EUROPEAN CHEMICALS AGENCY

Helsinki, 23 November 2017

Addressee: [redacted]

Decision number: CCH-D-2114379004-53-01/F
Substance name: 2-PYRROLIDONE
EC number: 210-483-1
CAS number: 616-45-5
Registration number: [redacted]
Submission number: [redacted]
Submission date: 02/09/2010
Registered tonnage band: Over 1000

DECISION ON A COMPLIANCE CHECK

Based on Article 41 of Regulation (EC) No 1907/2006 (the REACH Regulation), ECHA requests you to submit information on:

1. **In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.; test method: OECD TG 476 or TG 490) with the registered substance;**

2. **Pre-natal developmental toxicity study (Annex X, Section 8.7.2.; test method: EU B.31./OECD TG 414) in a second species (rabbit), oral route with the registered substance;**

3. **Extended one-generation reproductive toxicity study (Annex X, Section 8.7.3.; test method: EU B.56./OECD TG 443) in rats, oral route with the registered substance specified as follows:**
   - **Ten weeks premating exposure duration for the parental (P0) generation;**
   - **Dose level setting shall aim to induce some toxicity at the highest dose level;**
   - **Cohort 1A (Reproductive toxicity);**
   - **Cohort 1B (Reproductive toxicity) without extension to mate the Cohort 1B animals to produce the F2 generation;**
   - **Cohorts 2A and 2B (Developmental neurotoxicity)**

You have to submit the requested information in an updated registration dossier by **1 June 2020**. You also have to update the chemical safety report, where relevant. The timeline has been set to allow for sequential testing.

The reasons of this decision are set out in Appendix 1. The procedural history is described in Appendix 2 and advice and further observations are provided in Appendix 3.

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Appeal

This decision can be appealed to the Board of Appeal of ECHA within three months of its notification. An appeal, together with the grounds thereof, has to be submitted to ECHA in writing. An appeal has suspensive effect and is subject to a fee. Further details are described under: http://echa.europa.eu/regulations/appeals.

Authorised[1] by Kevin Pollard, Head of Unit, Evaluation E1

[1] As this is an electronic document, it is not physically signed. This communication has been approved according to ECHA's internal decision-approval process.

Appendix 1: Reasons

I. TOXICOLOGICAL INFORMATION

In accordance with Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at more than 1000 tonnes per year must contain, as a minimum, the information specified in Annexes VII to X to the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.

1. *In vitro* gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.)

An "*In vitro* gene mutation study in mammalian cells" is an information requirement as laid down in Annex VIII, Section 8.4.3. of the REACH Regulation, "if a negative result in Annex VII, Section 8.4.1. and Annex VIII, Section 8.4.2." is obtained. ECHA notes that the registration dossier contains negative results for both these information requirements.

You have sought to adapt this information requirement according to Annex XI, Section 1.5., of the REACH Regulation by providing a study record for an *in vitro* mammalian cell gene mutation assay (OECD TG 476) with the analogue substance 1-ethylpyrrolidin-2-one (EC no 220-250-6).

However, you have not provided any justification to support your read-across adaptation according to Annex XI, Section 1.5.

REACH Annex XI, Section 1.5., requires a structural similarity among the substances within a group or category such that relevant properties of a substance within the group can be predicted from the data on reference substance(s) within the group by interpolation.

ECHA considers that structural similarity alone is not sufficient to predict human health effects from data for a reference substance within the group by interpolation to other substances in the group. It has to be justified why such prediction is possible in view of the identified structural differences and factual evidence has to be provided in support of such explanation. In particular, the structural dis-similarities must be linked to a scientific explanation of how and why a prediction is possible.

With regard to this, ECHA notes that the target and selected source substances consist of five-membered lactam ring. The source substance also contains additional ethyl group attached to the nitrogen atom which locks the amide-bond, whereas the target substance is in tautomeric keto-enol equilibrium in suitable media. Therefore, it is important that your read across justification - among others – particularly includes justification on how prediction of *in vitro* gene mutation is possible in the presence of such structural difference. As explained above, you have not provided justification to support your adaptation according to Annex XI, Section 1.5. Hence, your adaptation of the information requirement is rejected. Therefore, the information provided on this endpoint for the registered substance in the technical dossier does not meet the information requirement. Consequently, there is an information gap and it is necessary to provide information for this endpoint.

