Committee for Risk Assessment

RAC

Annex 1

Background document

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

N-ethyl-2-pyrrolidone (NEP)

ECHA/RAC/CLH-O-0000002192-83-01/A1

EC number: 220-250-6
CAS number: 2687-91-4

Adopted

29 November 2011

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PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

Substance name: N-ethyl-2-pyrrolidone

EC number: 220-250-6

CAS number: 2687-91-4

Registration number(s):

Purity: generally > 97% based on information found in MSDS

Impurities: gamma-butyrolactone, free amines and water are listed in some MSDS

Proposed classification based on Directive 67/548/EEC:

Repr. Cat. 2; R61

Proposed classification based on CLP:

Repr. 1B – H 360D

Proposed labelling:

Based on Directive 67/548/EEC:		
	Indication of danger:	T
	Risk phrases:	R61
	Safety phrases:	S(1/2)-36/37-45-53
Based on Regulation 1278/2008 (EC):		
	Pictogram:	GHS 08
	Signal word:	Dgr
	Hazard statements:	H 360

Proposed specific concentration limits (if any):

None

Proposed notes (if any):

None

JUSTIFICATION

1 IDENTITY OF THE SUBSTANCE AND PHYSICAL-CHEMICAL PROPERTIES

1.1 Name and other identifiers of the substance

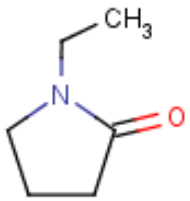
Chemical Name:	N-ethyl-2-pyrrolidone
EC Number:	220-250-6
EC Name:	1-ethylpyrrolidin-2-one
CAS Number:	2687-91-4
IUPAC Name:	1-ethylpyrrolidin-2-one

1.2 Composition of the substance

The following information is based from literature and MSDS. Data from the registration dossiers are included in the confidential Annex I to the CLH report (separate file).

Constituent

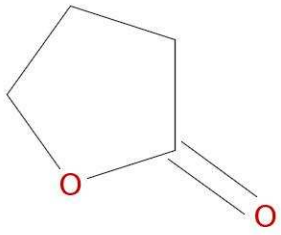
Chemical Name:	N-ethyl-2-pyrrolidone
EC Number:	220-250-6
EC Name:	1-ethylpyrrolidin-2-one
CAS Number:	2687-91-4
IUPAC Name:	1-ethylpyrrolidin-2-one
Molecular Formula:	$C_6H_{11}NO$
Structural Formula:	



Molecular Weight:	113.16 $g.mol^{-1}$
Typical concentration (% w/w)	Information not known
Concentration range (% w/w)	> 97 %

Impurities

Chemical Name:	Gamma-butyrolactone
EC Number:	202-509-5
CAS Number:	96-48-0
IUPAC Name:	Dihydrofuran-2(3H)-one
Molecular Formula:	$C_4H_6O_2$
Structural Formula:	



Molecular Weight:	86.09 $g.mol^{-1}$
Typical concentration (% w/w)	Not known
Concentration range (% w/w)	Not known
Classification	No harmonised classification

Chemical Name:	Water
EC Number:	231-791-2
CAS Number:	7732-18-5
IUPAC Name:	Water
Molecular Formula:	H_2O
Structural Formula:	H-O-H
Molecular Weight:	18$g.mol^{-1}$
Typical concentration (% w/w)	Not known
Concentration range (% w/w)	Not known
Classification	No harmonised classification

Free amines are also listed in some MSDS.

1.3 Physico-Chemical properties

Table 1 Summary of physico-chemical properties

REACH ref Annex, §	Property	Value	Reference
VII, 7.1	Physical state at 20 C and 101.3 KPa	Clear colourless to pale yellow liquid with a characteristic amine odour	INRS 2008
VII, 7.2	Melting / freezing point	Melting point: <-75°C	INRS 2008
VII, 7.3	Boiling point	212-213°C	INRS 2008
VII, 7.4	Relative density	0.998 at 20°C	INRS 2008
VII, 7.5	Vapour pressure	50Pa at 32°C	INRS 2008
VII, 7.6	Surface tension	36 dynes/cm	Taminco, 2005a
VII, 7.7	Water solubility	115 000 mg/l at 25°C	SRC, 2010
VII, 7.8	Partition coefficient n-octanol/water (log value)	-0.2	INRS 2008
VII, 7.9	Flash point	90.5°C	INRS 2008
VII, 7.10	Flammability	No data	
VII, 7.11	Explosive properties	Vapors can form explosive mixtures with air	INRS 2008
VII, 7.12	Self-ignition temperature	No data	
VII, 7.13	Oxidising properties	No data	
VII, 7.14	Granulometry	Not relevant (liquid)	
IX, 7.15	Stability in organic solvents and identity of relevant degradation products	Soluble in organic solvents	INRS 2008
IX, 7.16	Dissociation constant	No data	
IX, 7.17	Viscosity	2.09 mPas (99% purity)	Taminco, 2005a
	Auto flammability	245-250°C	INRS 2008
	Reactivity towards container material	No data	
	Thermal stability	Thermal decomposition may yield carbon oxides	Taminco, 2005b
	Reactivity	Could react with oxidants and strong acids	INRS 2008

2 MANUFACTURE AND USES

NEP is used as a solvent, catalyst and cationic surfactant in industry.

3 CLASSIFICATION AND LABELLING

3.1 Classification in Annex VI of CLP

No current harmonised classification in Annex VI of CLP

3.2 Self classification(s)

No data available.

4 ENVIRONMENTAL FATE PROPERTIES

Not covered in this dossier.

5 HUMAN HEALTH HAZARD ASSESSMENT

5.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

No data available.

5.2 Acute toxicity

Not covered in this dossier.

5.3 Irritation

Not covered in this dossier.

5.4 Corrosivity

Not covered in this dossier.

5.5 Sensitisation

Not covered in this dossier.

5.6 Repeated dose toxicity

5.6.1 Repeated dose toxicity: oral

A 90-day study has been conducted in rats by oral route (**BASF 2006**) according to OECD 408. Groups of 10 male and 10 female Wistar rats received doses of 0, 100, 300 and 1000 mg/kg bw/d of NEP (purity 99.8%) over a period of 3 months via their diet. Detailed clinical examinations in an open field were conducted prior to the start of the administration period and weekly thereafter. A functional observational battery (FOB) and measurement of motor activity was carried out. Vaginal smears for estrus cycle determination of all female animals were prepared and evaluated each day during the last 4 weeks of the study. Clinicochemical, hematological examinations and urinalyses were performed towards the end of the administration period. Ophthalmological examinations were performed before and towards the end of the administration period. All animals were assessed by gross pathology, followed by histopathological examinations. Additionally, sperm parameters were determined immediately after necropsy and organ weight determination.

At 1000 mg/kg, food consumption was significantly reduced in males (-21%) and females (-23%) and body weight was significantly reduced in both sexes from day 7 till 91 (up to -18% in males and -18% in females). At 300 mg/kg, food consumption was decreased in both sexes, statistically

significant on several days (variation not given) and body weight was significantly reduced from day 21 in females and day 28 in males (up to -9% in both sexes).

Grip strength was reduced at 1000 and 300 mg/kg/day and motor activity at 1000 mg/kg. Some effects on clinical chemistry and hematology parameters observed at 1000 mg/kg/day (Increased platelets, inorganic phosphate, calcium, cholesterol and triglycerides in both sexes, shortened prothrombin time and decreased creatinine in both sexes, decreased chloride, glucose and total bilirubin in males, decreased total protein and albumin in females). These effects were limited to a decrease in total bilirubin in males and in total protein and albumin in females at 300 mg/kg.

Target organs were the liver in both sexes and the kidney in males. Centrilobular hypertrophy of hepatocytes was observed in both sexes at 1000 mg/kg and in males at 300 mg/kg and 100 mg/kg and an increase in liver weights was observed in both sexes at 1000 mg/kg and in males at 300 mg/kg and 100 mg/kg. The centrilobular hypertrophy of hepatocytes is indicative for an enzyme induction of the cytochrome P450 system and is considered to be a detoxifying, adaptive effect (no signs for cytotoxicity recorded). In males at all doses, kidney weight was increased and an increase in basophilic tubules and accumulation of hyaline droplets (alpha 2µ globuline) were observed.

Sperm analysis revealed an increased number of sperms with abnormal heads in males at 1000 mg/kg but administration did not affect the number of homogenization resistant spermatids, epididymal sperm count and sperm motility. The most common sperm head anomalies were abnormal hooks and amorphous heads. This effect was not associated with any histopathological changes in the testis.

5.6.2 Repeated dose toxicity: inhalation

No data available.

5.6.3 Repeated dose toxicity: dermal

No data available.

5.6.4 Summary and discussion of repeated dose toxicity

Information on repeated dose toxicity is reported here for information only, so as to provide a general toxicological profile on NEP and assist evaluation of developmental effects.

5.7 Mutagenicity

Not covered in this dossier.

5.8 Carcinogenicity

Not covered in this dossier.

5.9 Toxicity for reproduction

5.9.1 Effects on fertility

Not covered in this dossier.

5.9.2 Developmental toxicity

5.9.2.1 Developmental studies in rabbits

A prenatal developmental study was performed in rabbit by dermal route (**BASF 2010**). The study was performed according to GLP and to OECD 414 guideline. NEP (purity: 99.8%) was administered dermally to 25 artificially inseminated female Himalayan rabbits per group from gestation day 6 to 28 at doses of 0, 100, 300 and 1000 mg/kg bw. The dosing solution was a 33.3% aqueous solution of NEP. Deionized water or ascending volume of NEP dosing solution were administered onto an intact shaven dorsal skin, covered by semi-occlusive gauze patch for six hours and subsequently washed off and dried. All female were observed daily for clinical signs of toxicity. Maternal food consumption was measured daily and body weights every 2 or 3 days. Animals were killed on gestation day 29 (post-insemination). Gross pathology was performed and ovary, uterine content and fetuses were examined for external anomalies. Corpora lutea were determined and number and distribution of implantation sites were determined. Fetuses were examined for external and visceral changes and for skeletal anomalies.

Maternal parameters are summarized in Table 2. One low dose group animal died prematurely (cause of death not given) and one mid dose group animal had to be sacrificed after abortion on day 29 post insemination (PI). Orange or reddish discolored urine was recorded in all high-dose females from GD 8. This finding reflects the systemic availability of NEP but is not considered to reflect an adverse toxic effect. No other significant clinical sign was reported in does. The skin was free from any notable findings.

Statistically significant decreases of daily food consumption were observed from GD 6 to 17 in the high dose group compared to controls. Increases in food consumption were also noted at the end of the gestation and were significant at GD 27-28. Over the whole treatment period, food consumption was 17% lower in the high dose group than in controls (no statistical analysis reported).

A loss of body weight was noted in high-dose females on GD 6-9 when treatment was started but there was no statistical difference between the body weight of control and treated animals during the administration period. The consequent body weight difference with controls was maintained approximately stable until the terminal sacrifice (2%).

A statistically significant decrease of weight gain was also observed in high-dose females on GD 6 to 9 but it did not attain statistical significance over the whole administration period (-21%). No significant effect on the corrected weight gain was observed and corrected maternal weight was similar across groups.

