ECHA
EUROPEAN CHEMICALS AGENCY

Helsinki, 9 December 2019

Addressee: [redacted]

Decision number: TPE-D-2114493112-55-01/F
Substance name: Calcium cyanamide
EC number: 205-861-8
CAS number: 156-62-7
Registration number: [redacted]
Submission number: [redacted]
Submission date: 08/03/2019
Registered tonnage band: Over 1000

DECISION ON A TESTING PROPOSAL

Based on Article 40 of Regulation ((EC) No 1907/2006) (the REACH Regulation), ECHA examined your testing proposals and decided as follows.

Your following testing proposals are rejected:

1. **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 using the registered substance;**
2. **Extended one-generation reproductive toxicity study (Annex X, Section 8.7.3.; test method: OECD 443) in rats, oral route, using the registered substance.**

The reasons for 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.

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 Ofelia Bercaru, Head of Unit, Hazard Assessment

[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

The decision of ECHA is based on the examination of the testing proposal you submitted and scientific information submitted by third parties.

0. Grouping of substances and read-across approach

Article 13(1) of the REACH Regulation requires information on intrinsic properties of substances on human toxicity to be generated whenever possible by means other than vertebrate animal tests, including from information from structurally related substances (grouping or read-across), *"provided that the conditions set out in Annex XI are met"*.

According to Annex XI, 1.5 there needs to be structural similarity among the substances within a group or a category and furthermore, it is required that the relevant properties of a substance within the group can be predicted from the data for reference substance(s) by interpolation, and the data should be adequate for the purpose of classification and labelling and/or risk assessment. ECHA has considered first the scientific validity of the read-across hypothesis (preliminary considerations below), before assessing the testing proposed (sections 1 and 2, below).

Description of read-across approach in the registration dossier prior to March 2019

ECHA understands that you intend to use available test results of the source substance (cyanamide; EC number 206 992-3, CAS RN 420-04-2) for read-across covering only selected, but not all human health endpoints of the registered (target) substance (calcium cyanamide). ECHA further notes that in your registration dossier you provided a read-across justification document for environmental endpoints (28 September 2018), and considered that data from cyanamide could be used for environmental hazard assessment for the target substance.

You have also provided a hydrolysis study (OECD TG 111, non GLP, [redacted] 1987) conducted with the registered substance. This study shows that calcium cyanamide (technical grade) is quickly transformed to hydrogen cyanamide at low pH in a buffered aqueous solution. You further note that *"results from the hydrolysis study at pH 1.2 and 5 clearly show that hydrogen cyanamide is quantitatively released from its calcium salt within a few minutes. Available data for cyanamide show that the substance was hydrolytically stable at 25 °C, regardless of the pH values."* ECHA notes that the OECD TG 111 does not address testing of hydrolysis in other than aqueous solutions.

This claim is also supported by the reference to the toxicokinetics study you submitted on the registered substance, which demonstrates that calcium cyanamide is quantitatively hydrolysed to cyanamide in one hour under simulated gastric conditions in rat and that this hydrolysis is required for absorption, as calcium cyanamide is insoluble in aqueous solutions (review article *"Disposition and Pharmacokinetics of Disulfiram and calcium Carbimide (Calcium Cyanamide), Brien, J. F.; Loomis, C. W., Drug Metabolism Reviews, 14(1), 113-126 (1983)*). You also state in the Chemical Safety Report (CSR), page 87, that *"calcium cyanamide is hydrolysed both in vitro and in vivo, producing cyanamide which is readily absorbed after oral administration and rapidly excreted via urine as its major metabolite acetylcyanamide"*.

Nonetheless, in the CSR, you conclude that the toxicokinetics of the target and source substance (an hydrolysis product of the target substance) are different and that appropriateness of using data from cyanamide for extrapolation of adverse effects must be considered on case-by-case basis: *"Calcium cyanamide is composed of approx.* [redacted] *(w/w)* [redacted]. *In aqueous solution* Ca^{2+} *ions are release, and* CN_2 *transforms into hydrogen*

cyanamide (H_2CN_2) with hydrogen covalently bound to the CN_2 moiety. Dissolution kinetics, including dependency on the aqueous milieu, is difficult to predict for calcium cyanamide. Though cyanamide will be released from calcium cyanamide, the appropriateness of using cyanamide data for extrapolation of adverse effects must be done on a case-by-case basis, not a default assumption. Dissolution kinetics also determine if and at what speed cyanamide is released from calcium cyanamide. A delayed release would lead to lower systemic concentrations that may not exceed the metabolic capacities of protective pathways or repair mechanisms, compared to a bolus effect that might occur after application of cyanamide".

