EUROPEAN CHEMICALS AGENCY

~~Confidential~~

Helsinki, 11 February 2021

Addressees
Registrant of DETU_Joint_Submission as listed in the last Appendix of this decision

Date of submission of the dossier subject to this decision
29/11/2019

Registered substance subject to this decision ("the Substance")
Substance name: 1,3-diethyl-2-thiourea
EC number: 203-308-5
CAS number: 105-55-5

Decision number: Please refer to the REACH-IT message which delivered this communication (in format CCH-D-XXXXXXXXXX-XX-XX/F)

DECISION ON A COMPLIANCE CHECK

Under Article 41 of Regulation (EC) No 1907/2006 (REACH), you must submit the information listed below, by the deadline of ***20 February 2023*** *from the date of the decision.*

Requested information must be generated using the Substance unless otherwise specified.

A. Information required from all the Registrants subject to Annex VIII of REACH

1. Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.; test method: EU B.63/OECD TG 421 or EU B.64/OECD TG 422) by oral route, in rats.

2. Activated sludge respiration inhibition testing (Annex VIII, Section 9.1.4.; test method: EU C.11/ OECD TG 209).

B. Information required from all the Registrants subject to Annex IX of REACH

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

   OR

   Transgenic rodent somatic and germ cell gene mutation assay (Annex IX, Section 8.4., column 2; test method: OECD TG 488 from 2020[1]) in transgenic mice or rats, oral route on the following tissues: liver and glandular stomach; germ cells and duodenum must be harvested and stored for up to 5 years. Duodenum must be analysed if the results of the glandular stomach and of the liver are negative or inconclusive.

[1] The updated OECD TG 488, adopted on 26 June 2020, is available on OECD website at https://www.oecd-ilibrary.org/docserver/9789264203907-en.pdf?expires=1596539942&id=id&accname=guest&checksum=D552783C4CB0FC8045D04C88EFFBFA66.

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

3. Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.; test method: OECD TG 414) by oral route, in one species (rat or rabbit).

Reasons for the request(s) are explained in the Appendices entitled "Reasons to request information required under Annexes VIII and IX of REACH", respectively.

Information required depends on your tonnage band

You must provide the information listed above for all REACH Annexes applicable to you, and in accordance with Articles 10(a) and 12(1) of REACH:

- the information specified in Annexes VII, VIII and IX to REACH, for registration at 100-1000 tpa

How to comply with your information requirements

To comply with your information requirements you must submit the information requested by this decision in an updated registration dossier by the deadline indicated above. You must also update the chemical safety report, where relevant, including any changes to classification and labelling, based on the newly generated information.

You must follow the general testing and reporting requirements provided under the Appendix entitled "Requirements to fulfil when conducting and reporting new tests for REACH purposes". In addition, you should follow the general recommendations provided under the Appendix entitled "General recommendations when conducting and reporting new tests for REACH purposes". For references used in this decision, please consult the Appendix entitled "List of references".

Appeal

This decision, when adopted under Article 51 of REACH, may be appealed to the Board of Appeal of ECHA within three months of its notification to you. Please refer to http://echa.europa.eu/regulations/appeals for further information.

Failure to comply

If you do not comply with the information required by this decision by the deadline indicated above, ECHA will notify the enforcement authorities of your Member State.

Authorised[2] under the authority of Christel Schilliger-Musset, Director of Hazard Assessment

[2] 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 A: Reasons to request information required under Annex VIII of REACH

1. Screening for reproductive/developmental toxicity

A Screening for reproductive/developmental toxicity study (test method: EU B.63/OECD TG 421 or EU B.64/OECD TG 422) is a standard information requirement under Annex VIII to REACH, if there is no evidence from analogue substances, QSAR or *in vitro* methods that the Substance may be a developmental toxicant. There is no information available in your dossier indicating that your Substance may be a developmental toxicant.

According to Annex VIII, Section 8.7., Column 2, first paragraph, fourth indent, the study does not need to be conducted if a pre-natal developmental toxicity study (OECD TG 414) is already available.

You justified the adaptation by stating that a prenatal developmental toxicity study is available and, therefore an EU B.63/OECD TG 421 or EU B.64/OECD TG 422 study does not need to be conducted.

However, the provided prenatal developmental toxicity (PNDT) studies do not fulfil the information requirement for the PNDT study. As further explained under request B.3. the key study (Saillenfait et al. 1991) is rejected as your grouping and read-across approach fails. In addition, the supporting study ([redacted] 1973) is rejected as your adaptation according to Annex XI, Section 1.2.1. fails as explained under request B.3.

Based on the above, the information you provided do not fulfil the information requirement.

Information on study design

A study according to the test method EU B.63/OECD TG 421 or EU B.64/OECD TG 422 must be performed in rats with oral[3] administration of the Substance.

