ECHA
EUROPEAN CHEMICALS AGENCY

Helsinki, 14 November 2019

Addressee: [redacted]

Decision number: TPE-D-2114488717-30-01/F
Substance name: Zinc bis(diethyldithiocarbamate)
EC number: 238-270-9
CAS number: 14324-55-1
Registration number: [redacted]
Submission number: [redacted]
Submission date: 25/05/2018
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 proposal(s) and decided as follows.

Your testing proposal is modified and you are requested to carry out:

1. **Extended one-generation reproductive toxicity study (Annex X, Section 8.7.3.; test method: 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 systemic 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); and**
   - **Cohort 3 (Developmental immunotoxicity)**

You have to submit the requested information in an updated registration dossier by ***22 November 2021***. You shall also update the chemical safety report, where relevant.

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.

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

Pursuant to Article 40(3)(b) of the REACH Regulation, ECHA may require the Registrant to carry out the proposed test under modified conditions.

The basic test design of an extended one-generation reproductive toxicity study (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 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 an extended one-generation reproductive toxicity study according to OECD TG 443 by the oral route in the rat, to be performed with the registered substance, according to the basic study design. You have provided the following justification, according to the criteria described in column 2 of Section 8.7.3 of Annex X:

"- Inclusion/exclusion of extension of Cohort 1B: [...] Since the available data do not trigger the inclusion of extension of cohort 1B, only 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 proposed.
- Termination time for F2: Not applicable: As an extension of Cohort 1B is not proposed, no F2 generation is included and the termination time does not need to be determined.
- Inclusion/exclusion of developmental neurotoxicity Cohorts 2A and 2B and/or developmental immunotoxicity Cohort 3: [...] the available data do not trigger an extension to include the extension of Cohort 1B, Cohorts 2A/2B, and/or Cohort 3. Furthermore, assessment of neurobehavior effects (functional observation battery and motor activity assessment) were included in the available OECD 408 study. [...] Thus, the OECD 408 study did not indicate neurotoxic potential of the substance.
Therefore, 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, and without Cohorts 2A, 2B and 3) is proposed.
- Route of administration: The oral route is proposed because this is a possible route of human exposure. In addition, the dose levels are determined based on the available data from a subchronic repeated dose toxicity test (according to OECD 408) and a prenatal developmental toxicity study (according to OECD 414) in which the animals were also administered via the oral route."

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(s) for which testing is proposed. ECHA has taken these considerations into account.

ECHA considers that the proposed study design requires modification to fulfil the information requirement of Annex X, Section 8.7.3. of the REACH Regulation. The following refers to the specifications of this required study.

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.

You did not specify the duration for premating exposure but proposed to follow the OECD test guideline 443. ECHA considers that 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[2].

You further proposed that "*Available data from a subchronic repeated dose toxicity test (according to OECD 408) and a prenatal developmental toxicity study (according to OECD 414) will be considered*" for dose-level setting. ECHA agrees with your proposal and reminds you that the highest dose level shall aim to induce systemic toxicity, but not death or severe suffering of the animals, to allow comparison of reproductive toxicity and systemic toxicity. The dose level selection should be based upon the fertility effects with the other cohorts being tested at the same dose levels. Hence any data, e.g from a pre-natal developmental toxicity study conducted on rats should be considered. If there is no relevant data to be used for dose-level setting, it is recommended that a range-finding study (or range finding studies) is performed and that its results are reported with the main study. This will support the justifications of the dose-level selections and interpretation of the results.

In your comments to the draft decision, you agreed with ECHA's conclusion that the extension of the Cohort 1B to produce an F2 generation is not triggered. Furthermore you agreed with the premating exposure duration and dose levels selection.

Species and route selection

You proposed testing in rats by the oral route. ECHA agrees with your proposal.
Cohorts 2A and 2B

Subsequent to a Proposal for Amendment (PfA), 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 Section 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.

One of the triggers set out in Annex X section 8.7.3. is that there is existing information on effects caused by substances structurally analogous to the substance being studied suggesting effects or mechanims/ modes of action with an association to neurotoxicity.

You proposed not to include Cohorts 2A and 2B. You further explained that "*assessment of neurobehavior effects (functional observation battery and motor activity assessment) were included in the available OECD 408 study ([redacted] 2017). Treatment-related findings*

[2] ECHA Guidance *on information requirements and chemical safety assessment*, Chapter R.7a, Section R.7.6 (version 6.0, July 2017)

included sliding with the ventral parts of the head and neck over the bottom of the open field, dyspnoea, grunting respiration, sniffing, piloerection, salivation, serous nasal discharge, low arousal, soft and/or mucoid faces, diarrhoea, soiled perineum and soiled fur. No neurotoxic effects of treatment were observed from motor activity assessment in any of the dose groups during the 30-minute test period. Thus, the OECD 408 study did not indicate neurotoxic potential of the substance".