Therefore, you propose to test calcium cyanamide (technical grade) for reproductive toxicity.

ECHA analysis of the application of the read-across approach to reproductive toxicity

Validity of read-across hypothesis

First, the information you provided on the hydrolysis of calcium cyanamide (technical grade) in the registration dossier prior to March 2019 (see above) indicates the formation of cyanamide "*within a few minutes*".

You updated the registration dossier in March 2019 and provided new studies on water solubility, pH and hydrolysis conducted with the registered substance. You claimed that "u*pon dissolution in water calcium cyanamide technical grade is transformed to (hydrogen) cyanamide. Therefore, it is technically not possible to determine the water solubility of calcium cyanamide technical grade itself*".

This new information provided in the dossier further substantiates the fact that when dissolved in water, calcium cyanamide will dissociate rapidly into its ions, Ca^{2+} and cyanamide, excluding systemic presence of the parent compound. In particular, this has been demonstrated in the newly submitted "Transformation of calcium cyanamide in water_2019" study, which indicates that calcium cyanamide transforms to cyanamide and dicyanamide in contact with water.

You further highlighted that both substances are different and pointed at different pHs (indicating pH for calcium cyanamide in water equal as 12.5 while for cyanamide in water, pH equal of 4.8). ECHA notes that the formed anion of cyanamide is a very strong base, and since the testing medium was not a buffer solution, the pH raised to 12 as it reacted with water molecules to form hydroxide ions. ECHA further notes that this change of pH would not happen in body fluids such as blood, which is a buffered medium, and the alkaline behaviour of the cyanamide anion would be compensated.

You nevertheless do not extend your read-across adaptation to these standard information requirements by referring to dissolution kinetics of the registered substance in aqueous media.

ECHA notes that dissolution kinetics, if different, may delay predicted adverse effects but not have an impact on the intrinsic hazardous properties of the substance. Furthermore, you have not provided information on dissolution kinetics on other than aqueous media, i.e. no information on biologically relevant media, and your claim of dissolution kinetics for the purpose of reprodutive toxicity is thus not substantiated.

Second, ECHA notes that the main difference between the two substances is the metal cation Ca^{2+}, an natural endogenous cation being present in the target (registered) substance and absent in the source, while the main toxicity of both substances is driven by cyanamide ion.

Third, you highlighted the registration dossier in March 2019 that, according to the new data, the physicochemical properties between two substances are substantially different, in particular pH.

ECHA considers that, based on the data available for both substances, all relevant physico-chemical properties are similar and both substances dissolve well in a water: target substance's water solubility is 29.4 g/L at 20°C, and cyanamide is considered as very water soluble (at > 800 g/L pH 3.8 (20°C).
Based on the data available in the updated dossier, ECHA further considers that , all relevant physico-chemical properties are similar and both substances are miscible in water (on the difference of pH see above). These similarities support the hypothesis that both substances have similar reproductive toxicity properties,

Fourth, ECHA considers that both target and source substance possess similar toxicological effects. Both substances have harmonised classification for acute oral toxicity, cause skin irritation (target) or are corrosive to skin (source) and both are skin sensitisers. Regarding systemic toxicity, especially thyroid toxicity is seen both for the target substance in a rat study and for the source substance in rat and dog studies when administered orally either via gavage in water or dietary route.

