternal body weight gains during the treatment period occurred at all dose levels. A dose-dependent increase in the percentage of resorptions per litter and a decrease in the foetal body weight were noted. There were one foetus with imperforate anus and absence of tail at 500 mg/kg, and three foetuses with anasarca at 625 mg/kg. <u>Main study</u> (125, 250, 500 and 750 mg/kg) - Maternal toxicity : No adverse effects at 125 and 250 mg/kg. At 500 and 750 mg/kg : No clinical signs of toxicity. Decreases in maternal body weight gain and food consumption (throughout treatment at the high dose), and reduction in absolute weight gain (38, 40, 34, 28 and 28 g, respectively at 0, 125, 250, 500, and 750 mg/kg). - Developmental toxicity : The incidence of resorptions was significantly higher than control at 500 mg/kg, and rose to 91% at 750 mg/kg (17/25 litters completely resorbed). The number of live fetuses was reduced at the high dose. The fetal body weight was depressed at doses ≥ 250 mg/kg (10, 30, and 47 % less than control at 250, 500, and 750 mg/kg, respectively). The overall incidence of malformed fetuses/litter and the percentage of litters containing at least one malformed foetus were significantly increased at 500 and 750 mg/kg. A number of external, visceral and skeletal malformations occurred only in NMP-treated groups, and a consistent dose-dependent trend was found in the incidence of these defects. NMP treatment was associated with an increased incidence of 2 types of external malformations: anasarca, and anal atresia associated with absent or vestigial tail. One or both were observed in <u>1 fetus at 250 mg/kg</u>, in 11 fetuses from 9 different litters at 500 mg/kg, and in 1 fetus at 750 mg/kg. Single instances of omphalocele, and of proboscis and cleft palate were also detected at 125 and 750 mg/kg, respectively. Heart and/or great vessels malformations (mostly persistent truncus arteriosis) were observed in 10	

					fetuses from 9 litters at 500 mg/kg, and in 6 fetuses from 4 litters at 750 mg/kg. Their incidence was significantly increased at these two doses. There was a significant increase in the incidence of fetuses and litters with skeletal malformations at 500 and 750 mg/kg. No individual skeletal malformation was statistically different from control. The most prevalent malformations were fusion or absence of cervical arches. In addition to missing caudal vertebrae, one fetus from the 500 mg/kg dose group showed no sacral centra, and another from a different litter exhibited missing thoracic, lumbar and sacral vertebrae and missing ribs. No external, visceral or skeletal malformation occurred at 125 mg/kg The percentage of fetuses with skeletal variations was significantly higher at 500 and 750 mg/kg. This was largely due to increased incidences of poorly ossified skull bones (frontals, parietals, and/or supraoccipital) and sternebrae. Although not significantly different, extra lumbar ribs was also observed more frequently. NOAELs for maternal toxicity and for developmental toxicity were 250 and 125 mg/kg/day, respectively. This study was conducted according to the current OECD and EU guidelines.	

Classification is justified for developmental toxicity, given that developmental effects consisted in embryolethal, teratogenic, and fetotoxic effects after oral (gavage) and dermal administration in rats, and after oral (gavage) treatment in rabbits.
In addition, fetal toxicity, expressed as reduced fetal body weight, occurred in the absence of significant maternal toxicity in rats treated by gavage. A fetus showing the characteristic malformations elicited by NMP was also observed at this dose level.

The developmental effects were specific and severe in regard to the maternal toxicity (ECBI/33/02).
It was considered unlikely that the embryolethality and the foetal malformations could have been secondary to the general toxicity of NMP.
Therefore, a classification **Repr. Cat.2; R 61** as a substance causing developmental toxicity is proposed.

Solomon et al. (1996) reported a human case of delayed foetal development followed by a stillbirth at week 31 of gestation. The mother worked in the electronics industry where she had used NMP and small amounts of acetone and methanol for the first 20 first weeks of pregnancy. Levels of exposure to NMP (presumably via dermal contact and inhalation) were not exactly known. Although these authors claimed that it raised concern, they could not conclusively link the exposure to NMP with this single case of stillbirth (Bower, 1997; Solomon et al, 1997).

ADDITIONAL DATA : TOXICOKINETIC/METABOLISM :
In rats, NMP is rapidly absorbed via inhalation, ingestion and dermal administration (Wells and Digenis 1988, Midgley et al. 1992, E.I. du Pont de Nemours 1995). Approximately 40 % of a topically applied dose (10 mg/kg, shaved rats) was absorbed. After inhalation exposure (nose-only), 7 - 9% of the exposed dose (10 or 100 ppm, vapours) were absorbed (E.I. du Pont de Nemours 1995). Once absorbed, NMP is widely distributed throughout the body, metabolized, and primarily eliminated in the urine (the majority within 24 hours after treatment), with negligible tissue residues remaining after 4-5 days post-dose. The major urinary NMP metabolite identified is 5-hydroxy-NMP, whatever route of administration.

NMP was shown to reach the foetus after whole body inhalation exposure of pregnant rats to 150 ppm NMP on GD 19 or 20. Comparable maternal and foetal blood levels of NMP were found (Ravn-Jonsen et al., 1992).

In vitro studies indicate that NMP has a high permeability through both human and rat skin (Ursin et al. 1995, Priborsky and Mühlbachova 1990).

Studies in workers and human volunteers have shown that NMP is readily absorbed by the inhalation, ingestion and dermal routes. NMP is extensively metabolized and only a minimal fraction of unchanged NMP is eliminated in urines. A metabolic pathway has been suggested for humans : NMP is first hydroxylated to 5-hydroxy-N-methyl-2-pyrrolidone (5-HNMP), and then oxidised to N-methylsuccinimide, which in turn is hydroxylated to 2-hydroxy-N-methylsuccinimide. 5-HNMP is the major urinary metabolite of NMP (Akesson and Paulsson 1997, Akesson and Jönsson 1997, 2000, Anundi et al. 2000, Jönsson and Akesson 2001).

B. ***Annex to Revision of the French proposal for harmonised classification, 2002*****.**
Relevant parts concerning reproductive toxicity of 1-methyl-2-pyrrolidone were copied.

E.C. Classification and Labelling Proposal : N-methyl-2-pyrrolidone

Reproduction toxicity :

1. Fertility:

1-1-Data from specific studies

A multigeneration study in rats by the oral route was reported with few details by OEHHA (1999) : NMP treatment was associated with a reduction in male and female fertility of the F1 generation. Clear toxic effects were found at 500 mg/kg.

In two-generation studies, the pup toxicity was confirmed at 350 and 500 mg/kg/day. No effects in relation to male and female fertility were observed at 350 mg/kg (BASF 1999, Huntingdon Life Science 1999).

In a two-generation inhalation study in rats, no effect on body, testis and ovary weights, or on reproduction ability of males and females was observed, up to 116 ppm (i.e. 478 mg/m³) (vapours) (Solomon et al. 1995).

In an inhalation developmental toxicity in rats, increased pre-implantation loss was attributed to NMP after exposure initiated before implantation (from GD 3, 165 ppm NMP/6hr/day, i.e. 680 mg/m³/6hr/day) (Hass et al. 1995).

1-2- Data from RTD studies

Studies with multiple administrations have been conducted in rats and mice by the oral (feed) and inhalation routes. Evaluations were limited to changes in the morphology and/or weights of the reproductive organs. No information on sperm parameters, stage specific analysis of the testis, and oestrous cycle is available. In general, when effects were observed, they occurred at high doses (> 1000 mg/kg by the oral route).

Oral

Histological changes in the testicular seminiferous tubules and decrease in the weight of testes were observed in male rats fed with 2.019 mg NMP/kg (i.e. 30000 ppm) for 4 weeks. These alterations were judged secondary to the reduction in the male body weight and food consumption (33 % and 31 %, respectively) that occurred at this dose (Malek et al. 1997). However, they may not be disregarded.

Male rats administered 2060 mg NMP/kg by gavage for 4 weeks showed clear testicular alterations. Body weight gain was also decreased (16 %) and symptoms of general toxicity were registered. Testicular effects were not reported at 1028 mg/kg (BASF 1978 cited in HSE 1997).

In 90-day studies, no pathological changes were found in the reproductive organs of male and female rats fed with 18000 ppm NMP (i.e. 1057 and 1344 mg/kg for males and females, respectively), or in mice fed with 7500 ppm NMP (i.e. 1931 mg/kg). Toxic effects, including reduced body weight and body weight gain, were observed in rats at this dose. (Malley et al. 1999).

In a two-year feeding study in rats, males given 678 mg NMP/kg showed lesions in reproductive tract and an increased incidence of small testes. Other toxic effects included reduced body weight and body weight gain, and severe chronic progressive nephropathy. No morphological changes were noted in the male reproductive organs at lower doses (i.e. 66.4 and 207 mg/kg) (Malley et al. 2001).

In a sub-chronic toxicity study (13 weeks) in dogs, feeding at levels up 250 mg/kg resulted in no pathological effects, nor changes in the weights of the testes and the ovaries (Becci et al. 1983).

Inhalation

In a two-year inhalation study, no treatment-related pathological lesions were found in the reproductive organs of male and female of rats exposed to 400 mg NMP/m³/6hr/day, 5 days/week (aerosol) (Lee et al. 1987).

In a 13-week study, rats exposed head only to 3000 mg/m³ NMP aerosol/vapour mixture showed decreased body weight gain (34 %) and absolute testes weight. Cell loss in germinal epithelium was also noted. Effects were not reversible after a 4-week recovery period. Such effects were not seen at 1000 mg/m³ (BASF 1994).

No clear-cut conclusions can be drawn from the studies available. However, there are limited, but appealing findings in some of the rat studies (e.g. abstract from OEHHA for male and female fertility, 4-week and 2-year toxicity studies for male fertility). Therefore, a classification of NMP for Toxic for Reproduction, category 3 may be considered.

