EUROPEAN CHEMICALS AGENCY

Helsinki, 21 February 2019

Addressee: [redacted]

Decision number: CCH-D-2114461492-49-01/F
Substance name: C,C'-azodi(formamide)
EC number: 204-650-8
CAS number: 123-77-3
Registration number: [redacted]
Submission number: [redacted]
Submission date: 3 April 2017
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. **Transgenic rodent somatic and germ cell gene mutation assays (Annex X, Section 8.4., column 2; test method: EU B.58/OECD TG 488) in transgenic mice or rats, oral route, on the following tissues: liver and glandular stomach with the registered substance. Germ cells and duodenum shall be harvested and stored for up to 5 years. Duodenum shall be analysed if the results of the glandular stomach and of the liver are negative or inconclusive. The test material used should be freshly prepared;**

   OR

   ***In vivo* mammalian alkaline comet assay (Annex X, Section 8.4., column 2; test method: OECD TG 489) in rats, oral route, on the following tissues: liver, glandular stomach and duodenum, with the registered substance;**

2. **Sub-chronic toxicity study (90-day), oral route (Annex IX, Section 8.6.2.; test method: OECD TG 408) in rats with the registered substance;**

3. **Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.; test method: OECD TG 414) in a first species (rat or rabbit), oral route with the registered substance;**

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

You are required to submit the requested information in an updated registration dossier by **30 August 2021**. You shall also 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.

This decision does not address the information requirement of the Extended one-generation reproductive toxicity study according to Annex X, Section 8.7.3. of the REACH Regulation.

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 C4

[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

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. **Transgenic rodent somatic and germ cell gene mutation assays (Annex X, Section 8.4., column 2) OR *In vivo* mammalian alkaline comet assay (Annex X, Section 8.4., column 2)**

"Mutagenicity" is an information requirement as laid down in Annex VIII, Section 8.4. of the REACH Regulation. Column 2 of Annex X, Section 8.4. provides that "If there is a positive result in any of the *in vitro* genotoxicity studies in Annex VII or VIII and there are no results available from an *in vivo* study already, a second *in vivo* somatic cell test may be necessary, depending on the quality and relevance of all the available data."

The technical dossier contains the following *in vitro* studies:

- Experimental study by [redacted] (1988). Bacterial reverse mutation test performed according to a guideline similar to OECD TG 471 and 472 with the registered substance. Results showed clear evidence of mutagenic activity with and without metabolic activation. You flagged this study as "key study".

- Experimental study by [redacted], (1984). Bacterial reverse mutation test performed according to OECD TG 471 with the registered substance. Results were positive with and without metabolic activation. You flagged this study as "key study".

- Published studies by HLS, (1984), Hachiya, (1987) and Hachiya, (1987). These were not fully reported in the technical dossier, but all were positive in bacterial reverse mutation tests. You flagged these studies as "supporting studies".

- Experimental study by [redacted] (1989). *In vitro* chromosomal aberration test performed according to OECD TG 473 with the registered substance. Results showed evidence of both clastogenic and polyploidy-inducing activity. You flagged this study as "key study".

- Experimental study by [redacted] (1984). *In vitro* gene mutation on mammalian cells conducted according to a protocol similar to OECD TG 476 with the registered substance. Results were negative. You flagged this study as "key study".

The positive results obtained in the five *in vitro* gene mutation studies in bacteria and in the *in vitro* chromosomal aberration test ([redacted] 1989) indicate that the substance is inducing gene mutations and chromosomal aberrations under the conditions of the tests. The technical dossier also contains two *in vivo* studies performed according to OECD TG 474 [redacted] 1988) and OECD TG 475 ([redacted] 1984) with the registered substance. Both studies show negative results. ECHA considers that these studies are adequate to follow-up on the positive results obtained in the *in vitro* chromosomal aberration test [redacted] 1989). However, an appropriate second *in vivo* genotoxicity study to follow up the concern on gene mutations is not available in the dossier but is necessary to

meet the information requirements. Consequently there is an information gap and it is necessary to provide information for this endpoint.

According to the ECHA *Guidance on information requirements and chemical safety assessment* (version 6.0, July 2017) Chapter R.7a, Section R.7.7.6.3, the transgenic rodent somatic and germ cell gene mutation assays ("TGR assay", OECD TG 488) and the *in vivo* mammalian alkaline comet assay ("comet assay", OECD TG 489) are suitable to follow up a positive *in vitro* result on gene mutation.

In case you decide to perform the TGR assay according to the test method EU B.58/OECD TG 488, the test shall be performed in transgenic mice or rats and the substance is usually administered orally.

