CLH report

Proposal for Harmonised Classification and Labelling

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

Chemical name:

2-pyrrolidone; pyrrolidine-2-one

EC Number: **210-483-1**

CAS Number: **616-45-5**

Index Number:

Contact details for dossier submitter:

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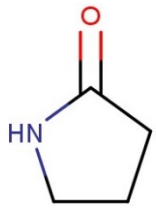
Version number: 2 **Date: 07.09.2023**

CONTENTS

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

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

Name(s) in the IUPAC nomenclature or other international chemical name(s)	pyrrolidin-2-one
Other names (usual name, trade name, abbreviation)	Butyrolactam G-butyrolactam Gamma-butyrolactam 2-pyrrolidinone 2-pyrrolidon 2-pyrrolidone butyrolactam 2-azacyclopentanone 2-p 2-ketopyrrolidine Alpha-pyrrolidone Piperidinic acid lactam 2-oxopyrrolidine 2-pyrol 2-pyrol4-aminobutyric acid lactam 4-aminobutanoicacidlactam 4-amino-butanoicacilactam 4-aminobutyric acid lactam 4-aminobutyricacidlactam 4-butyrolactam C4H7NO Pyrrolidin-2-one PYRROLIDONE 2 TPR-7
ISO common name (if available and appropriate)	
EC number (if available and appropriate)	210-483-1
EC name (if available and appropriate)	2-pyrrolidone
CAS number (if available)	616-45-5
Other identity code (if available)	
Molecular formula	C4H7NO
Structural formula	
SMILES notation (if available)	C1CC(=O)NC1
Molecular weight or molecular weight range	85.105

Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	
Description of the manufacturing process and identity of the source (for UVCB substances only)	
Degree of purity (%) (if relevant for the entry in Annex VI)	

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)
2-pyrrolidone	>=80 - <=100 % (w/w)	-	Repr 1B, H360 (Number of notifiers 232, 07.09.2023, only self-classification for Repr 1B included)

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

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
Not relevant				

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

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
Not relevant					

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5:

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitter's proposal	TBD	2-pyrrolidone; pyrrolidin-2-one	210-483-1	616-45-5	Repr 1B	H360D	GHS08 Dgr	H360D			

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

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

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

2-pyrrolidone has a number of self-classifications, notably as a reproductive toxicant (Repr 1B). There are 232 notifiers for this endpoint, amongst which is also the registrant's. The registrants have however also set a specific concentration limit of 3%.

The two new major studies that are described in this dossier were performed due to a compliance check decision. In this decision the required information was a pre-natal developmental toxicity study in a second species (rabbit) by the oral route, and an extended one-generation reproductive toxicity study in rats by the oral route with cohorts 1A and 1B (without extension to produce the F2 generation) and cohorts 2A and 2B (developmental neurotoxicity).

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

There is no requirement for justification that action is needed at Community level.

The substance has CMR properties (reproductive toxicity). Harmonised classification and labelling for CMR is a community-wide action under article 36 of the CLP regulation.

5 IDENTIFIED USES

This substance is used by consumers, in articles, by professional workers (widespread uses), in formulation or re-packing, at industrial sites and in manufacturing.

2-pyrrolidone is used in ink and toners, in coatings, in imaging and printing mixtures and as a laboratory chemical. It is also used as a catalyst in polymerisation.

"It is used as a cosolvent and plasticiser in aqueous coatings. As a setting agent, it is used for acrylic emulsions and acrylic/styrene copolymers in floor polishes. It also used as a co-solvent for water-based ink formulations.

It is an organic synthesis intermediate in the pharmaceutical industry and as a solvent for animal injection.

2-Pyrrolidone is a precursor of N-vinylpyrrolidone (NVP). It also finds application as a solvent in the manufacture of membrane filters for e.g. sterile filtration of drugs such as pharmaceutical proteins, wine filtration systems."[1]

6 DATA SOURCES

REACH registration dossier.

Use information: BASF website 2-Pyrrolidone | CAS No.:616-45-5 | (basf.com)

Full study reports of the pre-natal developmental toxicity study in rabbits, and the dose range finding study performed before the pre-natal developmental toxicity study in rabbits.

Full study reports for the extended one-generation reproductive toxicity study (EOGRTS) in rats, and the range finding study for the EOGRTS.

Full study report for the pre-natal developmental toxicity study in rats.

[1] Taken from the BASF website: 2-Pyrrolidone | CAS No.:616-45-5 | (basf.com)

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	liquid	ECHA Dissemination site, 2023	Colourless, yellow
Melting point	25 °C	ECHA Dissemination site, 2023	Atm. Press. 1 013 hPa
Boiling point	251.2 °C	ECHA Dissemination site, 2023	Atm. Press. 1 013 hPa
Relative density	1.11 g/cm³	ECHA Dissemination site, 2023	At 20 °C
Vapour pressure	0.018 hPa at 24.6 °C	ECHA Dissemination site, 2023	0.164 hPa at 49.9 °C 1.2 hPa at 79.3 °C
Surface tension	48.8 mN/m	ECHA Dissemination site, 2023	At 21°C
Water solubility	679.7 g/L	ECHA Dissemination site, 2023	At 20 °C
Partition coefficient n-octanol/water	log Pow -0.71	ECHA Dissemination site, 2023	At 25 °C
Flash point	138 °C	ECHA Dissemination site, 2023	Closed cup method
Flammability	Non-flammable	ECHA Dissemination site, 2023	It is not easily flammable liquid; the ignition point is over 100 °C.
Explosive properties	Non-explosive	ECHA Dissemination site, 2023	Lower expl limit 1,8 Vol.-% Upper expl limit 16,6 Vol.-%
Self-ignition temperature	395 °C	ECHA Dissemination site, 2023	
Oxidising properties	Slight fire potential when exposed to heat or flame. Can react with oxidising materials.	ECHA Dissemination site, 2023	
Granulometry	-	ECHA Dissemination site, 2023	the study does not need to be conducted because the substance is marketed or used in a non solid or granular form

Property	Value	Reference	Comment (e.g. measured or estimated)
Stability in organic solvents and identity of relevant degradation products	-	ECHA Dissemination site, 2023	the study does not need to be conducted because the stability of the substance is not considered to be critical
Dissociation constant	pKa 14.7	ECHA Dissemination site, 2023	At 20 °C
Viscosity	16.4 mPa s at 25.8°C 7.86 mPa s at 50°C	ECHA Dissemination site, 2023	

8 EVALUATION OF PHYSICAL HAZARDS

Not performed for this substance.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Not performed for this substance.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Not performed for this substance.

10.2 Acute toxicity - dermal route

Not performed for this substance.

10.3 Acute toxicity - inhalation route

Not performed for this substance.

10.4 Skin corrosion/irritation

Not performed for this substance.

10.5 Serious eye damage/eye irritation

Not performed for this substance.

10.6 Respiratory sensitisation

Not performed for this substance.

10.7 Skin sensitisation

Not performed for this substance.

10.8 Germ cell mutagenicity

Not performed for this substance.

10.9 Carcinogenicity

Not performed for this substance.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 8: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Extended one generation reproductive toxicity study (EOGRTS) with both developmental neuro- and immunotoxicity cohorts (Cohorts 1A, 1B without extension, 2A, 2B, 3 and 3A (control)) OECD TG 443, GLP study Crl:CD(SD) rats, 30 males and 30 females 03 Dec 2018 - 03 March 2020	2-pyrrolidone Purity 99.5% Doses: 0, 1500, 4000 and 8000 ppm, in drinking water. Dosing 70 days before mating until euthansia. To the offspring in F1: from weaning until euthanasia.	Systemic toxicity. Body weight changes: F0 generation; NOAEL: 4000 ppm for males and 8000 ppm for females. F1 generation; 4000 ppm for males and females. Neurotoxicity and immunotoxicity: F1 generation; NOAEL 8000 ppm for males and females. Reproductive effects in the F1-generation NOAEL: 4000 ppm based on several statistically significant effects in the offspring in the high dose group, including vaginal patency and reduced cauda epididymis sperm concentration. **F0 generation**: Mortality and clinical findings: no test substance related findings. Body weight: statistically significantly reduced body weight in a dose response-manner, observed especially in the high dose males, but also in the high dose females and also some statistically significant effect in the mid-dose males and females. This is the basis for the NOAEL for parental animals. See Table 11 for details. Food consumption and efficiency, water consumption: some sporadic findings of lower food consumption in the high dose males. Reproductive parameters: Sperm production and sperm concentration was statistically significantly lower in the high and low dose groups, but not in a dose response-manner and therefore assumed not to be test-substance related. No test substance related findings on other parameters observed. See Table 12 and Table 13 for details. Organ weights: no changes in organ weight could clearly be attributed to the test substance. **F1 generation, prior to weaning**: Litter data, postnatal survival, observations: no differences in litter data, general physical condition or clinical observations, except for a higher number of pups in the mid and high dose group that were described as having a "small stature" compared to control group, correlating with the lower body weight in these groups. Body weight: Statistically significant lower body weight observed in the	Unnamed, 2020

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		high dose group males (post-natal day (PND) 4 and 7), mid dose group males (PND 4), and in high dose females (PND 1-4). Lower birth weight was also observed for males and females in mid and high dose groups, although not statistically significant. See Table 15 for more details. **F1 generation, following weaning**: Mortality and observations: no test substance-related findings. Body weights: statistically significant lower body weight change and absolute body weight was observed for males in the two highest dose groups. A dose related decrease in body weight gain was observed in all dose groups. A similar, but less pronounced, trend for females was observed. See Table 16 for more details. Food consumption and efficiency and water consumption: Males had reduced food consumption and efficiency in a dose related manner in all dose groups. Food consumption for females was slightly reduced in all dose groups. In the high dose females, food efficiency was clearly reduced but not in a statistically significant degree. For water consumption, there were statistically significant differences in the high dose females (PND 21-28), compared to the control group. Reproductive parameters (balanopreputial separation, vaginal patency, oestrous cycle, sperm evaluations): Balanopreputial separation (males) Age of attainment was not statistically different from control group, but the body weight at attainment was statistically significantly lower in the high dose group. Vaginal patency (females) Age of attainment was statistically significantly higher in high dose females compared to control group. Oestrous cycle (females) No differences was observed in the test groups that were statistically significant compared to control. Sperm evaluations (males) A statistically significant difference was observed between cauda epididymal sperm concentration in the high dose group and the control group. Lower cauda epididymal weight in the same males was also observed, although not statistically significantly lower compared to control. See Table 17 for details. Thyroid hormone analysis, haematology, urinalysis, coagulation, and serum chemistry (cohort 1A): No test substance-related findings. Sensory function and neurobehavioural testing (cohort 2A): Lower Peak-value in all male dose groups compared to control with a dose-response relationship was observed in the first measurement. Low and high dose group showed statistically significant differences compared to control, but not in a dose-response manner. In females, differences were less marked, and showed no dose-response. See Table 19 for details. Functional observational battery (cohort 2A): Although some differences	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		were observed, none of the findings were deemed to be test substance related. Gross pathology (cohort 1A and 1B): There were no test substance-related findings. Organ weights (cohort 1A and 1B): There were no clear test substance related changes in cohort 1A, although statistically significant changes in organ weights relative to body weight was observed in the high dose group for both males and females in cohort 1A. In cohort 1B, this was seen for the high- and mid-dose males. Histopathology (cohorts 1A): There were no histological changes that could be attributed to the test-substance. Immunophenotyping (cohort 1A): Statistically significantly lower B lymphocyte count, characterised as "moderate" was observed in the high-dose group. See Table 20 for details. Neuropathology (cohorts 2A and 2B): No test substance related findings for macroscopic examination, brain weights and measurements or neurohistopathological examination. In several measurements in the morphometric analysis, there were statistically significant differences, but they were inconsistent and did not follow dose-response and was thus not considered treatment related. T-cell dependent antibody response (TDAR) Assay (cohort 3): Changes in the TDAR response compared to control group was observed, but they were not statistically significant and did not follow dose-response.	
Dose range finding study OECD TG 421, GLP study Rats, Crl:DC(SD) F0 and F1 generations: 10/sex/group April-December 2018	2-pyrrolidone Purity 99.5% Doses: 0, 3000, 8000 and 12000 ppm, in drinking water (LD 21). F0: dosing 13 days before mating until euthanasia. F1: from weaning until euthanasia (PND 35).	Based on the results of this study, dosage levels up to 8000 ppm were considered to be acceptable for an extended one-generation reproductive toxicity study (EOGRTS). **F0 and F1 generation**: Mortality and clinical findings: No test substance related findings. One F0 female in the high dose group had a total litter loss on lactation day (LD) 1 and was euthanised. Body weight: In the F0 generation, statistically significantly reduced body weight in a dose response-manner, observed especially in the high dose males, but also in the high dose females and also some statistically significant effect in the mid-dose males and females. In the F1 generation, lower mean offspring body weights and body weight gains were noted in the mid and high dose groups prior to weaning and which continued post-weaning. See Table 22 for details. Food consumption and efficiency, water consumption: In the F0 generation, significantly reduced food and water consumption before mating, during gestation and lactation, especially for the high dose females, but also seen in the mid dose females. In the F1 generation, no test substance related findings. Reproductive parameters, including litter data, postnatal survival, observations: A lower mean number of implantation sites was seen in the high dose group. A lower mean number of F1 pups born, live litter size on	Unnamed, 2019a