2. Developmental toxicity:

2. 1. Inhalation

Five prenatal development toxicity studies were conducted (Three whole body with NMP vapours, and one whole body and another head only with NMP as an aerosol/vapour mixture).

After exposure of rats to NMP vapours, developmental effects consisted in slightly delayed ossification and/or decrease in foetal body weight. These effects were observed at concentrations approaching the highest reliable vapour concentration technically possible (116-165 ppm, i.e. 478-680 mg/m³) (Hass et al. 1995, Solomon et al. 1995, Saillenfait et al.2001).

No developmental toxic effects were reported in rats exposed whole body to NMP aerosols up to 360 mg/m³/6hr/day. Nevertheless, concentrations producing evidence of maternal toxic levels were not reached (Lee et al. 1987). In rabbits exposed head only to aerosols of NMP, an increase in accessory ribs, a common finding, was observed at 1000 mg/m³. Increased post-implantation loss occurred at 2000 mg/m³, while there was no evidence of maternal toxicity (BASF 1991, 1993)

There was no evidence of teratogenic effects.

2.2. Dermal

Developmental effects, including teratogenicity and embryolethality, were observed after dermal application (non occlusive) of 750 mg NMP/kg/day to rats. Thus, NMP treatment resulted in increased incidence of resorptions, fewer live fetuses, and skeletal malformations, including fused atlas and exoccipital bones, and split/fused ribs.
This dose also caused developmental delay, as evidenced by reduced fetal weight, and incomplete ossification of sternebrae, vertebrae, hyoid and skull bones.
The maternal weight gain was depressed during pregnancy at 750 mg/kg. However, it is not clear weather this decrease was related to maternal toxic effects and/or was consecutive to the reduced number of live foetuses and mean foetal weight (Becci et al. 1982).

Developmental toxic effects limited to an increase in 13th accessory ribs were reported in rabbits at the highest dose tested, 1000 mg/kg/day. However, this dose did not cause maternal toxicity either, and the authors used 40% aqueous NMP (BASF 1993).

2.3. Oral (gavage)

Rats
Unequivocal evidence of embryolethal and teratogenic effects were reported in rats in a well-designed and conducted study (Saillenfait et al. 2002).
The adverse effects of NMP on embryonic viability appeared at 500 mg/kg. Then, the dose-response for prenatal mortality appeared to be steep. Thus, the incidence of resorptions rose from 10% at 500 mg/kg to 91% at 750 mg/kg. These findings are sustained by the results of the pilot study in which rats showed 4, 18, 68, and 96 % resorptions at 0, 500, 625 and 750 mg/kg, respectively.

The incidence of malformed foetuses and of litters with malformed foetus was significantly increased at 500 and 750 mg/kg. NMP elicited specific external, visceral, and skeletal malformations, including anasarca, anal atresia associated with absent or vestigial tail, cardiovascular malformations (mainly persistent truncus arteriosis), and various malformations of the spinal column (mainly fusion and/or absence of cervical arches). The spectrum of external malformations was consistent across the pilot and definitive studies.
At 250 mg/kg, a single foetus showed defects similar to those observed at higher doses, and they were attributed to NMP.

Attention should be paid to the excessive embryolethality observed at doses > 500 mg/kg that may mask the teratogenic effects of NMP (thus, at 750 mg/kg, the number of malformed foetuses is limited, but the % affected/live fetuses is high).

Growth retardation, evidenced by decrease in fetal body weight, was seen at doses ≥ 250 mg/kg. This effect appeared in the absence of significant maternal toxicity. Retarded development was also reflected by reduced ossification at 500 and 750 mg/kg.

Regarding maternal toxicity, decrease in maternal body weight gain, food consumption , and absolute weight gain (26% lower than control) were observed at 500 and 750 mg/kg. There were no death of dam or clinical signs of toxicity.

The NOAELs for maternal and developmental toxicity were 250 and 125 mg/kg/day, respectively.

These results are supported by a limited study in which severe embryolethality was observed in rats at 997 mg/kg in the presence of maternal toxicity (EPA 1987). In addition, in two two-generation studies, there was a reduction in the live birth index, in the postnatal survival, and in the pup weight in the F1 and/or F2 generations at 350 - 500 mg NMP/kg in feed (EPA 1999, BASF 1999).
Other studies provide additional evidence of moderate maternal toxicity associated with oral exposure to NMP. In a developmental toxicity study, administration of 40, 125 or 400 mg/kg during days 6-15 by gavage caused no clinical changes, or reduction in food consumption or corrected weight gain (Exxon, 1992). In a two-generation study, there was minimal maternal toxicity in the F1 generation given 350 mg/kg NMP in diet, while the F2 pup surviving was decreased (BASF 1999).

Studies in non-pregnant rats have shown that the NOAEL after dietary exposure over 4 weeks was 18 000 ppm (i.e. 1548 mg/kg) (Malek et al 1997).

Thus, the developmental toxic effects of NMP cannot be considered as a secondary consequence of maternal toxicity. They were specific and severe in regard to the maternal toxicity.

Rabbits
Increased incidence of resorptions, cardiovascular malformations and malformed skull bones were also mentioned in a brief report of the OEHHA (1999). The NOAEL for maternal toxicity was 55 mg/kg/day and the NOAEL for developmental toxicity was 175 mg/kg/day.

Thus, NMP has been proved teratogenic and embryolethal in two species (rats and rabbits) and by two routes of administration in rats (oral, dermal). The spectrum of the malformations raises high concern.
In addition, foetal toxicity was observed at a maternally non-toxic dose in rats given NMP by gavage.
Nevertheless, NMP doesn't occur toxic effects for development by inhalation.

Therefore, there is sufficient evidence to support a category 2 classification for developmental toxicity: "substance causing developmental toxicity" and "substance which should be regarded as if it causes developmental toxicity in humans". Phrase of risk R61: "May cause harm to the unborn child".

C. *Minutes of the meeting of the Commission Working Group on the Classification and Labelling of Dangerous Substances, Ispra, 17-19 November 2003.*

Relevant parts concerning 1-methyl-2-pyrrolidone were copied.

1-methyl-2-pyrrolidone (F034)
(CAS No: 872-50-4, EC No: 212-828-1, Annex I Index No: 606-021-00-7)

Classification proposal: [Repr. Cat. 2; R61] - R36/37/38
Classification in Annex I, 19th ATP: Xi; R36/38 spec. conc. Limits: C≥10% Xi; R36/38

ECBI/47/02	F, Classification Proposal for re-classification of 1-methyl-2-pyrrolidone
ECBI/47/02 Rev. 1	F, Final proposal for classification of 1-Methyl-2-Pyrrolidone
ECBI/47/02 Add. 1	IND, Classification proposal for 1-methyl-2-pyrrolidone for Developmental toxicity, fertility, Inhalation toxicity and respiratory irritation.
ECBI/47/02 Add. 2	IND, Two generation Study for reprotoxicity of 1-methyl-2-pyrrolidone, wistar rats.
ECBI/47/02 Add. 3	IND, Two generation Study for reprotoxicity of 1-methyl-2-pyrrolidone, Sprague dawley rats.
ECBI/47/02 Add. 4	Letter sent by industry, explaining which studies were valid and which were not.
ECBI/47/02 Add. 5	Presentation from IND about classification of 1-methyl-2-pyrrolidone (NMP) for developmental effects.
ECBI/47/02 Add. 6	**IND, Comments on the classification of 1-methyl-2-pyrrolidone**

In January 2003 the **Group** agreed to propose a classification for the substance with **Xi; 36/37/38** but not to add **R48/20** as suggested by F. **The Group** postponed the discussion on developmental toxicity to their next meeting. They agreed not to propose any classification for fertility, but Member States were still invited to give their opinion during the Follow up period if they still had a concern for fertility.
Follow up: BE and NL did not think there was a concern for fertility. No other MS asked for further discussion. Therefore only the discussion on Developmental toxicity was postponed to the next meeting.
In May 2003 there was no discussion due to time constraints.

Developmental toxicity

IND said that there were no effects observed by the inhalation route. There were two dermal studies, one with rats and one with mice. Dermal absorption in the rat was in the range of about 70 percent. *In vitro* rat skin test data indicated that in human skin there was two fold less absorption. This would exceed the limit of 100 mg/ kg bodyweight. Furthermore, the compound had been

rubbed into the skin of the rats, increasing absorption. The HSL laboratory (UK) had performed absorption studies of the substance and also in Germany studies had been performed.

FR referred to dermal assays. The rabbit study was negative but the study has not been conducted until maternal toxicity was reached. Only 40 % 1-methyl-2-pyrrolidone had been used. In the second study the maternal toxicity was high at the dose where malformations were seen, and it was not obvious if the mother had an altered weight gain. Concerning other aspects pointed out by IND, **FR** had no specific criticism but found that it was more a risk assessment than a hazard assessment. **FR** was still in favour of category 2 for development.

DE supported the FR view as IND presented a risk assessment that was not appropriate for C&L. IND often claims that the oral route has to be considered as default route and now they say the opposite. **DE** supported classification in category 2.

N supported fully FR and DE.

NL was also puzzled by all explanations for different routes of exposure given by IND. For C&L this should not be taken into account. This was neither contained in Annex VI nor in the GHS. The Group had to stick to intrinsic properties. **NL** agreed with other MS that had pronounced their opinion that the substance was a clear category 2.

UK agreed that very clear effects had been seen via the oral route but that they occurred at very high concentrations. They thought that category 3 would be more appropriate.

DE said that if the Group considered that this dose was too high they would have to declassify all phthalates. **NL** mentioned that there could possibly be specific concentration limits applied instead of applying a lower category.