The test shall be performed by analysing tissues from the liver as slowly proliferating tissue and primary site of xenobiotic metabolism, and glandular stomach and duodenum as rapidly proliferating tissue and site of direct contact. There are several expected or possible variables between the glandular stomach and the duodenum (different tissue structure and function, different pH conditions, variable physico-chemical properties and fate of the substance, and probable different local absorption rates of the substance and its possible breakdown product(s)). In light of these expected or possible variables, it is necessary to analyse both tissues to ensure a sufficient evaluation of the potential for mutagenicity at the site of contact in the gastro-intestinal tract. However, duodenum shall be stored (at or below -70 ºC) until the analysis of liver and glandular stomach is completed; the duodenum shall then be analysed only if the results obtained for the glandular stomach and for the liver are negative or inconclusive. Moreover, ECHA notes that according to the OECD TG 488 the tissues (or tissue homogenates) can be stored under specific conditions and used for DNA isolation for up to 5 years. Hence, in order to limit additional animal testing male germ cells shall be collected at the same time as the other tissues (liver, glandular stomach and duodenum), and stored up to 5 years (at or below -70 ºC). This duration is sufficient to allow you or ECHA, in accordance to Annex X, Section 8.4., column 2, to decide on the need for assessment of mutation frequency in the collected germ cells. This type of evidence may be relevant for the overall assessment of possible germ cell mutagenicity including classification and labelling according to the CLP Regulation.

In case you decide to perform the comet assay according to the test method OECD TG 489, the test shall be performed in rats. Having considered the anticipated routes of human exposure and adequate exposure of the target tissue(s), performance of the test by the oral route is appropriate.

The test shall be performed by analysing tissues from the liver as primary site of xenobiotic metabolism, and glandular stomach and duodenum as sites of contact. There are several expected or possible variables between the glandular stomach and the duodenum (different tissue structure and function, different pH conditions, variable physico-chemical properties and fate of the substance, and probable different local absorption rates of the substance and its possible breakdown product(s)). In light of these expected or possible variables, it is necessary to analyse both tissues to ensure a sufficient evaluation of the potential for genotoxicity at the site of contact in the gastro-intestinal tract.

In your comments to the draft decision, you indicated that the data in the dossier does not clearly describe the genotoxicity potential of the registered substance. In addition, you stated that the OECD 474/475/476 studies are useful in gaining an understanding of the

genotoxicity potential of the registered substance, and you propose to amend the corresponding IUCLID entries.

As explained above, there is no *in vivo* genotoxicity study to address the information requirement for gene mutation originating from the positive *in vitro* gene mutation tests. Therefore an *in vivo* genotoxicity study is requested to meet the information requirements.

Furthermore, ECHA will not take dossier updates into account during the decision making. Any new data will be taken into account in the follow-up evaluation according to Article 42 of REACH.

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:

Transgenic rodent somatic and germ cell gene mutation assays (test method: EU B.58/OECD TG 488) in transgenic mice or rats, oral route on the following tissues: liver and glandular stomach, germ cells and duodenum shall be harvested and stored for up to 5 years. Duodenum shall be analysed if the results of the glandular stomach and of the liver are negative or inconclusive. The test material used should be freshly prepared.

or

In vivo mammalian alkaline comet assay (test method: OECD TG 489) in rats, oral route, on the following tissues: liver, glandular stomach and duodenum.

Notes for your consideration

You are reminded that according to Annex X, Section 8.4., column 2 of the REACH Regulation, if positive results from an *in vivo* somatic cell study are available, the potential for germ cell mutagenicity should be considered on the basis of all available data, including toxicokinetic evidence. If no clear conclusions about germ cell mutagenicity can be made, additional investigations shall be considered.

In case you decide to perform the comet assay, you may consider examining gonadal cells in addition to the other aforementioned tissues, as it would optimise the use of animals. ECHA notes that a positive result in whole gonads is not necessarily reflective of germ cell damage since gonads contain a mixture of somatic and germ cells. However, such positive result would indicate that the substance and/or its metabolite(s) have reached the gonads and caused genotoxic effects. This type of evidence may be relevant for the overall assessment of possible germ cell mutagenicity including classification and labelling according to the CLP Regulation.

2. Sub-chronic toxicity study (90-day), oral route (Annex IX, Section 8.6.2.)

A "sub-chronic toxicity study (90 day)" is a standard information requirement as laid down in Annex IX, Section 8.6.2. of the REACH Regulation. Adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

In the technical dossier you provided the following information:

- Published study by Medinsky (1990). This is a sub-chronic inhalation study in rats with the registered substance. NOAEC was considered to be 200 mg/m^3. In lower dose groups, males rats had small but significant increase in sperm counts and an increase in T3/T4 levels. You flagged this study as "key".

- Published study by Medinsky (1990). This is a sub-chronic inhalation study in mice with the registered substance. NOAEC was considered to be 100 mg/m^3. No other effects noted. You flagged this study as "key".