ECHA considers that the *in vitro* mammalian cell gene mutation tests using the *Hprt* and *xprt* genes (OECD TG 476) and the *in vitro* mammalian cell gene mutation tests using the thymidine kinase gene (OECD TG 490) are appropriate to address the standard information requirement of Annex VIII, Section 8.4.3.

In your comments on the draft decision according to Article 50(1) of the REACH Regulation you have agreed to conduct the requested test. ECHA acknowledges that you agreed to perform the requested test.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: *In vitro* mammalian cell gene mutation test (test method: OECD TG 476 *or* OECD TG 490).

2. Pre-natal developmental toxicity study (Annex X, Section 8.7.2.) in a second species

Pre-natal developmental toxicity studies (test method EU B.31./OECD TG 414) on two species are part of the standard information requirements for a substance registered for 1000 tonnes or more per year (Annex IX, Section 8.7.2., column 1, Annex X, Section 8.7.2., column 1, and sentence 2 of introductory paragraph 2 of Annex X of the REACH Regulation).

The technical dossier contains information on a pre-natal developmental toxicity study in rats by the oral route using the registered substance as test material ([redacted], 1990; [redacted], 1971).

In the technical dossier you have provided study records for non-guideline developmental toxicity studies in mice as a second species with the registered substance via oral gavage ([redacted] 1970) and intraperitoneal administration ([redacted], 1970).

However, both studies in mice are pre-guideline studies and do not provide the information required by Annex X, Section 8.7.2. nor cover key parameters of this test, because of lower number of animals (12 instead 20 animals per dose) and shorter exposure duration (gestation days 11-15 instead as minimum days 5-15) that does not cover the whole period of organogenesis. Furthermore, the foetuses were examined only macroscopically for malformation without appropriate examination for skeletal and soft tissue alterations as required in OECD TG 414. In addition, the intraperitoneal exposure route used in the second mice study was identified as "*unsuitable application route (i.p.) to detect developmental toxicity*".

Therefore, the information does not comply with Article 13(3) and (4), the adaptation, if any, does not meet the requirements of Annex XI, section 1.1.2 of the REACH Regulation, and the information provided on this endpoint for the registered substance in the technical dossier does not meet the information requirement. Consequently there is an information gap and it is necessary to provide information for this endpoint.

The test in the first species was carried out by using rat. According to the test method EU B.31./OECD 414, the rabbit is the preferred second species. On the basis of this default assumption, ECHA considers that the test should be performed with rabbit as a second species.

ECHA considers that the oral route is the most appropriate route of administration for substances except gases to focus on the detection of hazardous properties on reproduction as indicated in ECHA *Guidance on information requirements and chemical safety assessment* (version 5.0, December 2016) Chapter R.7a, Section R.7.6.2.3.2. Since the substance to be tested is a liquid, ECHA concludes that testing should be performed by the oral route.

In your comments on the draft decision according to Article 50(1) of the REACH Regulation you have agreed to conduct the requested test. ECHA acknowledges that you agreed to perform the requested test.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: Pre-natal developmental toxicity study (test method: EU B.31./OECD TG 414) in a second species (rabbit) by the oral route.

3. Extended one-generation reproductive toxicity study (Annex X, Section 8.7.3.)

The basic test design of an extended one-generation reproductive toxicity study (test method EU B.56./OECD TG 443 with Cohorts 1A and 1B, without extension of Cohort 1B to include a F2 generation, and without Cohorts 2A, 2B and 3) is a standard information requirement as laid down in column 1 of 8.7.3., Annex X. If the conditions described in column 2 of Annex X are met, the study design needs to be expanded to include the extension of Cohort 1B, Cohorts 2A/2B, and/or Cohort 3. Further detailed guidance on study design and triggers is provided in the ECHA *Guidance on information requirements and chemical safety assessment* Chapter R.7a, Section R.7.6 (version 6.0, July 2017).

Adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

a) The information provided

You have not provided any study record of an extended one-generation reproductive toxicity study in the dossier that would meet the information requirement of Annex X, Section 8.7.3.

