COMPILED COMMENTS ON CLH CONSULTATION

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Last data extracted on 18.12.2023

Substance name: 2-pyrrolidone; pyrrolidin-2-one
CAS number: 616-45-5
EC number: 210-483-1
Dossier submitter: Norway

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
15.12.2023	Spain	<confidential>	Company-Downstream user	1
Comment received				
see documentation attached. The document (1) includes explain the rest of documents contents and it's justification. ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Contribution to the 2-P Public consultation.zip				

Date	Country	Organisation	Type of Organisation	Comment number
14.12.2023	Japan		Individual	2
Comment received				
The dossier submitter has proposed Repr. 1B, H360D in the CLH report - Proposal for Harmonized Classification and Labelling. In addition, in 10.10.10 Conclusion on classification and labeling for reproductive toxicity, they claim that “no SCL should be set for 2-pyrrolidone and the GCL should be applied.” It means that the specific concentration limits (Concentration range (%): 3 - 100, Hazard categories: Repr. 1B) that were set when registering this substance under the REACH regulation should not be set. With the following two points, we advocate that adopting GCL based solely on the opinion of the dossier submitter may lead to over-classification, and that the SCL needs to be reconsidered. Firstly, the dossier submitter argues against the rabbit study referenced by the registrant as below: “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.”				

However, we have doubts about this claim. In the first instance, there is no discussion about whether the incidence of malformations in the control group is truly rare. Therefore, this assertion alone does not support the argument that the study lacks adequacy and reliability. It is not uncommon for malformations to occur in control groups. Under conditions where malformations occur in the control group, it is reasonable that the chemically treated group would be calculated to have a higher ED10 value.

Secondly, the dossier submitter set the ED10 value as 372 mg/kg with the reasoning for the reduced rabbit foetal weight.
This value is indeed classified as Group 2 medium potency in ”Guidance on the Application of the CLP Criteria (Table 3.14 of page 415).” However, this value is close to “above 400 mg/kg bw/day”, which should be classified as Group 3 low potency. Considering the dispersion of test results as well as the existence of the test results regarding the rabbit malformation mentioned in the first argument, we have to say that it would be too hasty to set GCL, not SCL, only with this value as a reasonable reason. Furthermore, if focusing only on Female foetal weights in Table 29, it becomes “above 400 mg/kg bw/day”, which would be classified as Group 3 potency. There is a possibility that the dose setting was inappropriate for the calculation of ED10. Considering from this, we believe that it is premature to adopt GCL with the only basis from this test result. In page 408 of the guidance, it says “3.7.2.6.3. Determination of the ED10 value …The ED10 value (as used for reprotoxicity SCLs) is 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 (see in Section 3.7.2.6.3.2); however, the test results showing weight loss include a large margin of error. With this in mind, it cannot be avoided to say that it would be a conclusion with lack of thought to easily adopt the value of this test result as the only basis for the argument.

Based on the above, we believe that the adoption of GCL should be carefully considered, and the setting of SCL should be reexamined to avoid forcing over-classification. It is very important to take into account not only the results of studies on weight loss in rabbit foetal, but also the possibility of future studies for determining SCL. SCL needs to be reconsidered and reexamined with much carefulness based on scientific evidence and enough time.

Date	Country	Organisation	Type of Organisation	Comment number
12.12.2023	Germany		MemberState	3
Comment received				
DE-CA supports the proposal for classification of 2-pyrrolidone for developmental toxicity.				