- Published study *Oser et al.*, (1965). Chronic one year feeding study in rats with registered substance and metabolite (biurea). NOAEL considered to be 10% in diet, (5714 mg/kg bw/day). You flagged this study as "key".

- Published study Oser *et al.*, (1965). Combined oral repeat dose toxicity and carcinogenicity two-year feeding study in rats with the registered substance and metabolite. NOAEL considered to be 7.5 ppm for registered substance and NOAEL for metabolite (biurea) 7500 ppm (450 mg biurea mg/kg bw/day). You flagged this study as "key".

None of the oral studies provided in the technical dossier can be considered as equivalent of a Sub-chronic toxicity study (90-days; according to OECD TG 408). The published studies by Oser (1965) are non-GLP, non-guideline studies; they do not include all the key parameters foreseen to be investigated in an OECD TG 408; in addition the studies were conducted with a metabolite and an unconventional route of administration (i.e. bread baked from flour treated with the registered substance) was performed.

The Medinsky (1990) inhalation studies, although conducted similar to OECD TG 413 have the following issues and therefore do not sufficiently address this endpoint:

- Dose selection used in the definitive study based on the range finder may not be adequate. Ideally, the highest of three dose levels should be chosen with the aim to induce toxicity but not death. The range finder did not identify a high enough dose suitable for the definitive study since no/little toxicity induced. The studies do not allow clear target organs to be identified or a gradation of toxic effects between the low and high dose groups.
- Based on the results of the rat study, there seems to be sperm effects (increase in sperm count) in 50 and 100 mg/m^3 dose groups which are not addressed. Further, a dose-dependent increase with respect to T3 and T4 levels observed but not addressed.
- No clinical chemistry parameters were measured and therefore it is difficult to assess the relevance of the changes in T3, T4 levels seen in rats but not in mice.
- The mice study performed with an insufficient number of animals compared to the recommended OECD TG and some key parameters were not examined such as food/water consumption and ophthalmoscopic examinations. Further, not all the standard clinical pathology parameters were investigated as recommended in OECD TG.

ECHA concludes that you have not provided any study record of a sub-chronic toxicity study (90 day) in the dossier that would meet the information requirement of Annex IX, Section 8.6.2.

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.

ECHA has evaluated the most appropriate route of administration for the study. Based on the information provided in the technical dossier and/or in the chemical safety report, ECHA considers that the oral route - which is the preferred one as indicated in ECHA *Guidance on information requirements and chemical safety assessment* (version 6.0, July 2017) Chapter R.7a, Section R.7.5.4.3 - is the most appropriate route of administration.

In your comments to the draft decision you indicated that inhalation is the most likely route of human exposure and that [redacted] mg/m^3 was the highest technically achievable concentration in an acute inhalation study. You further commented that you do not consider the effect on sperm counts as relevant. ECHA acknowledges your comments and considers that the oral route is also an appropriate route, and the preferred route in ECHA Guidance (see reference above), of administration for repeated dose toxicity testing in experimental animals and that the exposure in the sub-chronic inhalation study was limited to 200 mg/m^3, whereas [redacted] mg/m^3 would have been technically possible.

Hence, the test shall be performed by the oral route using the test method OECD TG 408.

According to the test method OECD TG 408 the rat is the preferred species. ECHA considers this species as being appropriate and testing should be performed with the rat.

In your comments to the draft decision you agreed to perform a sub-chronic toxicity study by the oral route according to OECD TG 408, to include a hormone analysis (T3 and T4) and to combine this study with the combined repeated dose toxicity study with the reproduction/ developmental toxicity screening test according to OECD TG 422. You also indicated that inhalation is the most likely route of human exposure.

ECHA notes, that the measurement of thyroid hormones is required in the OECD TG 408 that was updated 25 June 2018. ECHA further notes that it is at your discretion to perform the intended combination with OECD TG 422 as long as it does not interfere with the examinations according to test method OECD TG 408. However, you are reminded that the proposed extension of this study does not fulfil the standard information requirements in the registration dossier for reproductive toxicity set out in Annex IX and X, Section 8.7.2. or 8.7.3. (see below).

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: Repeated dose 90-day oral toxicity study (test method: OECD TG 408) in rats.

Notes for your consideration

The Extended one-generation reproductive toxicity study (EOGRTS) according to Annex X, Section 8.7.3. is not part of this decision because the results of the Sub-chronic toxicity study (90-day) are considered crucial to inform on the study design of the EOGRTS. Therefore, the results of the Sub-chronic toxicity study (90-day) should be used, among other relevant information, to decide on the study design of the EOGRTS.

ECHA may therefore launch a separate compliance check at a later stage addressing the

EOGRTS information requirement.