> Target substance: decreased body weight, decreased food consumption, thyroid hyperplasia, thyroidectomy cells and decrease of acidophilic cells in the anterior pituitary, post-necrotic liver cirrhosis and dental disorders in Sherman rats (key study: one-year feeding study; doses 0, 2.75, 11, 45 and 180 mg/kg bw/day (pure substance); LOAEL 11 mg/kg bw/day; no guideline, not GLP, [redacted] 1960).
> Source substance: decreased body weight, decreased food consumption, thyroid follicular cell hyperplasia, increased incidence of biliary hyperplasia in liver, and increased incidence of pigmented macrophages in spleen of Sprague-Dawley rats (28-day oral gavage with doses of 5, 10, 20, 40 mg/kg bw/day in water; LOAEL 10 mg/kg bw/day; OECD 407, GLP, [redacted] 1988). Similar effects in thyroid gland were also seen in the 90-day oral feeding study in Wistar rats (0, 0.5, 1.5 and 4.5 mg/kg bw/day; non-GLP; LOAEL 4.5 mg/kg bw/day; [redacted] 1975), in the supporting 90-day and 1-year oral gavage dog studies (vehicle not reported, NOAEL 0.6 mg/kg bw/day (highest dose tested 6.0 mg/kg bw/day, [redacted] 2009; NOAEL 1 mg/kg bw/day, highest dose tested 5.0 mg/kg bw.day; [redacted] 1989)

Therefore, the read-across approach appears to be valid for reproductive toxicity.

In your comment to the draft decision, you provided an additional comparison on toxicological properties and current classification of both substances and pointed to the different toxicological potentials, concluding that read-across approach should not be applied for toxicological endpoints. These differences are, however, limited: the registered substance showed lower toxicological potency which is also reflected in the current harmonized classification and is consistent with the read-across hypothesis.

The difference in effects seen in pre-natal developmental studies in a first species does not either affect the read-across hypothesis (see section 1 below).

More importantly, ECHA notes that you also agreed that, regarding systemic toxicity, the NOAELs of both substances are similar and that the toxicological properties of the registered substance are dominated by cyanamide. Therefore, as outlined above, ECHA considers that the toxicological profiles of the target and source substances are sufficiently similar and that data generated with cyanamide can be used to fulfil standard information requirements for the registered substance.

Adequacy and reliability of available data on source substance

You have marked the above studies with source substance as "disregarded" and with a reliability score 4 without further explanation or justification. Some of these studies are indeed not conducted according the OECD test guidelines or under GLP and reporting provided in the dossier is limited (e.g. missing individual data, tabular results).

ECHA considers, however, that information from these studies can be used to support the read-across approach (see sections 1 and 2 below).

More specifically, ECHA notes that you did not provide any explanations on why you disregarded the OECD 407 study, conducted according to GLP [redacted] 1988). For this study, you only provided a general statement that "*For several (eco)toxicological endpoints, cyanamide is not considered a suitable read across partner for calcium cyanamide. This conclusion is drawn based on the composition and on the comparison of toxicological studies from endpoints, where experimental results are available for both compounds, calcium cyanamide and cyanamide. The differences between cyanamide and calcium cyanamide are pronounced for any test where classification is based on a limit dose or for any test with oral application. In the latter case, dissolution kinetics will determine if the compound is already resorbed in the oral cavity (i.e. in feeding studies), in the stomach, or not until it reaches the intestines. Dissolution kinetics also determines if and at what speed cyanamide is released from calcium cyanamide. A delayed release will lead to lower systemic concentrations that may not exceed the metabolic capacities of protective pathways or repair mechanisms, compared to a bolus effect that might occur after application of cyanamide.*"

With regard to another study conducted with the source substance [redacted], 1975), you referred to the following deficiencies: limited information on dietary preparations, no individual data; limited or missing clinical and histopathological investigations. However, you nevertheless concluded that the NOAEL (1.5 mg/kg bw/day pure active substance cyanamide, equivalent to 30 ppm in the diet) is based on the histopathological findings in the thyroid at 4.5 mg/kg bw/day cyanamide (equivalent to 90 ppm in the diet) in males and females.
ECHA further considers that, despite of limited reporting and other deficiencies, the information provided is sufficient to perform an assessment and to conclude that there were similar adverse effects on the thyroid at similar dose levels observed in different rat strains (both for target and source substance). These findings were also supported by observations of hypothyroidism in dog studies conducted on the source substance.