FR could not understand the reasoning of classification in category 3. They thought that this was clearly a category 2 or nothing. **BE** supported the FR standpoint.

The Group agreed to classify the substance with Repr. Cat. 2; R61

UK still said that they were not completely convinced about the classification as category 2. No other MS expressed the same concern.

IND wanted to make a proposal for specific concentration limits. **ECB** welcomed such proposal in the Follow up period. **ECB** said that if MS did not support such a proposal for specific concentration limits these would not be added during the follow up period.

Conclusion:
The Group agreed that the classification proposal to be sent to DG ENV for inclusion in a future ATP for **1-methyl-2-pyrrolidone** was: **Repr. Cat. 2; R61 – Xi; R36/37/38** (Symbol: T, R-phrases: 36/37/38-61 and S-phrases: 53-45.
Follow-up: IND sent a proposal for the application of specific concentration limits. Several Member States made comments and it was agreed to discuss the specific concentration limits at the next meeting.

D. ***Minutes of the Meeting of the Technical Committee C&L on the Classification and Labelling of Dangerous Substances, Arona, 15-18 March 2005.***

Relevant parts concerning 1-methyl-2-pyrrolidone were copied.

1-methyl-2-pyrrolidone (F034)
(CAS No: 872-50-4, EC No: 212-828-1, Annex I Index No: 606-021-00-7)

Classification proposal: [Repr. Cat. 2; R61] - R36/37/38
Classification in Annex I, 19th ATP: Xi; R36/38 spec. conc. Limits: C≥10% Xi; R36/38

ECBI/47/02	F, Classification Proposal for re-classification of 1-methyl-2-pyrrolidone
ECBI/47/02 Rev. 1	F, Final proposal for classification of 1-Methyl-2-Pyrrolidone
ECBI/47/02 Add. 1	IND, Classification proposal for 1-methyl-2-pyrrolidone for Developmental toxicity, fertility, Inhalation toxicity and respiratory irritation.
ECBI/47/02 Add. 2	IND, Two generation Study for reprotoxicity of 1-methyl-2-pyrrolidone, wistar rats.
ECBI/47/02 Add. 3	IND, Two generation Study for reprotoxicity of 1-methyl-2-pyrrolidone, Sprague dawley rats.
ECBI/47/02 Add. 4	Letter sent by industry, explaining which studies were valid and which were not.
ECBI/47/02 Add. 5	Presentation from IND about classification of 1-methyl-2-pyrrolidone (NMP) for developmental effects.
ECBI/47/02 Add. 6	IND, Comments on the classification of 1-methyl-2-pyrrolidone
ECBI/47/02 Add. 7	IND, Concentration limits for substances classified as toxic to reproduction/ developmental toxicity in preparations.
ECBI/47/02 Add. 8	FR, support for discussing specific concentration limits for substances toxic to reproduction.
ECBI/47/02 Add. 9	NL, support for discussing specific concentration limits for substances toxic to reproduction.
ECBI/47/02 Add. 10	IND, reaction on Add. 9
ECBI/47/02 Add. 11	IND, reaction on Add. 9
ECBI/47/02 Add. 12	**IND, Comments on follow-up actions of the CMR meeting in Riga, 12-14 May 2004, N-Methylpyrrolidone (NMP)**
ECBI/47/02 Add. 13	**IND, Concentration limits for substances classified as toxic to reproduction/development toxicity in preparation, NMP**
ECBI/47/02 Add. 14	**S, proposal to delete this substance from the agenda and postpone the discussion until after the SE meeting on reproductive toxicity and potency**

In January 2003 the **Group** agreed to propose a classification for the substance with **Xi; 36/37/38** but not to add **R48/20** as suggested by F. **The Group** postponed the discussion on developmental toxicity to their next meeting. They agreed not to propose any classification for fertility, but Member States were still invited to give their opinion during the Follow up period if they still had a concern for fertility.
Follow up: BE and NL did not think there was a concern for fertility. No other MS asked for further discussion. Therefore only the discussion on Developmental toxicity was postponed to the next meeting.
In May 2003 there was no discussion due to time constraints.

In November 2003, the Group agreed that the classification proposal to be sent to DG ENV for inclusion in a future ATP for 1-methyl-2-pyrrolidone was: Repr. Cat. 2; R61 – Xi; R36/37/38 (Symbol: T, R-phrases: 36/37/38-61 and S-phrases: 53-45. During the follow-up period **IND** sent a proposal for the application of specific concentration limits. Several Member States made comments and it was agreed to discuss the specific concentration limits at the next meeting.
In May 2004 the Group confirmed the classification as already agreed at an earlier meeting of the Group. **In Sept 2004** the discussion was postponed due to time constraints
At the meeting in March 2005 the discussion of specific concentration limits should be finalised.

FR said that that substance has been on the agenda since a long time.The only remaining issue was the concentration limits.

S wanted to await a general solution for consideration of potency of reproductive toxic compounds and wanted to await the planned discussion by the Specialised Experts, meanwhile the general default limit would apply. **FR** was worried about any further delay of the decision to include the substance in Annex I, which the **UK** agreed to.

DK supported the S proposal to add the default limits now and add the specific concentration limits later when there was an agreement on the method to be used.
The **UK** would be content to go with the majority view.

NL and **DE** agreed to the proposed specific concentration limit of 5%.

S and **DK** disagreed with the proposed specific concentration limit and instead preferred the default value.

FIN could accept the majority view. However, FIN would like to stress that the derived concentration limit is obtained by an approach that could easily be scientifically challenged. Therefore FIN could see a problem with this approach but was prepared to accept the majority view in this case.

ECB wanted more MS to speak up to clearly find out what the majority view was.

BE was not objecting to the suggested limit in this case but did not agree to the rationale on how to do it. For example, an inhalation study had been the basis for the proposal for the specific conc. limit but the classification was not based on that. BE would follow the majority view in this case but was not convinced that the method should be applied also in the future.
CZ and **EST** was also not sure but would go with the majority.
DK preferred the default value since they saw a problem with applying the method, which they did not support. **DE, EL, FR, IR, HUN, NL, AT, PL, PT, SK** upported the proposed limit of 5%. **N** agreed with the S comments but could go along with 5% in this particular case, if no precedence would be set. **SL** agree with the majority view. **FIN** also agreed with the majority view but as said before, the way of reaching the 5% could be scientifically challenged. **S** was concerned that this method should not be applied in general and supported the default value. The **UK** could live with 5% but had actually come to another figure of 2 or 3 %. The UK did not share the reservations of the unscientific approach of the method used. It may not be a general method but the UK did not think any rules of toxicology had been broken by applying the method, using algorithms, which seemed to be perfect to them. And it did not have to be a general method to be used every time.

ECB concluded that the majority of MS supported the suggested specific concentratin limit of 5% for Repr. Cat. 2; R61, with the reservations from some MS (DK, S, FIN and BE) for the method used. DK and S also had reservations on the value of 5% and instead supported the default value of 0.5%.

The substance should be included in the next ATP with the classification of Repr. Cat. 2; R61 – Xi; R36/37/38 and a specific conc.limit of 5% for R61. (Symbol: T, R-phrases: 36/37/38-61 and S-phrases: 53-45).

Annex 2

Determination of the ED10-value

The ED_{10} value (as used for reprotoxicity SCLs) is the lowest dose which induces reproductive toxic effects which fulfill the criteria for classification for reproductive toxicity with an incidence or magnitude of 10% after correction for the spontaneous incidence. According to the ECHA guidance (ECHA, 2012) the ED_{10} may be obtained either directly or by linear interpolation from experimental data or estimated using benchmark dose (BMD) software. The use of BMD software will result in a more precise estimate of the ED_{10} because all data from the dose-response curve are used. Here, we will derive the ED_{10} using the benchmark dose software PROAST, which is developed by RIVM and available at www.rivm.nl/proast.

The application of the BMD approach is performed according to the guidelines as set by EFSA (2009) and involves the following steps:
1. Specification of type of dose-response data
2. Specification of the relevant (benchmark) response (BMR)
3. Selection of candidate dose-response model(s)
4. Identification of acceptable models
5. Estimating the ED_{10}

These steps are discussed below.

1. Specification of type of dose-response data
Endpoints not showing dose response relationships are normally not used for deriving a BMD. The decision to disregard endpoints has been done by visual inspection of the data. Response data may be of various types: as an incidence (quantal data, non-parametric data), a magnitude (continuous data, parametric data) or both (ordinal data). The distinction between data types is important for statistical reasons (such as assumption of underlying statistical distribution), but also for the interpretation of the BMR.

In the case of NMP several effects on reproduction are observed in various studies. Effects fulfilling the classification criteria for reproductive toxicity were selected. These effects are all quantal data and are analyzed accordingly. For quantal data the number of affected individuals and the sample size are needed for each dose group.

2. Specification of the relevant (benchmark) response (BMR)
For quantal data the BMR is defined in terms of an increase in the incidence of the lesion / response scored, compared with the background response. The common way of doing this is either by additional risk or extra risk. According to ECHA guidance (ECHA, 2012), the relevant BMR is 10%, which is in the case of NMP defined in terms of extra risk. The dose corresponding to the 10% extra risk is termed BMD_{10} or ED_{10}.

3. Selection of candidate dose-response models
Different models, which fit the data equally well, can result in different ED_{10}s, reflecting model uncertainty. To take this aspect of uncertainty into account, various models need to be fitted to the same dataset. The usual suit of quantal models, containing the two-stage, log-logistic, Weibull, log-probit, gamma, logistic, probit, exponential and Hill models, is applied here. To avoid the models having undesirable properties, certain constraints are imposed on the model parameters. For instance, since quantal responses are usually between 0% and 100% response, the background response parameter (a) is constrained to be between (fractions) 0 and 1. For more details see EFSA (2009).