HEALTH HAZARDS – Reproductive toxicity

Date	Country	Organisation	Type of Organisation	Comment number
22.11.2023	Italy	<confidential>	Company-Downstream user	4
Comment received				
We substantially agree with the proposed classification of 2-pyrrolidone as Repr. 1B, H360D. However we noted that all registrants proposed a Specific Concentration Limit (SCL) of 3% (page 7). This SCL was discussed and rejected in the CLH report (page 45-46). But 3.0% is the limit of 2-pyrrolidone in Povidone (polyvinylpyrrolidone) for pharmaceutical use, according the harmonized monograph USP/EP/JP [no limit was set in Reg.231/2012 for PVP for food use (E1201)]. As consequence, no SCL on 2-pyrrolidone classification means that also the polymer				

polyvinylpyrrolidone should be classified as Repr. 1B, H360D.
This is in obvious contradiction with recent toxicological assessments, which stated the safety of PVP for food use (EFSA, 2020):
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7416752/
and for cosmetic use: https://journals.sagepub.com/doi/10.1177/109158189801700408
Its safety made PVP one of the most versatile pharmaceutical excipients:
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7416752/
Therefore the classification of PVP as Repr. 1B, H360D (as consequence of no SCL on 2-pyrrolidone) is in contradiction with the EU chemicals strategy for sustainability, i.e."One substance, one assessment".

Date	Country	Organisation	Type of Organisation	Comment number
15.12.2023	Spain	<confidential>	Company-Downstream user	5

Comment received

We agree with a harmonized classification of 2-Pyrrolidone as toxic for reproduction category 1B (H360D) and defend the Specific Concentration Limit proposed by the lead registrant following ECHA's regulatory guidance. A risk assessment was conducted that demonstrated the safety of our uses at concentrations at 3 and well above it.

ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Contribution to the 2-P Public consultation.zip

Date	Country	Organisation	Type of Organisation	Comment number
14.12.2023	Belgium	Taminco BV (Eastman) as Lead Registrant on behalf of the BDO & Derivatives REACH Consortium	Company-Manufacturer	6

Comment received

Comments on the open hazard classes:

Reproductive toxicity:

General: We agree with the CLH proposal to classify 2-pyrrolidone as Category 1B for developmental toxicity based on the observed malformations in two species at the highest tested dose levels of 1000 and 1900 mg/kg bw/day, in rabbits and rats respectively. We also agree to non-classification for effects on sexual function and fertility based on a 2020 extended one generation toxicity study according to OECD TG 443. Additionally, we agree to no classification for effects on or via lactation.

However, we do not agree with the proposed derivation of the Generic Concentration Limit (GCL) for developmental toxicity. Instead, we suggest setting a Specific Concentration Limit (SCL) of 3% due to the calculated ED10 values for effects in rats and rabbits relevant for classification in Category 1B for developmental toxicity (malformations), which are clearly above the threshold for placing in the low potency group (> 400 mg/kg bw/day).

Calculation of an SCL for developmental toxicity:

We disagree with the dossier submitter's proposal to consider the moderate decrease in fetal weights in the mid-dose (-14.5% in males and -11.6% in females) as the pivotal effect for calculating the most relevant ED10 value. This decrease in fetal weight was likely due to a drastic reduction in food intake during the treatment period (see below, e.g. up to -74% d13- 14; -33.6% d7-29). It has been reported that feed restrictions that produce substantial reductions in maternal body weight gain can result in developmental toxicity expressed by abortion, reduced fetal weight, and alterations in ossification (1,2). Therefore, moderate effects on fetal weight, especially when associated with maternal toxicity, usually do not result in classification in Category 1B for developmental toxicity. According to ECHA's guidance document (ECHA 2017, page 633), only the critical effects that trigger the assignment to the corresponding classification category should be taken into account when calculating the ED10 value:

“For substances where there is a difference in the LOAEL overall (lowest dose with any effect on reproduction) versus the LOAEL classification (lowest dose with an effect in reproduction fulfilling the classification criteria), this is in most cases due to non-significant increases in lethalities or malformations or decreases in foetal body weight at the LOAEL overall versus significant increases in lethalities or malformations at the LOAEL classification” and “ overall, the use of the ED10 for effects warranting classification is proposed as the most appropriate estimate for the potency. The advantage of this parameter is that it is a dose level with a specified level of effects of at least a certain severity”.