Table 2 Maternal parameters

	Dose (mg/kg/day)			
GD	*0*	*100*	*300*	*1000*

Body weight:	*GD0*	2408±115	2414±125	2414±137	2409±135
	GD6	2478±140	2489±175	2482±173	2473±155 (-0.2%)
	GD29	2794±163	2782±198	2793±1205	2726±145 (-2%)
Body weight changes:	*0-6*	69.9±75.41	75.2±78.69	67.9±76.49	64.3±84.31 (-8%)
	6-29	293.0±86.47	269.0±101.26	278.0±136.82	232.3±84.63 (-21%)
	0-29	386.0±118.68	373.8±116.12	382.5±140.57	317.1±99.76 (-18%)
Corrected weight gain[a]		-51.7±93.04	-46.7±85.91	-31.7±79.43	-73.4±102.49 (-42%)
Corrected weight GD29[b]		2426.5±160	2441.1±170.05	2444.6±178.47	2400.0±99.71 (-1%)
Food consumption[c]:	*0-6*	119.1±7.46	123.6±6.63	121.2±8.98	116.9±4.30
	6-29	108.1±9.98	107.5±12.22	105.7±9.39	89.7±13.72 (-17%)
	0-29	109.8±10.87	110.1±13.54	108.3±11.65	95.9±16.50 (-13%)

* p<0.05, **p<0.01

[a] weight of the carcass at GD29 after removal of the gravid uterus minus day 6 body weight

[b] weight of the carcass at GD29 after removal of the gravid uterus (grams).

[c] Mean per day and per animal. No statistical analysis reported for food consumption calculated over several days.

Main reproductive parameters are summarised in Table 3. 21-24 pregnant rabbits per group had implantation sites. No significant effect was observed on reproductive parameters and in particular on post-implantation loss or fetal weight.

Table 3 Gestational parameters

	Dose (mg/kg/day)			
	0	*100*	*300*	*1000*
% post-implantat° loss/ litter	5.8±10.71	5.2±9.79	8.9±13.11	9.6±14.48
% dead fetuses / litter	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
% resorptions / litter	5.8±10.71	5.2±9.79	8.9±13.11	9.6±14.48
% early resorptions/ litter	4.6±9.08	3.1±7.31	6.8±11.02	5.5±13.58
% late resorptions / litter	1.2±3.87	2.1±7.47	2.1±5.32	4.0±6.37
Fetal body weight (g)	39.1±2.49	37.9±5.18	39.4±3.97	36.8±3.89 (-6%)

* p<0.05, **p<0.01

Malformations and variations in the foetuses are reported in Table 4.

External malformations were reported in one foetus at the high dose (cleft palate). Cleft palate has been reported in historical controls of the laboratory (from 24 studies) with a foetal incidence of 0.06% (range: 0-0.6%) and although rare, this malformation is therefore observed with an incidence within the historical control range. There was no increase of external variations in the treated animals (data not shown in Table 4).

No significant increase in overall or individual incidences of visceral malformations and no dose-response are observed. Several malformations of the cardiovascular system are however reported in treated animals. In particular, absent subclavian is observed in one mid-dose and one high-dose foetus, whereas it has not been reported in historical controls despite the large size of the database (24 studies). Membranous ventricular septum defect and dextrocardia also exceed historical control range at the high dose, although it is noted that the three foetuses with dextrocardia are in the same litter. There was no visceral variation attributed to treatment (data not shown in Table 4).

No significant increase in overall or individual incidences of skeletal malformations and no dose-response are observed. The vast majority of the noted skeletal variations appeared without a dose response. Only the increase in incidence of supernumerary 13th rib (cartilage not present) was

observed at the high dose above historical controls (2.5 to 13.9% of foetuses and 8.0 to 52.2% of litters affected) and was statistically significant when litter incidence and incidence of affected foetuses by litter were considered (data not shown in the Table 4).

Table 4 Foetal incidence of malformations and variations

	Dose (mg/kg/day)				*Historical controls*[b]
	0	*100*	*300*	*1000*	
Number of foetuses (litters) evaluated	155 (23)	155 (23)	134 (21)	144 (23)	
% foetus with any malformations [c]	1.9%	6.5%	5.2%	4.9%	4.07% (1.27-7.64%)
% litters with any malformations	13%	29%	24%	22%	23.40% (8.00-43.48%)
% foetus with external malformations [c]	0%	0%	0%	0.7%	0.3% (0-2.6%)
% litters with external malformations	0%	0%	0%	4.3%	2.0% (0-20.0%)
% foetus with visceral malformations [c]	0.6%	3.9%	4.5%	3.5%	2.6% (0-7.2%)
% litters with visceral malformations	4.3%	13%	19%	13%	15.5% (0-43.5%)
Cardiovascular malformations[a]*:*	0	1.3%	0.7%	3.5%	-
Absent subclavian	0	0	0.7%	0.7%	0
Membranous ventricular septum defect	0	0.6%	0	1.4%	0.06% (0-0.7%)
Dextrocardia	0	0	0	2.1%	0
% foetus with skeletal malformations [c]	1.3%	2.6%	0.7%	0.7%	1.9% (0.6-3.1%)
% litters with skeletal malformations	8.7%	17%	4.8%	4.3%	12.8% (4.0-21.7%)
% foetus with skeletal variations [c]	65%	62%	61%	69%	60.9% (51.5-77.8%)
% litters with skeletal variations	100%	96%	95%	100%	96.6% (80.0-100%)
Supernumerary rib (13th); cartilage not present	7.7%	0.6%	12%	16%	6.9% (2.5-13.9%)

* $p<0.05$,

[a] incidence of foetuses with cardiovascular malformations, i.e. absent subclavian, aortic arch atresia, ventricular septum defect (membranous) and dextrocardia altogether.

[b] mean fetal incidence (range) in historical control data from 24 studies performed in the same laboratory from 2003 to 2009.

[c] no statistical analysis for this parameter

Overall, NEP by dermal route in rabbits induced significant but transient maternal toxicity at 1000 mg/kg/d at the beginning of substance administration (GD6-9) as evidenced by a loss of maternal body weight and statistically significant decreases in maternal food consumption. Over the whole treatment period, no significant difference in maternal corrected body weight was noted.

In this study, NEP had no significant effect on post-implantation loss, foetal weight and incidence of external or skeletal malformations. An increase in cardiovascular malformations was however observed and in particular the incidence of absent subclavian, membranous ventricular septum defect and dextrocardia were above historical control range in the high dose foetuses.

Two prenatal developmental studies were performed in rabbit by gavage (NEP purity: 99.8%). The studies were performed according to GLP and to OECD 414 guideline. NEP was administered by gavage to 25 artificially inseminated female Himalayan rabbits per group from gestation day 6 to 28. All female were observed daily for clinical signs of toxicity. Maternal food consumption was measured daily and body weights every 2 or 3 days. Animals were killed on gestation day 29 (post-insemination). Blood was taken from all surviving females and maternal blood and serum parameters were evaluated. Gross pathology was performed and ovary, uterine content and fetuses were examined for external anomalies. Maternal liver, spleen and kidneys were also weighted. Corpora lutea were determined and number and distribution of implantation sites were determined. Fetuses were examined for external and visceral changes and for skeletal anomalies. In the first study (**BASF 2007a**), dams were exposed to 0, 20, 60 or 200 mg NEP/kg bw as an aqueous solution. At scheduled necropsy, 22 to 23 females/group had implantation sites. Maternal parameters are summarized in Table 5. One low dose group animal had to be sacrificed after

abortion on day 29 post insemination (PI) and one high dose group animal died prematurely on GD 23 after gavage error. Orange or reddish discolored urine was recorded in all high-dose females from GD 8 and one mid-dose dam from GD 27. This finding reflects the systemic availability of NEP but is not considered to reflect an adverse toxic effect. No other significant clinical sign was reported in does.

A slight loss of weight in high dose females was noted on GD 6 when gavage was started and the consequent slight difference (not significant) between high dose and control animals was maintained until the terminal sacrifice (up to 3%). This was reflected by a statistically significant decrease of weight gain in high dose females between GD 6 to 9 but the weight gain over the whole gavage period was not significantly affected (affected only over the whole gestation). Similarly the maternal corrected body weight was statistically not different between groups. Statistically significant decreases of daily food consumption compared to controls were observed on GD 6 to 17 in the high dose group. Increases in food consumption were also noted on some days between GD 22 to 27 in the mid-dose group.

Table 5 Maternal parameters

		Dose (mg/kg/day)			
	GD	*0*	*20*	*60*	*200*
Body weight:	*GD0*	2456±180	2451±198	2480±224	2467±184
	GD6	2545±205	2526±189	2566±245	2540±193 (-0.2%)
	GD29	2800±197	2790±180	2822±168	2713±181 (-3%)
Body weight changes:	*0-6*	88.7±45.88	75.7±47.70	85.8±60.08	73.0±42.73 (-18%)
	6-29	236.5±85.83	246.1±98.79	240.3±105.39	173.2±88.57 (-27%)
	0-29	343.6±82.49	339.9±133.80	341.7±100.45	**246.0±106.66** (-28%)**
Corrected weight gain[a]		-86.4±86.79	-66.3±96.96	-75.9±105.90	-104.6±118.03 (-27%)
Corrected weight GD29[b]		2458.5±169.13	2476.3±137.97	2490.2±223.54	2435.7±129.35 (-1%)
Food consumption[c]:	*0-6*	122.9±2.24	120.4±5.30	124.0±2.95	124.3±4.79 (+1%)
	6-29	96.8±12.33	98.5±10.41	100.1±6.71	82.9±7.27 (-14%)
	0-29	101.6±15.75	102.6±13.29	104.6±11.83	91.5±18.27 (-10%)

* $p<0.05$, ** $p<0.01$

[a] weight of the carcass at GD29 after removal of the gravid uterus minus day 6 body weight

[b] weight of the carcass at GD29 after removal of the gravid uterus (grams).

[c] Mean per day and per animal. No statistical analysis reported for food consumption calculated over several days.

Absolute maternal weights of the liver, spleen and kidneys were not significantly affected but an increase in relative liver ($p<0.01$) and kidney ($p<0.05$) weights were observed at the high dose. Analysis of blood parameters revealed an increase of enzymatic activities of the alanine transferase from the mid-dose (0.96±0.57 µkat/l in controls vs 1.17±0.47* at mid-dose and 1.41±0.78** at the high dose) and of the γ-glutamyl transferase at the high dose (83±19 nkat/l in controls vs 118±45** at the high dose). The levels of calcium (3.04±0.20 mmol/l in controls vs 3.15±0.25* at mid-dose and 3.21±0.18** at the high dose) and inorganic phosphate (1.31±0.14 mmol/l in controls *vs* 1.44±0.13** at the high dose) were also respectively increased from the mid-dose or at the high dose. The authors considered that the slight increase in alanine aminotransferase activity in the high dose group are indicative of mild liver damage, because liver weights in this group were increased correspondingly. The elevated γ-glutamyltransferase activities are also assessed as being treatment-related and are a consequence of microsomal enzyme induction in the liver. Although the slight

increases in calcium and inorganic phosphate were not particular marked, these findings are considered to be test substance-related, too. However, due to the isolated occurrence of both effects and due to the low magnitude of change, the increases in inorganic phosphate and calcium are difficult to interpret in their pathogenesis.
Gross pathology revealed no substance-related observations in the dams. Microscopic examination of the organ was not performed and it is not possible to know whether microscopic lesions were present in the organs.
Main reproductive parameters are summarised in Table 6. No significant effect was observed on reproductive parameters and in particular on post-implantation loss or fetal weight.