4. Identification of acceptable models
The PROAST software takes care of fitting a model, which means finding the values of the unknown parameters in the model that make the associated dose-response curve approach the data as closely as

possible. This is called the best fit and is achieved by maximizing the log-likelihood. The BMD approach does not aim to find the single statistically best estimate of the BMD but rather all plausible values that are compatible with the data; therefore, the goal is not to find the single best fitting model, but rather to find those models with an acceptable fit.

The acceptance of a fitted model is based on two principles. The first principle is that from a nested family of models (i.e. the exponential and Hill families) only one member is accepted, by comparing the log-likelihoods of the various members in that family, using the likelihood ratio test. When a member with fewer parameters does not show a significantly poorer fit, then this member will be preferred.
The second principle is that any fitted model should provide reasonable description of the dose-response data, according to a goodness-of-fit test with a P value greater than 0.05. There are several types of goodness-of-fit tests. The likelihood ratio test is the recommended choice here. In the likelihood ratio test, the log-likelihood value associated with a fitted model is compared with, and tested against, the log-likelihood value associated with the so-called "full model". The full model simply consists of the observed (mean) responses at each applied dose. Hence, the number of parameters equals the number of dose groups. If a model's fit is not significantly worse than that of the full model, then the model may be accepted. The likelihood ratio test may be used to test if additional parameters in nested models result in a significant improvement of the fit. See Slob (2002) or EFSA (2009) for more details.

5. Estimating the ED_{10}

For each identified critical endpoint, the set of models is applied. Subsequently, for each of the accepted models the ED_{10} is derived. The lowest ED_{10} from this range can be considered to be the overall ED_{10}.

When the experimental data provide sufficient information on the dose-response relationship, the various models that fit the data will have similar shapes and will yield a narrow range of ED_{10} values. In some circumstances, the dose response relationship may not be well defined. For instance, there may be large gaps between consecutive response levels. In such datasets the various models that fit the data (according to the statistical criteria discussed above) may assume different shapes, and consequently the ranges of ED_{10} values obtained may be wide. These ED_{10} values would not provide a secure basis for establishing an SCL. Criteria to judge the adequacy of the dose-response data on the basis of the range of ED_{10} values obtained have so far not been established. As a general rule, dose-response data should not result in a range of ED_{10} values from different accepted models that substantially exceeds one order of magnitude. When this value is exceeded, several options are available and should be considered on a case-by-case basis, e.g. re-evaluating the set of models.

Results

For each study the dose response data of the critical endpoints are analyzed and reported by one table and one figure. In the table the number of parameters (npar) and loglikelihood (loglik) are given for the identification of acceptable models by the likelihood ratio test (see 4. above). The results of the null and full models are included for this reason as well. The lowest ED_{10} obtained from the accepted models is printed in bold.
The $ED_{10}L$ and $ED_{10}U$, reported for the accepted models, are the lower 5^{th} and upper 95^{th} percent confidence limits of the ED_{10} and are equivalent to the BMDL and BMDU. These confidence limits are indicative for the quality of the experimental data. Poor experimental data will result in a large confidence interval.
The figure illustrates the dose-response data (including 90%-CI) and curve of the model providing the lowest ED_{10}.

Rat, oral (Saillenfait AM et al., 2001/2002)

Table 1: ED_{10}s obtained from the postimplantation loss data in rat (Saillenfait, 2001/2002)

model	npar	loglik	accept	BMD	BMDL	BMDU
null	1	-912.09	--	NA	NA	NA
full	117	-275.19	--	NA	NA	NA
one-stage	2	-654.79	no	108	NA	NA
two-stage	3	-654.79	no	108	NA	NA
log-logist	3	-386.6	yes	523	505	547
Weibull	3	-386.6	yes	532	507	564
log-prob	**3**	**-386.6**	**yes**	**520**	**504**	**540**
gamma	3	-386.6	yes	521	505	542
logistic	2	-484.95	no	342	NA	NA
probit	2	-509.41	no	292	NA	NA
E3-	3	-386.79	yes	531	507	540
H3-	3	-428.41	no	428	NA	NA

BMR: 0.1 extra risk
P-value GoF: **1.00E-09**
constraint: no

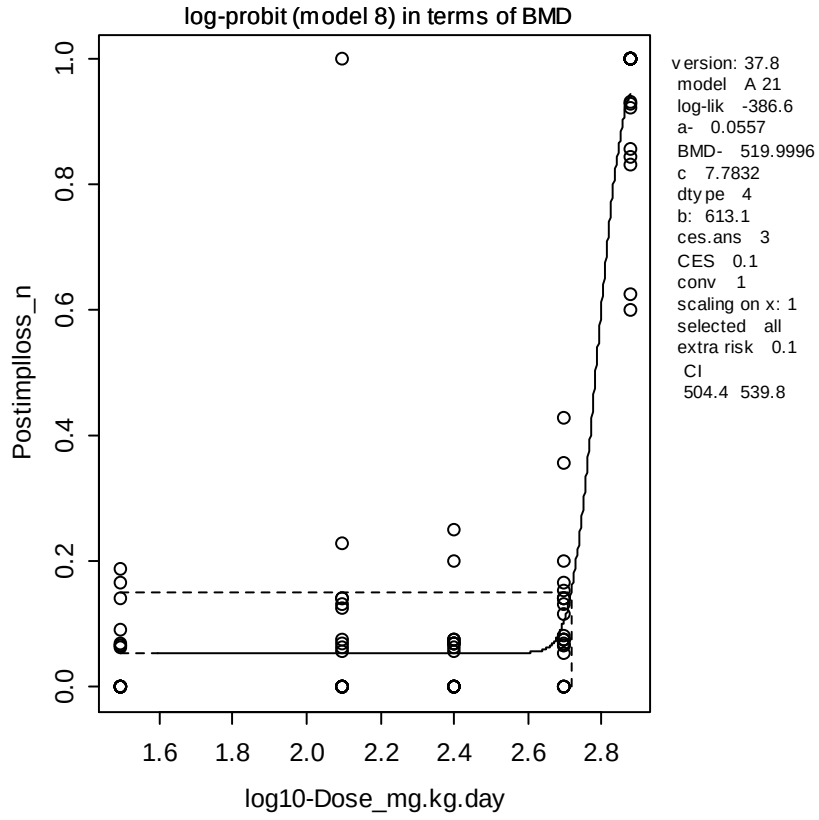


Figure 1: Dose response curve (log-probit model) of postimplantation loss in rats. The horizontal dashed line represents 10% extra risk and the vertical dashed line is located at the ED_{10}. Data are from Saillenfait (2001/2002).

Table 2: ED_{10}s obtained from the cardiac malformation data in rat (Saillenfait, 2001/2002)

model	npar	loglik	accept	BMD	BMDL	BMDU
null	1	-73.5	--	NA	NA	NA
full	5	-43.88	--	NA	NA	NA
one-stage	2	-58.9	no	895	NA	NA
two-stage	3	-58.9	no	895	NA	NA
log-logist	3	-43.93	yes	533	500	570
Weibull	3	-43.98	yes	539	502	576
log-prob	**3**	**-43.88**	**yes**	**528**	**499**	**561**
gamma	3	-43.88	yes	530	499	564
logistic	2	-44.35	yes	543	508	582
probit	2	-43.94	yes	535	502	562
E2-	2	-43.97	yes	534	502	571
H3-	3	-43.88	yes	529	499	563

BMR: 0.1 extra risk
P-value GoF: 0.05
constraint: no

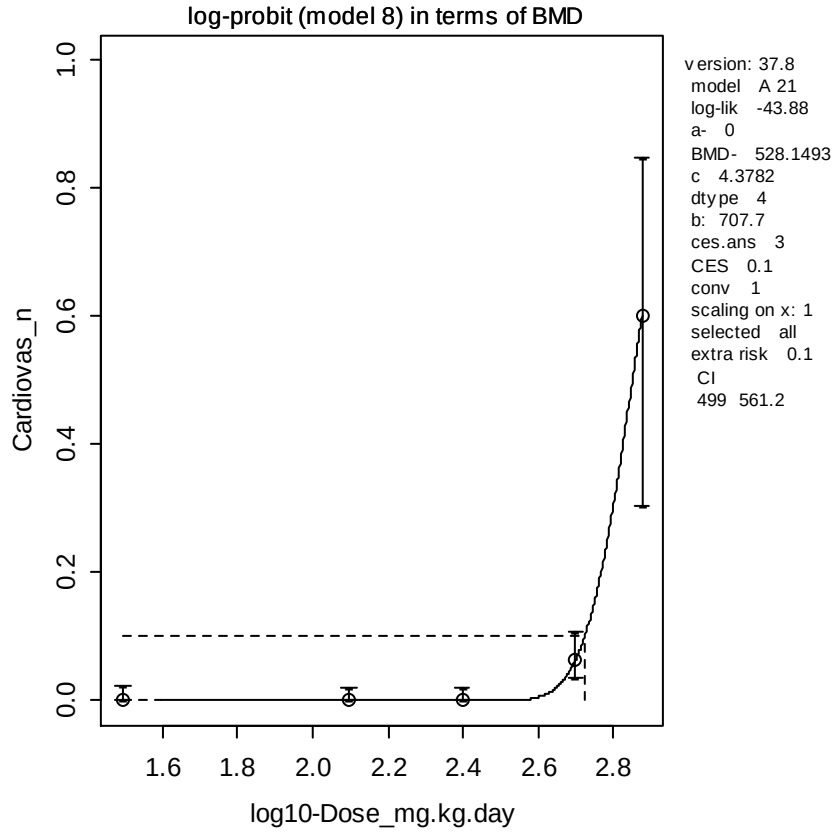


Figure 2: Dose response curve (log-probit model) of cardiac malformations in rats. The horizontal dashed line represents 10% extra risk and the vertical dashed line is located at the ED_{10}. Data are from Saillenfait (2001/2002).