ECHA notes that existing information on substances structurally analogous to the registered substance (ziram; zinc bis dimethyldithiocarbamate; "ZDMC"; (EC no 205-288-3)) derived from available *in vivo* studies [OECD 453 dietary study in rat (1994); available at: https://echa.europa.eu/registration-dossier/-/registered-dossier/2153/7/8/?documentUUID=6d8e62dc-bb38-4f27-8351-0ee210c50e76] show evidence of effects suggesting specific mechanisms/modes of action of the structurally analogous substance with an association to (developmental) neurotoxicity, i.e. relevant changes in thyroidal hormone levels. Specifically, T3, T4 and TSH were dosage-related decreased in week 4. The reduction in T4 values was still apparent for treated males at weeks 13 and 26. Moreover, this was associated with adverse effects, including a dose-related increase in thyroid weight in females at interim and terminal kills, and prominent thyroid ultimobranchial cysts (male and female).

ECHA notes that existing information on the registered substance itself derived from available *in vivo* studies [90-day study in rat ("7.5.1, Key, 1, [redacted] 2017, Repeated dose toxicity: oral rats_subchronic") and the publication Blackwell Smith Jr. R, Finnegan JK, Larson PS, Sanyoun PF, Dreyfuss ML, Haag HB (1953) Toxicologic studies on zinc and disodium ethylene bisdithiocarbamates. J. Pharmacol. Exp. Ther. 109, 159-166, partly referenced in your dossier as ("7.5.1, Sup, 2, Blackwell Smith, 1953, Repeated dose toxicity: oral rats_chronic")] show evidence of adverse effects (thyroid growth) consistent with the same mode of action as observed with the structurally analogous substance (i.e. relevant changes in thyroid hormone levels associated with adverse effects). Specifically, in the 90-day study the relative weight of the thyroid was higher than in controls in males of the mid- and high-dose group, but in the high-dose group statistical significance was not obtained. In the paper by Blackwell Smith et al. (1953), relative thyroid weight was statistically-significantly increased after 10, 20 and 30 days of treatment in both male and female rats, and an abnormal degree of hyperplasia was seen in high dose animals after 30 days of treatment. In the two year feeding study, there was a clear and dose-dependent increase in the incidence and severity of thyroid hyperplasia, with no animals in the control group having a severity greater than 2+, through to all animals in the high dose group having hyperplasia scored at 3+ or above. While ECHA considers that the effects seen on thyroid weight and hyperplasia are adverse, the more important consideration is that they are evidence of a physiological effect of the relevant changes in thyroidal hormone levels. Given that the relevant changes in thyroidal hormone levels can affect the physiology of the thyroid gland in adult animals, and that thyroidal hormones have a key role in brain development, there is a reasonable expectation that such changes in pregnant animals and offspring could affect the developing brain and the substance could be a developmental neurotoxicant.

In your comments to the PfA, you provided the comments which you submitted in response to ECHA's original draft decision, arguing the following:
(1) for the study of Blackwell Smith, 1953, the two year time period is too long and so is not relevant;
(2) since lesions in this study were found in the control group, the increase in incidence and severity of effect is mainly due to aging of the animals and not by direct thyroid toxicity;
(3) in Blackwell Smith, 1953, there is an effect of chronic exposure on the thyroid gland, but they cannot be interpreted as a particular concern on developmental neurotoxicity;

(4) in the [redacted] (2017), effects on thyroid weight were only seen in males and not females, and this is inconsistent with Blackwell Smith (1953);
(5) the relative weight increase in thyroid is caused by a decrease in body weight;
(6) the effect on thyroid weight in [redacted] (2017) and more generally are not adverse;
You also made comment about brain esterase inhibition, lack of clarity about a study Lindsey et al., 1994, and interpretation of developmental neurotoxicity studies on ziram. These comments are not relevant to the basis put forward for requesting the DNT study in the PfA.
In addition you argued that (7) in the OECD 453 study on ziram, the changes in T4 levels are considered to be minor with no adverse effect on homeostasis of the HPT axis; and (8) that the effects on the thyroid gland in the study by Blackwell Smith *et al.* (1953) are considered largely effects of age with potentially a repeat dose related effect of zineb.

ECHA's considerations are numbered according to your scheme:
(1) the studies in Blackwell Smith (1953) provide convincing evidence of thyroid perturbation because this effect was observed from 10 days through to 2 years and is biologically relevant;
(2) the biologically significant differences in incidence and severity between control and treated groups provides direct evidence of thyroid toxicity;
(3) ECHA agrees the substance causes an effect on thyroid after short-term and chronic exposure, and these findings provide evidence for physiological perturbation of the thyroid gland by the registered substance, in agreement with evidence of a mechanism/ mode of action from a structurally analogous substance, and thereby justify a concern for developmental neurotoxicity;
(4) the effects seen on thyroid in [redacted] (2017) are evidence of physiological perturbation of the thyroid gland by the registered substance. Minor differences between the studies do not remove the commonality of evidence of thyroid perturbation;
(5) the effects on body weight decrease do not correlate with the statistically significant increase in thyroid weight, and so ECHA rejects this hypothesis;
(6) these effects indicate a concern which is sufficient to trigger the cohort, ECHA is not seeking to demonstrate adversity per se;
(7) both T3 and T4 were reduced at the week 4 sampling. This was dose-related and there was evidence of physiological response in the thyroid (increased female thyroid weight). ECHA considers that these effects are statistically and biologically significant;
(8) the effects of the registered substance on the thyroid are modulated by length of exposure and, more importantly, the evidence of effects on thyroid by the registered substance is statistically significant and dose-related. The effects of the substance on the thyroid are consistent with the mechanism/ mode of action of thyroid hormone perturbation known for the structurally analogous substance, ziram.