Table 6 Gestational parameters

	Dose (mg/kg/day)			
	0	*20*	*60*	*200*
% post-implantat° loss / litter	6.0±12.31	4.2±9.61	10.7±11.45	13.8±16.61
% dead fetuses / litter	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
% resorptions / litter	6.0±12.31	4.2±9.61	10.7±11.45	13.8±16.61
% early resorptions/ litter	4.9±11.37	4.2±9.61	8.6±10.43	9.0±12.88
% late resorptions / litter	1.2±3.97	0.0±0.0	2.1±4.57	4.8±9.99
Fetal body weight (g)	38.0±3.60	38.4±4.13	38.7±4.58	36.4±3.68 (-4%)

* $p<0.05$, ** $p<0.01$

Malformations and variations in the foetuses are reported in Table 7.

External malformations were reported in one foetus at mid dose (spina bifida) and in one foetus at high dose (meningocele). These findings have never been reported in historical controls of the laboratory (from 9 studies). Three incidences of encephalocele (0.26% of litter and 0.039% of foetuses) and 1 incidence of meningocele (0.09% of litter and 0.013% of foetuses) are reported in the literature in 1136 litters of Himalayan rabbits (Viertel 2003). These malformations are therefore considered as rare. There was no increase of external variations in the treated animals (data not shown in Table 7).

No significant increase in visceral malformations is observed. It is noted that 2 foetuses (1.6%) have a cardiovascular malformation (ventricular septum defect) at high dose. One control foetus also have this defect and the incidence at high dose is in the range of historical controls of the laboratory (range of foetal incidence: 0 – 2.3%) and it may not be related to treatment. There was no visceral variation attributed to treatment (data not shown in Table 7).

The incidence of litters with foetuses having skeletal malformations was statistically increased at the high dose. Statistical analysis of the foetal incidence was not performed. Both litter (35%) and foetal (6.3%) incidences for skeletal malformations were above historical control range (0 to 17.4% of litters and 0 to 2.8% of foetuses affected in historical controls). Skeletal malformations affected sternebrae, vertebral column, ribs and/or skull bones. In particular, the incidence of foetuses per litter with misshapen cervical vertebra was statistically significant at high dose (data not shown in the table). The vast majority of the noted skeletal variations appeared without a dose response. Increases in misshapen sacral vertebra and supernumerary 13^{th} rib were however observed at the high dose above historical controls (misshapen sacral vertebra: 0 to 1.3% of foetuses and 0 to 8% of litters affected; supernumerary 13^{th} rib: 2.5% to 12.7% of foetuses and 16.0% to 52.2% of litters affected) and were both statistically significant when incidence of affected foetuses by litter was considered (data not shown in the Table 7).

Table 7 Foetal incidence of malformations and variations

	Dose (mg/kg/day)				*Historical controls*[b]
	0	*20*	*60*	*200*	
Number of foetuses (litters) evaluated	154 (23)	135 (22)	138 (22)	126 (23)	
% foetus with any malformations [c]	2.6%	3.0%	1.4%	9.5%	4.16%
% litters with any malformations	17%	18%	9.1%	**48%***	20.98%
% foetus with external malformations [c]	0%	0%	0.7%	0.8%	0.2% (0-0.8%)
% litters with external malformations	0%	0%	4.5%	4.3%	1.0% (0-5.0%)
% foetus with visceral malformations [c]	1.3%	2.2%	0.7%	3.2%	2.9% (0.6-4.9%)
% litters with visceral malformations	8.7%	14%	4.5%	17%	15.6% (4.0-24.0%)
Cardiovascular malformations[a]*:*	0.6%	0	0	1.6%	-
Muscular ventricular septum defect	0.6%	0	0	1.6%	0.7% (0-2.3%)
% foetus with skeletal malformations [c]	1.3%	0.7%	0.7%	6.3%	1.5% (0-2.8%)
% litters with skeletal malformations	8.7%	4.5%	4.5%	**35%***	8.8% (0-17.4%)
Severely malformed skull bones	0	0	0	0.8%	0
Misshapen cervical vertebra	0	0	0	2.4%	0.08% (0-0.7%)
Cervical hemivertebra	0	0	0	0.8%	0.08% (0-0.7%)
Small cervical arch	0	0	0	0.8%	0
Absent lumbar vertebra	0	0.7%	0	0.8%	0.2% (0-0.7%)
Splayed lumbar arch	0	0	0.7%	0	0
Misshapen lumbar vertebra	0.6%	0	0	0.8%	0.2% (0-0.7%)
Sternebrae severely fused	0	0	0.7%	0	0.08% (0-0.7%)
Branched rib	0	0.7%	0	0	0
% foetus with skeletal variations [c]	66%	82%	79%	73%	68.4% (57.2-82.8%)
% litters with skeletal variations	91%	100%	100%	96%	97.6% (82.4-100%)
Misshapen sacral vertebra	0	1.5%	0.7%	4.0%	0.2% (0-1.3%)
Supernumerary rib (13th); cartilage not present	5.2%	3.0%	5.8%	17%	6.3% (2.5-12.7%)

* $p<0.05$,

[a] incidence of foetuses with cardiovascular malformations, i.e. ventricular septum defect.

[b] mean fetal incidence (range) in historical control data from 9 studies performed in the same laboratory from 2003 to 2006.

[c] no statistical analysis for this parameter

Overall in this study, NEP induced a slight maternal toxicity at the high dose with transient significant effects on food consumption and body weight gain at the beginning of treatment. However, effects were not significant over the whole treatment period (non significant decreases of food consumption of -14% and of body weight gain of -27%). This body weight changes needs to be related to the overall weight of the animal and no statistically significant decrease of maternal body weight was observed at the end of treatment (-3%). Besides, the corrected maternal body weight was not affected (-1%). Signs of mild hepatic damage were noted.

NEP had no significant effect on post-implantation loss, foetal weight but NEP induced a statistically significant increased incidence of malformations at the high dose. It consisted mainly in skeletal malformations. Besides, rare external malformations of the neural tube and of the cardiovascular system were reported.

The second supplementary study (**BASF 2007b**) was performed to allow a sound assessment of the results from the previous study, which was considered to show some borderline effects. Dams were

exposed to 0 or 220 mg/kg bw as an aqueous solution according to a similar protocol. At scheduled necropsy, 24 to 25 females had implantation sites.

Maternal parameters are summarized in Table 8. No dam mortality was observed. Orange or reddish discolored urine was recorded in all treated females from GD7. This finding reflects the systemic availability of NEP but is not considered to reflect an adverse toxic effect. No other significant clinical sign was reported in does.

A lower daily food consumption was observed in the treated dams during the administration period and the difference was statistically significant on GD 6 to 16 and GD21-22. Food intake in treated dams was in particular very low on GD7 to 8.

A loss of body weight in treated females was noted on GD 6-9 when gavage was started but there was no statistical difference between the body weight of controls and treated animals across the whole administration period. The maternal corrected body weight was also similar across groups.

A statistically significant decrease of weight gain was also observed in treated females on GD 6 to 9 and during the overall administration period (-38%). No significant effect on the corrected weight gain was observed although it was lower in treated dams (-23%).

Table 8 Maternal parameters

		Dose (mg/kg/day)	
	GD	*0*	*220*
Body weight:	*GD0*	2606±162.7	2607±169.7
	GD6	2651±169.9	2641±171.4 (-1%)
	GD29	2869±177.5	2791±147.2 (-3%)
Body weight changes:	*0-6*	44.7±31.90	34.7±26.90 (-22%)
	6-29	208.3±71.55	**129.0±86.54** (-38%)**
	0-29	263.6±96.40	**184.2±91.72** (-30%)**
Corrected weight gain[a]		-115.1±66.56	-142.1±79.37 (-23%)
Corrected weight GD29[b]		2535.4±164.97	2499.4±149.49 (-1%)
Food consumption[c]:	*0-6*	106.9±5.48	101.2±6.48 (-5%)
	6-29	90.6±7.84	73.4±7.60 (-19%)
	0-29	93.6±10.17	79.1±13.50 (-15%)

* p<0.05, **p<0.01

[a] weight of the carcass at GD29 after removal of the gravid uterus minus day 6 body weight

[b] weight of the carcass at GD29 after removal of the gravid uterus (grams).

[c] Mean per day and per animal. No statistical analysis reported for food consumption calculated over several days.

Absolute and relative maternal weights of the liver were significantly increased in treated females (p<0.01). Analysis of blood parameters revealed a decrease in the clotted time (17.8±0.8 in controls vs 17.2±1.4* in treated dams) and in the enzymatic activity of the alkaline phosphatase (0.64±0.15 µkat/l in controls vs 0.54±0.11* in treated dams) and an increase of enzymatic activities of the alanine transferase (0.82±0.21 µkat/l in controls vs 1.09±0.44* in treated dams) and of the γ-glutamyl transferase (76±22 nkat/l in controls vs 109±23** in treated dams). The levels of inorganic phosphate (1.12±0.15 mmol/l in controls vs 1.23±0.14* in treated dams), urea (4.57±0.54 mmol/l in controls vs 5.02±0.64* in treated dams), triglycerides (0.32±0.08 mmol/l in controls vs 0.43±0.08** in treated dams) and cholesterol (0.13±0.06 mmol/l in controls vs 0.19±0.07** in

treated dams) were also respectively increased. Albumin (31.85±3.14 g/l in controls vs 29.79±3.28* in treated dams) and magnesium (0.98±0.10 mmol/l in controls vs 0.92±0.09* in treated dams) were significantly decreased. The authors considered that the higher γ-glutamyltransferase (GGT) activity as well as the increased triglyceride and cholesterol values are due to a microsomal induction of the phase II enzymes in the hepatocytes. Even the lower albumin values as well as the shortened prothrombin time in the dose group are hints of a changed liver cell metabolism according to this enzyme induction. A slightly higher serum alanine-aminotransferase (ALT) activity in the serum of the treated rabbits is an indication of a mild liver damage.
Gross pathology revealed no substance-related observations in the dams. Microscopic examination of the organ was not performed and it is not possible to know whether microscopic lesions were present in the organs.

Main reproductive parameters are summarised in Table 9. No significant effect was observed on reproductive parameters and in particular on post-implantation loss but the fetal weight was significantly lower in the test group (-15%). Considering that the maternal food consumption was reduced (-19%, not significant) in the test group during the gavage period, the link between the two effects can be questionned. However, the absence of effect on maternal corrected body weight at sacrifice (-1% in the test group) does not point toward an effect due to maternal toxicity but indicate that decreased food consumption could be in part secondary to the limited fetal weight development.