Instead, you have sought to adapt this information requirement. While you have not explicitly claimed an adaptation, you have provided information that could be interpreted as an attempt to adapt the information requirement according to Annex XI, Section 1.2. Hence, ECHA has evaluated your adaptation with respect to this adaptation. You provided the following justification for the adaptation "*There is sufficient information on reproductive performance from the subchronic drinking water study ([redacted], 1998a) and the developmental toxicity/teratogenicity study ([redacted], 1990). In the sub-chronic drinking water study, gross pathology and histopathology of reproductive organs did not reveal effects on gonads ([redacted], 1998a). Beyond this, female estrus cycle was not influenced by pyrrolidone ([redacted] 1998b). In the OECD 414 study ([redacted], 1990), NOAEL of 600 mg/kg bw for developmental effects was higher than NOAEL for maternal toxicity (190 mg/kg bw). Developmental malformations were observed only at the highest dose level (1990 mg/kg bw). Older studies confirmed this outcome. Similar results were obtained in the studies with rats ([redacted], 1971) and with NMRI mice ([redacted] 1970). Therefore, 2-generation study will not deliver additional information and considered to be superfluous (aspects of animal welfare)".*

To support your weight of evidence adaptation you have provided the following sources of individual information under IUCLID section 7.8.1:

1. Key study: Sub-chronic oral toxicity study with 2-Pyrrolidone in Wistar rats; oral drinking water (OECD TG 408, GLP) with the registered substance ([redacted] 1998; [redacted], 2003), reliability 1.
2. Supporting study: "*Study for clarification of thymus findings in female Wistar rats; Administration in drinking water up to 13 weeks*"; oral drinking water (guideline not reported, GLP) with the registered substance ([redacted], 1998), reliability 2.

In addition you have provided under IUCLID section 7.8.2., the following information:

1. Key study: Pre-natal developmental toxicity study in Sprague-Dawley rats; oral gavage (OECD TG 414, GLP) with registered substance ([redacted], 1990), reliability 1.
2. Supporting study: "*FDA guidelines for reproductive toxicity*" study in Sprague-Dawley rats; oral gavage (not GLP) with registered substance ([redacted], 1971), reliability 2.
3. Supporting study: "[redacted] *test: internal, standardized test method to determine developmental toxicity after peroral administration of the test substance in mice*" study in mice; oral gavage (not GLP) with registered substance ([redacted], 1970), reliability 2.
4. Supporting study: "[redacted] *test: internal, standardized test method to determine developmental toxicity after ip administration of the test substance in mice*" study in NMRI mice; intraperitoneal (not GLP) with registered substance [redacted], 1970), reliability 2.

b) ECHA's evaluation and conclusion of the provided information

Criteria applied

An adaptation pursuant to Annex XI, Section 1.2. requires sufficient weight of evidence from several independent sources of information leading to the assumption/conclusion that a substance has or has not a particular dangerous (hazardous) property with respect to the information requirement in question including an adequate and reliable documentation.

Your weight of evidence adaptation needs to address the specific dangerous (hazardous) properties of the registered substance in respect to investigations in an extended one-generation reproductive toxicity study (EU B.56./OECD TG 443) as requested in this decision. ECHA considers that this study provides, in addition to information to general toxicity, information in particular on two aspects, namely on sexual function and fertility in P0 and F1 generations (further referred to as 'sexual function and fertility') and on development and toxicity of the offspring from birth until adulthood due to pre- and postnatal and adult exposure in the F1 generation (further referred to as 'effects on offspring').

Relevant elements for 'sexual function and fertility' are in particular functional fertility (oestrous cycle, sperm parameters, mating behaviour, conception, pregnancy, parturition, and lactation) in the parental generation after sufficient pre-mating exposure duration and histopathological examinations of reproductive organs in both P and F1 generations. Relevant elements for 'effects on offspring' are in particular peri- and post-natal investigations of the F1 generation up to adulthood including investigations to detect certain endocrine modes of action, sexual development, and investigations on developmental neurotoxicity. Also the sensitivity and depth of investigations to detect effects on 'sexual function and fertility' and 'effects on offspring' needs to be considered.

Sexual function and fertility

With respect to the aspect of 'sexual function and fertility', you have provided information from a sub-chronic oral study on histopathological integrity of the reproductive organs, and information on estrous cycle. However, in the provided study, the fixation method used (formalin) is not recommended anymore for reproductive organs and lacks the sensitivity of the modern methods. Hence, the histopathological information – specially on the male reproductive organ- are considered as not sufficiently reliable.