ECHA's consideration is also supported by the Scientific Committee on Health and Environmental Risks (SCHER) as outlined in their final opinion on potential risks to human health and the environment from the use of calcium cyanamide as fertiliser (EC DG SANTE, 22 March 2016[2]): "*based on kinetic data available, the SCHER concludes that calcium cyanamide hydrolysed to cyanamide after oral administration to experimental animals as well as to humans and that toxic effects observed for calcium cyanamide were mainly attributable to cyanamide toxicity. <..> After subchronic and chronic exposure, values for NOAEL seemed to be in the same range for both substances. <...> For cyanamide, the main target organs following repeated oral administration were the thyroid (rat, dog) and the testes (dog) as well as the red blood cells (dog). <...> For calcium cyanamide, effects were comparable after repeated exposure with the thyroid and the reproductive system reported to be main target organs affected. <...>* ".

Finally, ECHA notes that the source substance cyanamide has been classified[3] as STOT RE Cat 2, H373 (May cause damage to organs <or state all organs affected, if known> through

[2] https://ec.europa.eu/health/scientific_committees/environmental_risks/docs/scher_o_169.pdf
[3] https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:32017R0776&from=EN

prolonged or repeated exposure <state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard>.) and that target organ is thyroid. ECHA further notes that the abovementioned studies on the source substance have been asssessed, considered as appropriate and adverse findings were taken into account for deciding on a harmonised classification for the thyroid toxicity.

Therefore, ECHA concludes that the submitted studies on the source substance are adequate and reliable for the purpose of the read-across approach.

In your comments to the draft decision you committed to provide an explanation on why certain studies conducted with cyanamide should be considered as disregarded. In the updated dossier of March 2019, you explained that Klimish score should not be applied to disregarded studies conducted with cyanamide. You further explained, in a similar way for the disregarded studies,that "*the reliability of the original study report and endpoint record is Klimisch 1 (or 2) but as read-across for this endpoint is not supported, Klimisch rating is not applied.<..>.For this specific endpoint, read-across from cyanamide to calcium cyanamide is not appropriate. <....> This endpoint study record is nevertheless copied to the calcium cyanamide dossier to demonstrate full consideration of all cyanamide study data in the substance assessment and classification of calcium cyanamide.*" However, you have provided more detailed explanations for OECD TG 416 study ([redacted] 1986). You scored it as Klimisch 3 and claimed that it should be disregarded due to lack of individual or historical control data, inappropriate reporting (without specification), dietary administration which is considered as not so reliable than application by gavage. ECHA acknowledges the shortcomings of that study. However, as also emphasised in the RAC opinion, the results of that study also support adverse effects on fertility; the study is still considered as an appropriate supporting study, Despite of a different route of administration and low doses, a slightly reduced litter size effects on thyroid and histopathological findings in testes were in line with adverse effects on fertility, noted in other studies. There was sufficient, consistent reporting to make an independent assessment of the study for the purpose to consider findings as a supplementary supporting information. ECHA acknowledges that different routes of administration may introduce further some uncertainties in extrapolation of findings, but these are limited and do not affect the overall conclusion; furthermore, and, as outlined in OECD TG 416, dietary administration is a standard route for a two-generation reproductive toxicity study and hence considered as appropriate and reliable.

The read-across approach is, however, appropriate and the study is reliable for the reasons provided above.

- You further doubted that due to the different composition of calcium cyanamide technical grade and cyanamide there is enough differences in the toxicological profile to question the adequacy of transferring the CLP classification from the suspected reprotoxic compound cyanamide (Repr 2, H361) to calcium cyanamide technical grade and emphasised the uncertainties of the reproductive toxicity tests used for the RAC opinion on harmonised classification of cyanamide. You therefore conclude that "*based on the different composition of calcium cyanamide technical grade and cyanamide together with the equivocal data that resulted in the classification of cyanamide as Repr 2*," the proposed studies (to be conducted according recently updated OECD test guidelines) will provide a clear picture on the different toxicological profile (and lack of reproductive effects) and will provide additional toxicological information, especially for the assessment of potential endocrine active substances, for calcium cyanamide technical grade. However, as outlined in the RAC Opinion[4], the data available is not equivocal. Indeed, "*notwithstanding that there are significant methodological*

[4] For further discussion of the validity of the studies:
https://echa.europa.eu/documents/10162/8014e72c-0933-1d38-50ec-87c73bfe1d5c

limitations in two of these studies, the evidence for an adverse effect is clear and convincing" and *"the data are hence considered by the RAC to support classification in category 2 (Repr 2, H361f)."* Therefore, the information available is sufficient to meet the information requirements under consideration and further *in vivo* testing will not address real information needs.

