Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

1-methyl-2-pyrrolidone (NMP)

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

CLH-O-0000004066-78-03/F

Adopted

6 June 2014

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON N-METHYL-2-PYRROLIDONE; 1-METHYL-2-PYRROLIDONE

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in this table as submitted by the webform. Please note that some attachments received may have been copied in the table below. The attachments received have been provided in full to the dossier submitter and RAC.

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Substance name: N-methyl-2-pyrrolidone; 1-methyl-2-pyrrolidone
CAS number: 872-50-4
EC number: 212-828-1
Dossier submitter: the Netherlands

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
11.10.2013	United Kingdom		MemberState	1
Comment received				
The report clearly outlines the proposal to remove the existing SCL of 5%. It might however be useful if further information regarding the derivation of the existing SCL, which was agreed at TCC&L, was included in the dossier.				
Dossier Submitter's Response				
The justification of the classification for developmental toxicity including the setting of the current SCL of 5% for 1-methyl-2-pyrrolidone can be found in the Annex 1 (A-D) of the CLH report. These annexes also include the minutes of the TCC&L-meeting at which the SCL was established. Further details are available in document 42/02-add.13 which is the industry proposal for setting SCLs. However, the data as presented in the current CLH dossier clearly indicate the need for removal of the current SCL of 5% which results in a GCL of 0.3%. Information on the establishment of the current SCL is in the opinion of the dossier submitter considered of minor importance.				
RAC's response				
Noted, and agree that information regarding the establishment of the current SCL is of minor importance.				

Date	Country	Organisation	Type of Organisation	Comment number
04.10.2013	Norway		MemberState	2
Comment received				
Norway would like to thank the Netherlands for the proposal for harmonised classification and labeling of 1-methyl-2-pyrrolidone, CAS- no. 872-50-4.				
Dossier Submitter's Response				
Thank you for the support.				
RAC's response				

Noted.

Date	Country	Organisation	Type of Organisation	Comment number
10.10.2013	Spain		MemberState	3

Comment received

In our opinion the report is appropriate and clear enough.
We consider that it should be stressed that when the current specific concentration limit of 5% was established in 2005 (Meeting of the Technical Committee C&L on the Classification and Labelling of Dangerous Substances, Arona, 15-18 March 2005), four MS had reservations concerning the procedure for setting that specific concentration limit and two MS supported the default value of 0.5% (according to Directive 1999/45/EC).
Since the Guidance on the Application of the CLP Criteria (Version 3.0 - November 2012) proposes the use of the ED10 for effects warranting classification as the most appropriate estimate for the potency of the substances, the ED10 method should be considered as a good option for setting SCL for reprotoxicity of 1-methyl-2-pyrrolidone. Thus, we agree with the CLH proposal, i.e., the GCL of 0.3% should be assigned to 1-methyl-2-pyrrolidone.

Additional information
A new study (not included in the CLH Report) has recently been published that corroborates the developmental toxic effects of NMP administered orally to rats; besides, impaired female fertility was shown when NMP was administered at 450 and 1000 mg/kg (Sitarek, Stetkiewicz and Wasowicz, 2012).

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

Dossier Submitter's Response

Thank you for the support.

The study of Sitarek et al. (2012) was evaluated. A summary of this study is provided below. In addition, it was evaluated whether the results of this study would affect the potency group and concentration limit.

Summary of Sitarek et al. (2012):
In this study, female Wistar rats were exposed 5 days/week for about 9 weeks (2 weeks before mating and 1 week of mating, 3 weeks of gestation, and 3 weeks of lactation) to NMP by oral gavage at dose levels of 0, 150, 450 and 1000 mg/kg bw/day. After 2 weeks of exposure, females from each exposure group were mated overnight with unexposed males (2 females to 1 male). On the first postnatal day (PND 1), the live and dead pups were counted, weighted and their gender was determined. On day 4 after birth, the litters were culled to eight animals each and balanced for gender (four females and four males) to the extent possible. From birth (PND 1) to weaning (PND 21), the offspring was assessed for the general appearance, litter weight, mean pup weight, and mortality. Females from group 0, 150, and 450 mg/kg were necropsied after 3 weeks of lactation and from group 1000 mg/kg/day with no delivery—at 25th day post mating. Integral indices of toxicity, including body weight on the day of dissection, hematocrit, macroscopic and microscopic evalulation of the internal organs, absolute and relative weight of the internal organ were determined.