Table 3: ED_{10}s obtained from the data on persistent truncus arteriosus in rat (Saillenfait, 2001/2002)

model	npar	loglik	accept	BMD	BMDL	BMDU
null	1	-38	--	NA	NA	NA
full	5	-27.13	--	NA	NA	NA
one-stage	2	-31.95	no	2090	NA	NA
two-stage	3	-31.95	no	2090	NA	NA
log-logist	3	-27.26	yes	632	566	827
Weibull	3	-27.28	yes	636	570	825
log-prob	**3**	**-27.15**	**yes**	**626**	**558**	**865**
gamma	3	-27.18	yes	628	560	833
logistic	2	-27.73	yes	641	580	761
probit	2	-27.37	yes	630	567	NA
E2-	2	-27.37	yes	631	567	768
H2-	2	-29.04	yes	908	696	1260

BMR: 0.1 extra risk
P-value GoF: 0.05
constraint: no

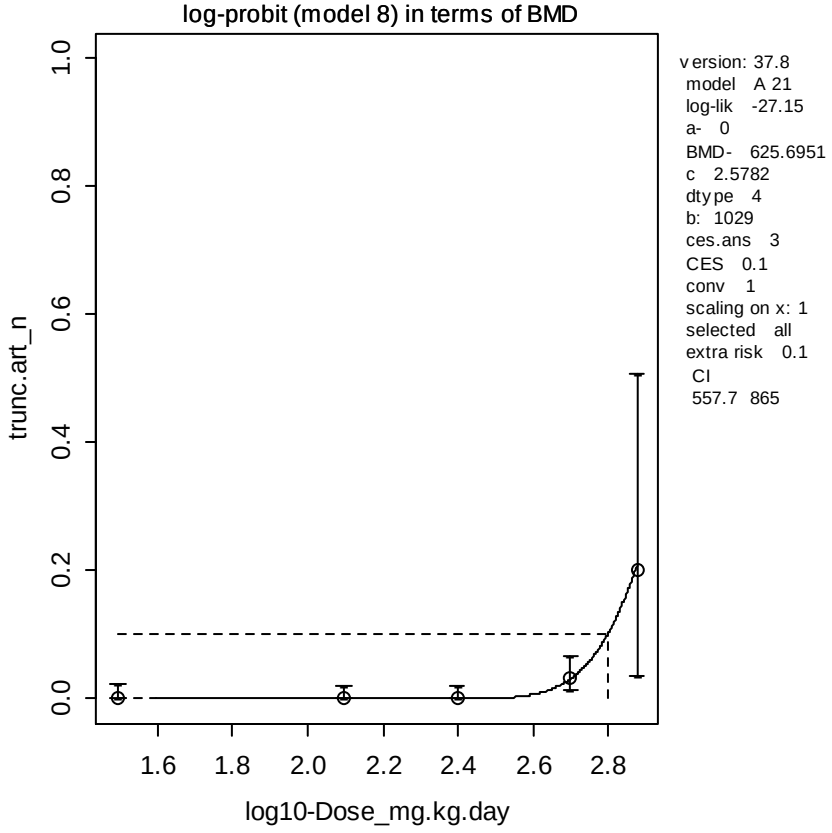


Figure 3: Dose response curve (log-probit model) of persistent truncus arteriosus in rats. The horizontal dashed line represents 10% extra risk and the vertical dashed line is located at the ED_{10}. Data are from Saillenfait (2001/2002).

Rabbit, oral (IRDC, 1991)

Table 4: ED_{10}s obtained from the data on postimplantation loss in rabbit (IRDC, 1991)

model	npar	loglik	accept	BMD	BMDL	BMDU
null	1	-204.56	--	NA	NA	NA
full	72	-113.29	--	NA	NA	NA
two-stage	3	-187.56	no	205	NA	NA
log-logist	3	-187.62	no	179	NA	NA
Weibull	3	-187.52	no	181	NA	NA
log-prob	3	-187.9	no	165	NA	NA
gamma	3	-187.5	no	178	NA	NA
logistic	2	-187.71	yes	303	257	375
E2-	2	-187.66	yes	287	241	364
H2-	**2**	**-187.6**	**yes**	**225**	**171**	**319**

no covariate
BMR: 0.1 extra risk
constraint: no
P-value GoF: 1.00E-07

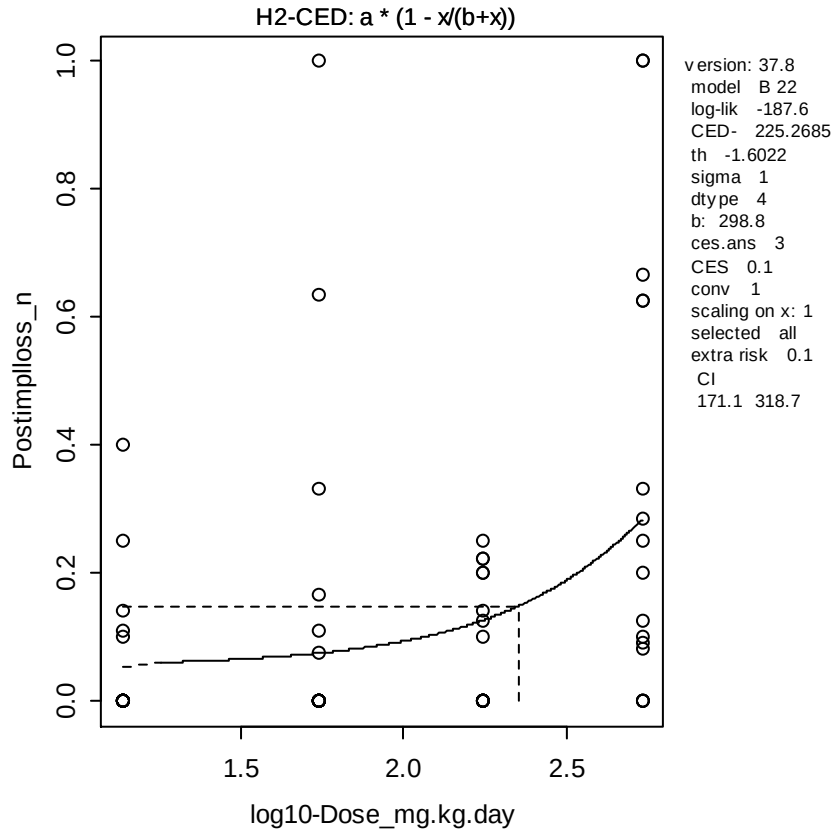


Figure 4: Dose response curve (H2 CED model) of postimplantation loss in rabbits. The horizontal dashed line represents 10% extra risk and the vertical dashed line is located at the ED_{10}. Data are from IRDC (2001).

Table 5: ED_{10}s obtained from the data on interventricular septal defect in rabbit (IRDC, 1991)

model	npar	loglik	accept	BMD	BMDL	BMDU
null	1	-106.73	--	NA	NA	NA
full	4	-70.64	--	NA	NA	NA
one-stage	2	-79.89	no	267	NA	NA
two-stage	3	-79.89	no	267	NA	NA
log-logist	3	-71.85	yes	363	287	NA
Weibull	3	-71.86	yes	371	295	NA
log-prob	**3**	**-71.82**	**yes**	**337**	**269**	**475***
gamma	3	-71.83	yes	354	285	534
logistic	2	-72.28	yes	386	342	430
probit	2	-72.56	yes	359	313	409
E2-	2	-72.56	yes	359	314	408
H3-	3	-71.89	yes	373	291	475

BMR: 0.1 extra risk
P-value GoF: 0.05
constraint: no
*BMDU from 1000 bootstraps

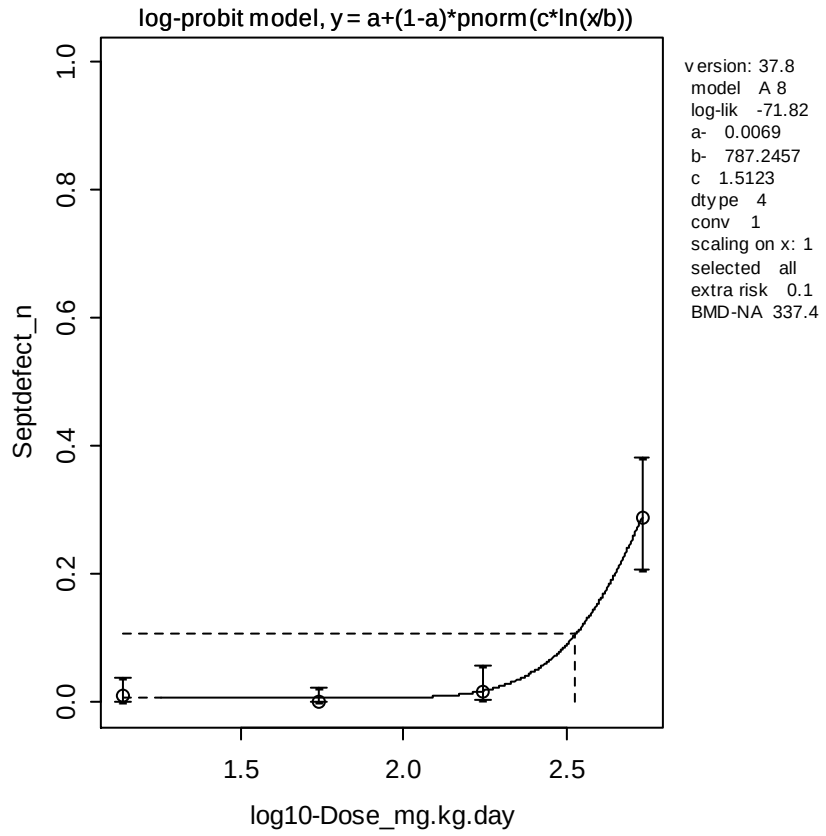


Figure 5: Dose response curve (log-probit model) of interventricular septal defects in rabbits. The horizontal dashed line represents 10% extra risk and the vertical dashed line is located at the ED_{10}. Data are from IRDC (2001).