The registrants calculation of the lowest ED10 value of 1489 mg/kg bw/day in accordance to ECHA’s Guidance (2017) based on the 2020 developmental study with rabbits is as follows: (for further details of the calculations please see attachment Rationale_SCL_2_Pyr 2023_11_28)

ED10 rate (% fetuses per litter with any malformations): 14.1% (= 4.1% in controls + 10%), interpolation between NOAEL (4.4% at 500 mg/kg bw/day) and LOAEL (9.3% at 1000 mg/kg bw/day) leads to an ED10 of 1489 mg/kg bw/day.

The dossier submitter questioned the reliability of the rabbit study due to a high number of rare malformations in the control group which would set a high starting point for the calculations of the ED10 value for malformations (%fetuses per litter incidences with any malformations were 4.1% in controls of the study versus 3.01% (mean of studies) in the historical control data). However, even if this argument is followed and as a worst case the historical control data (HC data) for malformations would be used or artificially set to 0 for the calculations, the ED10 is 858 mg/kg bw/day assuming 0% malformations in the control group and the low dose of 250 mg/kg bw/day to be the no-effect-level concerning malformations.

ED10 rate: 10% (=0% in controls + 10%), interpolation between NOAEL (0.5% at 250 mg/kg bw/day) and LOAEL (4.4% at 500 mg/kg bw/day) leads to an ED10 of 858 mg/kg bw/day.

(Calculation: 500 (LOAEL) - 250 (NOAEL)) mg/kg bw/day / (4.4-0.5) % = 64 mg/kg bw/day per % (steepness). Going from 0.5% to 10% requires an addition of 9.5%. 9.5% x 64 mg/kg bw/day per % = 608 mg/kg bw/day plus 250 mg/kg bw/day = 858 mg/kg bw/day

The ED10 values for 2-pyrrolidone calculated in the REACH registration dossier followed the same procedure as for the structurally and toxicologically related compound 1-methyl-2-pyrrolidone (NMP; CAS No. 872-50-4), which is also classified in Category 1B for developmental toxicity and which was assigned to the medium potency group for

developmental toxicity due to its higher reproductive toxicity potency by the RAC in 2014 (see calculations in the CLH Dossier, the RAC Opinion and the Background document to the RAC Opinion) (3, 4, 5). According to the RAC and the dossier submitter, the most severe findings for NMP were malformations, post-implantation loss, complete litter loss and decreased pup viability. Specifically, for the three oral key studies in rats and rabbits, ED10 values for NMP were derived for the most severe developmental toxic effects. These effects can be considered the most relevant for the classification of NMP in Category 1B for developmental toxicity. Neither the RAC nor the dossier submitter considered in the ED10 calculations a decrease in fetal weights (of about – 10% in absence of maternal toxicity). The reduction in fetal body weight was observed at a lower dose (250 mg/kg bw/day) compared to the dose level which induced malformations and losses after implantation (500 mg/kg bw/day) (6).

For reasons of coherence, the ED10 values for 2-pyrrolidone should be derived using the same procedure as for NMP, considering only the most severe developmental effects (increased incidence of malformations, but not a decrease in fetal weights) in the ED10 calculations as these were the key findings and the basis for classification in Category 1B for developmental toxicity.

Therefore, and in view of the available developmental toxicity studies in rats and rabbits, which demonstrate the low potency of the substance, we conclude that a SCL of 3% for developmental toxicity is justified in line with the provisions in the CLP regulation and the ECHA Guidance on the Application of the CLP criteria.

Reliability and validity of the rabbit OECD TG 414 study:

The CLH proposal raises concerns about the quality of the rabbit OECD 414 study due to the presence of rare malformations, including persistent truncus arteriosus and small heart ventricles, in the control group. We consider the study to be fully valid. First, it is completely normal for malformations of all types to occur in control animals. Untreated and vehicle-treated litters have a background incidence of death and abnormalities and it is important to consider this background incidence when interpreting the results of the study to accurately determine the effects of the treatment (7). In fact, laboratories started to keep historical control data to track the frequency of such occurrences. If malformations did not occur in controls, there would be no need for a historical control or even a control group at all. Also, the OECD 414 guideline has no consideration that malformations in the control might invalidate the study.