Some maternal toxic effects were observed such as reduced body weight during gestation in all exposure groups, reduced food and water consumption during the first week of mating (water only) and on days 0, 13 (food only) and 20 of gestation in the 1000 mg/kg bw/day exposed animals (Table 1).
The effect of NMP-exposure on reproductive performance of female rats and viability of their offspring is presented in Table 2. A reduced number of live pups was observed in the high dose group. Fertility index (percent of pregnant females in mating females group) was reduced in the 450 and 1000 mg/kg bw/d groups. Further, the percentage of pups that survived was significantly reduced in the 150 and 450 mg/kg bw/d groups.
In the 1000 mg/kg bw/d group, of the 22 inseminated females, 15 were pregnant and eight pups were delivered by only 7 pregnant females (with three live-born and five stillborn). All pups died within 4 days after birth, which prevented further observation of the offspring of the 1000 mg/kg bw/d exposure group. Early resorptions were found in the other eight inseminated females after necropsy at day 25 after insemination, indicating a substantial intrauterine mortality. Further, microscopically endometritis and foci of resorption were noted in uterus in these 8 females, and in four of these females the number of corpora lutea in the ovaries was reduced when compared to controls.
Reduced bodyweight was observed in offspring at day 4 (150 and 450 mg/kg bw/day exposure groups), 7, 14, 21 (all 450 mg/kg bw/d exposure group). See Table 3.

Table 1
Body Weight (gm), Daily Food (gm/dam), and Water (ml/dam) Consumptions During Exposure of Female Rats Receiving *N*-Methyl-2-Pyrrolidone Orally*

Parameters	Daily dose of NMP (mg/kg)	No. of dams per group	Weeks before mating		Days of gestation				Days of lactation[c]
			First	Second	0	6	13	20	21
Body weight (gm)	0	22	203.5 ± 10.7[a]	211.0 ± 13.2	215.1 ± 16.2[a]	254.2 ± 15.0	284.5 ± 18.1	321.3 ± 29.0	253,6 ± 11.8
	150	24	198.4 ± 10.2	204.2 ± 11.7	206.5 ± 10.8	223.7 ± 8.7[b]	243.9 ± 10.5[b]	299.6 ± 11.1[b]	254.7 ± 12.1
	450	20	200.0 ± 11.0	201.9 ± 13.8	204.0 ± 12.6	224.0 ± 14.6[b]	243.0 ± 16.5[b]	284.3 ± 40.4[b]	251.3 ± 21.0
	1000	15	206.1 ± 16.8	208.7 ± 17.4	216.4 ± 16.8	245.7 ± 19.9	253.2 ± 17.6[b]	259.7 ± 17.9[b]	–
Food consumptions (gm/dam)	0	22	17.8 ± 0.9	19,6 ± 1.4	18.8 ± 1.9	20.8 ± 2.7	22.7 ± 2.8	20.4 ± 3.4	62.4 ± 16.4
	150	24	16.9 ± 1.7	16.8 ± 2.0	17.6 ± 2.2	19.3 ± 1.8	20.5 ± 2.5	20.2 ± 3.1	60.2 ± 23.1
	450	20	15.9 ± 1.7	15.8 ± 2.0	16.3 ± 1.8	18.8 ± 1.5	20.2 ± 2.3	21.5 ± 2.1	55.1 ± 14.1
	1000	15	15.7 ± 2.6	18.2 ± 2.1	14.9 ± 1.6[b]	19.2 ± 2.0	15.8 ± 2.8[b]	14.1 ± 2.7[b]	–
Water consumptions (ml/dam)	0	22	20.2 ± 2.9	25.0 ± 4.0	32.8 ± 2.2	34.0 ± 4.6	34.9 ± 4.9	38.8 ± 5.3	103.8 ± 25.3
	150	24	23.1 ± 3.5	30.0 ± 2.2	31.8 ± 5.9	33.0 ± 3.1	34.1 ± 4.4	38.3 ± 5.4	96.9 ± 19.4
	450	20	22.8 ± 4.9	26.4 ± 5.7	32.2 ± 4.7	33.4 ± 4.7	33.9 ± 4.5	39.6 ± 3.9	89.9 ± 23.1
	1000	15	18.8 ± 5.3[b]	24.7 ± 5.3	28.8 ± 4.3[b]	32.1 ± 5.1	34.9 ± 5.9	31.6 ± 6.1[b]	–

*Observations concern only pregnant females.
[a]Mean ± SD.
[b]Significantly different ($p < 0.05$) from control value.
[c]Observations of mothers from group 1000 mg/kg have not been conducted because their offspring survived only until the four PND.