- You emphasised shortcomings in the SCHER report. According to you, *"SCHER has prepared a report in order to show the hazardous properties of calcium cyanamide technical grade."* You *"identified some significant shortcomings in SCHER opinion which were submitted to the EU Commission. Thus the EU Commission did not use this opinion for regulatory action"*. You therefore concluded that *"the fact that EU Commission asked ECHA to prepare a restriction dossier indicates that SCHER report was not considered appropriate"*.
  The Commission request to prepare a restriction dossier is, however, targeted to determine risks for environment and, therefore, your allegation, which is related to human health properties, is rejected. Further, you did not specify the identified "significant shortcomings". Independently from ECHA's assessment and conclusion on the need to generate further data, SCHER opinion also states that data on hydrogen cyanamide can be considered where appropriate, that toxic effects of calcium cyanamide are mainly attributable to cyanamide toxicity and that both substance pose similar systemic toxicity. Therefore, SCHER report as an appropriate source of supporting information in the context of application of read-across approach.

Conclusion

Based on the hydrolysis and toxicological data submitted for both the target and source substances on repeated dose toxicity (above) and prenatal developmental toxicity (section 1), ECHA considers that the toxicity of both substances is sufficiently similar, differences can be explained, and therefore the data available on cyanamide (source) can be used to fulfil the standard information requirements for calcium cyanamide (target) according the requirements of Annex XI, Section 1.5.

In response to your comments, ECHA concludes that available studies on the source substance are adequate and reliable and further *in vivo* studies are not justified.

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

Pursuant to Article 40(3)(d) of the REACH Regulation, ECHA may reject a proposed test.

Pre-natal developmental toxicity studies on two species are part of the standard information requirements for 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 dossier contains three pre-natal developmental toxicity studies in rats as first species.

- OECD TG 414 study ([redacted], 2014) with the target/ registered substance, dose levels 0, 7, 21, 49 mg/kg bw/day in Sprague-Dawley rats (oral gavage, vehicle: corn oil, GLP, reliability 1). NOAEL for maternal toxicity 7 mg/kg bw/day based on reduced bodyweight gain and food consumption. In foetuses slight reduced body weights in the same groups.
- OECD TG 414 study ([redacted] 1989) with the source substance, dose levels 0, 5, 15 and 45 mg/kg bw/day in Crl:CD Br rats (oral gavage, no vehicle reported, GLP, reliability 4) NOAEL for maternal toxicity 5 mg/kg bw/day. Mean foetal weights were significantly reduced and an increase in diaphragmatic hernia and skeletal

malformations (mainly of the vertebrae) and different variations of ossification in foetuses were observed (NOAEL: 15 mg/kg bw/day).

- OECD TG 414 study ([redacted] 2014) with the source substance, dose levels 0, 3, 15 and 45 mg/kg bw/day in Sprague-Dawley rats (oral gavage, vehicle: water, GLP, reliability 4). You reported similar effects on foetuses as in the study of [redacted] (visceral and skeletal malformations associated with marked maternal toxicity at high dose) and NOAEL of 3 mg/kg bw/day for maternal and developmental toxicity.

You have submitted a testing proposal for a pre-natal developmental toxicity study in a second species (rabbits) according to EU B.31./OECD TG 414 "*to be conducted by the oral route with the registered substance*" to cover the information gap for Annex X, Section 8.7.2.

ECHA requested your considerations for alternative methods to fulfil the information requirement for Reproductive toxicity (pre-natal developmental toxicity). ECHA notes that you provided your considerations concluding that there were no alternative methods which could be used to adapt the information requirement for which testing is proposed. ECHA has taken these considerations into account and notes that you state that there is no reliable data available on the registered substance.