Alternatively, you may also consider submitting a testing proposal for an Extended one-generation reproductive toxicity study together with the results of the requested Sub-chronic toxicity study (90-day). The testing proposal should include a justification for its study design following ECHA *Guidance on information requirements and chemical safety assessment* Chapter R.7a, Section R.7.6 (version 6.0, July 2017), taking into account the results of the Sub-chronic toxicity study (90-day).

3. Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.) in a first species

A "pre-natal developmental toxicity study" (test method OECD TG 414) for a first species is a standard information requirement as laid down in Annex IX, Section 8.7.2. of the REACH Regulation. Adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

In the technical dossier under this endpoint you have provided the following information: *"Based on the existing data, there is no evidence of reproductive and developmental toxicity. According to the Guidance on Information Requirements and Chemical Safety Assessment (section R7A Reproductive and Developmental toxicity, p370), no further data is required for the assessment of the developmental toxicity of ADCA."*
The existing data that you refer to are as follows:

- Published study (Hatano Research Institute, 2000), One-generation oral study in rats. Results, no alert on reproductive toxicity.
- Published study (Oser *et al.*, 1963, Oser *et al.*, 1965, secondary sources: SIDS 2001, IPCS 1999). Three-generation study in rats. No effect on fertility, reproduction or lactation in any generation.
- Published study (Medinsky, 1990). A 13 week inhalation study in rats and mice caused no significant change in vaginal cytology. In males rats, an increase in sperm count was observed and a dose-dependent increase in T3 and T4 levels. In mice, no effects observed.

None of the studies listed above are designed to investigate developmental toxicity and do not cover the key parameters foreseen to be investigated in a pre-natal developmental toxicity according to OECD TG 414. In particular, the information provided in the technical dossier does not address pertinent information on substance-induced effects on growth and survival of the foetuses, and increased incidences in external, skeletal and soft tissue malformations and variations in foetuses. Therefore, the existing data referred to in your dossier is not adequate for meeting the standard infromation requirement for a pre-natal developmental toxicity study (OECD TG 414; Annex XI, Section 8.7.2).

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.

According to the test method OECD TG 414, the rat is the preferred rodent species and the rabbit the preferred non-rodent species. On the basis of this default assumption ECHA considers testing should be performed with rats or rabbits as a first 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 6.0, July 2017) Chapter R.7a, Section R.7.6.2.3.2. Since the substance to be tested is a solid, ECHA concludes that testing should be performed by the oral route.

In your comments to the draft decision you refer to your proposal to perform a modified OECD 408 study including additional reproductive parameters to fulfil the information requirement for a PNDT study in a first species.

ECHA notes that the proposed modified OECD 408 to include additional parameters of an OECD 422 study cannot be considered equivalent to an OECD 414 study. Key parameters such as induced effects on growth and survival of the foetuses, and increased incidences in external, skeletal and soft tissue malformations and variations in foetuses would not be assessed. Furthermore, the number of animals assessed would not be sufficient leading to inadequate statictical power compared to an OECD 414 study. In addition, information on additional fertility endpoints and hormonal measurements in the dams would not be possible.

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: OECD TG 414) in a first species (rat or rabbit) by the oral route.

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

Pre-natal developmental toxicity studies (test method 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).

As explained above under Issue 3, the technical dossier does not contain information on a pre-natal developmental toxicity study.

According to the test method OECD TG 414, the rat is the preferred rodent species and the rabbit the preferred non-rodent species. On the basis of this default consideration, ECHA considers that testing should be performed with rabbits or rats as a second species, depending on the species tested in the first pre-natal developmental toxicity study.

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 solid, ECHA concludes that testing should be performed by the oral route.

In your comments to the draft decision you indicated that you will address the need for a PNDT study in a second species once the results from the modified OECD 408 study are available.

As explained under request 3 above, the proposed modified OECD 408 study cannot be considered equivalent to an OECD 414 study in a first species, and therefore, a PNDT study

in a second species cannot be adapted based on the proposed modified OECD 408 study. However, the need to perform a PNDT study in a second species is to be considered once the results of the requested PNDT study in a first species are available, as explained below under "Notes for your consideration". The deadline has been set to allow sequential testing.

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: OECD TG 414) in a second species (rabbit or rat) by the oral route.

Notes for your consideration

You are reminded that before performing a pre-natal developmental toxicity study in a second species you must consider the specific adaptation possibilities of Annex X, Section 8.7., column 2 and general adaptation possibilities of Annex XI. If the results of the test in the first species with other available information enable such adaptation, testing in the second species should be omitted and the registration dossier should be updated containing the corresponding adaptation statement.

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 7 August 2018.

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).

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

ECHA received proposals for amendment and did not modify the draft decision.

ECHA invited you to comment on the proposed amendments.

ECHA referred the draft decision to the Member State Committee.

You did not provide comments on the proposed amendment(s).

The Member State Committee reached a unanimous agreement on the draft decision during its MSC-63 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.