Table 9 Gestational parameters

	Dose (mg/kg/day)	
	0	*220*
% post-implantat° loss / litter	12.0±15.17	11.0±11.93
% dead fetuses / litter	0.0±0.0	0.0±0.0
% resorptions / litter	0.8±0.83	0.8±1.01
% early resorptions / litter	0.6±0.65	0.5±0.88
% late resorptions / litter	0.2±0.50	0.3±0.64
Fetal body weight (g)	39.9±2.92	**34.1±2.53** (-15%)**

* p<0.05, **p<0.01

Malformations and variations in the foetuses are reported in Table 10.

External malformations were reported in two foetuses from two litters in the test group. They showed severe multiple malformations (gastroschisis, cleft palate, meningocele, misshapen head, malrotated fore- and hindlimbs, forelimbs micromelia and ectrodactily in one fetus, acephaly, thoracogastroschisis, absent claw, forelimb paw hyperflexion in the other). These findings are rare as only 1 foetus (0.08% of foetuses) was reported to have multiple external malformations and 1 malrotated limb in historical controls of the laboratory (from 9 studies). There was no significant increase of external variations in the treated animals (data not shown in Table 10).

A statistically significant increase in visceral malformations was observed in the test group and they exceed historical control data. The two foetuses with multiple external malformations also showed multiple severe visceral malformations. When considering each type of malformation one by one, the incidences of small spleen and of absent gallbladder in the test group were slightly above the historical control upper range but were not statistically significant. The incidence of absent subclavian was above historical controls and was statistically significantly above incidence in controls when considering the incidence of foetuses per litter ($p<0.05$, data not shown in the table). When considering the incidence of the different malformations of the cardiovascular system altogether, it is observed that 2 foetuses were affected in controls vs 7 in the test group (no statistical analysis and no historical control data available for this calculated value). A statistical increase in the incidence of visceral variations was also noted but the incidence was in the historical control range.

The incidence of foetuses per litter with skeletal malformations was statistically increased in treated group and exceeded the historical control upper range (data not shown in Table 10). Malformations affect different foetal components and these findings did not form a distinct malformation pattern. Skeletal variations were also increased (statistically significant when considering incidence of foetuses per litter, data not shown in the table). In particular, the incidence of incomplete ossification of cervical centrum, extra ossification site between cervical arches, fused sternebra, misshapen sacral vertebra and supernumerary rib (13th) were above historical controls and statistically significant when considering incidence of foetuses per litter (data not shown in the table).

Table 10 Foetal incidence of malformations and variations

	Dose (mg/kg/day)		*Historical controls*[a]
	0	*220*	
Number of foetuses (litters) evaluated	150 (25)	144 (24)	
% foetus with any malformations [b]	6.0%	15%	3.74% (1.27-7.64%)
% litters with any malformations	32%	**67%***	19.21% (8.0-28.0%)

% foetus with external malformations [b]	0%	1.4%	0.2% (0-0.8%)
% litters with external malformations	0%	8.3%	1.0% (0-5.0%)
% foetus with visceral malformations [b]	4.0%	12%	2.6% (0.6-4.9%)
% litters with visceral malformations	24%	**54%***	13.8% (4.0-21.7%)
Small spleen	0	1.4%	0.08% (0-0.8%)
Absent gallbladder	2.0%	4.9%	1.5% (0-2.8%)
Cardiovascular malformations[b,c]	1.4%	4.9%	-
Absent subclavian[f]	0	2.1%	0.08% (0-0.6%)
Persistent truncus arteriosus [e]	0	0.7%	0
Muscular ventricular septum defect[f]	0.7% (4%)	0	0.6% (0-2.3%)
% foetus with visceral variations [b]	3.3%	17%	15.3% (5.6-28.1%)
% litters with visceral variations	20%	**50%***	47.3% (11.8-78.3%)
% foetus with skeletal malformations [b]	2.7%	6.9%	1.3% (0-2.8%)
% litters with skeletal malformations	16%	38%	7.9% (0-17.4%)
Small hyoid	0	0.7%	0
Severely malformed vertebra, sternum and/or ribs	0	0.7%	0
Misshapen cervical vertebra	0.7%	0	0
Cervical hemivertebra	0.7%	0	0
Fused cervical arch	0	0.7%	0
Misshapen thoracic vertebra	0	0.7%	0.08% (0-0.7%)
Absent lumbar vertebra	0	0.7%	0.2% (0-0.7%)
Misshapen lumbar vertebra	0.7%	1.4%	0.2% (0-0.7%)
Sternebrae severely fused	0.7%	1.4%	0.08% (0-0.6%)
Malpositioned and bipartite sternebra	0	0.7%	0
Small forepaw phalang[h]	0	0.7%	0
% foetus with skeletal variations [b]	62%	81%	68.8% (57.2-82.8%)
% litters with skeletal variations	100%	100%	96.6% (82.4-100%)
Incomplete ossify. of cervical centrum	1.3%	15%	2.6% (0-4.9%)
Extra ossif. site between cervical arches	0	3.5%	0.08% (0-.08%)
Fused sternebra	5.3%	13%	5.6% (2.8-10.7%)
Misshapen sacral vertebra	1.3%	4.9%	0
Supernumerary rib (13th); cartilage not present	13%	29%	5.4% (2.5-11.7%)

* $p<0.05$,

[a] mean fetal incidence (range) in historical control data from 9 studies performed in the same laboratory from 2003 to 2006.

[b] no statistical analysis for this parameter

[c] incidence of foetuses with cardiovascular malformations, i.e. absent subclavian, persistent truncus arteriosus, ventricular septum defect and mishappen heart altogether.

[d] do not include one additional foetus in the test group with multiple visceral malformations including absent subclavian and one with multiple visceral malformations including malpositioned subclavian branch.

[e] do not include one foetus in controls and two additional foetuses in the test group with multiple visceral malformations including persistent truncus arteriosus

[f] do not include one additional foetus in controls and two foetuses in the test group with multiple visceral malformations including ventricular septum defect.

[g] do not include one additional foetus in the test group with multiple skeletal malformations including small forepaw and hindpaw phalanges.

Overall in this study, NEP induced a slight maternal toxicity with transient significant effects on food consumption and body weight gain, in particular at the beginning of treatment. Body weight change was also significantly affected over the whole treatment period. However, the maternal body weight did not differ significantly between control and treated animals and the

corrected maternal body weight was also not affected (-1%). Signs of mild hepatic damage were noted.

NEP had no significant effect on post-implantation loss. However, a significantly lowered foetal weight was observed and NEP induced a significantly increased incidence of malformations. It consisted mainly in skeletal malformations. Besides, two severe external and visceral malformations were reported and the incidence of cardiovascular malformations was also elevated in treated animals. In particular the incidence of absent subclavian, was above historical control range and statistically significantly increased in treated foetuses.

5.9.2.2 Developmental studies in rats

A prenatal developmental study was performed in rat by dermal route (**BASF 2005**). The study was performed according to GLP and to OECD 414 guideline. NEP (purity: 99.8%) was administered dermally to 25 presumed pregnant female Wistar rats per group from gestation day 6 to 19 at doses of 0, 200, 400 and 800 mg/kg bw. The dosing solution was a 33.3% aqueous solution of NEP. Deionized water or ascending volume of NEP dosing solution were administered onto an intact shaven dorsal skin, covered by semi-occlusive gauze patch for six hours and subsequently washed off and dried. All female were observed daily for clinical signs of toxicity. Maternal food consumption was measured daily and body weights every 2 or 3 days. Animals were killed on gestation day 20 (post-insemination). Gross pathology was performed and ovary, uterine content and fetuses were examined for external anomalies. Corpora lutea and the number and distribution of implantation sites were determined. Fetuses were examined for external and visceral changes and for skeletal anomalies.

Maternal parameters are summarized in Table 11. At scheduled necropsy, 21 to 24 females/group had implantation sites. No mortality was observed in dams. Orange or reddish discolored urine was recorded in all mid-dose and high-dose females from GD 7 or 8. This finding reflects the systemic availability of NEP but is not considered to reflect an adverse toxic effect. Vaginal hemorrhage was occasionally observed between GD 13 to 15 (in 3, 1, 4 and 7 dams at 0, 200, 400 and 800 mg/kg) without clear relation to treatment.No other significant clinical sign was reported in dams. The skin was free from any notable findings.

Statistically significant decreases of daily food consumption were observed at GD 6-8 in the mid-dose group and at GD 6-8 and 8-10 and 17-19 in the high dose group compared to controls. Over the whole gavage period, food consumption was 10% lower in the high dose group than in controls (no statistical analysis reported for cumulative food consumption).

A loss of body weight was noted in the mid-dose and high-dose females on GD 6-8 when administration was started and it resulted in a statistically lower body weight gain than in controls but maternal body weight change on GD 8 to 10 was significantly increased in mid-dose and high-dose females. Over the whole treatment period, the body weight change was significantly lower in the high-dose animals than in controls (-22%) and corrected body weight gain was significantly decreased at mid- and high doses.

Similarly, the maternal body weight was significantly lower than controls at GD 6-8 in the mid-dose group and at GD 6-8, 8-10 and 17-19 in the high-dose group. The maternal corrected weight gain was significantly decreased only in the high dose group (-5%).

Table 11 Maternal parameters

		Dose (mg/kg/day)			
	GD	*0*	*200*	*400*	*800*
Body weight:	*GD0*	163.7±6.62	162.8±7.88	161.1±6.69	164.9±9.07
	GD6	192.6±9.08	191.9±8.31	191.2±7.42	194.1±9.45
	GD20	266.3±16.11	267.7±15.14	258.2±17.76 (-3%)	**252.5±19.00*** (-5%)
Body weight changes: 0-6		29.0±5.32	29.2±3.79	30.0±3.69	29.3±4.16
6-19		64.7±10.84	65.2±10.84	58.5±10.89 (-10%)	**50.7±12.33**** (-22%)
0-19		102.7±14.99	105.0±12.59	97.1±15.56 (-5%)	**87.6±14.11**** (-15%)
Corrected weight gain[a]		29.4±6.82	27.5±6.18	**23.3±7.47*** (-21%)	**16.7±7.85**** (-43%)
Corrected weight GD20[b]		222.0±12.0	219.4±11.67	214.4±11.14 (-4%)	**210.8±13.05**** (-5%)
Food consumption [c]: *0-6*		15.5±2.45	15.9±2.03	16.5±1.84	16.0±2.44
6-19		20.0±1.86	20.3±1.73	19.2±2.34	18.1±3.70 (-10%)
0-19		18.8±2.93	19.1±2.75	18.5±2.48	17.7±3.26 (-6%)

* p<0.05, **p<0.01

[a] weight of the carcass at GD20 after removal of the gravid uterus minus day 6 body weight

[b] weight of the carcass at GD20 after removal of the gravid uterus (grams).

[c] Mean per day and per animal. No statistical analysis reported for food consumption calculated over several days.

Main reproductive parameters are summarised in Table 12. 21-24 pregnant rats per group had implantation sites. One dam in the high dose group had full resorption. No significant effect was observed on reproductive parameters and in particular on post-implantation loss. However, fetal weight was significantly decreased in the high dose group.