First, you have disregarded the prenatal developmental toxicity studies with source substance and stated: "*The differences between cyanamide and calcium cyanamide are pronounced for any test where classification is based on a limit dose, or for in vitro tests, where test systems are generally sensitive to pH shifts and osmolarity, or for any test with oral application. In the latter case, dissolution kinetics will determine if the compound is already resorbed in the oral cavity (i.e. in feeding studies), in the stomach, or not until it reaches the intestines. Dissolution kinetics also determines if and at what speed cyanamide is released from calcium cyanamide. A delayed release will lead to lower systemic concentrations that may not exceed the metabolic capacities of protective pathways or repair mechanisms, compared to a bolus effect that might occur after application of cyanamide. For developmental toxicity, the result from an OECD 414 study in rat was used for classification of cyanamide as Repro 2 (s. RAC opinion, CLH-O-0000001412-86-67/F, from 5.6.2015, based on diaphragmatic hernias reported in [redacted] (1989)). A comparable study with calcium cyanamide was clearly negative [redacted] 2014]. Therefore, cyanamide is not considered suitable for read across.*"

With respect to your assumption on limit-dose *in vivo* testing and pH / osmolarity dependent *in vitro* testing, ECHA notes that you have not explained why relevant differences between cyanamide and its calcium salt exist and how they affect the possibility to predict by read-across.

ECHA disagrees with your considerations based on dissolution kinetics (see section above) and with your considerations of the differences between the two prenatal development toxicity studies for the source and the target substances (see below).

As explained above, the target substance hydrolyses quickly in aqueous environment but no hydrolysis rate has been reported in corn oil. It is possible that corn oil changes the bolus dosing, reduces the peak concentration in serum and consequently reduces the developmental toxicity. The reason for not observing malformations in the OECD TG 414 study with the target substance is likely the vehicle used; corn oil.

ECHA notes also that, according to OECD TG 414 and *ECHA Guidance on information requirements and chemical safety assessment, Chapter R.7a, Section R.7.6* (version 6.0, July 2017), for use of all other vehicles except of water a justification is needed and has to be documented. Furthermore, when using a vehicle, consideration should be given on the effects on the absorption, distribution, metabolism and retention or excretion of the test chemical.

You have not provided any justification on why did you use corn oil as a vehicle and what impact this vehicle might have had on the hydrolysis and absorption of the administered substance.

The hazard classification for reproductive toxicity/developmental toxicity is based on intrinsic properties of the substance, thus, the systemic availability of the substance should be maximised. Based on the considerations above and the lack of justification provided on the selection of the vehicle, ECHA considers that the result from the OECD TG 414 study in rats with the targed substance can be explained by the vehicle used and, thus, it does not weaken the possibility to apply read-across.

In your comments and the updated dossier, you explained that "*the most important reason was to have a stable suspension of all constituents, including graphite and calcium oxide*" and to preserve the integrity of the test item (calcium cyanamide technical grade) in corn oil. You further explained that corn oil was used to ensure the stability and further uptake of the test item. You added that "*the toxicokinetics of calcium cyanamide in corn oil is unknown, but it is known that calcium cyanamide technical grade is bioavailable and enters systemic circulation when administered in corn oil*" and that the requested study has been performed according to GLP and met all requirements of the OECD TG 414.

However, according to the OECD TG 414, "*if a vehicle or other additive is used to facilitate dosing, consideration should be given to the following characteristics: effects on the absorption, distribution, metabolism, and retention or excretion of the test chemical; effects on the chemical properties of the test chemical which may alter its toxic characteristics; and effects on the food or water consumption or the nutritional status of the animals*".In this respect, you did not provide any data on hydrolysis or toxicokinetics of calcium cyanamide in oil.

Further, the use of corn oil could prevent the investigation of the developmental toxicity of the registered substance since it would hamper hydrolysis, which occurs in the body.

Finally, you did not provide any justification that graphite and calcium oxide would be relevant and the use of corn oil would be justified to have them in stable suspension. Hence, the result from the OECD TG 414 study in rats with the target substance does not invalidate a read-across approach.

Second, your registration already contains data from a GLP pre-natal developmental study conducted with rabbit on the analogue substance cyanamide (intraoesophagic intubation, doses 0, 2, 6 and 18 mg/kg bw/day, [redacted] 1989; reliability 4) which you disregarded due to "*major methodological deficiencies*" without detailed explanation or justification.
The information provided, however, does not suggest any major methodological deficiency and, therefore, your unsubstantiated claim of major deficiencies cannot be accepted and the study is considered to be valid.