Further, you have not provided any information on functional fertility (mating behaviour, conception, pregnancy, parturition, and lactation) and histopathological examinations of reproductive organs in F1 animals in adulthood, sexual maturation, and investigations related to hormonal modes of action.

Thus, the information you provided is not sufficient to support your conclusion that the substance does not have a dangerous property with respect to sexual function and fertility.

Effects on offspring

ECHA notes that the provided pre-natal developmetal toxicity studies only provide information on pre-natal effects of the substance. However, the provided information does not address key elements of offspring toxicity observable peri- and postnatally, such as survival, growth and sexual development. Thus, the information you provided does not allow a conclusion on the hazardous property of the registered substance with respect to development and offspring toxicity observable peri- and post-natally.

Developmental neurotoxicity

Based on the currently available information there is concern for potential (developmental) neurotoxicity effect of the substance in adults and consequently the developmetal neurotoxicity cohort is triggered and included in the extended one-generation study design for the reasons explained under section "*a*" below. However, you have not provided information on the potential effect of the substance on developing nervous system. Thus, the information you provided does not allow a conclusion on the hazardous property of the registered substance with respect to developmental neurotoxicity.

Conclusion

Hence, the information you provided to support your adaptation, considered individually or together, lacks information on critical elements of reproductive toxicity and do not allow to assume/conclude that the substance does not have the particular dangerous (hazardous) property addressed by the information requirement of Annex X, Section 8.7.3.

As explained above, the information provided on this endpoint for the registered substance in the technical dossier does not meet the information requirement. Consequently there is an information gap and it is necessary to provide information for this endpoint. Thus, an extended one-generation reproductive toxicity study according Annex X, Section 8.7.3. is required. The following refers to the specifications of this required study.

c) The specifications for the study design

Premating exposure duration and dose-level setting

To ensure that the study design adequately addresses the fertility endpoint, the duration of the premating exposure period and the selection of the highest dose level are key aspects to be considered. According to ECHA Guidance, the starting point for deciding on the length of premating exposure period should be ten weeks to cover the full spermatogenesis and folliculogenesis before the mating, allowing meaningful assessment of the effects on fertility.

Ten weeks premating exposure duration is required because there is no substance specific information in the dossier supporting shorter premating exposure duration as advised in the ECHA *Guidance on information requirements and chemical safety assessment* Chapter R.7a, Section R.7.6 (version 6.0, July 2017).

The highest dose level shall aim to induce some toxicity to allow comparison of effect levels and effects of reproductive toxicity with those of systemic toxicity. The dose level selection should be based upon the fertility effects with the other cohorts being tested at the same dose levels.

If there is no existing relevant data to be used for dose level setting, it is recommended that results from a conducted range-finding study (or range finding studies) are reported with the main study. This will support the justifications of the dose level selections and interpretation of the results.

Cohorts 2A and 2B

The developmental neurotoxicity Cohorts 2A and 2B need to be conducted in case of a particular concern on (developmental) neurotoxicity as described in column 2 of 8.7.3., Annex X. When there are triggers for developmental neurotoxicity, both the Cohorts 2A and 2B are to be conducted as they provide complementary information.

According to column 2 of Annex X 8.7.3. a trigger includes "*existing information on effects caused by substances structurally analogous to the substance being studied, suggesting such effects or mechanisms/modes of action*".
ECHA notes that existing information on the registered substance itself and/or substances structurally analogous to the registered substance derived from available *in vivo* studies shows effects in the nervous system.More specifically, single administration of the registered substance to mice affected the convulsions induced by beta-methyl-beta-ethylglutarimide (anticonvulsive ED50=150 mg/kg bw) or pentylenetetrazole (anticonvulsive ED50 = 200 mg/kg bw) but not the electroshock induced convulsions (Hawkins and Sarett 1957).

Intravenous administration of the registered substance to mice resulted in anti-strychnine activity at 875 and 1750 mg/kg but not at higher doses (Lightowler and MacLean 1963).
In another study with the registered substance via intraperitonal administration to mice, rats, rabbits, cats or guinea pigs resulted in significant lower movements, a reduced ability for coordination and a reduced spontaneous activity in the brain (Sieroslawska 1965). The author stated that 2-pyrrolidone functioned as physiological tranquillizer at doses of 1/20 of the LD50 and more.