Table 12 Gestational parameters

	Dose (mg/kg/day)			
	0	*200*	*400*	*800*
% post-implantat° loss/ litter	5.2±6.79	5.8±7.84	7.7±18.28	8.2±21.57
% dead fetuses / litter	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
% resorptions / litter	5.2±6.79	5.8±7.84	7.7±18.28	8.2±21.57
% early resorptions/ litter	4.0±5.25	5.1±6.56	6.6±18.6	3.6±6.73
% late resorptions / litter	1.3±4.48	0.8±2.51	1.1±3.56	4.5±21.32
Fetal body weight (g) [a]	3.5±0.16	3.4±0.24	3.4±0.19	**3.1±0.19**** (-11%)

* p<0.05, **p<0.01

[a] mean fetal weight of 3.5 g (range 2.8 to 4.2 g) in historical control data from 18 studies performed in the same laboratory with the same strain from 2001 to 2003.

Malformations and variations in the foetuses are reported in Table 13.

External malformations were reported in three foetuses from one litter in controls (omphalocele). No other external malformations or variations were reported. No visceral malformation and no significant incidence of visceral variations were observed.

Skeletal malformations were observed in one control foetus (misshapen lumbar vertebra), one mid-dose foetus (malpositioned bipartite sternebra) and one high-dose foetus (misshapen lumbar vertebra). The majority of the noted skeletal variations appeared without a dose response. Only significant increases in incidence of incomplete ossification of basisphenoid (incidence of affected

foetuses per litter significant and above historical controls at high dose), unossified sternebra (incidence of affected foetuses per litter significant from low-dose group but absence of dose-response and within the historical control incidences), unilateral ossification of sternebra (incidence of affected foetuses per litter significant and above historical control incidences in the low-dose and high-dose groups but absence of dose-response) and supernumerary 14th rib (incidence of affected foetuses per litter significant and above historical control incidences at high dose) were observed.

Table 13 Foetal incidence of malformations and variations

	Dose (mg/kg/day)				
	0	*200*	*400*	*800*	*Historical controls*[b]
% foetus with any malformations[a]	2.0%	0%	0.6%	0.5%	0.82% (0-2.70%)
% litters with any malformations	8.3%	0%	4.8%	4.8%	7.07% (0-25.0%)
Number of foetuses (litters) evaluated	197 (24)	211 (23)	178 (21)	189 (21)	
% foetus with external malformations[a]	1.5%	0%	0%	0%	0.2% (0-0.5%)
% litters with external malformations	4.2%	0%	0%	0%	1.5% (0-4.5%)
Number of foetuses (litters) evaluated	91 (24)	99 (23)	83 (20)	89 (21)	
% foetus with visceral malformations[a]	0%	0%	0%	0%	0.06% (0-1.1%)
% litters with visceral malformations	0%	0%	0%	0%	0.2% (0-4.2%)
Number of foetuses (litters) evaluated	106 (24)	112 (23)	95 (21)	100 (21)	
% foetus with skeletal malformations[a]	0.9%	0%	1.1%	1.0%	1.3% (0-5.1%)
% litters with skeletal malformations	4.2%	0%	4.8%	4.8%	6.1% (0-25.0%)
% foetus with skeletal variations[a]	96%	99%	98%	100%	94.6% (88.0-99.2%)
% litters with skeletal variations	100%	100%	100%	100%	100%
Incomplete ossification of basisphenoid	6.6%	4.5%	12%	26%	6.7% (0-15.7%)
Unossified sternebra (unchanged cart.)	0.9%	14%	12%	20%	10.6% (3.4-35.7%)
Unilateral ossification of sternebra	0.9%	7.1%	4.2%	8.0%	1.3% (0-3.7%)
Supernumerary rib (14th): cart. present	4.7%	8.0%	6.3%	17%	4.0% (0-8.7%)

* $p<0.05$,

[a] no statistical analysis for this parameter

[b] mean fetal incidence (range) in historical control data from 18 studies performed in the same laboratory from 2001 to 2003.

Overall, NEP by dermal route in rats induced slight but significant maternal toxicity as evidenced by the significantly decreased maternal corrected weight of 5% at 800 mg/kg/d.

In this study, NEP had no significant effect on post-implantation loss and incidence of malformations. A decreased foetal weight (-11%) was observed at 800 mg/kg/d but in presence of decreased maternal corrected weight and food consumption, it is not possible to attribute this effect to either direct foetotoxicity of NEP or to an effect secondary to maternal toxicity. The incidence of some skeletal variations was also increased.

In another study **(Saillenfait, 2007),** NEP (purity 99%, impurities not given) was administered daily by gavage on GD 6-20 to 19-24 pregnant Sprague-Dawley rats per group. Based on a dose-range

finding study, the dose levels were 0, 50, 250, 500 and 750 mg/kg/d. Dosing solutions were formulated in distilled water as vehicle. All female were observed daily for clinical signs of toxicity. Maternal food consumption and body weights were measured every 3 days from GD 6. Animals were killed on GD 21 and ovary, uterine content and fetuses were examined for external anomalies. The number of pregnant dam at euthanasia was respectively 20, 19, 23, 24 and 23. Half of the fetuses were examined for visceral changes and half for skeletal anomalies. This study was consistent with OECD guideline 414 except that the age of the dams is not given in the publication. However, the weight of the dams when supplied was consistent with Sprague-Dawley females aged 8 to 9 weeks, which is in agreement with OECD 414 requirement to use young adult animals.

Maternal parameters are summarized in Table 14. No clinical signs were reported except that urine of dams given NEP was bright yellow. Maternal body weight gain was significantly reduced at all doses on GD 6-9 as well as on GD 15-18 from 250 mg/kg onward, on GD 18-21 from 500 mg/kg and on GD 12-15 at the highest dose. No significant effect on corrected maternal body weight was however noted and the reduction in weight gain during late gestation was probably mainly due to post-implantation loss (see Table 15) at the two highest doses. Decrease in weight gain was accompanied by a significant decrease of food consumption in all groups on GD 6-9 as well as in the 500 and 750 mg/kg groups on GD 9-12. During late gestation, significantly decreased food consumption was observed only at the highest dose on GD 15-18 and 18-21.

Table 14 Maternal parameters

	Dose (mg/kg/day)				
	0	*50*	*250*	*500*	*750*
Body weight changes GD 6-9	13±2	**2±7****	**0±8 ****	**-4±5 ****	**-4±6 ****
GD 9-12	19±6	19±5	18±7	19±5	15±6
GD12-15	18±9	20±7	20±8	16±5	**8±6****
GD15-18	41±11	39±8	**34±10***	**28±8****	**13±6****
GD18-21	47±13	43±8	41±12	**37±11****	**12±10****
GD 0-21	170±36	154±25	**142±31* (-17%)**	**128±28** (-25%)**	**74±22** (-56%)**
Corrected weight gain[a]	66±23	60±26 (-9%)	52±16 (-21%)	55±13 (-17%)	58±12 (-11%)
Corrected weight GD 21[b]	292	287 (-2%)	279 (-5%)	282 (-3%)	283 (-3%)
Food consumption: *GD 6-9*	24±2	**19±4****	**18±3 ****	**17±2 ****	**15±3 ****
GD 9-12	26±2	25±3	25±3	**24±2****	**22±3****
GD12-15	26±3	26±4	27±3	26±2	25±2
GD15-18	28±4	29±74	28±4	27±3	**25±2***
GD18-21	26±4	26±4	25±3	24±2	**24±3***
GD0-21	26±2	25±3	24±2	**24±2* (-8%)**	**22±2** (-15%)**

[a] body weight gain during GD0-21 minus gravid uterine weight
[b] body weight at GD 21 minus gravid uterine weight. Calculated based on results given in the publication, no statistical analysis performed
* p<0.05, **p<0.01

The number of implantation sites was similar across groups. A significant dose-related increase in post-implantation loss was observed at the two highest doses (see Table 15). It consisted mainly of an increase in resorptions at these two doses as well as a small non significant increase in dead fetuses per litter at the highest dose. A dose-related increase in late resorptions was observed from 500 mg/kg/d onward whereas early resorptions were significantly induced only at 750 mg/kg/d.

A significant and dose-related decrease in fetal weight was also observed at 250 mg/kg and higher doses (respectively –7, -28 and –42% of control). The decrease in foetal weight was more important than the corresponding decrease of maternal corrected weight.

Table 15 Gestational parameters

	Dose (mg/kg/day)				
	0	*50*	*250*	*500*	*750*
% post-implantat° loss / litter	9.1±22.5	3.7±5.9	5.6±11.4	**20.8±25.9 ***	**88.3±22.9 ****
% dead fetuses / litter	0.0±0.0	0.0±0.0	0.0±0.0	0.9±2.3	5.1±8.8
% resorptions / litter	9.1±22.5	3.7±5.9	5.6±11.4	**19.9±26.0 ***	**83.2±25.2 ****
% early resorptions / litter[a]	8.7±22.2	3.7±5.8	5.6±11.4	7.1±11.1	**66.2±34.0****
% late resorptions / litter[a]	0.5±2.0	0.0±0.0	0.0±0.0	**12.8±22.9***	**16.9±25.6****
Fetal body weight (g)	5.58±0.26	5.61±0.33	**5.19±0.37 ** (-7%)**	**3.99±0.19 ** (-28%)**	**3.19±0.40 ** (-42%)**

* p<0.05, **p<0.01

[a] Personal communication from AM Saillenfait, attached in Appendix I.

The incidence of malformation in foetuses and in litters was statistically significantly increased at the two highest doses (see Table 16). The increase was significant for external malformations, skeletal malformations and skeletal variations at 500 and 750 mg/kg/d but only at 750 mg/kg/d for visceral malformations. Main results are summarized in Table 16. External malformations essentially consisted of oedema and anal atrasia associated with the absence of tail. Visceral evaluation revealed heart and great vessels malformations. Skeletal malformations primarily involved the axial skeleton. The most common were fused cervical arches. Skeletal variations were significantly increased from 250 mg/kg/d onward and consisted mainly of retarded ossification of the skull bones and sternebrae and supernumerary ribs. All reported malformations were rare malformations and exceeded the historical control data from the same laboratory.