In light of the acceptable read-across (see section 0 above)and quality of the studies available on the source substance ECHA considers that information from the available rabbit study on the source substance is adequate to fulfil the information requirement of the Annex X, Section 8.7.3 for the prenatal toxicity in the second species.

Therefore, pursuant to Article 40(3)(d) of the REACH Regulation, your testing proposal for a pre-natal developmental toxicity study in a second species (rabbits), oral route using the registered substance (test method: EU B.31/OECD TG 414) is rejected.

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

a) Examination of the testing proposal

Pursuant to Article 40(3)(d) of the REACH Regulation, ECHA may reject a proposed test.

The basic test design of an extended one-generation reproductive toxicity study (EOGRTS) is a standard information requirement as laid down in column 1 of Section 8.7.3., Annex X of the REACH Regulation, whereas column 2 defines when the study design needs to be expanded. The information on this endpoint is not available for the registered substance but needs to be present in the technical dossier to meet the information requirements. Consequently there is an information gap and it is necessary to provide information for this endpoint.

You have submitted a testing proposal for a basic design extended one-generation reproductive toxicity study according to (EU B.56./OECD TG 443) by the oral route to be performed with the registered substance.

ECHA requested your considerations for alternative methods to fulfil the information requirement for Reproductive toxicity (extended one-generation reproductive toxicity study). ECHA notes that you provided your considerations concluding that there were no alternative methods which could be used to adapt the information requirement for which testing is proposed. ECHA has taken these considerations into account and notes that you state that there is no reliable data available on the registered substance.

More specifically, you state that "*calcium cyanamide partly dissociates into hydrogen cyanamide. However, because of pronounced differences in the toxicological profile, uncertainties in the toxicokinetics, and uncertainties in the hazard assessment of cyanamide as source compound, a read-across approach is not considered justified.*"

In your registration dossier, you have provided records of two-generation reproductive toxicity studies conducted with source substance cyanamide following the OECD TG 416:

- OECD TG 416 study [redacted] 1987) dose levels 0, 2, 7, 25 mg/kg bw/day in CD SD rats (oral gavage, no vehicle reported, non GLP, some deviations from guideline; reduced fertility in females reported;
- OECD TG 416 study ([redacted] 1986) dose levels 0, 0.81, 2.25, 7.55 mg/kg bw/day in Wistar rats (oral, dietary route, GLP, with some deviations from the guideline); histopathological findings in thyroid; reduced litter size, reduced post-natal survival, reduced birth weight;
- OECD TG 416 study ([redacted] 1990) in Crl: CD SD rats via oral gavage (doses prepared in water), GLP compliant, doses 0, 2.5, 7.5 and 30.0 mg/kg bw/day for 12 weeks and then reduced to 1.25, 3.75 and 15.0 mg/kg bw/day (due to the toxicity observed). Systemic toxicity and reduced fertility in F0 and F1 animals reported.

You have given all these studies a reliability score of 4 (not assignable) due to "*major methodological deficiencies*". However you did not further specify what these methodological deficiencies were.

In your comments to the draft decision, you committed to provide an appropriate justification on why these studies should be disregarded. In the updated dossier, you kept studies labelled as disregarded, but explained that regarding both studies of [redacted] (1987) and [redacted] et al (1990)," *the reliability of the original study report and endpoint record is Klimisch 1, but*

as read-across for this endpoint is not supported, Klimisch rating is not applied". You also assigned Klimisch score 3 for the study of [redacted] (1986). As explained above, you highlighted that this study has to be disregarded due to lack of individual or historical control data, inappropriate reporting (without specification), dietary administration which is considered as not so reliable than application by gavage.
However, as already indicated above, the adverse effects reported in these studies are sufficiently relevant and reliable to conclude whether the substance has or has not a hazardous property. Therefore, ECHA maintains its conclusion that the submitted studies on the source substance are adequate and reliable for the purpose of the read-across approach.