Table 6: ED_{10}s obtained from the data on bulbous aortic arch in rabbit (IRDC, 1991)

model	npar	loglik	accept	BMD	BMDL	BMDU
null	1	-89.31	--	NA	NA	NA
full	4	-57.59	--	NA	NA	NA
one-stage	2	-65.67	no	333	NA	NA
two-stage	3	-65.67	no	333	NA	NA
log-logist	3	-58.19	yes	405	325	NA
Weibull	3	-58.19	yes	411	332	NA
log-prob	**3**	**-58.17**	**yes**	**379**	**304**	**495***
gamma	3	-58.18	yes	394	321	NA
logistic	2	-58.46	yes	427	381	472
probit	2	-58.68	yes	403	354	455
E2-	2	-58.68	yes	402	353	456
H3-	3	-58.2	yes	412	325	497

BMR: 0.1 extra risk
P-value GoF: 0.05
constraint: no
* BMDU from 1000 bts

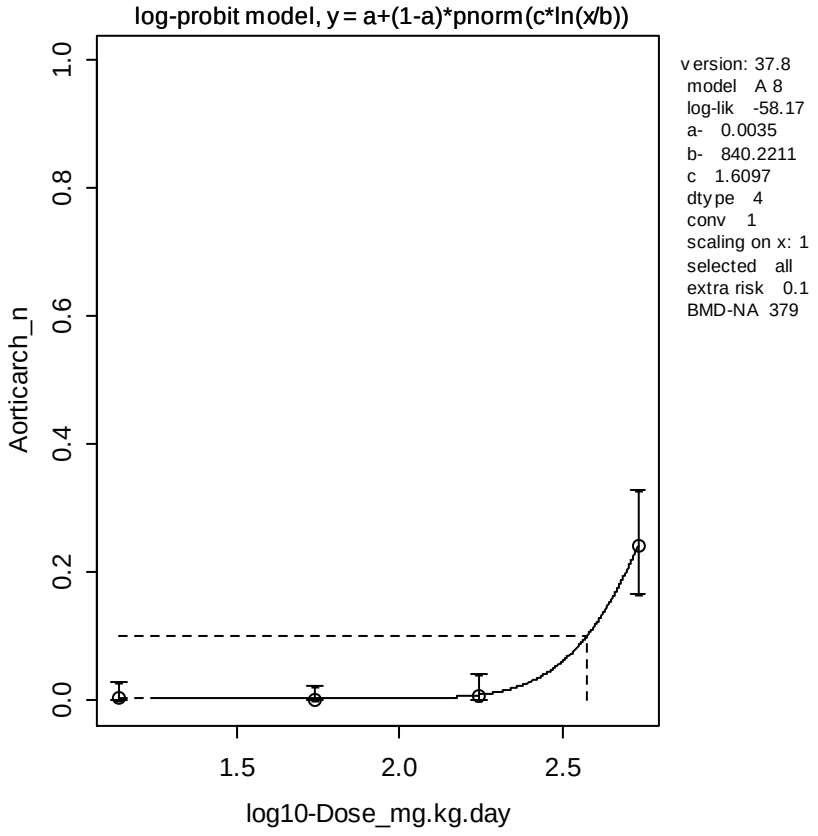


Figure 6: Dose response curve (log-probit model) of bulbour aortic arch in rabbits. The horizontal dashed line represents 10% extra risk and the vertical dashed line is located at the ED_{10}. Data are from IRDC (2001).

Table 7: ED_{10}s obtained from the data on pulmonary trunk stenosis in rabbit (IRDC, 1991)

model	npar	loglik	accept	BMD	BMDL	BMDU
null	1	-79.98	--	NA	NA	NA
full	4	-53.84	--	NA	NA	NA
one-stage	2	-60.42	no	395	NA	NA
two-stage	3	-60.42	no	395	NA	NA
log-logist	3	-54.44	yes	424	342	551
Weibull	3	-54.44	yes	429	348	529
log-prob	**3**	**-54.43**	**yes**	**401**	**321**	**521**
gamma	3	-54.43	yes	415	338	537
logistic	2	-54.68	yes	446	398	496
probit	2	-54.86	yes	428	372	490
E2-	2	-54.86	yes	425	372	487
H3-	3	-54.46	yes	432	342	514

BMR: 0.1 extra risk
P-value GoF: 0.05
constraint: no

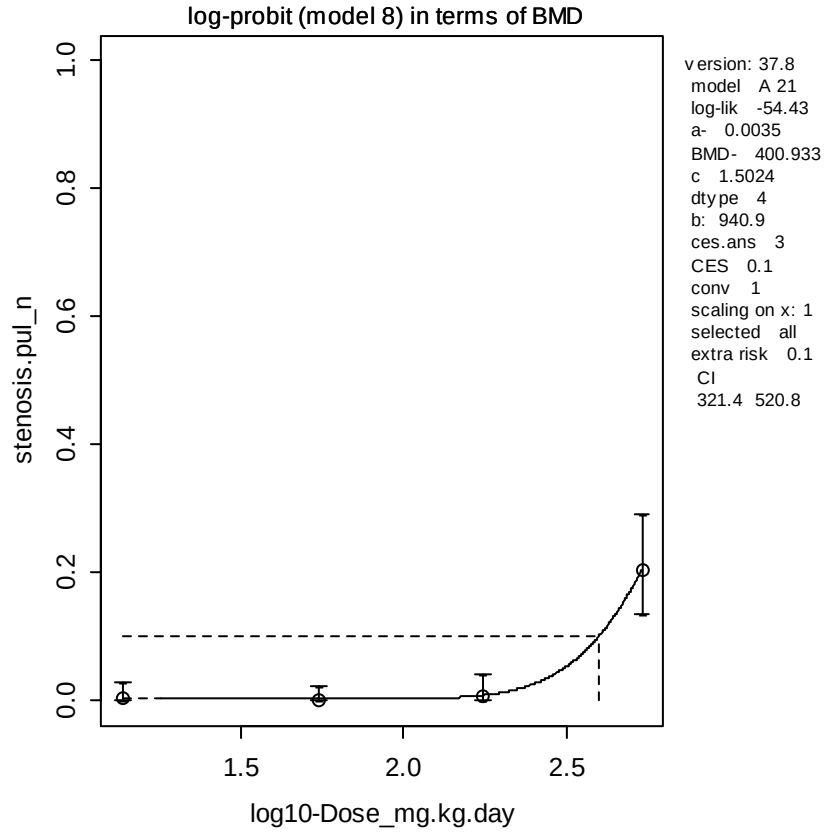


Figure 7: Dose response curve (log-probit model) of pulmonary trunk stenosis in rabbits. The horizontal dashed line represents 10% extra risk and the vertical dashed line is located at the ED_{10}. Data are from IRDC (2001).

Rat, oral 2-gen study (BASF 1999)

Table 8: ED_{10}s obtained from the data on pup mortality (F1a-generation) in rat (BASF, 1999)

model	covar	npar	loglik	accept	BMD	BMDL	BMDU	level
null	NA	1	-1074.83	--	NA	NA	NA	--
full	NA	8	-667.73	--	NA	NA	NA	--
one-stage	ab	4	-774	no	54.4	NA	NA	F1a
two-stage	ab	5	-774	no	54.4	NA	NA	F1a
log-logist	--	3	-676.95	yes	361	346	379	--
Weibull	--	3	-676.97	yes	364	345	385	--
log-prob	**--**	**3**	**-676.95**	**yes**	**360**	**347**	**374**	**--**
gamma	**--**	**3**	**-676.95**	**yes**	**360**	**346**	**375**	**--**
logistic	ab	4	-697.38	no	160	NA	NA	F1a
probit	ab	4	-703.26	no	7.00E-04	NA	NA	F1b
E3-	--	3	-677.25	yes	365	347	370	--
H3-	b	4	-677.8	no	284	NA	NA	F1a

covariate: Generation
BMR: 0.1 extra risk
P-value GoF: 0.001
constraint: no

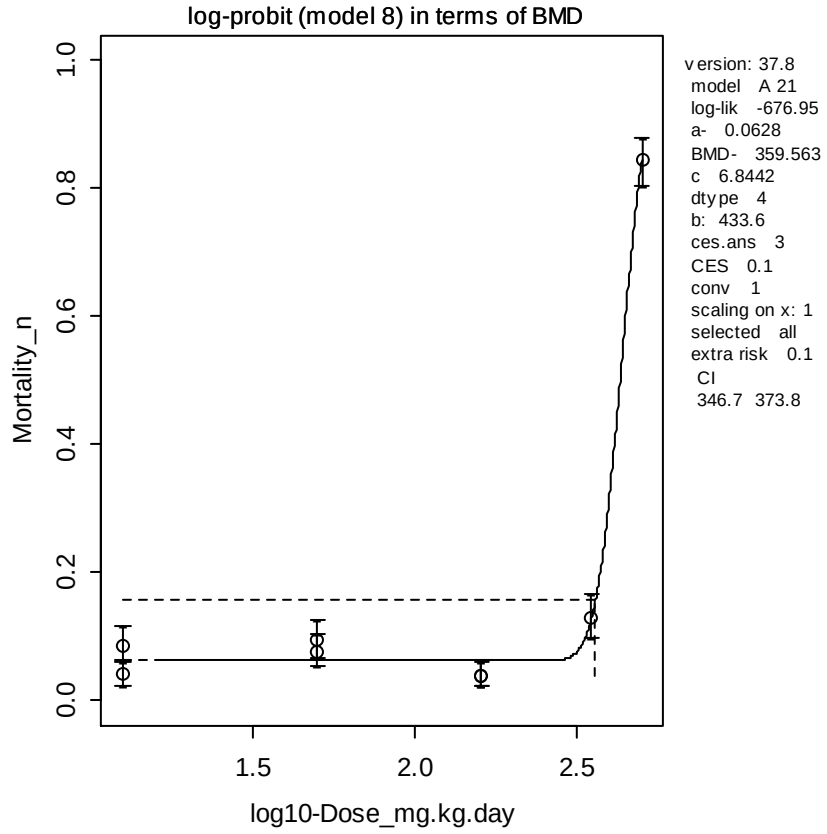


Figure 8: Dose response curve (log-probit model) of pup mortality (F1a-generation) in rats. The horizontal dashed line represents 10% extra risk and the vertical dashed line is located at the ED_{10}. Data are from BASF (1999).