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		PND 0, and postnatal survival (PND 0-1 and from birth to PND 4) were noted in the high dose group. See Table 23 for details. Hormone levels: In the F0 generation there was a dose related increase in T3 and TSH in all dose groups. However, in the F1 generation, lower T3 levels in the mid and high dose groups, and lower TSH levels in all dose groups were seen on PND 4. This difference was reduced at PND 21. See Table 24 for details. Organ weights: In the F0 generation lower absolute and relative spleen and thymus weights were seen in the females in all dose groups. This was considered test substance related. Lower absolute and relative ovary/oviduct weights and pituitary gland weights were seen in the females of the high dose group. Low thymus weight and moderate lymphoid depletion was seen in two high dose females. In the F1 generation there was lower absolute and relative spleen weight in all dose groups on PND 21. Other organ weight differences were only seen in the high dose group: absolute and relative liver and thymus (only females) weight on PND21. Absolute and relative spleen (only females) and thymus weights on PND 35. See Table 25 for details.	

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

One extended one-generation reproductive toxicity study (EOGRTS) in rats was performed with 2-pyrrolidone. In addition a range-finding study on rats was performed.

An extended one-generation reproductive toxicity study (anonymous 2020) was performed in rats according to OECD 443.

The study was performed with groups of 30 female and 30 male Crl:CD(SD) rats (F0) and with both developmental neuro- and immunotoxicity cohorts. F1 animals were subdivided into cohorts following weaning and specifically evaluated for the following: Cohort 1 for reproductive/developmental toxicity (including oestrous cycles and sperm evaluation), Cohort 2 for developmental neurotoxicity (including neurobehavior, neuropathology, and brain morphometry), Cohort 3 for immunotoxicity testing.

The following parameters and end points were evaluated in this study: clinical signs, body weights, body weight gains, food and water consumption, oestrous cycles, reproductive performance, parturition, litter viability and survival, anogenital distance, areolae/nipple anlagen, developmental landmarks, neurobehavior, thyroid hormones, clinical pathology, gross necropsy findings, sperm parameters, organ weights and measurements, histopathologic examinations, and neuropathologic and brain morphometric examinations.

Test substance administration

The F0 males were administered the test substance continuously in the drinking water for 70 consecutive days prior to mating and continuing until euthanasia. F0 females were administered the test substance continuously in the drinking water for 70 consecutive days prior to mating and continuing throughout mating, gestation and lactation, until euthanasia.

The F1 generation was potentially exposed to the test substance in utero, through nursing during lactation, and through potential direct exposure via drinking water prior to weaning. Following weaning, the offspring selected for the F1 generation were dosed until euthanasia (PND 22 - Cohort 2B; PND 59 - Cohorts 3 and 3A; PND 78 - Cohort 2A; PND 91 - Cohort 1A; PND 98 -Cohort 1B).

The doses administered were 0, 1500, 4000 and 8000 ppm. As the substance was administered through the water the actual intake of the substance varied quite a bit throughout the study. See Table 9 and Table 10.

Table 9 Mean test substance consumption F0 generation

Theoretical water concentration (ppm)	Mean test substance consumption (mg/kg/day)					
	Males F0		Females F0			
	Prior to mating	After mating	Prior to mating	Gestation	Lactation (LD 1-4)	Lactation (LD 4-14)
1500	176	105	211	191	241	139
4000	514	300	586	531	672	333
8000	1012	558	1203	1041	1321	707

Table 10 Mean test substance consumption F1 generation

Theoretical water concentration (ppm)	Mean test substance consumption (mg/kg/day)			
	Males F1		Females F1	
	PND 21-35	PND 42-98	PND 21-35	PND 42-98
1500	134	150	98	181
4000	396	423	273	530
8000	827	853	561	1002

The doses were selected based on a dose-range finding study (see description of the study below). However, it could be argued that the doses selected for the EOGRTS could have been even higher since the body weight reduction seen in the EOGRTS barely reached 10% for the males and did not reach 10% for the females.

Results

F0 generation:

Mortality and clinical findings:

There were no test substance-related effects on survival for F0 parental animals. No test substance-related clinical findings were noted during the generation at the daily examinations.

Body weight:

There was a treatment related effect on body weight for both males and females, however the effect was largest in the high dose males.

Males

Males had a dose related reduced body weight gain in all dose groups, but this was only statistically significant for the whole period for males in the high dose group. These males had also significantly reduced absolute body weight at the end of the treatment period. The difference was also significant in the pre-mating period for the mid-dose males, whereas the difference was not significant for low dose males except for intermittently during the treatment period.

Females

There was also an effect on female body weight, but less than on males. However, the difference in the mid- and high dose group was statistically significant pre-mating for body weight gain. There was also a lower body weight gain during gestation and absolute body weight was statistically significantly lower for the high dose group from gestation day (GD) 7 and until the end of gestation. There was clearly lower body weight at

the end of gestation for the mid-dose too but without statistical significance. There were no differences between dose groups in body weight gains during lactation, but the differences in absolute weight that had accumulated previously during treatment were still apparent at the end of the treatment period, resulting in an overall lower absolute body weight in the high dose group at the end of treatment.

Table 11 Body weight and body weight changes, males and females F0.

Body weight (g)	Control	1500 ppm	4000 ppm	8000 ppm
Males				
Absolute wt, day 70	546	538	**507****	**488****
% difference	-	-1.5	**-7.1**	**-10.6**
Absolute wt, day 131	652	650	619	593
% difference	-	-0.3	-5.1	-9.0
Body wt change premating, day 0-70	359	350	**325****	**305****
	-	-2.5	**-9.5**	**-15.0**
Body wt change	465	462	438	**410****
Day 0-131	-	-0.6	-5.8	**-11.8**
Females				
Absolute wt, day 0	149	150	150	151
% difference	-	+0.7	+0.7	+1.3
Absolute wt, day 70 (pre-mating period)	296	293	284	283
% difference	-	-1.0	-4.1	-4.4
Absolute wt, day 84 (end of mating period, lasting max 14 days)[2]	380	354	325	No data. All females had evidence of mating. Refer to gestation bw. data
% difference		-6.8	-14.5	
Absolute wt. GD1	292	294	282	279
% difference[3]		+0.7	-3.4	-4.5
Absolute wt, GD 20	431	433	423	**406***
% difference	-	0.5	-1.9	**-5.8**
Absolute wt, LD1	334	335	327	315
% difference		+0.3	-2.1	-5.7
Absolute wt, LD 21	343	355	340	333
% difference	-	3.5	-0.9	-2.9
Absolute wt, day 131	341	338	331	325
% difference	-	-0.9	-2.9	-4.7

[2] Only females who had not shown signs of mating were weighed. Only two females in each of the control, low- and mid-dose groups had not mated by the end of the 14 day mating period. All females in the high dose group had mated by day 84.

[3] Most dams had mated within four days of start of mating. GD1 was therefore within 1-4 days after the weight measured for day 70 (pre-mating).

Body wt change premating, day 0-70	146 -	143 -2.0	**134*** **-8.2**	**132*** **-9.6**
Body wt change, gestation, day 0-20	139 -	139 0	141 1.4	126 -9.4
Body wt change, lactation, day 1-21	13 -	20 53.8	14 7.7	18 38.5

* = Significantly different from the control group at 0.05 using Dunnett's test ** = Significantly different from the control group at 0.01 using Dunnett's test

Food consumption, food efficiency and water consumption

Males

There was some evidence of lower food consumption in the high dose males both pre- and post-mating. This was however only sporadically statistically significant. Food efficiency (defined as body weight gain as percent of feed consumed) was also intermittently lower in the high dose group. There were no differences in the other dose groups compared to the control group. There were only slight differences in the water consumption in the high dose group compared to the control and the other dose groups.

Females

The same tendencies were seen in the females but to an even lesser degree. There were very few differences in food consumption, food efficiency and water consumption in the females both pre-mating, during gestation and during lactation.

Reproductive parameters

There were no effects of the treatment with the test substances on the parameters in Table 12.

Table 12 F0 male and female reproductive parameters

Reproductive parameter	Control	1500 ppm	4000 ppm	8000 ppm	Historical control mean (range)[4]
Male mating index (%)	93.3	100.0	100.0	100.0	98.0 (83.3-100.0)
Female mating index (%)	93.3	100.0	100.0	100.0	98.0 (83.3-100.0)
Male fertility index (%)	90.0	90.0	93.3	93.3	93.9 (80.0-100.0)
Female fertility index (%)	90.0	90.0	93.3	93.3	93.9 (80.0-100.0)
Male copulation index (%)	96.4	90.0	93.3	93.3	95.7 (80.0-100.0)
Female conception index (%)	96.4	90.0	93.3	93.3	95.7 (80.0-100.0)
Oestrus cycle length (days)	4.1	4.2	4.1	4.2	4.2 (3.9-5.2)
Pre-coital interval (days)	3.4	2.5	2.9	2.1	2.7 (1.4-4.5)
Gestation length	22.0	21.9	21.9	22.0	21.8

Sperm production and sperm concentration was significantly lower in the low and high dose group, but not in the mid-dose group. In the mid-dose the results were comparable to the control. As these results did not show a dose-response relationship they are supposed to be not treatment related.

Table 13 Sperm parameters with significant difference from the control

Sperm parameter	Control	1500 ppm	4000 ppm	8000 ppm
Testis, left, concentration (millions/gram)	87.3	**78.4****	86.3	**77.3****
Sperm production rate (millions/gram/day)	14.3	**12.9****	14.2	**12.7****

[4] Charles River Ashland historical control data (version 2019.04)

** = Significantly different from the control group at 0.01

Hormone levels and haematology

There was a dose-dependent increase in TSH-levels and small decrease in T4 levels in all dose groups. T3 levels were not measured in the study. The differences were not statistically significant. There were no other test-related effects on haematology in any dose groups.

Table 14 Thyroid hormone levels, F0 generation

Thyroid hormone levels	Control	1500 ppm	4000 ppm	8000 ppm
TSH (ng/ml)	6.4	7.8	8.2	9.5
% difference	-	21.9	28.1	48.4
T4 (pg/ml)	47860	47030	44310	45210
% difference	-	-1.7	-7.4	-5.5

Organ weights

No changes in organ weight could be attributed to the test substance. The organs that showed statistical difference to the control were usually relative to the final body weight and seemed to mostly reflect the lower final body weight rather than an actual adverse organ weight. Few organ weight changes were significant when compared to brain weight. Those that were, included:

- lower spleen weight in males in the mid- (-10.4 %) and high dose (-13.9 %) group males, in a dose related manner.
- lower adrenal weight in low- (-9.3 %), mid- (-9.6 %) and high (-11.8 %) dose group females
- lower pituitary weight in high dose females (-12.2 %)

The registrant states that these organ weights were within historical control ranges, however we have not been able to check these as only historical control data for reproductive organ weights were included in the full study report. No microscopic effects in these organs were seen.

F1 generation, prior to weaning:

Litter data, postnatal survival and observations

There were no differences in mean number of pups born, litter size, percentage of each sex per litter at birth, and postnatal survival between the test groups and control group. Except for a higher number of pups in the high dose group that were characterised as having a "small stature" compared to the control group, correlating with the lower body weight seen in this group, there were no differences in general physical condition and clinical observations between test groups and control group.

Body weight

There was a lower birth weight both for males and females of the mid- and high dose groups. These differences were not statistically significant. However there was statistically significant lower body weight in the high dose group males at two different time intervals (PND 4 and 7) and for mid dose group males at one time interval (PND 4). The females of the mid and high dose groups also had lower body weight, but the difference never reached statistical significance. Body weight change was however statistically significantly lower in both males and females at high and mid dose at PND 1-4.