Furthermore, the toxicokinetic data available in the registration dossier shows that the registered substance passes the blood brain barrier and is metabolised to gamma aminobutyric acid which is a known inhibitory neurotransmitter in the central nervous system.

N-methyl-2-pyrrolidone (NMP) and *N*-ethyl-2-pyrrolidone (NEP) are considered substances structurally analogous to the registered substance 2-pyrrolidone for the following reasons:

In general, ECHA considers substances which are grouped together for read-across purposes (category or analogue approach in registration dossiers), have similar chemical groups, and which form relevant (bio)transformation products as structurally analogues of the substance being evaluated.

ECHA notes that the registrant proposed read-across from NMP and NEP and therefore these two substances are considered in this assessment. Moreover, the 2-pyrrolidone ring is a common structural feature of 2-pyrrollidone, NMP as well as NEP. Furthermore, 2-pyrrolidone has been reported as a metabolite of NMP in rat and human urine possibly formed by direct demethylation of NMP Lokajova et al., Cent. Eur. J. Chem., 9(5), 2011, 825-833; Carnerup et al., Food and Chemical Toxicology, 43, 2005, 1441-1447; Cernerup et al., Toxicology Letters, 162, 2006, 139-145). Furthermore, Koch et al. 2014 (Koch et al., Arch. Toxicol., 88, 2014, 893-899) state that "*2-Pyrrolidone would also be a possible NEP metabolite, however, common to both NMP and NEP.*" Therefore, NMP and NEP are considered structural analogues of the registered substance 2-pyrrolidone and information on these substances is used for triggering considerations.

Information from NEP for 13-weeks shows statistically significant decrease in grip strength forelimbs in male rats (33.0% at 300 mg/kg bw/day, and 40.1% at 1000 mg/kg bw/day), and motor activity in female rats at 1000 mg/kg bw/day ([redacted] 2006). Furthermore, the OECD TG 408 study on NMP reports neurobehavioral effects (e.g., increase in foot splay values, higher incidence of low arousal). Hence, both structural analogues NEP and NMP show relevant effects in the functional observational battery.

In your comments on the draft decision according to Article 50(1) of the REACH Regulation you have not agreed with the inclusion of Cohorts 2A and 2B in study design. You have reached to this conclusion after you have re-assessed the provided information in the registration dossier and also other additional information as presented below.

Re-evaluation of the neurotoxicity study results submitted in the dossier

For the data by Hawkins and Sarett (1957), you have explained that the neuroprotective property of the substance against the studied anticonvulsants was attained at high doses via the more sensitive intravenous ('i.v.') administration compared to oral administration and the equivalent oral doses would be higher than the limit dose level. These equivalent oral doses would exceed the maximum tolerated dose related to general toxicity by many times. You have further concluded that the registered substance has no obvious adverse effect on the nervous system.

Regarding the data by Lightowler and MacLean (1963), you have concluded that "*again, this study showed no neurotoxicity effects resulting from 2-pyrrolidone exposure. As already mentioned above, the i.v. route of administration is not a relevant route of exposure under REACH. Moreover, the doses of 875 mg/kg bw and 1750 mg/kg bw are again irrelevant for the registered substance because they also exceed the maximum tolerated dose related to general toxicity and are thus not applicable for the requested EOGRTS*".

Regarding the data by Sieroslawska (1965), you have stated that "*the author stated that 2-pyrrolidone reduced spontaneous activity in mice starting at doses of approximately 75 to 185 mg/kg bw following i.p. administration. The author postulates that because 2-pyrrolidone is a form of GABA it may function as a physiological tranquillizer*". In addition, you have cited the acute oral study by [redacted] (1999) and stated that no clinical signs (except for hunched posture) were observed in rats treated with the substance at 2000 mg/kg bw/day. You have further stated that "*this is explained by the fact that chemicals administered orally are subject to 'firstpass metabolism' meaning that systemic availability of parent chemicals is lower than chemicals administered by i.v. or i.p. routes. As a consequence, any potentially neuro-related responses will be significantly lower, if not absent, at relevant oral doses of chemicals (which in comparison to i.p. is a relevant exposure route under REACH).*

Again, the Registrants stress that potential neurobehavioral changes related to 2-pyrrolidone exposure observed in test animals generally appear as neuroprotective instead of neurodegenerative or neurotoxic".