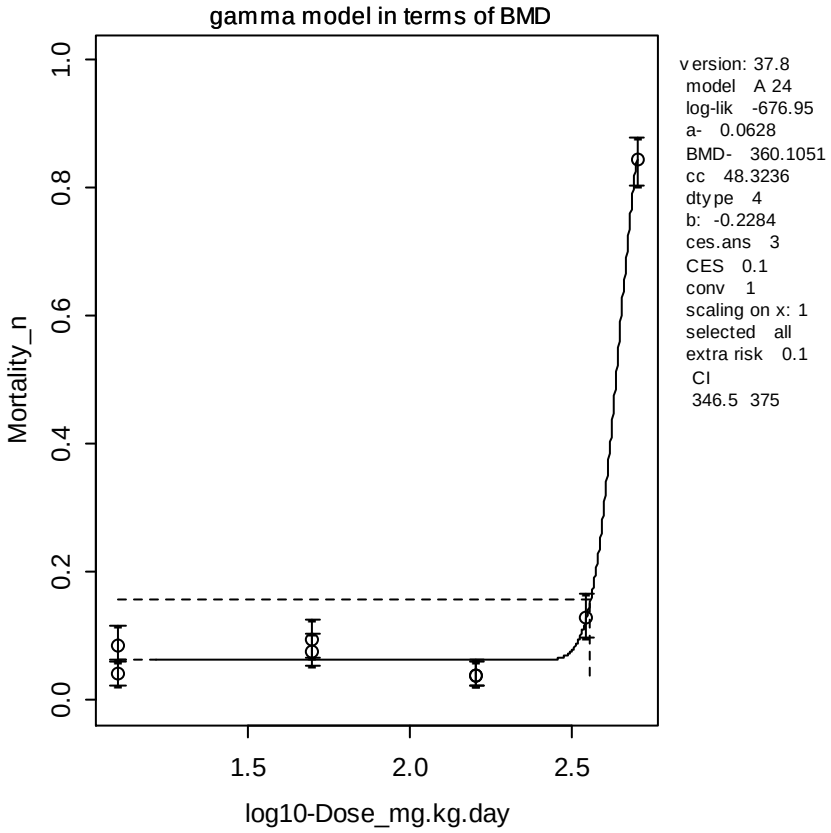


Figure 9: Dose response curve (gamma model) of pup mortality (F1a-generation) in rats. The horizontal dashed line represents 10% extra risk and the vertical dashed line is located at the ED_{10}. Data are from BASF (1999).

Table 9: ED_{10}s obtained from the data on complete litters lost at the end of the lactation period in rat (BASF, 1999)

model	covar	npar	loglik	accept	BMD	BMDL	BMDU	level
null	NA	1	-62.49	--	NA	NA	NA	--
full	NA	8	-17.65	--	NA	NA	NA	--
one-stage	b	3	-29.73	no	71	NA	NA	F1a
two-stage	b	4	-23.08	no	152	NA	NA	F1a
log-logist	--	3	-19.65	yes	472	372	498	--
Weibull	--	3	-19.65	yes	477	453	488	--
log-prob	--	3	-19.65	yes	449	369	466	--
gamma	--	3	-19.65	yes	451	370	486	--
logistic	**b**	**3**	**-20.47**	**yes**	**263**	**203**	**328**	**F1a**
probit	ab	4	-19.09	yes	500	NA	NA	F1a
E2-	ab	4	-19.1	yes	344	219	356	F1a
H3-	b	4	-19.68	yes	321	194	354	F1a

covariate: Generation
BMR: 0.1 extra risk
P-value GoF: 0.05
constraint: no

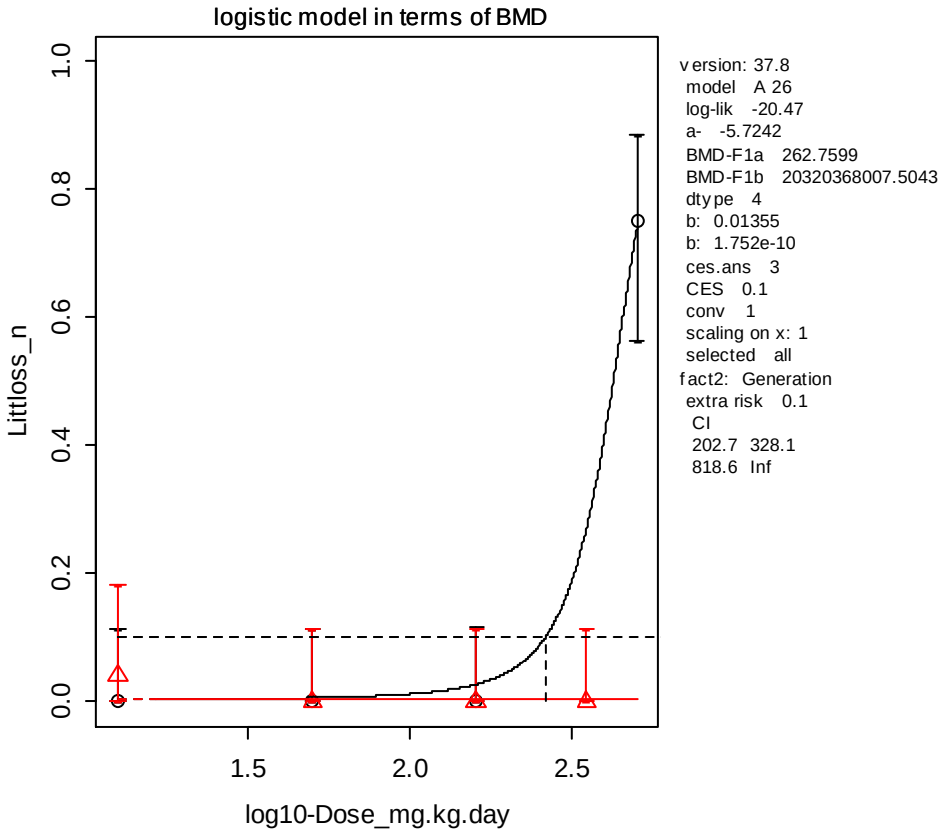


Figure 10: Dose response curve (logistic model) of complete litters lost at end of lactation period in rats. The horizontal dashed line represents 10% extra risk and the vertical dashed line is located at the ED_{10}. Data are from BASF (1999).

The BMD analyses was performed using both F1a and F1b. However, F1a was determinative for the BMD_{10}

Table 10: ED_{10}s obtained from the data on stillborn pups in rat (BASF, 1999)

model	covar	npar	loglik	accept	BMD	BMDL	BMDU	level
null	NA	1	-324.43	--	NA	NA	NA	--
full	NA	8	-302.93	--	NA	NA	NA	--
one-stage	b	3	-311.54	yes	851	582	1400	F1a
two-stage	b	4	-311.54	yes	851	582	1400	F1a
log-logist	a	4	-309.01	yes	51900	1850	Inf	F1a
Weibull	a	4	-309	yes	45900	1730	Inf	F1a
log-prob	a	4	-309.05	yes	149000	2590	Inf	F1a
gamma	**--**	**3**	**-311.83**	**yes**	**511**	**501**	**619**	**--**
logistic	b	3	-311.33	yes	634	519	861	F1a
probit	a	3	-310.97	yes	762	NA	NA	F1a
E2-	a	3	-310.97	yes	994	577	1230	-
H2-	a	3	-311.1	yes	1390	634	1600	-

BMR: 0.1 extra risk
P-value GoF: 0.001
constraint: no

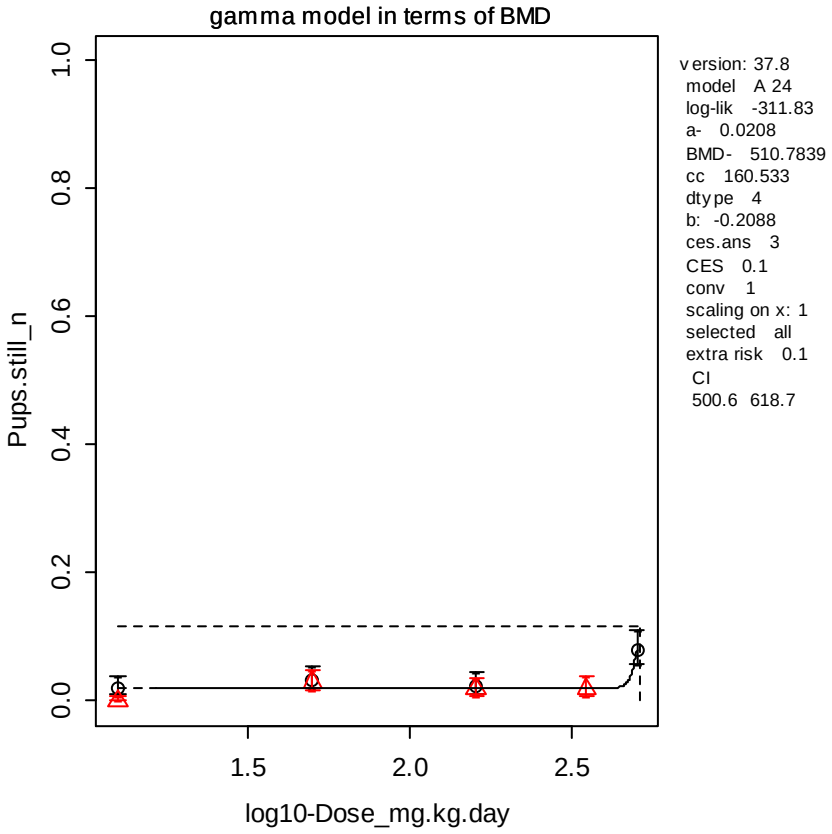


Figure 11: Dose response curve (logistic model) of stillborn pups in rats. The horizontal dashed line represents 10% extra risk and the vertical dashed line is located at the ED_{10}. Data are from BASF (1999).