Table 15 Body weights and body weight change, males and females F1 generation, prior to weaning

Body weight (g)	Control	1500 ppm	4000 ppm	8000 ppm
Males				
Absolute wt, PND 1	7.5	7.5	7.2	7.2
% difference	-	0.0	-4.0	-4.0

Absolute wt, PND 4	11.2	11.1	**10.3***	**10.3***
% difference	-	-0.9	**-8.0**	**-8.0**
Absolute wt, PND 7	18.4	18.3	17.6	**17.1***
% difference	-	-0.5	-4.3	**-7.1**
Body wt change PND 1-4	3.6	3.6	**3.1***	**3.0****
% difference	-	0.0	**-13.8**	**-16.7**
Females				
Absolute wt, PND 1	7.0	7.0	6.7	6.8
% difference	-	0.0	-4.3	-2.9
Absolute wt, PND 4	10.5	10.4	9.7	9.7
% difference	-	-1.0	-7.6	-7.6
Absolute wt, PND 7	17.3	17.4	16.6	16.2
% difference	-	0.6	-4.0	-6.4
Body wt change PND 1-4	3.5	3.4	**3.0***	**2.9***
% difference	-	-2.9	**-14.3**	**-17.1**

* = Significantly different from the control group at 0.05 using Dunnett's test

Developmental landmarks (see also under section 10.10.4, developmental effects)

Anogenital distance: there was a statistically significantly higher mean anogenital distance for mid and high dose males when evaluated as a function of the mean pup body weight (anogenital distance relative to cube root of pup body weight). According to the laboratory this was within historical control range. There was no difference with the control in the low dose group.

Thyroid hormone analysis

There were differences in TSH and T4 levels, but the levels varied, and it was not possible to see an obvious effect of the test substance. The levels were significantly different in the low dose and high dose females but did not follow a dose-response relationship.

Gross pathology and organ weights

There were no differences in the internal findings in the pups that were examined after unscheduled deaths, at necropsy for the pups euthanized at PND 4 and in pups euthanized at PND 21. The findings that were seen were noted infrequently and did not show dose response relationship. There was also no difference in organ weights in these pups.

F1 generation, following weaning:

Mortality and observations

There were no test substance-related effects on survival for F1 animals. No test substance-related clinical findings were noted for the F1 generation at the daily examinations.

Body weights

Males
There was a dose related decrease in body weight gain in all dose groups, resulting in statistically significant lower body weight change and absolute body weight in the two highest dose groups. For the high dose group the difference became significant at PND 28 and for the mid dose group the difference became significant from PND 63. The difference in the lowest dose group was less pronounced and did not reach statistical significance for absolute body weight, but the body weight changes for the periods PND 21-56 and PND 21-90 were

significantly lower than for the control.
The same body weight reductions were seen in cohort 2A, where the body weight was measured at PND 65.

Females
For the females the trend was the same as for the males but less pronounced. There was a reduced body weight gain which resulted in statistically significant reduced absolute body weight in the high dose group from PND 28. The difference did not reach statistical significance for the low and mid-dose groups, except for PND 77 in the mid-dose group. For the body weight changes the difference was statistically significant for PND 21-77 and PND 21-90 in the mid-dose group and for most time periods in the high dose group.
The same body weight reductions were seen in cohort 2A, where the body weight was measured at PND 65.

Table 16 Body weights and body weight change, males and females F1 generation, following weaning.

Body weight (g)	Control	1500 ppm	4000 ppm	8000 ppm
Males				
Absolute wt, PND 63	402	387	**381****	**362****
% difference	-	-3.7	**-5.2**	**-10.0**
Absolute wt, PND 77	473	458	**444****	**420****
% difference	-	-3.1	**-6.1**	**-11.2**
Absolute wt, PND 98	539	524	**499****	**481****
% difference	-	-2.8	**-7.4**	**-10.8**
Body wt change PND 21-90	462	**441***	**427****	**405****
% difference	-	**-4.5**	**-7.6**	**-12.3**
Body wt change PND 21-98	539	524	**499****	**481****
% difference	-	-2.8	**-7.4**	**-10.8**
Females				
Absolute wt, PND 63	242	240	233	**222****
% difference	-	-0.8	-3.7	**-8.3**
Absolute wt, PND 77	270	267	**257***	**248****
% difference	-	-1.1	**-4.8**	**-8.1**
Absolute wt, PND 98	297	293	284	**271****
% difference	-	-1.3	-4.4	**-8.8**
Body wt change PND 21-90	236	229	**223***	**214***
% difference	-	-3.0	**-5.5**	**-9.3**
Body wt change PND 21-98	243	238	231	**220****
% difference	-	-2.1	-4.9	**-9.4**
Cohort 2A, body weight measured in F1-generation at PND 65				
Body weight (g), N = 12	Control	1500 ppm	4000 ppm	8000 ppm
Absolute wt, males	407.8	388.6	**371.4***	**364.5****
% difference	-	-4.7	**-8.9**	**-10.6**
Absolute wt, females	239.3	231.0	230.2	**216.1****
% difference	-	-3.5	-3.8	**-9.6**

* = Significantly different from the control group at 0.05 using Dunnett's test ** = Significantly different from the control group at 0.01 using Dunnett's test

Food consumption, food efficiency and water consumption

Males

Food consumption and food efficiency was reduced in a dose related manner in all three dose groups and differences were intermittently significant in all groups. Food efficiency was also somewhat reduced and intermittently statistically significant, mostly in the high dose group but sometimes also in the low and mid dose group.

For the females the food consumption was only slightly reduced and was not statistically significant in any dose groups, however food efficiency was clearly reduced in the high dose group, and in a statistically significant manner in the first half of the measurements (PND 21- 56), and intermittently also in the mid and low dose group.

There were only slight differences in water consumption and the difference with the control was only statistically significant in the females in the high dose group PND 21-28.

Reproductive parameters – developmental landmarks

Balanopreputial separation: ages of attainment were not statistically different from the control group, however the body weight at attainment was statistically significantly lower in the high dose group. According to the laboratory this was within historical control.

Vaginal patency: ages of attainment was significantly higher in the high dose females compared to the control. This was also higher than the historical controls. The registrant considered the result to be spurious and not test substance related, with no more explanation. Vaginal patency is a sign of sexual maturation, and a late maturation is an important effect, but it is worth noting that lower body weight may have caused the effect, so that it is more of a secondary effect to the body weight rather than a specific effect of the test substance on the sexual maturation.

Oestrous cycle: there were no differences in the test groups from the control group that were statistically significant.

Sperm evaluations: there was a statistically significant difference between cauda epididymal sperm concentration in the high dose group and the control. The cauda epididymal weight was also lower in these males, but the weight was not statistically significantly lower than the control group. No other differences were seen.

Table 17 Reproductive parameters

Reproductive parameters	**Control**	**1500 ppm**	**4000 ppm**	**8000 ppm**
Balanopreputial separation (PND)	44.3	44.2	44.2	45.2
Weight at attainment (grams) % difference	246.2 -	236.0 -4.1	236.3 -4.0	**231.5*** **-6.0**
Vaginal patency (PND)	33.0	33.4	33.5	**35.2****
Weight at attainment (grams) % difference	124.4 -	124.6 0.2	123.0 -1.1	129.2 3.9
Cauda epididymus left, sperm concentration (millions/gram) % difference	468.2 -	409.1 -12.6	466.2 -0.4	**379.9*** -18.9

Weight, left cauda epididymus, grams	0.268	0.266	0.271	0.265
% difference	-	-1.1	1.2	-4.3

* = Significantly different from the control group at 0.05
** = Significantly different from the control group at 0.01

Thyroid hormone analysis, haematology, urinalysis, coagulation and serum chemistry (cohort 1A)

There were higher levels of TSH in all treated groups for both males and females. This was however attributed to abnormally low levels in the control group, corroborated with the historical control data. The high TSH levels did not correlate with lower T4 levels, as might be expected. T3 levels were not measured in the study. Apart from a lower creatinine level in males of all dose groups (dose-response relationship, only significant in the high dose group) there were no other differences in other analysed levels.

Table 18 TSH, T4 and creatinine level in males and females at PND 90

	Control	**1500 ppm**	**4000 ppm**	**8000 ppm**
	Males			
TSH ng/mL	5.2	11.1	12.3	14.6
% difference	-	113.5	136.5	180.8
T4 pg/mL	40120	43880	45700	42680
% difference	-	9.4	13.9	6.4
Creatinine mg/dL	0.32	0.31	0.29	**0.26****
% difference	-	-3.1	-9.4	-18.8
	Females			
TSH ng/mL	4.0	8.3	8.3	6.3
% difference	-	107.5	107.5	57.5
T4 pg/mL	35620	33100	34560	35720
% difference	-	-7.1	-3.0	0.3
Creatinine mg/dL	0.39	0.39	0.45	0.34
% difference	-	0.0	15.4	-12.8

* = Significantly different from the control group at 0.05 using Dunnett's test ** = Significantly different from the control group at 0.01 using Dunnett's test

Sensory function and neurobehavioural testing (cohort 2A)

Auditory startle response (cohort 2A): There was a lower Peak-value in all dose group males compared to control, with a dose-response relationship in the first measurement (-14, -17, -29%). In the overall results the effect was less in the mid-dose group (not significant) than in the low and high dose group (significant) and thus did not show dose-response, but all treated groups had lower peak-values than the control group.

The t-peak was higher in all male dose groups, but the values were only marginally different.

In the females the peak-value was also lower in the high dose group (-8%, not significant), but the differences were less marked than in the males and did not show dose-response. There were only marginal differences in the t-peak values between dose groups and control.

Table 19 Peak-value and t-peak

	Control	1500 ppm	4000 ppm	8000 ppm
	Males			
Peak-value, 1-10 trials	1767.5	1516.8	1463.2	1245.3
% difference	-	-14.2	-17.2	-29.5
Peak-value, all trials	1645.5	**1285.6***	1317.3	**1066.0***
% difference	-	**-21.9**	-19.9	**-35.2**
T-peak (milliseconds), all trials	26.7	27.2	27.8	27.5
% difference	-	1.9	4.1	3.0
	Females			
Peak-value, 1-10 trials	1360.8	1467.6	1320.3	1356.6
% difference	-	7.8	-3.0	-0.3
Peak-value, all trials	1176.54	1336.61	1177.77	1081.23
% difference	-	13.6	0.1	-8.1

* = Significantly different from the control group at 0.05 using Dunnett's test ** = Significantly different from the control group at 0.01 using Dunnett's test

Functional observational battery (cohort 2A): Home cage observations, handling observations, open field observations, sensory observations, neuromuscular observations, physiological observations, locomotor activity: There were very few differences between the control group and the test groups in all these tests on PND 65. Some differences were statistically significantly different from the control but were not deemed to be a result of the test substance (such as a higher incidence of females in the mid-dose group were lying on side or curled up). One significant difference was however the aforementioned lower mean body weights for males in the 4000 and 8000 ppm groups and females in the 8000 ppm group.

Gross pathology (cohorts 1A and 1B) and ovarian follicle counts (cohort 1A)

There were no observations that were considered to be associated with the test substance.

Organ weights (cohorts 1A and 1B)

There were no clear test substance related alterations in organ weights in animals of Cohort 1A. There were several statistically significant changes in organ weights in males and females, yet these changes were considered to reflect lower absolute group mean final body weights in the 1500, 4000, and 8000 ppm group males and females. However, there were statistically significant changes in organ weights relative to body weight which suggests that the change was not a mere reflection of changes in final body weight:

- Cohort 1A:
    - Males, high dose: the heart ↓, the kidneys ↑, SV/CG/ACC fluid ↑ (seminal vesicle/coagulating glands/and their fluid)
    - Females, high dose: kidneys ↑, liver ↑
- Cohort 1B:
    - Males, high- and mid-dose: epididymis left, right ↑, LA/BC (levator ani/bulbo cavernosus) muscle ↓, testis left, right ↑
    - Females: no statistically significant differences

Histopathology (cohorts 1A)

There were no histological changes that occurred at an incidence that could suggest that it was caused by the test substance.