Re-evaluation of the study results on toxicokinetics submitted in the dossier

In your comments, you have considered the information from Callery et al. (1979), Fasolato et al. (1988) and the report by EMEA (1998). You explained that "*the registered substance passes the blood brain barrier and may potentially be metabolised to gamma aminobutyric acid (GABA) in the brain which is a known inhibitory neurotransmitter in the central nervous system. However, this conversion appears to be effectively regulated by homeostatic mechanisms preventing uncontrolled GABA and glutamate formation*". In addition, you have stated that the "*relatively large*" intravenous dose of 200mg/kg bw did not alter brain steady state levels of GABA. In addition, you explained that GABA has neuroprotective role due to its inhibitory effect in the central nervous system which is supported by descibed studies.

Neurotoxicity data on structurally related substances NEP and NMP

In your comments, you have considered that the statistically significant decrease in grip strength at 300 and 1000 mg/kg bw/day in males is secondary to general toxicity (reduced body weight and food consumption) in the 90-day study by [redacted] (2006) with N-ethyl pyrrolidone (NEP). Furthermore, you have stated that the toxicological profile of NEP is different from the registered substance and the read-across between the two was not justified for the *in-vitro* mammalian gene mutation study and concluded that the read-across is not supported for the genetic toxicity or for reproductive toxicity endpoints.

In addition, you have provided information on structurally related substance N-methyl pyrrolidone (NMP) from the publication by Malley et al. (1999). You stated that neurobehavioural effects (higher incidence of arousal and slight palpebral closure) were shown which suggest a sedative effect or a general malaise, particularly since no morphological changes were evident in either the peripheral or central nervous system.

Furthermore, you have provided additional information on anticonvulsive, tranquilizing and/or anti-amnestic properties on the derivatives of the registered substance. The publication by Avetisyan and co-workers (1998) and Shorvon (2001) demonstrated that the structural differences (including the character and position of the substituents) results differences in the pharmacological activity.

ECHA has addessed your comments on the draft decision as follows:

Re-evaluation of the neurotoxicity study results submitted in the dossier

ECHA notes that the anticonvulsant effects reported in Hawkins and Sarett (1957) were observed with an ED50 of 150 - 200 mg/kg bw/day after oral administration of 2-pyrrolidone, and not after intravenous administration. The systemic toxicity was about 586 mg/kg (kidney effect and body weight reduction) and 600 mg/kgbw/day (body weight reduction) in the OECD TG 408 and OECD TG 414, respectively. Hence, the reported anticonvulsant effects by Hawkins and Sarett (1957) were attained at lower dose level than the systemic effects reported in the OECD TG 408 and OECD TG 414 studies.

ECHA also notes that the results reported in Lightowler and MacLean (1963) were not from oral administration and were at rather high doses. Based on the the toxicokinetic information in the dossier and also the report by EMEA (1998) shows that oral absorption of 2-pyrrolidone is complete. Hence, systemic availability seems to be the same after oral and intravenous administration. Therefore, the anti-strychnine activity at 875 mg/kgbw/day further suports the activity of 2-pyrrolidone in the nervous system.

In addition, ECHA considers that the clinical investigations seem to be limited to only hunched posture observation for [redacted] (1999), as reported in the registration dossier. In addition, your conclusion that "*no other clinical signs were observed in rats*" and "*the findings observed after i.v. study by Sieroslawska 1965, are not mirrored by doses administered orally*" seems to be inconsistent with the study report by [redacted] (1999).

Re-evaluation of the study results on toxicokinetics submitted in the dossier

ECHA notes that Fasolato et al. (1988) demonstrated that more GABA is formed in the brain after repeated oral exposure compared to single intravenous administration. Potential compensatory mechanisms are likely to exist in brain also during development, however, the increased concentration of GABA, although of limited magnitude, is still considered to support the concern for developmental neurotoxicity.

In addition, ECHA would like to emphasize that the report by EMEA (1998) is intended for the evaluation of medicinal products and is subjected to Regulation (EC) No. 726/2004 which is different from the intention of REACH where depending on the property of the substance and other information a maximum dose of up to 1000 mg/kg bw/day can be used to investigate intrinsic hazardous property of the substance. Hence, concentrations close to 1000 mg/kg bw/day may be relevant in the absence of severe other systemic toxicity. Hence, a dose of 200 mg/kg is not high for investigations of intrinsic hazardous properties of a substance under REACH.