References to Annex 2

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Annex 3. Calculations of ED10 values by linear interpolation.

Rat oral (Saillenfait, 2001)

- ***Postimplantation loss:***

Dose-level (mg/kg bw/day)	Postimplantation loss (%)
0	4.1 ± 6.1
125	9.3 ± 21.3
250	4.5 ± 6.6
500	10.6 ± 10.5
750	94.2 ± 11.2

- Effect-level at control is 4.1 %. Effect-level at ED10 is 14.1% (i.e. 4.1% + 10%)
- 500 mg/kg bw/day < ED10 < 750 mg/kg bw/day
- An increase in dosing of 250 mg/kg bw/day (i.e. 750-500 mg/kg bw/day) ~ an increase in postimplantation loss incidences of 83.6% (i.e. 94.2-10.6)
- 1% change in postimplantation loss ~2.99 mg/kg bw/day increase in dose
- 3.5% change (i.e. 14.1-10.6) in postimplantation loss ~11 mg/kg bw/day increase in dose
- ED10 = 500 + 11 = 511 mg/kg bw/day

- ***Cardiovascular malformations***

Dose-level (mg/kg bw/day)	Cardiovascular malformations (%)
0	0
125	0
250	0
500	6.4
750	60

- Effect-level at control is 0 %. Effect-level at ED10 is 10% (i.e. 0% + 10%)
- 500 mg/kg bw/day < ED10 < 750 mg/kg bw/day
- An increase in dosing of 250 mg/kg bw/day (i.e. 750-500 mg/kg bw/day) ~ an increase in cardiovascular malformation incidences of 53.6% (i.e. 60-6.4)
- 1% change in cardiac malformations incidences ~4.66 mg/kg bw/day increase in dose
- 3.6% change (i.e. 10-6.4%) in cardiac malformations ~17 mg/kg bw/day increase in dose
- ED10 = 500 + 17 = 517 mg/kg bw/day

- ***Truncus arteriosus***

Dose-level (mg/kg bw/day)	Truncus arteriosus (%)

0	0
125	0
250	0
500	3.2
750	20

- Effect-level at control is 0 %. Effect-level at ED10 is 10% (i.e. 0% + 10%)
- 500 mg/kg bw/day < ED10 < 750 mg/kg bw/day
- An increase in dosing of 250 mg/kg bw/day (i.e. 750-500 mg/kg bw/day) ~ an increase in incidences of truncus arteriosus of 16.8% (i.e. 20-3.2)
- 1% change in truncus arteriosus incidences ~14.88 mg/kg bw/day increase in dose
- 6.8% change (i.e. 10-3.2%) in truncus arteriosus incidences ~101 mg/kg bw/day increase in dose
- ED10 = 500 + 101 = 601 mg/kg bw/day

Rabbit oral (IRDC, 1991)

- ***Postimplantation loss:***

Dose-level (mg/kg bw/day)	**Postimplantation loss (%)**
0	5.5
55	10.9
175	10.0
540	25.9

- Effect-level at control is 5.5 %. Effect-level at ED10 is 15.5% (i.e. 5.5% + 10%)
- 175 mg/kg bw/day < ED10 < 540 mg/kg bw/day
- An increase of 365 mg/kg bw/day (i.e. 540-175 mg/kg bw/day) ~ an increase in postimplantation loss incidences of 15.9% (i.e. 25.9-10%)
- 1% change in postimplantation loss ~22.95 mg/kg bw/day increase in dose
- 5.5 % change in postimplantation loss (i.e.15.5-10%) ~126 mg/kg bw/day increase in dose
- ED10 = 175+126 = 301 mg/kg bw/day

- ***Bulbous aortic arch***

Dose-level (mg/kg bw/day)	**Bulbous aortic arch (%)**
0	0.6
55	0
175	0.9
540	24

- Effect-level at control is 0.6 %. Effect-level at ED10 is 10.6% (i.e. 0.6% + 10%)

- 175 mg/kg bw/day < ED10 < 540 mg/kg bw/day
- An increase in dosing of 365 mg/kg bw/day (i.e. 540-175 mg/kg bw/day) ~ an increase of bulbous aortic arch incidences of 23.1% (i.e. 24-0.9%)
- 1% change in bulbous aortic arch incidences ~15.8 mg/kg bw/day increase in dose
- 9.7 % change in bulbous aortic arch incidences (i.e. 10.6-0.9%) ~153 mg/kg bw/day increase in dose
- ED10 = 175+153=328 mg/kg bw/day

- ***Pulmonary trunk stenosis:***

Dose-level (mg/kg bw/day)	**Pulmonary trunk stenosis (%)**
0	0.6
55	0
175	0.9
540	20

- Effect-level at control is 0.6 %. Effect-level at ED10 is 10.6% (i.e. 0.6% + 10%)
- 175 mg/kg bw/day < ED10 < 540 mg/kg bw/day
- Increase in dosing of 365 mg/kg bw/day (i.e. 540-175 mg/kg bw/day) ~ an increase in pulmonary trunk stenosis incidences of 19.1% (i.e. 20-0.9%)
- 1% change in pulmonary trunk stenosis ~19.1 mg/kg bw/day increase in dose
- 9.7 % change in pulmonary trunk stenosis incidences (i.e. 10.6-0.9%) ~185 mg/kg bw/day increase in dose
- ED10 = 175+185 = 360 mg/kg bw/day

- ***Interventricular septal defect***

Dose-level (mg/kg bw/day)	**Interventricular septal defect (%)**
0	1.2
55	0
175	1.9
540	28.9

- Effect-level at control is 1.2 %. Effect-level at ED10 is 11.2% (i.e. 1.2% + 10%)
- 175 mg/kg bw/day < ED10 < 540 mg/kg bw/day
- Increase in dosing of 365 mg/kg bw/day (i.e. 540-175 mg/kg bw/day) ~ increase of 27% (i.e. 28.9-1.9%) incidences
- 1% change in interventricular septal defect incidences ~13.5 mg/kg bw/day increase in dose
- 9.3 % change in interventricular septal defect incidences (i.e. 11.2-1.9%) ~126 mg/kg bw/day increase in dose
- ED10 = 175+126 = 301 mg/kg bw/day

Rat, oral 2-generation study (BASF 1999)

- ***Complete litters lost incidences (%)***

Dose-level (mg/kg bw/day)	Complete litters lost (%)
0	0
50	0
160	0
500	75

- Effect-level at control is 0 %. Effect-level at ED10 is 10% (i.e. 0% + 10%)
- 160 mg/kg bw/day < 500 mg/kg bw/day
- Increase in dosing of 340 mg/kg bw/day (i.e. 500-160 mg/kg bw/day) ~ an increase of 75% (i.e. 75-0%) complete litters lost
- 1% change in complete litters lost incidences ~4.5 mg/kg bw/day increase in dose
- 10 % change in complete litters lost incidences (i.e. 10-0%) ~45 mg/kg bw/day increase in dose
- ED10 = 160+45 = 205 mg/kg bw/day

- ***Stillborn pups (% of total pups)***

Dose-level (mg/kg bw/day)	Stillborn pups (%)
0	2.1
50	3.3
160	2.5
500	8.1

- Effect-level at control is 2.1 %. Effect-level at ED10 is 12.1% (i.e. 2.1% + 10%)
- ED10 > 500 mg/kg bw/day
- Increase in dosing of 340 mg/kg bw/day (i.e. 500-160 mg/kg bw/day) ~ an increase of 5.6% (i.e. 8.1-2.5%) of stillborn pups
- 1% change in percentage stillborn pups ~60.7 mg/kg bw/day increase in dose
- 4 % increase in percentage stillborn pups (i.e. 12.1-8.1%) ~243 mg/kg bw/day increase in dose
- ED10 = 500+243=743 mg/kg bw/day

- ***Pup (F1a)-mortality incidences***

Dose-level (mg/kg bw/day)	Pup (F1a) mortality (%)
0	2.8
50	7.6
160	2.8
500	54

- Effect-level at control is 2.8%. Effect-level at ED10 is 12.8 (i.e. 2.8% + 10%)
- 160 mg/kg bw/day < ED10 < 500 mg/kg bw/day

- Increase in dosing of 340 mg/kg bw/day (i.e. 500-160 mg/kg bw/day) ~ an increase of 51.2% (i.e. 54-2.8%) of pup (F1a) mortality
- 1% change in pup mortality (F1a) incidences ~6.6 mg/kg bw/day increase in dose
- 10 % change in pup mortality (F1a) incidences (i.e. 12.8-2.8%) ~66 mg/kg bw/day increase in dose
- ED10 = 160+66 = 226 mg/kg bw/day