Immunophenotyping (cohort 1A)

There were minimal (25-30%) or mild (31-35%) differences in several of the immunophenotyping parameters between the control group and treated groups[5]. The only statistically significant difference was a moderately lower B lymphocyte count in the high dose group. There were no consistent or significant differences in TDAR Assay either, see table below.

Table 20 Immunophenotyping parameters. (Only results where the results were characterised as "mild" or "moderate" or were statistically significantly different from the control in at least one dose group are included in the table. These results are also marked in bold.)

Cells	**Control**	**1500 ppm**	**4000 ppm**	**8000 ppm**	**Control**	**1500 ppm**	**4000 ppm**	**8000 ppm**
	Males				**Females**			
% of cell count								
B lymphocytes, (CD3-CD45RA+)	40.7	36.3	38.4	**34.0****	37.7	35.2	35.0	**31.7****
% difference	-	-10.8%	-5.7%	**-16.5%**	-	-6.6	-7.2	**-15.9**
Absolute number of cells (X 10^6)								
Total T-lymphocytes, (CD3+)	98.86	87.3	84.58	**68.53**	80.09	69.59	65.86	64.84
% difference	-	-11.7	-14.4	**-30.7 %**	-	-13.1	-17.8	-19.0
Cytotoxic T lymphocytes, (CD3+CD8+)	39.53	34.81	28.77	**26.88**	32.87	26.65	23.48	27.01
% difference	-	-11.9	-27.2	**-32.0**	-	-18.9	-28.6	-17.8
B lymphocytes, (CD3-CD45RA+)	102.06	75.11	83.45	**55.92**	71.81	54.47	56.26	**45.19**
% difference	-	-26.4	-18.2	**-45.2**	-	-24.1	-21.7	**-37.1**
NK cells (CD3-CD161a+)	9.89	9.11	8.80	**6.57**	7.25	6.17	6.72	5.38
% difference	-	-7.9	-11.0	**-33.6**	-	-14.9	-7.3	-25.8
TDAR Assay (u/mL)								
sRBC IgM count	37360.89	**62916.84**	28512.34	**49340.06**	44905.37	44227.89	31937.61	**30976.36**
	-	**68.4**	-23.7	**32.1**	-	-1.5	-28.9	**-31.0**

* = Significantly different from the control group at 0.05 using Dunnett's test ** = Significantly different from the control group at 0.01 using Dunnett's test

[5] In the laboratory report changes in immunophenotypic parameters and TDAR assay are characterised as minimal (25-30%), mild (31-35%), moderate (36-70%) and marked (>70%) based on the percent difference from the control group mean values.

Neuropathology (cohorts 2A and 2B)

Macroscopic examination: There were no observations that were considered to be associated with administration of the test substance.

Brain weights and measurements (cohorts 2A and 2B): There were no statistically significant differences in the brain weights or measurements in Cohort 2B (PND 22) animals and in Cohort 2A (PND 78) animals compared to the control animals.

Neurohistopathological examination (cohorts 2A and 2B): There were no microscopic findings in the neurohistopathological tissue examinations in any of the treated groups in the Cohorts 2A (PND 78) or 2B (PND 22) animals.

Morphometric analysis (cohort 2A): There were statistically significant changes in several measurements, but the changes were inconsistent, did not follow dose-response and were therefore not considered treatment related. The changes were: higher S1 (thickness of frontal cortex) in 4000 and 8000 ppm females, higher S2 and S3 (thickness of parietal cortex and width of caudate-putamen, respectively) in 1500 and 4000 ppm females, lower S5 (thickness of hippocampus) in 4000 ppm males, and lower S6 (height of cerebellum) in 1500 and 8000 ppm males.

T-cell dependent antibody response (TDAR) Assay (cohort 3)

There were changes in the TDAR response compared to the control group (see Table 20), but the differences were not statistically significant and did not follow dose-response. The positive control group (cohort 3A, treated with 25 mg/kg/day cyclophosphamide for five days, PND 54-58) showed a quite clear effect with a -98.0% and -97.3% difference males and females respectively compared to the control and demonstrated the acceptability of the sRBC-IgM ELISA assay to detect a decrease in the T-cell mediated response to an antigen.

The NOAEL for systemic toxicity is set at 4000 ppm for males and 8000 ppm for females in the F0 generation based on effects on body weight changes. In the F1 generation the NOAEL is 4000 ppm for males and females also based on body weight changes. NOAEL for neurotoxicity and immunotoxicity in the F1 generation is set at 8000 ppm for males and females.

The NOAEL for reproductive effects in the F1-generation is 4000 ppm based on several statistically significant effects in the offspring in the high dose group, including vaginal patency and reduced cauda epididymis sperm concentration.

A range finding study (unnamed 2019a), was performed in rats according to OECD 421.

The study was performed in order to find the right dose levels to use in the EOGRTS described above. Groups of 10 females and 10 males Crl:CD(SD) rats in the F0 generation were mated. At PND 21 all pups of the F1 generation, except groups of 10 female and 10 male pups, were euthanised. The selected groups of pups were followed until PND 35.

The following parameters and end points were evaluated in this study: clinical signs, body weights, body weight gains, food consumption, water consumption, oestrous cycles, reproductive performance, parturition, litter viability and survival, anogenital distance, areolae/nipple anlagen, thyroid hormones, gross necropsy findings, organ weights, and histopathologic examinations.

Test substance administration

The F0 generation was exposed to the test substance through the drinking water from 13 days before mating and until euthanasia. For the males this was study day 28 and for the females lactation day (LD) 21. The selected pups in the F1 generation were administered the test substance from weaning on PND 21 until euthanasia on PND 35.

The doses administered were 0, 3000, 8000 and 12000 ppm. As the substance was administered through the water the actual intake of the substance varied quite a bit throughout the study. See Table 21.

Table 21 Mean test substance consumption F0 and F1 generation.

Theoretical water concentration (ppm)	Mean test substance consumption (mg/kg/day)					
	Males F0	Females F0			Males F1	Females F1
	Prior to mating[a]	Prior to mating[a]	Gestation	Lactation	PND 21- PND 35	
3000	333	488	321	604	595	588
8000	590	1082	834	1820	1590	1716
12 000	1251	1094	953	2313	2594	2593

a = study days 0-7 only

Results

Mortality and clinical findings in the F0 generation:

There were no F0 mortalities and no specific clinical observations in any dose groups during the study. One female in the high dose group had a total litter loss on LD 1 and was subsequently euthanized.

Body weight

F0 generation: Body weight was affected in the mid- and high dose groups especially. For the males there were no statistically significant differences compared to the control. The females also had reduced body weight gain which resulted in statistically significantly lower absolute body weight and body weight gain under gestation. The differences between the control group and the test groups were reduced during lactation, due to an unusually low body weight gain in the control group, but the high dose group still had statistically significantly lower absolute body weight at the end of the lactation period.

F1 generation: there was a decrease in body weight and body weight gain in all dose groups in a dose related manner. Statistically significant reduced body weight (high dose group) and body weight gain (mid and high dose groups) was seen as of day 10 in the postweaning period.

Table 22 Body weight and body weight gain in the F0 and F1 generation, males and females.

Body weight (g)	**Control**	**3000 ppm**	**8000 ppm**	**12 000 ppm**
F0 Males				
Absolute wt, day 28 (final study day)	483	465	465	456
% difference	-	-3.7	-3.7	-5.6
Body wt change, whole study	135	119	113	112
Day 0-28	-	-11.9	-16.3	-17.0
F0 Females				
Absolute wt, day 14, pre-mating	286	269	253	257
% difference	-	-5.6	-11.2	-9.8
Absolute wt, GD 20	446	427	**397****	**376****
% difference	-	-4.3	**-11.0**	**-15.7**
Absolute wt, LD 21	341	355	324	**304***
% difference	-	-1.8	-5.0	**-10.9**
Body wt change, gestation, day 0-20	164	160	**141***	**112****
% difference	-	-2.4	**-19.5**	**-31.7**
Body wt change, Lactation, day 1-21	12	24	34	28

% difference	-	200	283.3	233.3
F1 Males				
Absolute wt, PND 21	51.2	51.4	46.6	**43.5****
% difference	-	0.4	-9.0	**-15.0**
Body wt change, day 1-21#	44	44	39.6	36.5#
% difference	-	0.0	-10.0	-17.0
F1 Females				
Absolute wt, PND 21	50.2	49.2	45.4	**43.6***
% difference	-	-2.0	-9.6	**-13.1**
Body wt change, day 1-21#	43.2	42.1	38.8	37#
% difference	-	-2.5	-10.2	-14.4

* = Significantly different from the control group at 0.05 using Dunnett's test ** = Significantly different from the control group at 0.01 using Dunnett's test

= The laboratory did not do a statistical analysis of the body weight change day 1-21. Therefore, the results in the table are not highlighted in bold and marked with a star but would probably have reached statistical significance had the calculations been done.

Food and water consumption

F0 generation, males: there were no differences in food and water consumption between the control group and the test groups.

F0 generation, females: There was a slight, non-statistically significant lower intake of food for the females in the high dose group pre-mating. The lower food consumption was dose-response related and statistically significant in the mid- and high dose females during gestation (-15.4% and -19.2% respectively), and in the high dose group during lactation (-11.5%).

There was a lower water consumption in the high dose group pre-mating (-26.7%), although non-statistically significant. The lower water consumption continued during gestation in the high dose group and was statistically significant, -32.4%. During lactation water consumption in the high dose group was still lower than the control (-10.7%), and other test groups, but no longer statistically significant.

F1 generation: there were no differences in food and water consumption between the control group and the test groups.

Reproductive parameters

There were no statistically significant differences between test groups and control in the following reproductive parameters: mating index, fertility index, copulation index, estrous cycle length and pre-coital interval. There were no differences in gestation length and parturition.

A dose-response reduction in mean number of implantation sites and pups born was seen in the test groups. The number of implantation sites and pups born was however within the historical control even in the high dose group and the difference with the control were not statistically significant. There was also a reduced post-natal survival from birth to PND 4. None of the differences were statistically significant.

Table 23 Reproductive parameters, range finding study.

	0	**3000 ppm**	**8000 ppm**	**12 000 ppm**
Mean nr. of implantation sites	16.9	15.9	15.2	14.8
% difference	-	-5.9	-10.0	-12.4
Mean nr. of pups born	15.7	14.8	14.2	13.3

% difference	-	-5.7	-9.6	-15.3
Postnatal survival 0-PND 4 (% pr litter)	99.3	98.1	95.3	82.1
% difference	-	-1.2	-4.0	-17.3

Hormone levels

In F0 males a dose dependent increase in T3 and TSH hormone levels was seen in all dose groups. There was also a small increase in T4 levels in all dose groups. The differences were not statistically significant. The F0 females were also sampled for thyroid hormone analysis but the samples were never analysed. There was no explanation for this omission.

There were some differences in hormone levels in the pups in the dose groups compared to the control group also, but none that were statistically significant.

Table 24 Hormone levels, range finding study.

F0 males, day 28 (only male samples were analysed)	**0**	**3000 ppm**	**8000 ppm**	**12 000 ppm**
Total T3, pg/mL	603	634	667	694
% difference	-	5.1	10.6	15.1
Total T4, pg/mL	63540	69710	70500	69540
% difference	-	9.7	11.0	9.4
TSH, ng/mL	7.9	10.6	11.5	12.3
% difference	-	34.2	45.6	55.7
F1 pups, PND 4 (no distinction between males and females)	**0**	**3000 ppm**	**8000 ppm**	**12 000 ppm**
Total T3, pg/mL	158.3	192.9	145.5	127.8
% difference	-	21.9	-8.1	-19.3
Total T4, pg/mL	29550	30060	28480	28667
% difference	-	1.7	-3.6	-3.0
TSH, ng/mL	3.1	2.0	2.1	2.0
% difference	-	-35.5	-32.3	-35.5
F1 pups, PND 21 (males/females)	**0**	**3000 ppm**	**8000 ppm**	**12 000 ppm**
Total T3, pg/mL	921/918	1018/878	933/909	986/949
% difference	-	10.5/-4.4	1.3/-1.0	7.1/3.4
Total T4, pg/mL	63460/63060	68830/64580	58420/55200	59378/61813
% difference	-	8.3/2.4	-7.9/-12.5	-6.4/-2.0
TSH, ng/mL	5.0/4.6	3.5/3.5	5.0/3.7	4.9/4.2
% difference	-	-30/-23.9	0/-19.6	-2.0/-8.7

Organ weights

F0 males: Statistically significant organ weight differences in the test groups compared to the control group was a decrease in the relative (to brain) epididymis weight in the high and low dose groups. The weight was also decreased in the mid dose group but not in a statistically significant manner.