Neurotoxicity data on structurally related substances NEP and NMP

ECHA considers that the reported neuro-behavioural effect in male rats in [redacted] (2006) are relevant and may not be secondary to the statistically significant reduction of body weight and food consumption. Because the body weight reduction was shown in both sexes but the effect on neurobehaviour was only in male and not in females.
Furthermore, ECHA considers that similarity on the general toxicological profile between registered substance and structurally analogue substance (in this case NEP) is not a pre-requisite to trigger the inclusion of Cohorts 2A and 2B. Instead, according to Column 2 of Annex X, Section 8.7.3. a particular concern on developmental (neurotoxicity) from "*existing information on effects caused by substances structurally analogous to the substance being studied, suggesting such effects or mechanisms/modes of action*" associated to (developmental) neurotoxicity triggers the inclusion of Cohorts 2A and 2B. This means that the existing information from structurally analogous substances is sufficient to raise a concern for (developmental) neurotoxicity without a full read-across proposition for (developmental) neurotoxicity under Annex XI, Section 1.5 of the REACH Regulation.
In addition, difference in toxicological profile with respect to systemic toxicity should not hinder to clarify a concern with respect to neurotoxicity. More specifically, the neuro behavioural assessment for NEP shows neurotoxicity effects while such parameters were not investigated in the 90-day study with the registered substance. Therefore, the concern for neurotoxicity for the registered substance remains.

Regarding your comment on Avetisyan and co-workers (1998) and Shorvon (2001), ECHA notes that the potential pharmacological effects is not demonstrated for 2-pyrrolidone itself. Hence, your conclusion that "*it can be inferred that 2-pyrrolidone, would not possess pharmacologically relevant tranquilizing and anticonvulsive activity because the pyrrolidone moiety does not possess substituents*" is not supported by the information included in those publications. In addition, 2-pyrrolidone was active in the nervous system after intravenous/intraperitoneal administration according to Hawkins and Sarett (1957), Lightowler and MacLean (1963) and Sieroslawska (1965).

In summary, ECHA notes that 2-pyrrolidone has activity in the nervous system as shown by its effects in mice after a single injection (Lightowler and MacLean 1963 and Sieroslawska 1965) and single oral dose (Hawkins and Sarett 1957) while no clinical signs were reported in the sub-chronic toxicity study ([redacted] 1998) provided in the registration dossier. However, based on the study report, it seems that no neurobehavioual assessment was conducted. Hence, ECHA acknowledges that there is no clear evidence of neurotoxicity in adults. On the other hand, GABA levels increased in the adult brain in steady-state conditions (after repeated exposure) but not after a single dose (Fasolate et al. 1988). Thus, repeated administration seems to be relevant for an effect.

In addition, the findings from the structurally related substances NEP and NMP supports the cooncern for neurotoxicity. Furthermore, as GABAergic system has an essential role in the brain, potential changes in GABA levels during development rises a concern for developmental neurotoxicity.

Therefore, there is concern for developmetal neurotoxicity based on the following evidences:

1) Substance is active in adult brain
2) GABA levels is more after oral repeated dose
3) supportive information from structurally analogous substances NEP and NMP

Consequently, ECHA concludes that the developmental neurotoxicity Cohorts 2A and 2B need to be conducted because there is a particular concern on (developmental) neurotoxicity based on the results from the above-identified *in vivo* studies on the registered substance itself and/or substances structurally analogous to the registered substance.

The study design must be justified in the dossier and, thus, the existence/non-existence of the conditions/triggers must be documented.

Species and route selection

According to the test method EU B.56./ OECD TG 443, the rat is the preferred species. On the basis of this default assumption, ECHA considers that testing should be performed in rats.