It is important to note that malformations found in the control group have been observed and documented in the HC data of the testing facility, which demonstrates that they can occur spontaneously, albeit infrequently. It should also be noted that the multiple visceral malformations in the control group are occurring in the same fetuses (i.e. simultaneous occurrence of interventricular septal defects (VSD) and persistent truncus interiosus in 2 of the control fetuses). These findings are inter-related. The VSDs are listed for fetuses 5686-09 and 5691-11 because the testing laboratory almost always sees a VSD when the truncus arteriosus does not divide into the pulmonary artery and aorta (personal communication with Charles River Laboratories) (for further information see attachments: CRL historical control data_OECD 414 and Malformations by Fetus and Group_OECD 414 rabbits 2-Pyrrolidone).

Furthermore, the total percentage of observed malformations per litter indicated by the dossier submitter does not take into account the litter proportions. When considering the litter proportions, the total percentage per litter incidences in the current study are 4.1%,

0.5%, 4.4%, and 9.3%, as shown in the study report. The laboratory HC data have a mean of 3.01% ±1.818 (mean of study means with a min/max range of study means of 0.0 - 8.62%). This demonstrates that the control group as well as groups 2 (low dose) and 3 (mid dose) fell within the range of historical incidence and, therefore, there is no increase in the incidence of rare malformations in the mid- dose group.

The dossier submitter suggested setting the NOAEL for maternal toxicity at 1000 mg/kg bw/day due to the absence of adverse effects. However, it is evident from the study results that there were clear indications of maternal toxicity, such as initial body weight loss in the high and medium dose groups, along with the statistically significant and pronounced reduction in food intake. In the study, it was found that the high dose group showed a 49% reduction in food consumption between day 7-29 and the medium dose group showed a 34% reduction compared to the control group. Additionally, 17 rabbits in the high dose group showed essentially no food consumption from day 8 to 15. The effect on food consumption was less pronounced at 500 mg/kg bw/day, but still less than half of the control group. There were also 2 animals euthanized in extremis at 1000 mg/kg/day compared to none in the control. These findings support the determination of the systemic maternal NOAEL at the lowest dose level (250 mg/kg bw/day).

These maternal decreases in food consumption across the board also speak to potential decreases in fetal weights. We disagree with the author's conclusion that there is an obvious effect on fetal weights at the low dose. The authors state (on page 29 of the CLH report) that the HC data for fetal weight is in the range of 38.8-44.5g. Therefore, a mean combined fetal weight of 40.0g, is well within the range of HC data, and the 6% variation versus the control group is attributable to biological variability in this strain of laboratory rabbits.

Conclusions
- We agree to classify 2-pyrrolidone as Category 1B for developmental toxicity and to no classification for effects on sexual function and fertility as well for no classification for effects on or via lactation.
- The 2020 rabbit study according to OECD TG 414 is assessed as fully reliable and relevant for the classification and assessment of developmental toxicity of 2-pyrrolidone.
- The incidental occurrence of rare malformations in the control group is clearly within the laboratory historical control data.
- The reduced fetal weight in the medium and high dose group in the rabbit study can be attributed to the significantly reduced feed intake, the body weight losses of dams during the first 2 weeks of the treatment and the reduced bodyweight gain of the dams during the overall treatment period.
- Based on the occurrence of severe developmental toxicity in the form of an increased incidence of malformations outside the historical control data only at or above the limit dose of 1000 mg/kg bw/day, an assignment of 2-pyrrolidone to the low potency group is justified and an SCL of 3% for developmental toxicity is proposed.