F0 females: There was a dose-response decrease in absolute and relative weights of the ovaries/oviducts, spleen and thymus for all dose groups. The decrease in ovary weight was statistically significant for the high dose group, the decrease in thymus weight was statistically significant in the mid and high dose group, and the decrease in spleen weight was statistically significant in all dose groups (except for relative to body weight in the low dose group).

F1 males: the only statistically significant difference with the control group was reduced absolute liver weight in the high dose group, which corresponded with reduced body weight.

F1 females: there was a statistically significant reduction in absolute and relative liver weight, and absolute spleen weight in the high dose group.

Table 25 Organ weights

F0 males, organ weights	**0**	**3000 ppm**	**8000 ppm**	**12 000 ppm**
Epididymis, left (g/100 g brain)	30.958	**28.341***	28.953	**28.480***
% difference	-	**-8.5**	-6.5	**-8.0**
F0 females, organ weights	**0**	**3000 ppm**	**8000 ppm**	**12 000 ppm**
Ovaries/oviducts (g)	0.1647	0.1507	0.1436	**0.1225****
% difference	-	-8.5	-12.8	**-25.6**
Ovaries/oviducts (g/100 g brain)	8.239	7.357	7.404	**6.440****
% difference	-	-10.7	-10.1	**-21.8**
Spleen, absolute (g)	0.74	**0.65***	**0.59****	**0.50****
% difference	-	**-12.2**	**-20.3**	**-32.4**
Spleen, (g/100 g final body weight)	0.218	0.195	**0.183****	**0.166****
% difference	-	-10.6	**-16.1**	**-23.9**
Spleen, (g/100g brain)	36.736	**31.549***	**30.701****	**26.389****
% difference	-	**-14.1**	**-16.4**	**-28.2**
Thymus, absolute (g)	0.2353	0.1956	**0.1326****	**0.1019****
% difference	-	-16.9	**-43.6**	**-56.7**
Thymus, (g/100 g final body weight)	0.068	0.059	**0.041****	**0.033****
% difference	-	-13.2	**-39.7**	**-51.5**
Thymus, (g/100g brain)	11.757	9.604	**6.866****	**5.374****
% difference	-	-18.3	**-41.6**	**-54.3**
F1 males, organ weights	**0**	**3000 ppm**	**8000 ppm**	**12 000 ppm**
Liver, absolute (g)	2.3429	2.2892	2.1936	**1.9424***
% difference	-	-2.3	-6.4	**-17.1**
F1 females, organ weights	**0**	**3000 ppm**	**8000 ppm**	**12 000 ppm**
Liver, absolute (g)	2.2638	2.1601	2.1728	**1.8508***
% difference	-	-4.6	-4.0	**-18.2**
Liver (g/100 g brain)	162.401	148.851	157.897	**139.197***
% difference	-	-8.3	-2.8	**-14.3**
Spleen, absolute (g)	0.2454	0.2117	0.1988	**0.1881***
% difference	-	-13.7	-19.0	**-23.3**

* = Significantly different from the control group at 0.05 using Dunnett's test ** = Significantly different from the control group at 0.01 using Dunnett's test

The conclusion of the range-finding study was that doses up to 8000 ppm were considered appropriate in an EOGRTS.

10.10.3 Comparison with the CLP criteria

There are no epidemiological data to support classification of 2-pyrrolidone in Category 1A.

A new extended one generation toxicity study from 2020, performed according to OECD 443 and GLP shows reduced body weight change in the F0 and F1 animals and the NOAEL for the systemic toxicity is based on this effect. There were no effects on reproductive parameters in the parental generation. In the offspring, there were some statistically significant effects in the high dose group that are considered an effect on a reproductive parameter (late vaginal patency, higher mean anogenital distance in function of pup body weight and reduced cauda epididymis sperm concentration) and the reproductive NOAEL is therefore set at 4000 ppm. As there were very few effects in the parental animals, the effects in the offspring cannot be considered secondary to maternal effects. However, the late onset of vaginal patency could also be secondary to lower body weight, and the reduced cauda epididymis sperm concentration was not correlated to a statistically significant lower cauda epididymis weight. And according to the laboratory report the higher anogenital distance was within historical control data. All in all the effects cannot be considered clearly an adverse effect on sexual function and fertility caused by the treatment with 2-pyrrolidone. Therefore a classification of 2-pyrrolidone as a reproductive toxicant for sexual function and fertility in category 1B is not warranted.

In order to classify a substance as a category 2 reproductive toxicant there must be "some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1". Although there were effects seen on the offspring in this EOGRTS-study, as described above, it does not seem as though the effects had an adverse effect on sexual function and fertility. A classification as Repr 2 is therefore not considered justified for 2-pyrrolidone, and no classification for effects on sexual function and fertility is proposed.

10.10.4 Adverse effects on development

Table 26: Summary table of animal studies on adverse effects on development.

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Prenatal Developmental Toxicity Study OECD Guideline 414 GLP study Rabbits, New Zealand White 2019-06-05 - 2019-02-22 (experimental phase: 2018-	Dosage/Dose Level (mg/kg/day) 0, 250, 500 1000 Oral gavage, during gestation days (GD) 7-28. 24 females pr group.	Maternal NOAEL: 1000 mg/kg bw/day based on few signs of toxicity. Body weight reductions were seen but less than -10% compared to control. Developmental NOAEL: 250 mg/kg bw/day based on reduced foetal weight. There was also an increase in malformations seen in the mid- and high dose groups. However, the increase in malformations in the mid- dose group compared to the control was not very pronounced. Taking into consideration that the incidence of malformations in the control group was above the historical control data, especially for rare malformations, there may also be an increase in the mid dose group. **Maternal toxicity**: Moribundity:	Unnamed, 2019b

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
06-18 - 2018-08-20)		**Control and 500 mg/kg** bw/day dose groups: No mortality **250 mg/kg** bw/day dose group: 1 dam euthanized in extremis on GD 19 after weight loss of 9.8 % GD 14-19 due to reduced food intake after GD 13 (no food intake after GD15). Considered not substance related since no other effects were seen at 250 or 500 mg/kg groups. **1000 mg/kg** bw/day dose group: 2 dams euthanized in extremis, one on GD 15 and one on GD 28 due to "adverse occurrences of moribundity". Considered adverse and substance related by the laboratory. Both dams had viable pups in utero. The dam euthanized on GD 15 was hypoactive, had lower food consumption and body weight loss of 11%, and decreased defecation. The dam who was euthanized on GD 28 had red material in the urogenital and anogenital area and in the cage. She also experienced increased respiration rate and a prolapsed vagina. Abortions: **Control** group: one dam aborted on GD 29 after low food consumption and loss of body weight (7.8% GD 23-29). **1000 mg/kg** bw/day dose group: one dam aborted on GD 27 seven dead foetuses with no apparent malformations. The same dam also had a late resorption. The abortion happened after a body weight loss of 17% and lower food consumption. Clinical findings: The most common clinical finding was decreased defecation which seemed to occur in a dose-related manner and was also related to decreased food consumption. Food consumption: Food consumption was reduced in a dose-related manner. The consumption was lower than the control group in all three dose groups but was statistically significant only in the two highest groups (-12%, -33%, -45% food consumption compared to control in the low, middle, and highdose groups). See Table 27. Body weight: Body weight was reduced in a dose related manner. The terminal body weight and body weight change was lower in all dose groups but was only statistically significant in the two highest dose groups. Net body weight/corrected body weight was only statistically lower in the high dose group. Net/corrected body weight: -2.1%, -4.9%, -9.4% compared to control in the low, middle, and high dose groups. Only the high dose group was statistically significant. Terminal body weight: -1.8%, -5.9%, -8.1% compared to control in the low-, mid-, and high dose groups. Mid- and high dose significant. Although the body weight effects in the dams seem to be substance related, they were not very large and can barely be called adverse (less than 10% lower compared to control) especially since rabbits often	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		experience fluctuations of weight during pregnancy (CLP guidance, Annex I: 3.7.2.4.4.). See Table 28. Necropsy: Gross pathology: no findings in any groups were considered substance related or adverse. Mean liver weight was unaffected by the substance. **Foetal toxicity**: Foetal body weights: Mean foetal body weight was reduced in a substance related manner. The foetal body weight was lower in all dose groups but was only statistically significant in the two highest dose groups. The mean foetal weights were -6%, -14.1% and -20% compared to control in the low, middle, and high dose groups. Middle and high dose significant. However, there is an obvious effect also in the low dose group and an ED10 calculation shows that the ED10 value is below 400 mg/kg. Historical control data for foetal weight is in the range of 38.8-44.5 g. See Table 29. Resorptions: There was increase in late (6 %) and total (10.2 %) resorptions in the high dose group. See Table 30. Malformations: Malformations were observed in 7(6), 1(1), 8(5), and 16(8) foetuses (litters) in the control, 250, 500, and 1000 mg/kg/day groups, respectively, which in percents corresponds to 3.5%(26%), 0.5%(4.5%), 4%(20.8%) and 8.3%(40%) in the dose groups. The percentage of malformations in the control group was well above the historical control data which is stated to be a mean of 3.01 % per litter. *Visceral malformations* were observed in 4(4), 1(1), 6(3), and 11(6) foetuses (litters) in the control, 250, 500, and 1000 mg/kg/day groups, respectively. There were especially many visceral malformations of the heart (persistent truncus arteriosus, interventricular septal defect, small heart ventricle, and bulbous aorta) in 9 foetuses in 5 litters in the high dose group (5% of foetuses, 25% of litters). *External and skeletal malformations*: there was also an increase in external and skeletal malformations in the high dose group. See Table 31 for more details on malformations.	
A GLP dose-range finding study for OECD 414 Rabbits, New Zealand White	Dosage/Dose Level (mg/kg/day) 0, 100, 300, 700, 1000 Oral gavage, during	Maternal NOAEL: 1000 mg/kg bw/day based on few signs of toxicity. Body weight reductions were seen but less than -10% compared to control. Developmental NOAEL: 1000 mg/kg bw/day based on no effects. **Maternal toxicity**: No moribundity, no clinical signs. The only sign of toxicity was	Unnamed, 2018

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
2018-04-24 - 2018-11-20 (experimental phase: 2018-04-26 - 2018-05-18)	gestation days (GD) 7-28. 6 females pr group.	reduced body weight gain. The reduction was dose-related and was seen in all dose groups some days of the administration of the substance but was only significant in the high dose group the first days of dosing. The overall body weight gain reduction was not significant and not adverse. Food consumption was also reduced in all groups in a dose related manner. The difference was only statistically significant in the two top dose groups. Mean absolute and relative (to net body weight/corrected body weight) liver weight in the 1000 mg/kg/day group was higher (20.8% for absolute) than the control group; the difference for mean liver weight relative to net body weight/corrected body weight was statistically significant. **Foetal toxicity**: No external malformations or variations were noted at any dosage level. Foetal weights were not measured. The foetuses were only examined externally. There was an increase in late resorptions seen in the high dose group (7.9 % as opposed to 0 in all other groups).	
Extended one generation reproductive toxicity study (EOGRTS) with both developmental neuro- and immunotoxicity cohorts (Cohorts 1A, 1B without extension, 2A, 2B, 3 and 3 A (control)) OECD TG 443, GLP study Crl:CD(SD) rats, 30 males and 30 females 03 Dec 2018 - 03 March 2020	2-pyrrolidone Purity 99.5% Doses: 0, 1500, 4000 and 8000 ppm, in drinking water. Dosing 70 days before mating until euthansia. To the offspring in F1: from weaning until euthanasia.	**Developmental effects from the EOGRTS:** Developmental landmarks: for mid and high dose males, there was a statistically significantly higher mean anogenital distance when evaluated as a function of the mean pup body weight. Thyroid hormone analysis: clear differences in TSH and T4 levels were observed in the offspring prior to weaning in low dose and high dose females, but results were not statistically significant and not in a dose-response relationship. Gross pathology and organ weights: no test substance related findings. Sensory function and neurobehavioural testing (cohort 2A): Lower Peak-value in all male dose groups compared to control with a dose-response relationship was observed in the first measurement. Low and high dose group showed statistically significant differences compared to control, but not in a dose-response manner. In females, differences were less marked, and showed no dose-response.	Unnamed, 2020
Prenatal Developmental Toxicity Study OECD Guideline 414	Dosage/Dose Level (mg/kg/day) 0, 190, 600, 1900	Maternal NOAEL: 600 mg/kg bw/day based on few signs of toxicity. Body weight reductions were seen in the mid dose group but was either not statistically significant or less than -10% compared to control. Developmental NOAEL: 600 mg/kg bw/day based on reduced foetal weight. There was also an increase in malformations seen in the mid- and high dose groups. However, the increase in malformations in the	Unnamed, 1990