Table 2
Effect of *N*-Methyl-2-Pyrrolidone on the Reproductive Performance of Female Rats and Viability of Their Progeny

	Daily dose of NMP (mg/kg)			
	0	150	450	1000
Number of				
Mating females with males	24	26	28	22
Pregnant females	22	24	20	15
Died females*	0	0	0	2
Number of				
Live pups per litter	11.5 ± 3.5[a]	10.4 ± 2.6	10.5 ± 3.4	0.33 ± 0.82[b]
Dead pups per litter	0.18 ± 0.85	0	0.13 ± 0.34	0.80 ± 1.1[b]
Sex ratio (F : M)	132 : 125	112:137	105:107	5:3
Indices				
Fertility	91.7	92.3	71.4[b]	68.2[b]
Viability	94.0	86.4[b]	71.6[b]	0
Lactation	96.1	78.2[b]	43.4[b]	0
Body weight gain of mothers from 0 to 20 GD (percentage of control)	100	87.7	75.6	40.8

*Two nonpregnant females died in the 30th and in the 32nd day of experiment, respectively.
[a]Mean ± SD.
[b]Significantly different ($p < 0.05$) from control value.
F, female; M, male; GD, gestation day.
Index of fertility—percentage of pregnant females in mating females group.
Index of viability—percentage of pups born alive that survived to 4 days.
Index of lactation—percentage of pups alive at 4 days that survived to 21 days.

Table 3
Body Weight of Offspring Control and Exposed Prenatally and Postnatally to *N*-Methyl-2-Pyrrolidone

	Days of live				
Groups of NMP (mg/kg)	1	4	7	14	21
		Females			
0 ($n = 22$)	5.55 ± 0.51[a]	9.79 ± 1.42	12.14 ± 1.05	23.35 ± 1.95	32.17 ± 2.85
150 ($n = 24$)	5.47 ± 0.48	7.35 ± 1.20[b]	10.97 ± 2.08	21.57 ± 4.10[b]	32.31 ± 3.17
450 ($n = 20$)	4.93 ± 0.36	6.07 ± 0.83[b]	8.01 ± 1.63[b]	17.81 ± 3.77[b]	27.46 ± 4.66[b]
		Males			
0 ($n = 22$)	6.03 ± 0.78	11.05 ± 1.13	13.13 ± 1.63	24.28 ± 1.88	33.79 ± 2.77
150 ($n = 24$)	5.73 ± 0.49	7.70 ± 1.29[b]	11.55 ± 1.85	22.71 ± 3.55	32.83 ± 4.83
450 ($n = 20$)	5.13 ± 0.39	6.34 ± 1.02[b]	8.57 ± 1.85[b]	19.73 ± 3.07[b]	29.08 ± 2.50[b]

n, number of letters in the group.
[a]Mean ± SD.
[b]Significantly different ($p < 0.05$) from control values.

Evaluation of this study and conclusion with respect to the potency group and concentration limit.

The data of Sitarek et al. (2012) show that NMP at dose levels of 150 and 450 mg/kg bw/day reduces the survival of the pups, both during the first 4 days as well as between days 4 and 21 (i.e. indices of viability and lactation, respectively). At the highest dose of 1000 mg/kg bw/day, the total number of pups (live+dead) was reduced, the number of live pups was reduced, and the number of dead pups was increased. All live-born pups died within 4 days after birth. Further, resorptions were observed.

It should be noted that also some slight maternal toxicity was observed (i.e. reduced food intake and bw gain).

Resorption is an effect which fulfills the classification criteria for developmental toxicity. However, as this effect was only qualitatively presented and not quantitatively, this can not be considered for evaluating the ED_{10}.
Pup mortality is an effect which fulfills the classification criteria for developmental toxicity and can be considered for evaluating the ED_{10}. However, pup mortality was determined until postnatal day 21, and therefore exposure of the pups via lactation (as the mothers were also exposed to NMP during lactation period) might also have contributed to the observed pup mortality. This presents some uncertainty whether the effect of pup mortality can be considered a true developmental effect.
Calculating ED_{10} values by using linear intrapolation results in ED_{10} values of 199 and 84 mg/kg bw/day for indices of viability and lactation respectively.