ECHA considers that the oral route is the most appropriate route of administration for substances except gases to focus on the detection of hazardous properties on reproduction as indicated in ECHA *Guidance on information requirements and chemical safety assessment* (version 6.0, July 2017) Chapter R.7a, Section R.7.6.2.3.2. Since the substance to be tested is a liquid, ECHA concludes that testing should be performed by the oral route.

d) Outcome

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: Extended one-generation reproductive toxicity study (test method EU B.56./OECD TG 443), in rats, oral route, according to the following study-design specifications:

- Ten weeks premating exposure duration for the parental (P0) generation;
- Dose level setting shall aim to induce some toxicity at the highest dose level;
- Cohort 1A (Reproductive toxicity);
- Cohort 1B (Reproductive toxicity) without extension to mate the Cohort 1B animals to produce the F2 generation;
- Cohorts 2A and 2B (Developmental neurotoxicity)

Notes for your consideration

The conditions to include the extension of Cohort 1B are currently not met. Furthermore, no triggers for the inclusion of Cohort 3 (developmental immunotoxicity) was identified. However, you may expand the study by including the extension of Cohort 1B, and/or Cohort 3, if new information becomes available after this decision is issued to justify such an inclusion. Inclusion is justified, if the new information shows triggers which are described in column 2 of Section 8.7.3., Annex X and further elaborated in ECHA *Guidance on information requirements and chemical safety assessment* Chapter R.7a, Section R.7.6 (version 6.0, July 2017). You may also expand the study to address a concern identified during the conduct of the extended one-generation reproduction toxicity study and also due to other scientific reasons in order to avoid a conduct of a new study. The justification for the expansion must be documented. The study design must be justified in the dossier and, thus, the existence/non-existence of the conditions/triggers must be documented.

II. DEADLINE TO SUBMIT THE REQUESTED INFORMATION

In your comments on the draft decision according to Article 50(1) of the REACH Regulation you stated that you disagree with the timeline of 30 months to submit the requested information. Instead you proposed that 36 months from the date of the decision is appropriate: "*the Registrants want to highlight that conducting such an EOGRTS requires a time consuming protocol including a dose range finder study, the main study, as well as additional work arising from potential high-dose findings and discussions of results and reporting. Also the results of the requested pre-natal developmental toxicity study should be available before even commencing the EORGTS. Hence, the Registrants kindly request a time extension for submitting the requested information of 36 month*".

However, ECHA would like to emphasize that the provided timeline, 30 months to submit the requested information, takes into account the timeline for the dose-range finding study, main study, and other additional work needed to finalise the study report and submission. In addition, as already indicated in the decision, the timeline is set in order to allow for sequential testing.

Therefore, ECHA considers that the timeline of 30 months is sufficient to submit the requested information in the draft decision.

Appendix 2: Procedural history

For the purpose of the decision-making, this decision does not take into account any updates of your registration after the date when the draft decision was notified to you under Article 50(1) of the REACH Regulation.

The compliance check was initiated on 30 November 2016.

The decision making followed the procedure of Articles 50 and 51 of the REACH Regulation, as described below:

ECHA notified you of the draft decision and invited you to provide comments.

ECHA took into account your comments and did not amend the request(s) or the deadline.

ECHA notified the draft decision to the competent authorities of the Member States for proposals for amendment.

ECHA received proposal(s) for amendment and modified the draft decision.

ECHA invited you to comment on the proposed amendment(s).

ECHA referred the draft decision to the Member State Committee.

Your comments on the proposed amendment(s) were taken into account by the Member State Committee.

The Member State Committee reached a unanimous agreement on the draft decision during its MSC-56 meeting and ECHA took the decision according to Article 51(6) of the REACH Regulation.

Appendix 3: Further information, observations and technical guidance

1. This compliance check decision does not prevent ECHA from initiating further compliance checks on the present registration at a later stage.

2. Failure to comply with the requests in this decision, or to otherwise fulfil the information requirements with a valid and documented adaptation, will result in a notification to the enforcement authorities of your Member State.

3. In relation to the information required by the present decision, the sample of the substance used for the new tests must be suitable for use by all the joint registrants. Hence, the sample should have a composition that is suitable to fulfil the information requirement for the range of substance compositions manufactured or imported by the joint registrants.

   It is the responsibility of all joint registrants who manufacture or import the same substance to agree on the appropriate composition of the test material and to document the necessary information on their substance composition. In addition, it is important to ensure that the particular sample of the substance tested in the new tests is appropriate to assess the properties of the registered substance, taking into account any variation in the composition of the technical grade of the substance as actually manufactured or imported by each registrant.

   If the registration of the substance by any registrant covers different grades, the sample used for the new tests must be suitable to assess these grades. Finally there must be adequate information on substance identity for the sample tested and the grades registered to enable the relevance of the tests to be assessed.