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
GLP study Sprague-Dawley rats 1990	Oral gavage, during gestation days (GD) 6-15. 25 females pr group.	mid- dose group compared to the control was not very pronounced. **Maternal toxicity**: Moribundity and clinical signs: No mortality in any group. No clinical observations. Food consumption: Food consumption was reduced in a statistically significant manner parts of the treatment period for the mid dose group and for the whole treatment period for the high dose group ($P < 0.01$). Body weight: Body weight was reduced in a dose related manner. High dose: weight loss days 6-9 of treatment. Statistically significantly reduced body weight gain days 9-12 and reduced overall body weight for days 9-20. Also, statistically significant reduction in corrected body weight and corrected body weight gain. Mid dose: Statistically significantly reduced body weight gain days 9-12 and reduced overall body weight for days 9-20. Also, statistically significant reduction in corrected body weight. Corrected body weight gain was also reduced but not in a statistically significant manner. Low dose: slight reduction in body weight, body weight gain, corrected body weight and corrected body weight gain, but not in a statistically significant manner. See Table 34 Necropsy: Gross pathology: no findings in any groups were considered substance related or adverse. **Foetal toxicity**: Foetal body weights: There was a statistically significant reduction in foetal weight in the high dose group. There was also a slight reduction in foetal weight in the mid dose group, but not in a statistically significant manner. See Table 35. Malformations: There was a statistically significant increase in the incidence of litters and foetuses with *major malformations* in the high dose group. There was also a statistically significant increase in the incidence of foetuses with *minor visceral- and skeletal* anomalies in the high dose group. However, the incidence of these anomalies in the litters was not increased. There were no increases of malformations or anomalies in the mid- and low dose groups. See Table 36.	

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

Two prenatal developmental toxicity studies were performed with 2-pyrrolidone, one in rabbits and one in

rats. In addition, a range-finding study on rabbits was performed. Developmental endpoints from the EOGRTS are also included below.

A prenatal developmental toxicity study (Unnamed 2019b) was performed in rabbits according to OECD 414.

Groups of 24 female rabbits (New Zealand White [Hra:(NZW)SPF]) were treated with 2-pyrrolidone by oral administration daily (gavage) at three dose levels of 250, 500 and 1000 mg/kg bw/day from gestation day 7 up to and including gestation day 28. A control group of 24 females was given the vehicle, deionised water.

During the study, mortality was checked for and clinical observations were performed. Body weight and food consumption of the dams were also recorded. Scheduled euthanasia was performed on gestation day 29 and gross pathology was performed. The uterus was weighed, and the ovaries and uterus were examined for number and distribution of corpora lutea, implantation sites, live and dead foetuses, and early and late resorptions. The placentae were also examined. Foetal examinations were conducted without knowledge of treatment group. External, internal, and skeletal foetal findings were recorded as developmental variations or malformations. In total, there were 23(195), 22(193), 24(200) and 20(179) evaluated litters (foetuses) in the control, 250, 500 and 1000 mg/kg groups, respectively.

Maternal toxicity:

Moribundity

In the high dose group two dams were euthanised in extremis. One was euthanized on GD 15 due to hypoactivity on the day of euthanisation, body weight loss of 11% and lower food consumption day 7 to15 and in consequence decreased defecation. This dam had 7 normally developing implantations in utero. Compared to the other dams in the high dose group it seems that the only difference with this dam was the hypoactivity registered on GD 15.

The other dam was euthanised on GD 28 due to findings of red material in the urogenital/anogenital area and red material in the cage pan. The vagina was prolapsed, and she had increased respiration rate GD 27. She had normal body weight and food consumption all through the test period and 8 viable foetuses and 1 late resorption in utero with no apparent malformations.

These two deaths were considered by the registrant to be test substance related.

In the low dose group one dam was euthanised on GD 19 after a weight loss of 9.8% GD 13-19. This came after lower food consumption from GD 13. The dam stopped eating altogether as of GD 15. This dam had two early resorptions and six normally developing implantations in utero. This mortality was not considered test substance related by the registrant.

No dams died in the control or the mid dose group.

Abortions:

In the high dose group, 1 dam aborted on GD 27. This dam had experienced a body weight loss of 17% and lower food consumption GD 19 – 26, with corresponding decreased defecation. The dam aborted 7 dead foetuses. The foetuses that were not cannibalised appeared to have no malformations. This abortion was considered to be test substance related.

In the control group one dam aborted on GD 29. This dam had lost 7.8% of its body weight GD 23–29 with corresponding low food consumption (0–1 g/day) during GD 24–29.

Clinical findings:

The most common clinical finding was decreased defecation which seemed to occur in a dose-related manner and was also related to decreased food consumption.

Food consumption:

Food consumption was reduced in a dose-related manner. The consumption was lower in all three dose groups compared to the control group but was statistically significant only in the two highest groups.

Table 27 Summary of maternal food consumption

	0 mg/kg bw/day	250 mg/kg bw/day	500 mg/kg bw/day	1000 mg/kg bw/day
Day 7-29, g/animal/day	122	108	**81****	**63****
% difference	-	-11.5	**-33.6**	**-48.4**
Day 7-29, g/kg/day	34	30	**23****	**19****
% difference	-	-11.8	**-32.4**	**-44.1**

** = Significantly different from the control group at 0.01 using Dunnett's test
Nongravid weight(s) not included in calculation of mean

Body weight:

Body weight (bw) was reduced in a dose related manner and reflected the reduced food consumption in these same groups.

In the high dose group body weight loss was noted on GD 7-10 and 10-13. However, body weight gain was slightly higher than in the control group for the remainder of the treatment period, GD 13-20 and 20-29. The overall body weight gain was however lower for the high dose group due to the initial weight loss. Mean absolute body weight was 8.1% lower in the high dose compared to the control group.

In the mid dose group body weight loss was seen on GD 7-10 and 10-13, and slightly lower mean body weight was noted during GD 13-20 and 20-29. The initial weight loss resulted in a lower body weight gain for the whole treatment period compared to the control.

The terminal body weight and body weight change was lower also in the low dose group but was only statistically significant in the two highest dose groups. Net body weight/corrected body weight was only statistically significantly lower in the highest group.

Gravid uterine weight was not significantly lower in any dose groups.

Table 28 Maternal body weight, developmental toxicity study

	0 mg/kg bw/day	250 mg/kg bw/day	500 mg/kg bw/day	1000 mg/kg bw/day
Initial body wt	3325	3348	3352	3306
Terminal body wt	3742	3672	**3521***	**3436****
% difference	-	-1.8%	**-5.9%**	**-8.1%**
Gravid uterin wt	495.2	493.5	432.2	494.4
Net body wt /corrected body wt	3247.2	3178.6	3089.1	**2941.4****
% difference	-	-2.1%	-4.9%	**-9.4%**
Net body weight change/ corrected body wt change	-78.2	-169.0	**-262.5****	**-364.9****

* = Significantly different from the control group at 0.05 using Dunnett's test ** = Significantly different from the control group at 0.01 using Dunnett's test

The effects on the body weight changes were clearly dose-related. They were however not very large and only borderline adverse since the body weight parameters were less than 10% lower than the control at all time intervals, with the exception of three days (day 15, 16 and 17 where the difference in the high dose group with the control was respectively -10.2, -10,9 and -10.6%). This is especially true for rabbits that often experience fluctuations of weight during **pregnancy** (CLP guidance, Annex I: 3.7.2.4.4.).

Necropsy:

Gross pathology: no findings in any groups were considered substance related or adverse.

Of organs, only liver weights were measured. Mean liver weight was unaffected by the substance.

Foetal toxicity

The foetal body weights were reduced in all dose groups in a dose-related manner. Only the middle and high dose groups were statistically significantly different from the control. However, there is an effect also in the low dose group and an ED10 calculation shows that the ED10 value is below 400 mg/kg. Historical control data for the performing laboratory showed a foetal weight in the range of 38.8 – 44.5 g, with a median of 41.7 g.

Table 29 Foetal weight, developmental toxicity study

Foetal weights in grams	**0**	**250**	**500**	**1000**
Male foetal weights	43.3	40.4	**37.0****	**34.8****
% difference from control	-	-6.7	**-14.5**	**-19.6**
Female foetal weights	41.5	39.7	**36.7***	**33.3****
% difference from control	-	-4.3	**-11.6**	**-19.8**
Foetal weight, combined	42.6 g	40.0	**36.6****	**34.1****
% difference from control	-	-6%	**-14.1%**	**-20%**

* = Significantly different from the control group at 0.05 using Dunnett's test ** = Significantly different from the control group at 0.01 using Dunnett's test

ED10 calculation[6]:

(500-250)/(40.0-36.6) = 73.5; 40.0-38.34 = 1.66; 1.66 x 73.5 = 122.01; 250 + 122 = 372 mg/kg

According to the registrant intrauterine survival did not show statistically significant differences between the dose groups. However, there was a higher percentage of late resorptions and total resorptions in the high dose group compared to the other dose groups. According to historical control data the maximum % of late resorptions has been 5.5% with a median of 0.75 % for this laboratory (range 0-5.5 %). There was clearly a high incidence of late resorptions in the high dose group in this study even if it did not show statistical significance. The early resorptions were within the historical control data (range 0 – 9.65%, median 2.93%).

Table 30 Resorptions, developmental toxicity study

	0 mg/kg	**250 mg/kg**	**500 mg/kg**	**1000 mg/kg**
Early resorptions	4.7 %	7.2 %	3.8 %	4.2 %
Late resorptions	0.8 %	1.4 %	1.8 %	6 %
Total resorptions	5.5 %	8.6 %	5.6 %	10.2 %

Malformations

Malformations were observed in 7(6), 1(1), 8(5), and 16(8) foetuses (litters) in the control, 250, 500, and 1000 mg/kg/day groups, respectively. In percents this corresponds to 3.5%(26%), 0.5%(4.5%), 4%(20.8%) and 8.3%(40%) in the dose groups. In the historical control for the performing laboratory the mean % foetuses per litter is 3.01%. The incidence of malformations in the high dose group is clearly high and well above the control of the study and the historical control data.

Visceral malformations were observed in 4(4), 1(1), 6(3), and 11(6) foetuses (litters) in the control, 250, 500,

[6] According to CLP guidance, 3.7.2.6.3.3

and 1000 mg/kg/day groups, respectively.

There were especially many visceral malformations of the heart (persistent truncus arteriosus, interventricular septal defect, small heart ventricle, and bulbous aorta) in 9 foetuses in 5 litters in the high dose group (5% of foetuses, 25% of litters).

External and skeletal malformations: there was also an increase in external and skeletal malformations in the high dose group.

There was no correlation between the malformations seen and the individual foetal weights and so one cannot attribute the occurrence of the malformations to general growth retardation in the foetuses. Two of the foetuses with these findings had body weights that were below the mean of the litter. The remaining affected foetuses had higher body weights than the mean of the litter.

The registrant also agrees that adverse and test substance related occurrences of external malformations and visceral malformations were seen at the high dose level.

There is however also a higher number of malformations than expected in the control group. Rare and serious malformations are seen, especially visceral malformations of the heart.