References:
(1) Cappon et al., 2005. Effects of Feed Restriction During Organogenesis on Embryo-Fetal Development in Rabbit Birth Defects Research (Part B) 74:424-430
(2) Matsuoka et al., 2006. Effects of Feed Restriction During Organogenesis on Embryo-Fetal Development in Rabbit. Effects of Feed Restriction During Organogenesis on Embryo-Fetal Development in Rabbit

(3) CLH report. Proposal for Harmonised Classification and Labelling. Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2 Substance Name: 1-methyl-2-pyrrolidone, August 2013. https://echa.europa.eu/documents/10162/3ebc00fe-21ed-a63a-0d38-7a612b8b84ac Committee for Risk Assessment RAC Opinion proposing harmonised classification and labelling at EU level of 1-methyl-2-pyrrolidone (NMP). 6 June 2014. https://echa.europa.eu/documents/10162/355b86c1-5a0f-f104-0931-8ffdce4e1cbd Committee for Risk Assessment RAC. Annex 1. Background document to the Opinion proposing harmonised classification and labelling at Community level of 1-methyl-2-pyrrolidone (NMP). https://echa.europa.eu/documents/10162/ca607fe3-b439-58e4-f6bf-c4a6238e5bc6
(4) Saillenfait et al., 2002. Developmental toxicity of N-methyl-2-pyrrolidone administered orally to rats. Food and Chemical Toxicology 40, 1705-1712
(5) Wise et al., 2016. Numeric Estimates of Teratogenic Severity from Embryo–Fetal Developmental Toxicity Studies. Birth Defects Research (Part B) 107:60–70

ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment attachments for 2-pyrrolidone.zip

Date	Country	Organisation	Type of Organisation	Comment number
12.12.2023	Germany		MemberState	7

Comment received

The classification is justified by foetal effects in two species without clear adverse effects in the mothers.
Malformations were observed in two OECD TG 414 studies, in rats at 1900 mg/kg bw/day and in rabbits at 1000 mg/kg bw/day.
As no exceptional circumstances are given, setting an SCL is not justified.
In rabbits (oral gavage, 0, 250, 500, 1000 mg/kg bw/day) the developmental NOAEL of 250 mg/kg bw/day was based on reduced mean foetal weight: -6 %, -14.1 % and -20 % compared to control in low, middle, and high dose groups. The calculated ED10 value based on reduced foetal weight is 372 mg/kg bw/day and should be included in the data used as the basis to place 2-pyrrolidone in the medium potency group and the GCL should be applied.
An increase in late (6 %) and total (10.2 %) resorptions in the high dose group was observed. An increase in malformations was seen in the mid and high dose groups: in 7 (6), 1 (1), 8 (5), and 16 (8) foetuses (litters) in the control, 250, 500, and 1000 mg/kg/day groups, corresponding to 3.5 % (26 %), 0.5 % (4.5 %), 4 % (20.8 %) and 8.3 % (40 %). 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. Many visceral malformations of the heart (persistent truncus arteriosus, interventricular septal defect, small heart ventricle, and bulbous aorta) were seen in nine foetuses in five litters in the high dose group (5 % of foetuses, 25 % of litters). There was also an increase in external and skeletal malformations in the high dose group.
In rats (oral gavage, 0, 190, 600, 1900 mg/kg bw/day) the developmental NOAEL of 600 mg/kg bw/day was based on reduced foetal weight. An increase in the incidence of litters and foetuses with major malformations and in foetuses with minor visceral and skeletal anomalies in the high dose group was observed. Five foetuses in five litters were affected, and all had anal atresia and either acaudia or microcaudia. One of these five foetuses had absence of some thoracic and all lumbar, sacral and caudal vertebrae and absence of nine pairs of ribs.

CONFIDENTIAL ATTACHMENTS
1. Contribution to the 2-P Public consultation.zip [Please refer to comment No. 1, 5]
2. attachments for 2-pyrrolidone.zip [Please refer to comment No. 6]