Table 16 Malformations and variations

	Dose (mg/kg/day)					*Historical*
	0	*50*	*250*	*500*	*750*	*controls*[b]
% foetus with any malformations	0.4%	0.8%	0.3%	**7.0%****	**41.0%****	
% litters with any malformations	5.3%	10.5%	4.3%	**47.8%****	**88.9%****	
Number of foetuses (litters) evaluated	280 (19)	242 (19)	298 (23)	285 (23)		
% foetus with external malformations	0%	0%	0.3%	**2.8%***	**25.6****	0.08%
% litters with external malformations	0%	0%	4.3%	**30.4%***	**55.6%****	1.15%
Oedema[a]	0	0	0	1.0% (13%)	26% (55%)	0
Mand. micrognatia and cleft palate[a]	0	0	0	0.3% (4%)	0	0
Anal atresia and absent tail[a]	0	0	0	1.4% (17%)	0	0
Number of foetuses (litters) evaluated	140 (19)	121 (19)	149 (22)	143 (22)		
% foetus with visceral malformations	0.7%	1.7%	0%	4.9%	**25.0****	0.34%
% litters with visceral malformations	5.3%	10.5%	0%	22.7%	**50.0%***	2.30%
Cardiovascular malformations[a]*:*	0	0	0	4.2% (18%)	25% (50%)	0
Truncus arteriosus persistent[a]	0	0	0	1.4% (9%)	15% (25%)	0
Aorta, transposed[a]	0	0	0	0.7% (4%)	0	0
Aorta, origin abnormalities[a]	0	0	0	1.4% (4%)	0	0
Pulmonary artery, narrowed[a]	0	0	0	0.7% (4%)	5% (12%)	0
Interventricular septum defect, isolated[a]	0	0	0	0	5% (12%)	0
Number of foetuses (litters) evaluated	140 (19)	121 (19)	149 (23)	142 (23)		
% foetus with skeletal malformations	0%	0%	0%	**6.3%****	**26.3%****	0.17%
% litters with skeletal malformations	0%	0%	0%	**39.1%****	**57.1%****	1.16%
Mandible, small and split palatine[a]	0	0	0	0.7% (4%)	0	0
Cervical arches malformations[a]	0	0	0	4.9% (30%)	21% (43%)	0
Atlas and exoccipital, fused[a]	0	0	0	1.4% (9%)	0	0
Cervical arches, fused[a]	0	0	0	3.5% (22%)	16% (29%)	0
Cervical and first thoracic arches, fused[a]	0	0	0	0	5.3% (14%)	0

Absent[a]	0	0	0	1.4% (9%)	0	0
Ribs, thor., lumbar and sacral verteb., absent[a]	0	0	0	0.7% (4%)	0	0
Thoracic vertebral centra, absent[a]	0	0	0	0.7% (4%)	0	0
Ribs, fused[a]	0	0	0	0.7% (4%)	5.3% (14%)	0.08% (0.58%)
% foetus with skeletal variations	17.9%	23.1%	**32.2%****	**80.3%****	**94.7%****	
% litters with skeletal variations	73.7%	73.7%	78.3%	**100%***	100%	
Frontal and parietals, incomplete ossification[a]	0	0	2	28% (74%)	47% (86%)	
Supraoccipital, incomplete or bipartite ossif.[a]	0	0	0	18% (56%)	58% (100)	
Sternebrae : incompl. ossif. or unossif.[a]	0	1	6	18% (65%)	42% (71%)	
Ribs: 14th supernumerary all[a]	6	12	41	44% (83%)	74% (71%)	
14th supernumerary long[a]	1	0	4	22% (39%)	58% (71%)	
14th supernumerary short[a]	5	12	37	22% (70%)	16% (10%)	

* p<0.05, **p<0.01

[a] % of fetuses (litters) affected

[b] historical control data from 9 studies performed in the same laboratory from 2002 to 2009. Personal communication from AM Saillenfait, attached in Appendix I.

Overall, this study shows that NEP induces foetotoxic effects arising as post-implantation loss and reduction of fetal growth and teratogenic effects arising as external, visceral and skeletal malformations and skeletal variations in rats by oral route. These effects occur in presence of slight, transient maternal toxicity.
When correcting the significantly decreased maternal body weight change to take into account the post-implantation loss from 500 mg/kg/d onward and the reduction of foetal weight from 250 mg/kg/d onward, corrected maternal body weight gain was not significantly affected at any dose.
This is also supported by the absence of reduction of maternal food consumption during late gestation at 250 mg/kg/d and 500 mg/kg/d when foetal growth occurs. However, late post-implantation loss were observed at 500 mg/kg/d onward and foetal body weights were significantly reduced from 250 mg/kg/d onward. Besides, the decrease in foetal weight was more important than the corresponding decrease of maternal corrected weight.
Malformations induced by NEP are rare malformations observed above historical controls and with a statistical significance. Besides, the severity of malformations such as cardiovascular malformations, cleft palate or anal atresia does not allow linking them to maternal toxicity.

It is therefore considered that NEP induces foetotoxic and teratogenic effects that cannot be considered secondary to maternal toxic effects.

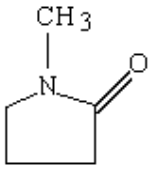
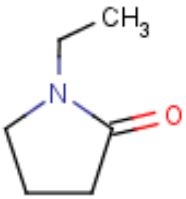
5.9.3 Human data

No data

5.9.4 Other data: developmental effects of a structurally close substance: NMP

NEP is structurally close to N-methyl-2-pyrrolidone (NMP) with only the length of carbon side chain being one carbon less in NMP. Identification information and main physical properties for these two substances are summarised in Table 17.

Table 17 Substance identity and physical properties of NEP and NMP

Substance name	N-methyl-2-pyrrolidone[a]	N-ethyl-2-pyrrolidone
CAS number	872-50-4	2687-91-4
Molecular formula	C_5H_9NO	$C_6H_{11}NO$
Structural formula		
Molecular weight	99 $g.mol^{-1}$	113.16 $g.mol^{-1}$
Physical state (20°C, 1013 hPa)	liquid	liquid
Melting/freezing point	-23.5°C	<-75°C
Boiling point (°C)	202°C (760 mm Hg)	212-213°C
Vapour pressure	32 Pa (20°C)	<100 Pa (20°C)
Relative density	1,027	0.998 (20°C)
Solubility in water	Miscible	Miscible
Partition coefficient (log Pow)	-0.46	-0.2

[a] data from OECD2007 and Classification proposal, 2002.

Some general toxicological properties were tested on both NMP and NEP by Ansell, 1988 and are summarized in Table 18 below.

Table 18 Summary of toxicological properties tested in Ansell, 1988

Substance name	N-methyl-2-pyrrolidone	N-ethyl-2-pyrrolidone
LD50 rat (mg/kg)	4 150	1 350
Dermal irritation rating	Minimal	Non irritating
Ocular irritation rating	Moderate	Moderate

NMP has developmental effects and its harmonised classification Repr. Cat. 2; R61 was included in the 31st ATP of Directive 67/548/EEC and in the 1st ATP to CLP. Several studies have investigated developmental toxicity of NMP and were considered in the recommendation of the classification. The developmental toxicity studies available on NMP are summarized in Table 19 as presented in the classification proposal (2002). They are presented here in the aim to compare developmental toxicity of NMP and NEP.

Table 19 Summary of main studies investigating developmental toxicity of NMP

Species	Route	Dose /Conc.	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	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. 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 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 foetuses and in the foetal 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 foetuses from 5 litters), and fusion of the exoccipital and atlas bones (4 foetuses from 4 litters). - No increase in the incidence of soft tissue variations or malformations. - No treatment-related effects at 75 and 237 mg/kg.	Becci et al. 1982

					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 foetal body weight.	
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, their foetal and litter incidences were 15% and 60%, respectively (historical value 8.4 and 40 %, respectively). There was no effect on foetal body weight, or on the incidence of external, soft tissue and skeletal malformations. No effects were observed at 300 mg/kg.	BASF 1993a, cited in HSE 1997
Rats (25/dose) (Crl:CD)	Whole body inhalation (100 % pure)	100 and 360 mg/m^3 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 foetal 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/m^3 (aerosol) (50% relative humidity)	6hr/day	GD 7 to 19	<u>Preliminary study</u> (5 animals/dose) (external foetal examination only) 300, 1000 and 2000 mg/m^3 : No signs of toxicity in dams. Slight but not significant changes in maternal liver weights. Increase in maternal clotting times at 1000 mg/m^3. Small but dose-related decrease in gravid uterine weight (99, 90, 82 and 71 g at 0, 300, 1000, and 2000 mg/m^3. Concomitant findings included a dose-related decrease in the number of foetuses (which attained statistical significance at the high dose), and a statistical increase in post-implantation loss at 2000 mg/m^3. <u>Main study</u> (15 animals/dose) 200, 500 and 1000 mg/m^3 :	BASF 1991, 1993b Cited in HSE 1997

					(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 foetuses, and foetal body weight. No increase in the incidence of external, visceral or skeletal malformations. Clear increase in the incidence of accessory ribs at 1000 mg/m^3. Smaller non-statistically significant increase in this parameter at 500 mg/m^3. This was considered a common finding in rabbits, although the incidence was much higher than that of the concurrent value (80% at 1000 mg/m^3 /32 % in control).	
Rats (14-16 litters/dose) (Mol :WIST)	Whole body inhalation (≥99.5 % pure)	151 ppm (i.e. 620 mg/m^3) with an additional control (clean air)	6 hr/day	GD 7 to 20 Behavioural developmental toxicity study	No effects on maternal weight gain during gestation, on the gestation 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.	Hass et al. 1994
Rats (20-23 pregnant females) (Mol :WIST)	Whole body inhalation (≥99.5% pure)	165 ppm (i.e. 680 mg/m^3) (highest technically possible concentration, 40-50 % relative humidity in the	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-	Hass et al. 1995

		inhalation chambers) with an additional control (air). Primarily vapour phase			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 foetuses and resorptions. Slight decrease in foetal 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.	
Rats (10 males and 20 females) (Crl:CD)	Whole body inhalation (vapours)	116 ppm (i.e. 478 mg/m^3) 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 Part of a two-generation study	Males: pre-mating and mating periods (total > 100 days) Females : pre-mating, mating, and GD 0 to 20	The maternal body weight was not affected. The foetal body weight was significantly lower than control (2%). Pup body weight at birth was also reduced in the two-generation study. There were no differences in the incidences of malformations and variations (external, visceral and skeletal).	Solomon et al. 1995
Rats Sprague-Dawley (20-25 pregnant)	Whole body inhalation (≥ 99.5% pure)	30, 60, 120 ppm (i.e. 124, 247, and 494 mg/m^3) with an additional control (air). Vapours (40% relative humidity in the inhalation chambers)	6 hr/day	GD 6 to 20	- Maternal toxicity : Body weight gain was reduced during the first half of exposure at 60 and 120 ppm. Exposure to 120 ppm also led to a significant decrease in food consumption on GD13-21. There were no significant differences in the absolute weight gain. - Developmental toxicity : The number implantations of live foetuses and the incidences of non-live implants and resorptions were comparable across groups Foetal body weights were decreased at 120 ppm (5-6 %). Examination of the foetuses was limited to external observations. Several common variations (club foot) were observed with no indication of any adverse effects related to NMP exposure. No malformations were noted in NMP exposed groups.	Saillenfait et al. 2003

					The NOAEL for maternal toxicity was 30 ppm/6hr/day. The NOAEL for developmental toxicity was 60 ppm/6hr/day.	
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 foetuses included reduction in foetal 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
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 : Body weight gain was depressed during treatment at 400 mg/kg (GD 6-9, GD 9-12, GD 6-15). 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.	Exxon 1992 cited in OECD 2007

					- Developmental toxicity : At 400 mg/kg: Reduced foetal body weight (10-11 %) and an increased incidence of stunted foetuses (foetuses: 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	
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	<u>Two dose-range finding studies</u> were conducted (10-16 pregnant rats/dose, foetal external examination only) <u>- First study:</u> 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). <u>- Second study:</u> 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	Saillenfait et al. 2002

					mg/kg. At 500 and 750 mg/kg: Decreases in maternal body weight gain and food consumption (throughout treatment at the high dose), and reduction in absolute weight gain. - Developmental toxicity : The incidence of resorptions was significantly higher than control at 500 mg/kg, and rose to 91% at 750 mg/kg (17/24 litters completely resorbed). The number of live foetuses was reduced at the high dose. The foetal 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 foetuses/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 1 foetus at 250 mg/kg, in 11 foetuses from 9 different litters at 500 mg/kg, and in 1 foetus 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 foetuses from 9 litters at 500 mg/kg, and in 6 foetuses 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 foetuses 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 foetus 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 foetuses 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 were 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.	