Table 31 Malformations, external and visceral

		Foetuses				**Litters**			
	Dose group	**0 mg/kg**	**250 mg/kg**	**500 mg/kg**	**1000 mg/kg**	**0 mg/kg**	**250 mg/kg**	**500 mg/kg**	**1000 mg/kg**
	Number examined	195	193	200	179	23	22	24	20
	Foetuses/litters with malformations, absolute number	7	1	8	15	6	1	5	8
	Total malformations, percentage	3.6	0.5	4	8.3	26	4.5	20.8	40
	Historical control data	91 datasets with 1930 litters and 17099 foetuses. % per litter: Mean: 3.01. Median: 2.82. Range 0 - 8.62. 25th quartile: 1.61. 75th quartile: 3.94							
External effects	**Foetuses/litters with external malformations**	1	0	0	3	1	0	0	2
	Omphalocele	1	-	-	1	1	-	-	1
	Open eyelid	1	-	-	-	1	-	-	-
	Spina bifida	-	-	-	2	-	-	-	1
	Short tail	-	-	-	2	-	-	-	1
	Localized foetal edema	-	-	-	1	-	-	-	1
Visceral effects	**Foetuses/litters with visceral malformations**	4	1	6	11	4	1	3	6
	Lungs- lobular agenesis	1	-	4	5	1	-	2	2
	Persistent truncus arteriosus (very rare malformation*)	2	-	1	1	2	-	1	1
	Heart- ventricle(s), small (very rare malformation*)	1	-	-	3	1	-	-	2
	Bulbous aorta	-	-	-	2	-	-	-	2

	Interventricular septal defect (very rare malformation*)	1	1	-	2	1	1	-	2
	Kidney(s)-malpositioned	-	-	1	-	-	-	1	-
Skeletal effects	**Foetuses/litters with skeletal malformations**	2	0	2	6	2	0	2	4
	Costal cartilage anomaly	1	-	-	2	-	-	2	2
	Vertebral anomaly with or without associated rib anomaly	-	-	-	1	-	-	1	1
	Rib anomaly	-	-	-	1	-	-	-	1
	Skull anomaly	-	-	-	1	-	-	-	1
	14th full rib(s)	-	-	-	1	-	-	-	1
	Vertebral centra anomaly	-	-	-	1	-	-	-	1
	Sternoschisis	-	-	-	1	-	-	-	1
	28 presacral vertebrae	-	-	1	-	-	-	1	-
	Sternebrae fused	1	-	-	-	1	-	-	-

* Very rare malformations. In historic control data the incidence for these malformations were:
Persistent truncus arteriosus: 7/17099 pups had this malformation
Heart- ventricle(s) small: 1/17099 had this malformation
Interventricular septal defect: 1/17099 pups had this malformation

The registrant has set a NOAEL of 250 mg/kg bw/day for both maternal and developmental toxicity. We propose however to set the NOAEL at 1000 mg/kg bw/day for maternal toxicity due to lack of adverse effects, and a NOAEL for developmental toxicity at 250 mg/kg bw/day mainly due to reduced foetal weight and a slight increase in malformations in the 500 mg/kg bw/day. The increase in malformations in the 500 mg/kg bw/day group was not large compared to the control, but taking into consideration that the control group had an unusual amount of rare malformations one should maybe rather compare the incidence in the 500 mg/kg bw/day with the mean of the historic control, in which case there was a slight increase in the medium dose group. The control group presented several malformations that are hardly ever seen, and it might put into question the quality of the study.

In the 1000 mg/kg bw/day there were two dams who were euthanised "in extremis". These were by the registrant considered to be substance related. However, the two dams had different symptoms. The dam euthanized on GD 15 was hypoactive, had lower food consumption and body weight loss of 11%, and decreased defecation. The dam who was euthanized on GD 28 had red material in the urogenital and anogenital area and in the cage. She also experienced increased respiration rate and a prolapsed vagina. There was also a euthanised dam in the 250 mg dose group. She had more or less the same symptoms as one of the dams in the high dose group (weight loss of 9.8 % GD 14-19). However, the registrant did not consider this substance related. It is not clear that the symptoms experienced by the two euthanised dams in the high dose group were adverse effects due to the test substance. Both dams had viable pups in utero.

The dams in the high dose group also had a significantly reduced body weight (-9.4%) at the end of the test period compared to the control group. However, in general, the difference in body weight should be at least 10% in order to be considered adverse. In addition, the CLP guidance says: "In rabbits, the body weight gain may not be useful indicators of maternal toxicity because of normal fluctuations in body weight during

pregnancy."[7]. It is therefore not obvious that the effects observed in the dams in the high dose group can be considered adverse and **we therefore set the NOAEL at 1000 mg/kg bw/day for maternal effects**.

For the foetuses/developmental effects we agree with the registrant to set the NOAEL at 250 mg/kg bw/day based on reduced foetal weight seen in a dose response manner in all dose groups but considered adverse in the two highest dose levels. In addition, an increase in malformations and late resorptions were seen in the high dose group.

A dose range finding study for OECD 414, was performed in rabbits (unnamed 2018).

Groups of 6 female New Zealand White rabbits were treated with 2-pyrrolidone by oral administration daily (gavage) at four dose levels of 100, 300, 700 and 1000 mg/kg bw/day from gestation day 7 up to and including gestation day 28. A control group of 6 females was given the vehicle, deionised water. The dams were euthanised on GD 29.

The following parameters and end points were evaluated in this study: clinical signs, body weights, body weight gains, gravid uterine weights, food consumption, clinical pathology parameters (haematology and serum chemistry), intrauterine growth and survival, gross necropsy findings, organ weights, and external foetal examinations.

Maternal toxicity:
Clinical signs: There were no mortalities in any of the test groups. In the high dose group there were instances of decreased defecation during GD 11-18, which corresponded with lower mean food consumption noted in this group. Other clinical observations were sporadic and did not occur in a dose-response manner.

Body weight and food consumption: There was body weight loss in the high dose group, and a dose-related reduction in body weight gain which was seen in all dose groups especially days 7-13 of gestation but which only reached statistical significance at certain time intervals in the high dose group. This was followed by a statistically significantly increased food consumption in the high dose group GD 13-20. The overall body weight- and body weight gain reduction was not statistically significant. Food consumption was also reduced in all groups in a dose related manner. The difference was only statistically significant in the two top dose groups.

Table 32 Summary of maternal body weight and food consumption

Doses, mg/kg bw/day	**0**	**100**	**300**	**700**	**1000**
mg	**Summary of maternal body weight data in grams**				
Initial body weight	3233	3191	3245	3246	3242
Terminal body weight % difference	3599 -	3578 -0.6	3497 -2.8	3497 -2.8	3474 -3.5
Gravid uterine weight	457.7	454.3	474.2	444.4	469.7
Net body weight / corrected body wt % difference	3141.3 -	3123.3 -0.8	3022.8 -3.8	3052.8 -2.8	3003.9 -4.8
Net body wt change / corrected body wt change	-91.7	-67.3	-222.5	-193.6	-237.8
	Summary of food consumption during gestation				
Gestation day 7-29	131	117	108	**97***	**89****

[7]Guidance on the application of the CLP criteria: 58b5dc6d-ac2a-4910-9702-e9e1f5051cc5 (europa.eu)

* = Significantly different from the control group at 0.05 using Dunnett's test ** = Significantly different from the control group at 0.01 using Dunnett's test

Clinical pathology parameters (haematology and serum chemistry): the only statistically significant difference seen was a lower mean creatinine concentration in the 1000 mg/kg/day, although there was a dose-response reduction seen in all dose groups.

Organ weights/ Gross necropsy findings: Mean liver weight was increased in all dose groups but reached statistical significance only in the high dose group and only for liver weight relative to body weight (+26.6%). There were no specific findings in the maternal macroscopic examinations.

External foetal examinations / Intrauterine growth and survival: There was an increase in late resorptions in the high dose group (7.9 % as opposed to 0 in all other groups), and an increase in early resorptions in the 700 mg/kg/day group (12.1%, as opposed to 4% in the control and 0 in the other test groups). No external malformations or variations were noted at any dosage level. The foetuses were only examined externally. Foetal weights not measured.

Based on the effects seen in this study dosage levels of 250, 500, and 1000 mg/kg/day were selected for a prenatal developmental toxicity study of 2-pyrrolidone administered orally by gavage to New Zealand White rabbits.

Maternal NOAEL: 1000 mg/kg bw/day based on few signs of toxicity. Body weight reductions were seen but less than -10% compared to control.
Developmental NOAEL: 1000 mg/kg bw/day based on no effects.

An extended one-generation reproductive toxicity study (anonymous 2020) was performed in rats according to OECD 443.

Developmental landmarks (see also under section 10.10.1, fertility and sexual function)

Anogenital distance: there was a statistically significantly higher mean anogenital distance for mid and high dose males when evaluated as a function of the mean pup body weight (anogenital distance relative to cube root of pup body weight). According to the laboratory this was within historical control range. There was no difference with the control in the low dose group.

Table 33 developmental landmarks, developmental toxicity study

Anogenital distance	**Control**	**1500 ppm**	**4000 ppm**	**8000 ppm**
males	2.06	2.12	**2.14***	**2.14***
% difference	-	2.9	**3.9**	**3.9**
females	1.12	1.10	1.14	1.15
% difference	-	-1.8	1.8	2.7

* = Significantly different from the control group at 0.05 using Dunnett's test ** = Significantly different from the control group at 0.01 using Dunnett's test

Thyroid hormone analysis

There were differences in TSH and T4 levels in the offspring prior to weaning but the levels varied, and it was not possible to see an obvious effect of the test substance. The levels were significantly different in the low dose and high dose females but did not follow a dose-response relationship.

Gross pathology and organ weights

There were no differences in the internal findings in the pups that were examined after unscheduled deaths, at necropsy for the pups euthanized at PND 4 and in pups euthanized at PND 21. The findings that were seen were noted infrequently and did not show dose response relationship. There was also no difference in organ weights in these pups.

Auditory startle response (cohort 2A):

There was a lower Peak-value in all dose group males compared to control, with a dose-response relationship in the first measurement (-14, -17, -29%). In the overall results the effect was less in the mid-dose group (not significant) than in the low and high dose group (significant) and thus did not show dose-response, but all treated groups had lower peak-values than the control group.

The t-peak was higher in all male dose groups, but the values were only marginally different.

In the females the Peak-value was also lower in the high dose group (-8%, not significant), but the differences were less marked than in the males and did not show dose-response. There were only marginal differences in the t-peak values between dose groups and control. For more information see Table 19.

A prenatal developmental toxicity study (Unnamed 1990) was performed in rats according to OECD 414.

Groups of 25 female Sprague-Dawley rats were treated with 2-pyrrolidone by oral administration daily (gavage) at three dose levels of 190, 600 and 1900 mg/kg bw/day from gestation day 6 up to and including gestation day 15. A control group of 25 females was given the vehicle, water.

During the study mortality was checked for and clinical observations were performed. Body weight and food consumption of the dams were also recorded regularly, but not daily. Scheduled euthanasia was performed on gestation day 20 and gross pathology was performed. The uterus was weighed, and the ovaries and uterus were examined for number and distribution of corpora lutea, implantation sites, live and dead foetuses, and early and late resorptions. The foetuses were weighed and externally examined. An internal visceral examination was performed on about half of the foetuses. The remaining foetuses were eviscerated and were examined for skeletal abnormalities with Wilsons's technique. Abnormalities were classified as major malformations, minor visceral- or skeletal anomalies or common skeletal variants.

In total, there were 22(341), 25(388), 23(355) and 24(354) evaluated litters (foetuses) in the control, 190, 600 and 1900 mg/kg groups, respectively.

Maternal toxicity:

General toxicity

No animals died during the study. The only clinical sign seen was alopecia. This was seen in all dose groups but without dose related effect (0, 3, 1, 6 in the control, low-, mid- and high dose groups).

Body weight

Dams in the high dose group experienced weight loss days 6-9 of treatment. Body weight gain was reduced in a statistically significant manner in the mid dose group days 6-9 and in the high dose group days 9-12 ($P < 0.01$ for all results). This resulted in statistically significant lower body weight in the mid- ($P < 0.05$) and high dose groups for days 9-20 ($P < 0.01$). There was statistically significant ($P < 0.01$) decrease in the corrected body weights for mid and high dose groups by 5.4 % and 7.4 %, respectively, and the corrected body weight gain was decreased significantly ($P < 0.01$) in the 1900 mg/kg/day group.

The body weight changes were clearly dose-related. They were however not very large and only borderline adverse since most of the body weight parameters were less than 10% lower than the control. The corrected body weight change in the high dose group was however markedly reduced in the high dose group. The performing laboratory concludes that the maternal toxicity is "marked" at the mid dose level due to effects in the maternal body weight. However, although there was an effect in the mid dose group it can hardly be called "marked toxicity" and the NOAEL is therefore set at the mid dose level based on body weight effects seen in the high dose group. See table Table 34.

Food consumption

Over days 6-9 and 9-12 of gestation, food consumption in both the mid- and high dose groups was significantly ($P < 0.01$) reduced by maximum 7.8 and 17.8 %, respectively. Food consumption continued to be significantly ($P < 0.01$) reduced over days 12 to 15 of gestation in the high dose group (-11.2 %).