In your comments on the draft decision, you agree to perform the requested study.

2. Activated sludge respiration inhibition testing

Activated sludge respiration inhibition testing is an information requirement under Annex VIII to REACH (Section 9.1.4.).

You have adapted this information requirement under Section 9.1.4. Column 2 of Annex VIII and you provided the following supporting information: a ready biodegradability study as per OECD 301 D ([redacted], 2011, GLP).

We have assessed this information and identified the following issue:

Under Section 9.1.4., Column 2, third indent, Annex VIII to REACH, the study may be omitted if:

- the substance is found to be readily biodegradable, and
- the applied test concentrations are in the range of concentrations that can be expected in the influent of a sewage treatment plant.

ECHA Guidance Section R.7.8.19.1 further specifies that the information content of ready biodegradability tests can also be used to derive a NOEC when the toxicity control shows

[3] ECHA Guidance R.7a, Section R.7.6.2.3.2.

good degradation of a positive control substance (e.g. glucose, sodium acetate) in the presence of the test substance.

Your dossier provides an OECD TG 301D showing the following:

- 3% biodegradation in 28 days
- 61% biodegradation was reached in the positive control after 28 days,
- 47% biodegradation in the inhibition control after 14 days and no significant biodegradation was observed after day 14 of the inhibtion control test

You have used the results from the inhibition control (toxicity control) to derive a NOEC for activated sludge.

The data provided shows that the substance is not readily biodegradable (3% biodegradation after 28 days).

Therefore, your adaptation is rejected.

In addition, the data of the inhibition control indicates lower biodegradation of the reference substance ("positive control substance") when the test substance (the Substance) is present. As such, the results of the inhibition control with your substance shows some inhibitory effect, contrarily to the positive control (where 61% of biodegradation is reached with the reference substance alone). Therefore the inhibition control cannot be used to derive a valid NOEC.

On this basis, the information requirement is not fulfilled.

In your comments on the draft decision, you agree to perform the requested study.

Appendix B: Reasons to request information required under Annex IX of REACH

1. In vivo mammalian alkaline comet assay; or Transgenic rodent somatic and germ cell gene mutation assays

Under Annex IX to REACH, the information requirement for an appropriate *in vivo* somatic cell genotoxicity study is triggered if 1) there is a positive result in any of the *in vitro* genotoxicity studies in Annex VII or VIII and 2) there are no appropriate results already available from an *in vivo* somatic cell genotoxicity study.

In relation to the first condition, your dossier contains positive results for the *in vitro* gene mutation study in mammalian cells which raises the concern for gene mutation.

In relation to the second condition, your dossier contains the following *in vivo* studies:

i. *In vivo* mammalian erythrocytes micronucleus test according to OECD TG 474 and GLP via oral route in rats, a key study ([redacted] 2011)
ii. *In vivo* DNA fragmentation and DNA repair synthesis (mammalian Comet Assay) via oral route in rats, no test guideline and GLP followed, a key study (Mattioli et al. 2006)
iii. *In vivo* Drosophila sex-linked recessive lethal (SLRL) assay via oral route and by injection in Drosophila melanogaster, no test guideline followed, GLP not specified, a supporting study (Valencia et al. 1985).

We have assessed this information and identified the following issues:

A. Study (i)

Under ECHA Guidance R.7a, in order to justify that an *in vivo* somatic cell genotoxicity study does not need to performed in accordance with Annex IX, Section 8.4, column 2, the results of the available *in vivo* study must address the specific concern raised by the *in vitro* positive result.

However, the *in vivo* study (i) provided is not addressing the gene mutation concern raised by the *in vitro* data.

The provided *in vivo* test is not appropriate to address the concern identified by the *in vitro* gene mutation study in mammalian cells. Therefore, the conditions set out in Annex IX, Section 8.4, column 2 are met and the information requirement for an appropriate *in vivo* somatic cell genotoxicity study is triggered.

B. Study (ii)

To be considered adequate, the study has to meet the requirements of OECD TG 489, and the key parameters of this test guideline include:

a) The study must include a minimum of three doses/groups of treated animals as well as a negative control group and a positive control group.
b) Each group must have a minimum of 5 analysable animals (the test can be performed in either sex).
c) At least 150 cells must be analysed for each sample (per tissue, per animal).
d) Where increases in DNA migration are observed, an examination of one or more indicators of cytotoxicity (e.g. inflammation, cell infiltration, apoptotic or necrotic changes) must be performed, as target tissue toxicity may result in increases in DNA migration.