NMP induces resorptions in rats by dermal route and in rats and rabbits by gavage. Resorptions generally occur in presence of maternal toxicity. No effect was observed by inhalation.

A reduction of foetal body weight was identified in rats by dermal, respiratory and oral routes at maternal non-toxic doses. No effect was observed in rabbit.

In rat by dermal and oral routes and in rabbit by oral route, malformations were induced by NMP in presence of maternal toxicity. They consisted mainly in malformations of the skull bones such as fusion or absence of cervical arches and in fused or split ribs. By oral route, cardiovascular malformations such as truncus arteriosis were also observed and visceral rare malformations such as anasarca, anal atresia and cleft palate.

Developmental effects of NMP are regarded as specific and severe and are not considered as secondary to maternal toxicity.

NMP was classified Repr. Cat. 2; R61 in the 31st ATP of Directive 67/548/EEC and in the 1st ATP to CLP as Repr Cat 1B, H360.

5.9.5 Summary and discussion of reproductive toxicity

The data relevant for the assessment of developmental toxicity of NEP are summarised in Table 20 below.

Table 20 Summary of main findings in prenatal developmental studies on NEP

Study type (expo-	Species	Route	Doses in mg/kg/d	Maternal toxicity	Developmental toxicity	Refe-rence

sure)						
OECD 414 (GD 6-28)	Rabbit	Dermal	0, 100, 300, 1000	<u>1000 mg/kg :</u> ↓ food consumption (-17%) and bw gain (-21%) during administration (significant at the beginning of treatment only). No effect on maternal corrected weight.	<u>≥ 300 mg/kg/d:</u> observation of rare cardiovascular malformations above historical controls. <u>1000 mg/kg/d:</u> ↑supernumerary 13th rib (variation).	BASF 2010
OECD 414 (GD 6-28)	Rabbit	Gavage	0, 20, 60, 200	<u>200 mg/kg :</u> ↓ food consumption (-14%) and bw gain (-27%) during administration (significant at the beginning of gavage only). No effect on maternal corrected weight. ↑ relative liver and kidney weights, ↑ALAT and γ-GT.	<u>200 mg/kg/d:</u> ↑ (significant) of skeletal malformations, above historical controls.	BASF 2007a
OECD 414 (GD 6-28)	Rabbit	Gavage	0, 220	<u>220 mg/kg :</u> ↓ food consumption (-19%) and bw gain (-38%) during administration. No effect on maternal corrected weight. ↑ liver weight, ↑ALAT and γ-GT.	<u>220 mg/kg/d:</u> ↓ fœtal weight (-15%); 2 foetuses with severe multiple malformations; ↑ (significant) of visceral and skeletal malformations, above historical controls, in particular rare cardiovascular malformations.	BASF 2007b
OECD 414 (GD 6-19)	Rat	Dermal	0, 200, 400, 800	<u>800 mg/kg :</u> ↓ food consumption (-10%), bw gain (-22%) during administration and maternal corrected weight (-5%). <u>400 mg/kg :</u> ↓ bw gain (-10%) during	<u>800 mg/kg/d:</u> ↓ fœtal weight (-11%); ↑some skeletal variations.	BASF 2005

				administration. No effect on maternal corrected weight.		
Consistent with OECD 414 (GD 6-20)	Rat	Gavage	0, 50, 250, 500, 750	<u>≥ 250 mg/kg :</u> ↓ bw gain during gestation (-17, 25 and 56%). <u>≥ 500 mg/kg :</u> ↓ food consumption during gestation (-8 and 15%). No effect at any dose on maternal corrected weight.	<u>≥ 250 mg/kg/d:</u> ↓ fœtal weight (-7, 28 and 42%). <u>≥ 500 mg/kg/d:</u> ↑ (siginficant) in post-implantation loss ; ↑(significant) external and skeletal malformations; observation of rare cardiovascular malformations above historical controls. <u>750 mg/kg/d:</u> ↑(significant) visceral malformations variations.	Saillenfait 2007

In rabbits by dermal route (**BASF 2010**), NEP induced rare cardiovascular malformations (absent subclavian, ventricular septum defect and dextrocardia) that are observed above historical control range at 1000 mg/kg/d. A statistically significant increase in supernumerary 13th rib (skeletal variation) was also observed at this dose. At this dose transient maternal toxicity was observed as evidenced by significant decreases in food consumption and body weight gain mainly at the beginning of exposure. However, the effect was considered as slight and transient as no effect on maternal corrected weight was observed at the end of gestation and the cardiovascular malformations cannot be considered as secondary to maternal toxicity due to their specificity.

In rabbits by oral route (**BASF, 2007a and 2007b**), NEP induced skeletal and soft tissue malformations, in particular cardiovascular malformations at doses inducing a decrease in maternal food consumption and body weight gain and signs of mild hepatic damage. Besides, a decreased foetal weight was also observed at the highest dose tested (220 mg/kg). The corrected maternal body weight was however not significantly altered by treatment. The general maternal toxicity is therefore not considered to be linked to the induction of specific and severe malformations such as cardiovascular malformations. It also does not point toward a link with the observed decrease in foetal weight. Liver damage was mild and is not considered as a toxic effect in the meaning of the CLP. While maternal toxicity was clearly demonstrated, the possibility that the serious specific malformations and also developmental toxicity may be treatment-related cannot the discounted. Such an effect must be critically assessed irrespective of maternal toxicity. Such malformations and developmental toxicity cannot be considered as consequential on maternal toxicity.

In rats by dermal route (**BASF 2005**), NEP induced growth retardation as evidenced by a decrease in foetal body weight. The observation of skeletal variations such as delayed ossification can also reflect a retarded development. These effects were reported only at the highest dose of 800 mg/kg/d in presence of significant maternal toxicity. Maternal corrected body weight gain was reduced by 21% of controls and maternal corrected weight by 5% whereas foetal body weight was reduced by 11%. and it is possible neither to exclude nor to clearly establish a link between maternal toxicity and foetal growth retardation.

In rats by oral route (**Saillenfait, 2007**), foetal body weights were significantly reduced from 250 mg/kg/d. At these doses, maternal body weight change was significantly altered but it can be largely explained by induction of post-implantation loss from 500 mg/kg/d and reduction of foetal weight from 250 mg/kg/d: corrected maternal body weight gain was not significantly affected at any dose. This was also supported by the absence of reduction of maternal food consumption during late gestation at 250 mg/kg/d and 500 mg/kg/d when foetal growth occurs. Besides, the decrease in foetal weight was more important than the corresponding decrease in maternal corrected weight. It is therefore considered that NEP induces growth retardation that cannot be considered secondary to maternal toxic effects.

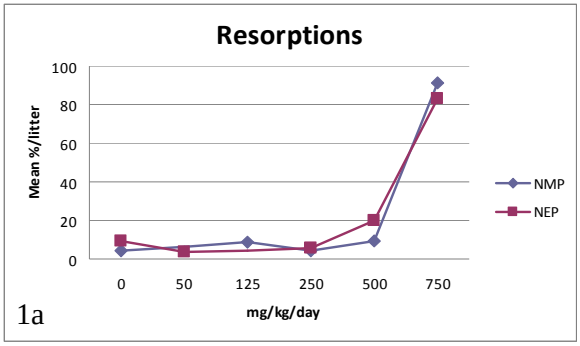
A dose-related increase in post-implantation losses reached the level of significance at a dose of 500 mg/kg/d. They consisted in significant late resorptions that are observed from 500 mg/kg/d in a dose related manner. At these doses, reduction of maternal body weight gain in particular during late gestation can largely be explained by foeto-toxic effects and corrected maternal body weight was not significantly altered. Besides, maternal food consumption during late gestation was not altered at 500 mg/kg/d. Late resorptions can therefore not be attributed to maternal toxicity.

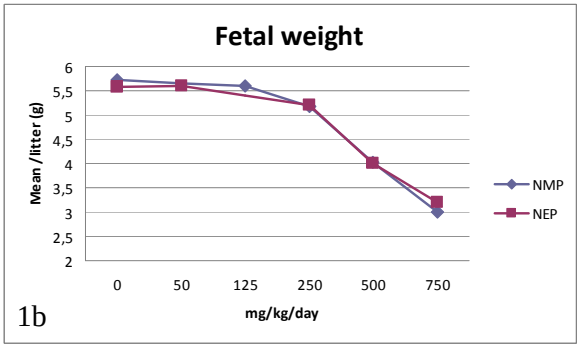
At 750 mg/kg/d, a large increase of early resorptions was also observed. Transient maternal toxicity was observed at the beginning of treatment during GD 6-9 with decreased food consumption and body weight gain that may not be explained by foetotoxic effects at this stage of gestation. An impact on early resorptions may therefore not be excluded. It should however be noted that this effect occurs with a substantial magnitude.

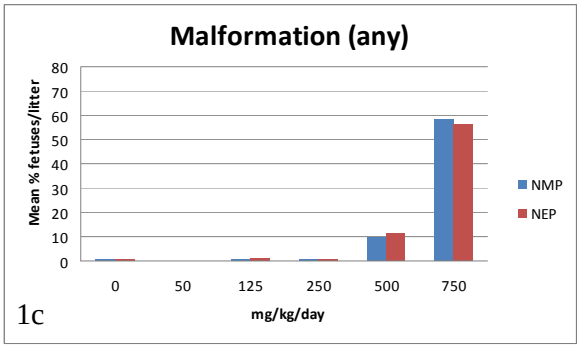
External, visceral and skeletal malformations were induced by NEP from 500 mg/kg/d and the number of foetuses affected was significantly increased from 500 mg/kg/d onward for external and skeletal malformations and at 750 mg/kg for visceral malformations. Reported malformations are rare and severe malformations and exceed historical control data and are therefore clearly related to NEP. Besides, it may be noted that teratogenicity of NEP may also have been partly masked by the high incidence of post-implantation loss at the highest dose (88.3%) and this may explain that a dose-response is not observed for each malformation individually.

The developmental profile of NEP is very similar to the developmental profile of NMP that is summarised in section 5.9.4. NMP induces resorptions, reduction of foetal weights and a pattern of malformation in rats and rabbits similar to what is seen in rat by oral route with NEP. Figure 1 below compares the outcomes of the study Saillenfait 2007 on NEP and Saillenfait 2002 on NMP under similar experimental conditions. It supports the fact that developmental effects of NEP are an intrinsic property of these structurally close compounds and are not secondary to maternal toxicity, in particular as regards to malformations.

Fig. 1 Comparison of resorptions (1a), reduction of fetal weight (1b) and malformations (1c) induced by NEP in Saillenfait 2007 and by NMP in Saillenfait 2002.







Rationale for classification in Repr. 1B:

The CLP criteria for classification in Repr. 1B are as follows:

"The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide ***clear evidence*** *of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is* ***considered not to be a secondary non-specific consequence of other toxic effects****. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate."*

Overall, based on animal studies NEP induces:

– adverse effects on foetal body weights in rabbits by oral route, in rats by dermal route and in rats by oral route

– effects on post-implantation loss and in particular late resorptions in rats by oral route.