Gross pathology

No findings in any groups were considered substance related or adverse.

Uterine findings

There were no statistically significant differences between control and treated groups concerning total corpora lutea, total implantation sites, sex ratio, number of live foetuses, number of dead foetuses, resorptions, and pre- and post-implantation losses.

There was a statistically significant lower ($P < 0.01$) gravid uterus weight in the high dose groups compared to the control group.

Table 34 Maternal body weight, developmental toxicity study in rats

Weights in grams	**0 mg/kg bw/day**	**190 mg/kg bw/day**	**600 mg/kg bw/day**	**1900 mg/kg bw/day**
Initial body weight	277.2	274.4	270.3	273.5
Terminal body weight	441.0	435.4	**419.2***	**403.5****
% difference	-	-1.2%	**-4.9%**	**-8.5%**
Gravid uterin weight	83.4	84.5	81.1	**72.2****
% difference	-	+1.3%	-2.75%	**-13.4%**
Net body weight/corrected body weight	357.7	351.0	**338.1****	**331.2****
% difference	-	-1.8%	**-5.4%**	**-7.4%**
Net body weight change /corrected body weight change	41.5	39.2	33.2	**22.0****
% difference	-	-5.5%	-20%	**-47%**

* = Significantly different from the control group at 0.05 using Dunnett's test ** = Significantly different from the control group at 0.01 using Dunnett's test

Foetal toxicity:

Foetal weight

Foetal weight was statistically significant reduced in the high dose group ($P < 0.01$). See Table 35.

Table 35 Foetal weight, developmental toxicity study, rats

Foetal weights in grams	**0 mg/kg bw/day**	**190 mg/kg bw/day**	**600 mg/kg bw/day**	**1900 mg/kg bw/day**
Male foetal weights	3.55	3.64	3.51	**3.21****
% difference from control	-	+2.5%	-1.1%	**-9.5%**
Female foetal weights	3.36	3.45	3.28	**3.04****
% difference from control	-	+2.7%	-2.3%	**-9.5%**
Foetal weight, combined	3.45	3.54	3.40	**3.12****
% difference from control	-	+2.6%	-1.4%	**-9.5%**

** = Significantly different from the control group at 0.01 using Dunnett's test

Malformations

There was a statistically significant increase in malformations in the high dose group. The laboratory classified the malformations as "major malformations" meaning "rare abnormalities judged to be likely to

result in death or to adversely affect postnatal development". There was also a statistically significant increase in minor visceral and skeletal anomalies.

There were five foetuses in five litters that were affected, and all had anal atresia and either acaudia or microcaudia. In addition, one of these five foetuses had absence of some thoracic and all lumbar, sacral and caudal vertebrae and absence of nine pairs of ribs. See Table 36.

Table 36 Malformations and anomalies, developmental toxicity study, rats

		Foetuses				Litters			
	Dose group	**0 mg/kg**	**190 mg/kg**	**600 mg/kg**	**1900 mg/kg**	**0 mg/kg**	**190 mg/kg**	**600 mg/kg**	**1900 mg/kg**
	Number examined	341	388	355	354	22	25	23	24
	Foetuses/litters with major malformations, absolute and %	0 -	1 0.26%	1 0.28%	5* 1.41%	0 -	1 4%	1 4.3%	5* 20.1%
	Foetuses/litters with minor visceral anomalies, absolute and %	1 0.29%	2 0.52%	1 0.28%	7* 1.9%	1 4.5%	2 8%	1 4.3%	5 20.1%
	Minor skeletal anomalies, absolute and %	82 24%	98 25.3%	60 16.9%	140*** 39.5%	19 86%	23 92	19 82.6%	23 95.8%
Major malformations	Foetuses/litters with external malformations	0	1	1	5	0	1	1	5
	Hydrocephalous	-	1	-	-	-	1	-	-
	Eventration of umbilicus	-	-	1	-	-	-	1	-
	Anal atresia	-	-	-	5	-	-	-	5
	No tail (acaudia)	-	-	-	3	-	-	-	3
	Short tail (microcaudia)	-	-	-	2	-	-	-	2
	Thoracic vertebrae/ribs (multiple anomalies) - fused centra, fused arches, ribs absent	-	1	-	-	-	1	-	-
	Fusion in 2nd thoracic vertebra; vertebrae absent below 3rd thoracic; ribs absent below 4th pair; 4th pair of ribs conjoined	-	-	-	1	-	-	-	1
Minor visceral anomalies	Dilatation of lateral ventricles (WT)	-	1	1	4	-	1	1	2
	Oval lens (WT)	1	-	-	-	1	-	-	-
	Unilateral hemorrhage under right eye (WT)	-	1	-	-	-	1	-	-
	Thorax: Absent inominate artery	-	-	-	2	-	-	-	2
Mi nor	Frontal bone(s) reduced ossification	10	10	6	26*	7	8	4	10

	Supraoccipital bone irregular ossification	12	16	6	33**	6	9	5	16
	Reduced no. of pre-sacral vertebrae	7	9	3	26**	3	3	3	7
	Ribs: Ossification center an 7th cervical	4	8	5	52***	3	5	3	14**
s k	Rib(s) absent	-	7*	1	1	-	5*	1	1

Significant difference from control group 1: *P < 0.05, **P < 0.01, ***P < 0.001 (Fisher's/Chi^2).

¤ only effects with statistically significant increase are included in the table.

Historical control data:

The historical control data comprised 6 datasets with 129 litters and 1821 foetuses from Charles River-Kingston laboratories in the USA. The data were collected within five years of this study (1985-1988) but the data are not from the same laboratory as the one performing the present study. In the six datasets, there was a total of four major malformations (1 litter in each of four datasets, two datasets had no recording of any malformations). None of the malformations reported in the historical control were the same as the ones reported in the present study.

Maternal NOAEL: 600 mg/kg bw/day based on few signs of toxicity. Body weight reductions were seen in the mid dose group but was either not statistically significant or less than -10% compared to control.

Developmental NOAEL: 600 mg/kg bw/day based on reduced foetal weight. There was also an increase in malformations seen in the mid- and high dose groups. However, the increase in malformations in the mid-dose group compared to the control was not very pronounced. This could also be due to the large gap between the doses in the mid- and high dose group (600 to 1900 mg/kg bw/day) and we do not know at what dose the increase in malformations start.

10.10.6 Comparison with the CLP criteria

There are no epidemiological data to support classification of 2-pyrrolidone in Category 1A.

A new developmental toxicity study with rabbits from 2019, performed according to OECD 414 and GLP shows effects in the foetuses in the two highest dose groups giving a NOAEL of 250 mg/kg bw/day. There was a decrease in foetal weight in all dose groups in a dose response manner, but the effect was only considered adverse in the two highest dose groups (500 and 1000 mg/kg bw/day). An ED10 calculation of this effect did however show that the ED10 level is below 400 mg/kg bw/day. A reduction in foetal weight was also seen in the EOGRTS rat-study reaching statistically significance in the mid- and high dose group (4000 and 8000 ppm) and in the developmental toxicity study in rats reaching statistically significance in the high dose group (1900 mg/kg bw/day). In the rabbit study there was also an increase in the frequency of visceral malformations in the high dose group compared to the other dose groups and which was also above the frequency for malformations in the historical control data. There was also an increase in major malformations in the developmental toxicity study in rats, in the high dose group. The frequency of late resorptions in the rabbit study was increased in the high dose group, although statistical significance was not shown with a conservative Dunn's test. In rabbits there were few effects in the maternal animals (decreased feed consumption, decreased body weight gain and decreased defecation) and they were not severe enough to be considered adverse. The NOAEL for maternal effects is therefore set at 1000 mg/kg bw/day in the rabbit study. In the rat developmental toxicity study the effects reported in the maternal animals in the high dose group was reduced body weight gain and reduced food consumption. The NOAEL for maternal effects is set at 600 mg/kg bw/day.

Since developmental effects were seen both in high and in mid dose foetuses, without clear adverse effects in the mothers, and in the absence of unequivocal evidence that the developmental effects are secondary to maternal toxicity, it is concluded that the developmental effects are direct effects and not secondary to maternal toxicity.

There is no information on a possible mode of action underlying the observed effects. In the absence of any information on a species-specific mode of action the effects are regarded as relevant for humans.

Based on foetal effects such as reduced foetal weight and malformations in two species and assuming relevance of the underlying mode of action for humans in the absence of any other information, the observed effects are considered evidence of an adverse effect on development. Therefore, classification for developmental toxicity in Category 1B is proposed.

Although the registrant has set another maternal NOAEL (250 mg/kg bw/day) in the rabbit study they agree that an effect is seen in the foetuses and has already proposed a classification of the substance as a developmental toxicant in Category 1B based on the effects described above.

10.10.7 Adverse effects on or via lactation

There are neither animal studies nor human data on effects on or via lactation or other studies relevant for effects on or via lactation available.

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

There are no human or experimental data available with respect to effects via lactation.

10.10.9 Comparison with the CLP criteria

In the absence of any data on possible effects on or via lactation this endpoint cannot be assessed.

10.10.10 Conclusion on classification and labelling for reproductive toxicity

Based on the available data 2-pyrrolidone should be classified as Repr 1B H360D according to the criteria laid down in the CLP Regulation.

The registrant has already proposed a classification of the substance as a developmental toxicant in Category 1B based on the effects described above, however the registrant is of the opinion that since the effects occur at a high dose level a higher specific concentration limit (SCL) is warranted.

The CLP guidance states:

> "In exceptional circumstances specific concentration limits may be set by the manufacturer, importer or downstream user where he has adequate, reliable and conclusive scientific information that a hazard of a substance classified as hazardous is not evident at a level above the concentrations set for the relevant hazard class in Part 2 of Annex I or above the generic concentration limits set for the relevant hazard class in Parts 3, 4 and 5 of that Annex."

To consider the setting of a SCL the following steps must be taken[8]:

- Determine ED10 using the available data
- Determine preliminary potency group
- Determine final potency group considering the modifying factors
- Determine SCL

The ED10 value must be calculated using "the lowest dose which induces reproductive toxic effects which fulfil the criteria for classification for reproductive toxicity with an incidence or magnitude of 10% after correction for the spontaneous incidence", as stated in the CLP guidance.

[8] Figure 3.6 in chapter 3.7.2.6.1 of the CLP guidance

As we cannot see that there are any modifying factors that can influence the determination of the potency group, the SCL must be determined either based on the ED10 value for malformations or the ED10 value for foetal weight.

The registrant has calculated an ED10 value for the malformations seen in the high dose group of the rabbit study and has arrived at a value of 1489 mg/kg bw/day. We agree with the calculations done by the registrant, however we question the adequacy and reliability of the study taking into account the high number of rare malformations in the control group. This high incidence also sets the starting point for the calculations of the ED10 value for malformations. The ED10 value is therefore calculated to be higher for malformations than if the incidence of rare malformations in the control group would have been as expected according to historic control data.

We have however also set a NOAEL in the rabbit study for developmental toxicity at 250 mg/kg bw/day, based on reduced foetal weight. As described above, the calculated ED10 value for this effect is 372 mg/kg bw/day. As we see an effect on the foetuses in the rabbit study at the mid-dose group and even to a certain degree at the low dose group, we argue that the ED10 based on reduced foetal weight should be included in the basis to determine the potency group.

The CLP guidance clearly states that a SCL should only be set in exceptional circumstances. In order to set a specific concentration limit we must be very confident that the study the ED10 value is set on, should be very solid. However, we are not confident that the developmental toxicity study on rabbits is as well performed as it should be, taking into account the high incidence of serious and rare malformations seen in the control group. We therefore argue that for 2-pyrrolidone the circumstances are not "exceptional". As a consequence 2-pyrrolidone should be placed in the medium potency group.

In a weight of evidence assessment, taking into account the reduced foetal weight in rabbits and rats, and increased malformations in the rabbit study, although acknowledging the low reliability of the rabbit study due to incidences of malformations also observed in the control group, no SCL should be set for 2-pyrrolidone and the GCL should be applied.

10.11 Specific target organ toxicity-single exposure

Not performed for this substance.

10.12 Specific target organ toxicity-repeated exposure

Not performed for this substance.

10.13 Aspiration hazard

Not performed for this substance.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not performed for this substance.

12 EVALUATION OF ADDITIONAL HAZARDS

Not performed for this substance.

13 ADDITIONAL LABELLING

Not relevant

14 REFERENCES

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15 ANNEXES

No annexes.