Based on the ED_{10} calculations as presented in the CLH report, the lowest ED_{10} value of all the studies for effects warranting classification (which is determinative for the overall ED_{10} of the substance) was the ED_{10} of 225 mg/kg bw/day for postimplantation loss in the developmental study in rabbits (IRDC, 1991) (see also table 19 of the CLH report).
When comparing the lowest ED_{10} value of the study of Sitarek et al. 2012 with this ED_{10} value of 225 mg/kg bw/day, it is noticed that the ED_{10} value of 84 mg/kg bw/day (based on the Sitarek et al. (2012) study) can be considered even lower.

This ED_{10} value of 84 mg/kg bw/day correspond to a medium potency group (i.e. boundaries: 4 mg/kg bw/day < ED_{10} value < 400 mg/kg bw/day) for NMP.
As this ED_{10} value is considered the lowest overall ED_{10} value and might therefore be the determinative for the concentration limit (although there are some uncertainties whether the effect of pup mortality was a true developmental effect), it was also evaluated whether modifying factors might by applied on this specific ED_{10}.

According to the 'Guidance on the Application of the CLP Criteria' (paragraph 3.7.2.5.5) modifying factors (i.e. for type of effect or severity, data availability, dose-response relationship, modes or mechanism of action, toxicokinetics, and bio-accumulation of substances) can be applied to account for case-specific data situations which indicate that the potency group for a substance as obtained by the preliminary assessment should be changed. It was already concluded in the CLH dossier of NMP that, based on the available data, adaptation of the potency group based on type of effect or severity, data availability, dose-response relationship, modes or mechanism of action, bio-accumulation of substances was not warranted. The study of Sitarek et al. (2012) does not present additional information with respect to these aspects.
However, the need for a modifying factor for toxicokinetics might be reconsidered as the study of IRDC (with the lowest ED_{10} value as mentioned in the CLH report) was performed in rabbits and the study of Sitarek et al. (2012) was performed in rats. Therefore, comparison of the kinetics of NMP after oral exposure in rats and humans (if known) should be taken into account when determining the potency group of NMP. For rats, a comparative kinetics study including oral, dermal, inhalation and intravenous exposure and an oral study focusing on the distribution and elimination are available. For humans limited information is available on the kinetics of NMP after oral exposure, with one oral study focused on the metabolic pathway of NMP. The available data indicate that both in humans and rats, NMP is well absorbed after oral exposure. Further, the rat data show that NMP is eliminated via urine with negligible tissue residues remaining after 4-5 days postdose. There is a difference observed in metabolic pattern, i.e. the major metabolite in humans is 5-hydroxy-*N*-methyl-2-pyrrolidone (5-HNMP), whereas in rats the major metabolite is 1-methyl-5-OH-2-

pyrrolidone. As no information is available on the mode of action of NMP for the induction of developmental effects, including info on whether the parent compound or one of its metabolites is the responsible toxic agens, the relevance of differences in the metabolic profile can not be assessed. Determining the potency group should be based on a comprehensive knowledge of all involved toxicokinetic factors and not on a single parameter. A full comparison between kinetics in humans and rats after oral exposure is, based on the available data, not possible. No adaptation of the potency group is needed. Taken all the available data, NMP can still be considered a medium potency reproductive toxicant, and the current SCL of 5% should be removed resulting in a GCL of 0.3%.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
10.10.2013	France		MemberState	4
Comment received				
FR agrees with the removal of the Specific Concentration Limit of 5% and then to apply the GCL of 0.3% based on developmental effects (post-implantation loss at 225 mg/kg bw/day) estimating an ED10 of mild potency (ED10 > 4 mg/kg/d and < 400 mg/kg/d).				
Dossier Submitter's Response				
Thank you for the support.				
RAC's response				
Noted.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
08.10.2013	Germany		MemberState	5
Comment received				
According to the newly developed Guidance on the Application of the CLP Criteria (draft), specific concentration limits (SCL) for reproductive toxicants are assigned based on their potency (i.e., high, medium and low). Several well-conducted guideline studies support the calculation of ED10 levels for developmental effects in the range of 4-400 mg/kg bw/day indicating that 1-methyl-2-pyrrolidone is of medium potency. For Category 1 reproductive toxicants of the medium potency group, a general concentration limit (GCL) of 0.3% is applicable. Thus, the proposal for removing the current SCL of 5% is justified and can be supported.				
Dossier Submitter's Response				
Thank you for the support.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
04.10.2013	Norway		MemberState	6
Comment received				