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 substances structurally analogous to the registered substance indicating a specific mechanism/ mode of action of the substance with an association to developmental neurotoxicity.

Cohort 3

The developmental immunotoxicity Cohort 3 needs to be conducted in case of a particular concern on (developmental) immunotoxicity as described in column 2 of 8.7.3., Annex X.

ECHA notes that existing information on the registered substance derived from the available *in vivo* study shows evidence of (developmental) immunotoxicity.

More specifically, the OECD TG 408 study [redacted] 2017; doses 0, 10, 50 and 250 mg/kg bw/day) showed the following adverse effects in relation to immunotoxicity in high dose group animals:

- an increase in absolute neutrophil counts and decrease in percentage of lymphocytes (males).
- a decrease of absolute thymus weight in both sexes (statistical significance only in males, amount of change not specified) with histopathological correlate (decreased cortical cellularity).
- increased relative spleen weights (not specified) with histopathological correlate (accumulation of brown pigment in spleen) were noted in both sexes. Based on the adverse effects observed e.g. in spleen, you concluded that " *Based on the data available, the test article is classified as STOT Rep. Exp. 2 H373: 'May cause damage to the liver and spleen prolonged or repeated exposure' in accordance with EU [...] (CLP) Regulation No. (EC) 1272/2008.*"

In addition, the registered substance has a harmonised classification as a Category 1 skin sensitiser, which, according to ECHA's Guidance, is considered as supportive factor for justifying inclusion of the developmental immunotoxicity cohort.

In your comments to the draft decision, you agreed with ECHA's conclusion that the triggering of developmental immunotoxicity cohort was justified. You further noted though that, based on the immunotoxicological effects and current classification of the registered substance, the inclusion of developmental immunotoxicity cohort might not provide relevant additional information.

ECHA notes that, as outlined in the column 2 of 8.7.3., Annex X, this cohort shall be proposed by the registrant or may be required by the Agency in case of particular concerns on (developmental) immunotoxicity, such as existing information on the substance itself derived from the available *in vivo* studies. As pointed out by you, the registered substance is already classified as a skin sensitizer and toxic to spleen. ECHA therefore considers that the conditions of the column 2 of 8.7.3., Annex X are met and maintains its request. ECHA emphasises that this cohort investigates developmental effects and not effects on adult animals after repeated exposure. Hence, developmental immunotoxicity findings in offspring are relevant for considering classifying for developmental effects.

ECHA concludes that the developmental immunotoxicity Cohort 3 needs to be conducted because there is a particular concern on (developmental) immunotoxicity based on the results from the above-identified *in vivo* studies on the registered substance.

Outcome

Therefore, pursuant to Article 40(3)(b) of the REACH Regulation, you are requested to carry out the modified study with the registered substance subject to the present decision: Extended one-generation reproductive toxicity study (test method OECD TG 443), in rats, by the oral route, as specified above.

While the specifications for the study design are given above, you shall also submit with the new endpoint study record a scientific justification on each of the following aspects: 1) length of the premating exposure duration and dose level selection, 2) reasons for why or why not Cohort 1B was extended, 3) termination time for F2 generation, and 4) reasons for why or why not Cohorts 2A/2B and/or Cohort 3 were included.

Notes for your consideration

The conditions to include the extension of Cohort 1B are currently not met. However, you may expand the study by including the extension of Cohort 1B if new information becomes available after this decision is issued to justify such an inclusion. Inclusion is justified if the available information, together with the new information, shows triggers which are described in column 2 of Section 8.7.3., Annex X and further elaborated in ECHA *Guidance*[3]. 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.

Appendix 2: Procedural history

ECHA received your registration containing the testing proposals for examination in accordance with Article 40(1) on 5 April 2018.

ECHA held a third party consultation for the testing proposals from 10 August 2018 until 24 September 2018. ECHA did not receive information from third parties.

This decision does not take into account any updates after **14 January 2019**, 30 calendar days after the end of the commenting period.

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 amended the request.

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 in its MSC-66 written procedure and ECHA took the decision according to Article 51(6) of the REACH Regulation.

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 will result in a notification to the enforcement authorities of the Member States.

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.