– malformations in rabbits by dermal and oral route and in rats by oral route. It consisted in significant increase in skeletal malformations by oral route in both rats and rabbits. Besides, rare cardiovascular malformations were observed above historical controls in rabbit by dermal and oral routes and in rats by oral route.

– Developmental effects of NEP and in particular the profile of malformations observed in the rat by oral route, are similar to the developmental effects observed with NMP, which strengthen the weight of evidence that the effects observed in the NEP studies are related to administration of the test substance.

On this basis, it is concluded that it provides **clear evidence** of teratogenic and foeto-toxic effects of NEP.

Besides:

- as discussed here-above, the decrease in foetal weight in the rat and in the rabbit by oral route, the induction of late resorptions in the rat by oral route and of malformations in rabbit by dermal and oral route and in rat by oral route cannot be correlated to a limited maternal toxicity, showing that the two phenomena have no direct link.
- The similarity of effects between NEP and NMP also support that these effects are an intrinsic property of these compounds.

Therefore, developmental effects of NEP are considered specific and severe and **cannot be considered secondary to maternal toxic effects**.

A classification **Repr. 1B –H360D** is therefore warranted (Repr. Cat. 2; R61 according to Directive 67/548/EEC). As no developmental study is available by inhalation, it is proposed not to specify route of exposure in the hazard statement. In absence of evaluation of potential effects of NEP on fertility and in agreement with section 3.7.4.1 of the guidance on CLP criteria, it is not possible to specify the hazard in the general hazard statement.

Classification in Repr 1A is not appropriate as it should be based on human data and no human data are available for NEP.

Classification in Repr 2 is not appropriate as the developmental studies available on NEP have no deficiency and the results from these studies are considered as reliable without restrictions. Besides, supporting evidences of developmental effects are provided by the similarity of profile with NMP. Considering the whole database in a weight of evidence approach, the level of evidence is considered as 'clear evidence' and not 'some evidence'.

Guidelines to set specific concentration limits (SCL) for reproductive toxicity are currently under discussion. In absence of adopted guidelines at this point in time, no SCL are proposed.

Final Assessment

A significant data base of experimental animal studies were submitted by the notifier and evaluated by the DS. These include both dermal (BASF 2010) and oral gavage (BASF 2007a/BASF 2007b) studies in the rabbit and oral gavage (Saillenfait 2007) and dermal (BASF 2005) studies carried out in the rat. All data were either compliant or consistent with current OECD guidelines. In addition, the developmental profile of a closely related substance N-methyl-2-pyrrolidone (NMP) currently classificed as Cat 1B H360 (1st ATP CLP) and Repr. Cat.2; R61 (31st ATP Directive 67/548/EEC and) was included for comparative purposes. Based on the animal studies carried out, it was clearly demonstrated that NEP induces:

~ -adverse effects on foetal body weights in rabbits by oral route, in rats by dermal route and in rats by oral route

-effects on post-implantation loss and in particular late resorptions in rats by oral route.

-malformations in rabbits by dermal and oral route and in rats by oral route.

There was a significant increase in skeletal malformations by oral route in both rats and rabbits. Rare cardiovascular malformations were observed at above historical control levels in rabbit by dermal and oral routes, and in rats by oral route.

Developmental effects of NEP and in particular the profile of malformations observed in the rat by oral route, are similar to those observed with NMP, which strengthens the weight of evidence for NEP.

On this basis, it is concluded that there is clear evidence of teratogenic and foeto-toxic effects of NEP.

It is noted that the decrease in foetal weight in the rat and in the rabbit by oral route, the induction of late resorptions in the rat by oral route and of malformations in rabbit by dermal and oral route and in rat by oral route cannot be correlated to a limited maternal toxicity. While maternal toxicity was clearly demonstrated, the possibility that the serious specific malformations may be treatment-related cannot the discounted. Such an effect must be critically assessed irrespective of maternal toxicity. Such malformations cannot be considered as consequential on maternal toxicity

The similarity of effects between NEP and NMP also support that these effects are an intrinsic property of these compounds.

A classification Repr. 1B –H360D is warranted (Repr. Cat. 2; R61 according to Directive 67/548/EEC). As no developmental study is available by inhalation, it is proposed not to specify route of exposure in the hazard statement. It was agreed by the RAC to assign the letter D to the developmental toxicity classification even though no fertility are available and this endpoint has not been discussed. This would be consistent with the labeling of other substances not yet studied for effects on fertility.

Guidelines to set specific concentration limits (SCL) for reproductive toxicity are currently under discussion. In absence of adopted guidelines at this point in time, no SCL are proposed.

The classification proposal of France (DS) for developmental toxicity was supported by all comments from member state and from the industry federation CEFIC. However, there has been a reference to new data relevant to the fertility endpoint during the Public Consultation (CEFIC 6/5/2011 Annex 2 Draft RCOM) It was stated that new data for exposure *via* the inhalation route relevant for fertility (28d study according to OECD412, experimental phase completed 21.03.2011 – final study report expected mid of 2011) are available. RAC does not support the proposal that the harmonized classification and labeling for the reprotoxicity of NEP should be delayed at this point because 1) A 28 day inhalation toxicity study is not considered to influence the fertility classification and 2) No additional information has been provided by the industry to the RAC (RAC 17) with regard to the proposed 2-generation study.

6 HUMAN HEALTH HAZARD ASSESSMENT OF PHYSICO-CHEMICAL PROPERTIES

Not covered in this dossier

7 ENVIRONMENTAL HAZARD ASSESSMENT

Not covered in this dossier

JUSTIFICATION THAT ACTION IS REQUIRED ON A COMMUNITY-WIDE BASIS

NEP has a CMR property, i.e. reproductive toxicity, which justifies a harmonised classification and labelling according to article 36 of CLP.

Information on repeated dose toxicity is reported here for information only, so as to provide a general toxicological profile on NEP and assist evaluation of developmental effects. This point is however not proposed for harmonisation.

OTHER INFORMATION

No other information

REFERENCES

Ansell JM and Fowler JA. (1988) The acute oral toxicity and primary ocular and dermal irritation of selected N-alkyl-2-pyrrolidones. Fd Chem Toxic 26(5), 475-479.

BASF (1991) Range finding study for the maternal inhalation toxicity of N-methylpyrrolidone in pregnant rabbits. Project Number: 21R0544/90049.

BASF (1993a) Study of the prenatal toxicity of N-methylpyrrolidone in rabbits after after dermal application. Project Number: 44R0544/90078.

BASF (1993b) Study of the prenatal toxicity of N-methylpyrrolidone in rabbits after inhalation of vapour-aerosol mixtures. Project Number: 41R0544/90100.

BASF (2005) N-ethyl-2-pyrrolidone. Prenatal developmental toxicity study in Wistar rats. Dermal application. Project Number: 34R0033/04006.

BASF (2006) N-ethyl-2-pyrrolidone. Repeated dose 90-day oral toxicity study in Wistar rats; administration in the diet. Project Number: 50S0033/04072.

BASF (2007a) N-ethyl-2-pyrrolidone. Prenatal developmental toxicity study in Himalayan rabbits. Oral administration (gavage). Project Number: 40R0033/04058.

BASF (2007b) N-ethyl-2-pyrrolidone. Prenatal developmental toxicity study in Himalayan rabbits. Oral administration (gavage). Project Number: 40R0033/04075.

BASF (2010) N-ethyl-2-pyrrolidone. Prenatal developmental toxicity study in Himalayan rabbits. Dermal application. Project Number: 44R0033/04110. Becci PJ, Knickbockrer MJ, Reagan EC, Parent RA and Llewellyn WB (1982) Teratogenicity study of N-methyl-2-pyrrolidone after dermal application to Sprague-Dawley rats. Fundamental and Applied Toxicology 2, 73-76.

Classification proposal (2002) 1-methyl-2-pyrrolidone (NMP). Classification and labelling of Dangerous substances. Commission of the European Communities, DG XI. Prepared by: Toxicology units, INRS, France. Doc ECBI/47/02Rev.1.

Environmental Protection Agency (EPA) (1987) Letter from BASF Corp to USEPA regarding the summary of two studies on the teratogenic potential of N-methylpyrrolidone. Microfiche OTSO513411.

Exxon (1992) Developmental toxicity study in rats with N-methyl-2-pyrrolidone. Project number: 136534. EPA-OTS Microfiche OTS0539109.

Hass U, Lund SP, Elsner J (1994) Effects of prenatal exposure to N-methylpyrrolidone on postnatal development and behavior in rats. Neurotoxicology and Teratology 16/3, 241-249.

Hass U, Jakobsen BM, Lund SP. (1995) Developmental toxicity of inhaled N-methylpyrrolidone. Pharmacology and Toxicology 76, 406-409.

Health and Safety Executive (HSE) (1997) N-methyl-2-pyrrolidone. Risk assessment document. EH 72/10.

Institut National de Recherche et de Sécurité (INRS) (2008), Fiche Toxicologique FT 270 : N-Ethyl-2-pyrrolidone, (N. Bonnard, M.-T. Brondeau, D. Jargot, D. Lafon, O. Schneider)

Lee KP, Chromey NC, Culik R, Barnes JR and Schneider PW. (1987) Toxicity of N-methyl-2-pyrrolidone (NMP): Teratogenic, subchronic, and two-year inhalation studies. Fundamental and Applied Toxicology 9, 222-235.

OECD (2007) SIDS initial assessment report for SIAM 24. 1-methyl-2-pyrrolidone.

Office of Environmental Health Hazard Assessment (OEHHA). California Environmental Protection Agency (April 23, 1999) Safe drinking water and toxic enforcement act of 1986 (proposition 65). Chemicals meeting the criteria for listing as developmental and reproductive toxicants (DARTs) via the authorative bodies mechanism : 4 chemicals identified by U.S. EPA.

Saillenfait AM, Gallissot F, Langonné I, Sabaté JP. (2002) Developmental toxicity of N-methyl-2-pyrrolidone administered orally to rats. Food Chem Toxicol. 40(11):1705-12

Saillenfait AM, Gallissot F, Morel G. (2003) Developmental toxicity of N-methyl-2-pyrrolidone in rats following inhalation exposure. Food Chem Toxicol. 41(4):583-8.

Saillenfait AM, Galissot F and Sabaté JP. (2007) Developmental toxic effects of N-ethyl-2-pyrrolidone administered orally to rats. J Appl Toxicol 27(5); 491-7

Solomon H.M., Burgess B.A., Kennedy G.L. and Staples R.E. (1995) 1-Methyl-2-pyrrolidone (NMP): reproductive and developmental toxicity study by inhalation in the rat. Drug and Chemical Toxicology 18/4, 271-293.

SRC. Physical properties database. Syracuse Research Corporation. http://www.syrres.com/what-we-do/databaseforms.aspx?id=386 accessed on 25/03/2010

Taminco (2005a) Ethylpyrrolidone. Technical Data Sheet 51063/12-2005.

Taminco (2005b) NEP (n-ethylpyrrolidone). Material Safety Data Sheet.12/05/05.