We support the proposal to remove the current specific concentration limit of 5% for developmental toxicity of 1-methyl-2-pyrrolidone. Analysis of the oral reproductive studies showed multiple ED10 levels for effects fulfilling the classification criteria for developmental toxicity with values between 4 and 400 mg/kg bw/day. The calculation of ED10 values performed by the Netherlands corresponds to a medium potency group for 1-methyl-2-pyrrolidone. In combination with classification as Repr. 1B - H360D, the GCL of 0,3% would be applicable for this substance. The current SCL of 5% can be removed.
Dossier Submitter's Response
Thank you for the support.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
11.10.2013	Sweden		MemberState	7
Comment received				
The Swedish CA supports the proposal to remove the current SCL (5%) for 1-methyl-2-pyrrolidone (CAS no 872-50-4), a compound that has a harmonized classification for reproductive toxicity in Cat 1B (H360D). According to the Guidance on the application of the CLP criteria the ED10 value (as used for reprotoxicity SCLs) is the lowest dose which induces reproductive toxic effects that fulfill the criteria for reproductive toxicity with an incidence of 10% after correction for spontaneous incidence. In the current dossier, the dossier submitter has calculated ED10 values for different developmental toxicity endpoints (postimplantation loss, malformations indicative of effects on the developing cardiovascular system and reduced pup survival) that all fulfill the criteria for classification. Overall, the lowest ED10 value identified (i.e the one that according to the guideline should be used for deciding on potency level) was 225 mg/kg bw/day (postloss in rabbit, using the benchmark dose (BMD) method) or 205 mg/kg bw/day (litter loss at end of lactation, using the linear interpolation method). Both these values are clearly within the range (4< ED10 < 400 mg/kg bw/day) that the guideline defines for a Repro Cat 1B compound of medium potency. In addition, the lowest ED10 values for effects on the development of the cardiovascular system was 337 (using BMD) or 301 mg/kg bw/day (using linear interpolation), both values being within the range for a compound of medium potency. The CLP Guideline also specifies that modifying factors such as severity of effect, differences in metabolism/toxicokinetics etc. should be taken into consideration when deciding on potency group. The developmental effects observed for the compound are all severe. However, the identified ED10 values are not close to the lower or upper boundaries of the medium potency group and therefore there is no need to modify the potency group due to the severity of the effect. There is no available data that indicate that there are species differences in terms of toxicokinetics/metabolism and therefore there is no need to adjust the assigned potency group. In conclusion, based on available data 1-methyl-2-pyrrolidone is a medium potency Cat 1B (H360D) developmental toxicant and according to the CLP guideline the SCL for this potency group is 0.3%, i.e. the general concentration limit for a Cat 1B reproductive toxicant, and thus there is no need to assign a SCL.				

Dossier Submitter's Response
Thank you for the support.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
11.10.2013	Belgium		MemberState	8
Comment received				
We support the removal of the specific concentration limit of 5% (SCL) for developmental toxicity , which result in a Generic Concentration Limit of 0,3%, according to the "Guidance on the Application of the CLP Criteria". We appreciate the clear justification of the DS for the establishment of ED 10 value. We agree with: - The developmental effects chosen that fulfill the classification criteria for the animal (oral) studies on reproductive toxicity, - The lowest ED10 chosen (225 mg/kg bw/day for postimplantation loss in the developmental study in rabbits) corresponding to the medium potency group (4 mg/kg BW/day< ED 10 value < 400 mg/kg BW/day). - No adaptation needed (no modifying factors – type of effects or severity, data availability, dose response relationships, mode or mechanism of action, toxicokinetics, and bio-accumulation of substances). All in all, we agree with the rationale that NMP is of medium potency and the current SCL of 5% for developmental toxicity should be reduced to a level of 0,3 %.				
Dossier Submitter's Response				
Thank you for the support.				
RAC's response				
Noted.				