Regarding the study of [redacted] 1990, under the same endpoint study record, you provided a contradictory statement under '*Overall remarks*': "*This study is assessed as appropriate and valid since it was not only performed according to internationally accepted testing guidelines under GLP conditions but also sufficient analyses were carried out and the reporting, assessment and data presentation in the study report was considered as appropriate. <...>. Finally, the most appropriate application route, i.e. oral by gavage was used since the dosing can be directly adjusted in respect to body weight development and is therefore considered as more reliable in comparison to dietary administration with its great variations in respect to substance intake during certain phase, palatability problems and occurrence of spilling.*"

Two-generation reproductive toxicity studies (B.35, OECD TG 416) that were initiated before 13 March 2015 shall be considered appropriate to address this standard information requirement according to the specific adaptations of column 2, Section 8.7.3 of Annex X of the REACH Regulation.

Furthermore, as outlined in Sections 0 and 1 above, ECHA considers that the toxicological profiles of the target and source substances are sufficiently similar and that the data generated with cyanamide can be used to fulfil the standard information requirements for calcium cyanamide. ECHA therefore considers that a two-generation reproductive toxicity study conducted with cyanamide according OECD TG 416 and GLP ([redacted], 1990) is sufficient to fulfil standard information requirement for this endpoint.

In your comments to the draft decision, you did not agree with ECHA's proposal to use data available on cyanamide and, based on the differences between calcium cyanamide technical grade and cyanamide, you claimed the right to conduct this study. As indicated above, ECHA considers that generation of further information on this endpoint for the registered substance is not justified as the information requirement can be fulfilled by using data available on cyanamide.

b) Consideration of the information received during third party consultation

ECHA received third party information concerning the testing proposal during the third party consultation. For the reasons explained below the information provided by third parties might be sufficient to fulfil this information requirement.

The third party provided their considerations and doubts of the necessity of the study and stated that based on the data available on cyanamide (EC 206-992-3, CAS RN 420-04-2), a read-across approach is scientifically justified. The third party also referred to the available and GLP compliant two-generation reproductive toxicity study (OECD TG 416) with cyanamide, and other supporting studies conducted with cyanamide. ECHA agrees with the third party that a read-across approach is acceptable and that there are data available for the analogue substance which fulfil this information requirement.

In your comments to the draft decision, you pointed out that the third party did not provide

any reasoning on why the read-across approach is acceptable. ECHA refers in this respect to the considerations provided above (Appendix 1, section 0).

c) Outcome

As explained in Sections 0 and 1 above, ECHA considers that the available information is sufficient to demonstrate that the information provided fulfills the requirements for grouping of substances and read-across approach in accordance with Annex XI, Section 1.5. for this endpoint.

Therefore, pursuant to Article 40(3)(d) of the REACH Regulation, your testing proposal for an extended one-generation reproductive toxicity study in rats, oral route using the registered substance (test method: OECD TG 443) is rejected.

Notes for your consideration

Following the considerations on the read-across approach provided above, you are recommended to consider the relevancy of the studies on the source substance for the purpose of classification and labelling of the registered substance.

The registered substance, as well the analogous substance, cyanamide, induces thyroid toxicity. This is a trigger indicating a particular concern for developmental neurotoxicity (ECHA *Guidance on information requirements and chemical safety assessment*, Chapter R.7a, Section R.7.6 (version 6.0, July 2017). This is not a standard information requirement, but you may consider conducting an OECD TG 426 study to address this concern under your own initiative.

Appendix 2: Procedural history

ECHA received your registration containing the testing proposals for examination in accordance with Article 40(1) on 30 October 2017.

ECHA held a third party consultation for the testing proposals from 28 February 2018 until 16 April 2018. ECHA received information from third parties (see Appendix 1).

This decision does not take into account any updates after **11 March 2019**, 30 calendar days after the end of the commenting period.

ECHA notified you of the draft decision and invited you to provide comments within 30 days of the notification. You updated your registration on 8 March 2019. ECHA took into account your comments and the above update and did not amend the draft decision.

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

As no amendments were proposed, ECHA adopted the decision under Article 51(3) of REACH.

Appendix 3: Further information, observations and technical guidance

1. This decision does not imply that the information provided in your registration dossier is in compliance with the REACH requirements. The decision does not prevent ECHA from initiating a compliance check on the 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 the Member States.