ECHA acknowledges that you provided an *in vivo* study (ii) "*In vivo DNA fragmentation and DNA repair synthesis (mammalian Comet assay) via oral route in rats"* performed with the Substance (no test guideline followed) in order to follow up the concern for gene mutation raised by the *in vitro* results. However, the above mentioned key parameter(s) are not met, because the reported data for the study do not include:

a) the appropriate number of doses (only 1 dose used) and positive control
b) the appropriate number of analysable animals (only 3 animals)
c) information how many cells were analysed
d) examination of indicator(s) of cytotoxicity

C. Study (iii)

To be considered adequate, the study must meet the requirements of OECD TG 488 or 489, and has to follow the key parameters of these test guidelines, including testing in mammalian cells (rodent species).

However, the information provided in study (iii) relates to tests different from OECD TGs 488 or 489 that do not address gene mutation in mammalian cells. The tests were performed with insects and not with mice or rats (as in OECD TGs 488 and 489).

The provided *in vivo* tests (ii) and (iii) are not adequate. Therefore, the conditions set out in Annex IX, Section 8.4, column 2 are met and the information requirement for an appropriate *in vivo* somatic cell genotoxicity study is triggered.

According to the ECHA Guidance Chapter R.7a[4], the transgenic rodent somatic and germ cell gene mutation assay ("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 comet assay according to the test method OECD TG 489, the test must 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.

In line with the test method OECD TG 489, the test must be performed by analysing tissues from liver as primary site of xenobiotic metabolism, 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 case you decide to perform the TGR assay, according to the test method OECD TG 488, the test must be performed in transgenic mice or rats and the test substance is usually administered orally.

Based on the recent update[5] of OECD TG 488, you are requested to follow the new 28+28d regimen, as it permits the testing of mutations in somatic tissues and as well as in tubule

[4] ECHA Guidance Chapter R.7a, Section R.7.7.6.3
[5] The updated OECD TG 488, adopted on 26 June 2020, is available on OECD website at https://www.oecd-ilibrary.org/docserver/9789264203907-en.pdf?expires=1596539942&id=id&accname=guest&checksum=D552783C4CB0FC8045D04C88EFFBFA66

germ cells from the same animals. This updated version provides for a transitional period for the new version. However, ECHA is aware that testing according to the updated OECD TG is already available from CROs and the new study design would provide meaningful germ cell data, so this decision requires the application of the new version.

According to the test method OECD TG 488, the test must be performed by analysing tissues from liver as slowly proliferating tissue and primary site of xenobiotic metabolism, 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 must be stored (at or below −70 ºC) until the analysis of liver and glandular stomach is completed; the duodenum must then be analysed only if the results obtained for the glandular stomach and for the liver are negative or inconclusive.

Germ cells

A subsequent germ cell genotoxicity study (TGR/OECD TG 488, or CA on spermatogonia/OECD TG 483) may still be required under Annex IX of REACH, in case 1) an *in vivo* genotoxicity test on somatic cell is positive, and 2) no clear conclusion can be made on germ cell mutagenicity.

> Therefore, in case you decide to perform the comet assay, you may consider to collect the male gonadal cells from the seminiferous tubules in addition to the other aforementioned tissues in the comet assay, as it would optimise the use of animals. You can prepare the slides for male gonadal cells and store them for up to 2 months, at room temperature, in dry conditions and protected from light. Following the generation and analysis of data on somatic cells, in accordance to Annex IX, Section 8.4., column 2, you should consider analysing the slides prepared with gonadal 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.
>
> Therefore, in case you decide to perform the TGR, you must collect the male germ cells (from the seminiferous tubules) at the same time as the other tissues, in order to limit additional animal testing. According to the OECD 488, the tissues (or tissue homogenates) can be stored under specific conditions and used for DNA isolation for up to 5 years (at or below −70 ºC). This duration is sufficient to allow you or ECHA, in accordance to Annex IX, 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 your comments on the draft decision, you agree to perform the *in vivo* mammalian alkaline comet assay in rats.

2. Sub-chronic toxicity study (90-day)

A Sub-chronic toxicity study (90 day) is a standard information requirement under Annex IX to REACH.

Although you do not explicitly claim an adaptation, ECHA understands that the information provided was submitted in order to meet the required information by way of adaptation under Annex XI, Section 1.1.2. You have provided the following key studies to support your adaptation:

i. A 7 weeks range-finding study via oral route (the Substance in diet) in mice (no test guideline followed, GLP not specified), a key study ([redacted] 1978);
ii. A 7 weeks range-finding study via oral route (the Substance in diet) in rats (no test guideline followed, GLP not specified), a key study (NCI 1978);

In addition you have also provided an adaptation according to Column 2 of Annex IX, Section 8.6.2. in your dossier. In support of your adaptation, you provided a justification *"A sub-chronic toxicity study (90 days) does not need to be conducted because a reliable chronic/carcinogenicity toxicity study is available, conducted with an appropriate species (rat and mice) and appropriate route of exposure (oral)."* and the following key and supporting studies:

iii. A 103 week carcinogenicity study via oral route (the Substance in diet) in mice (no test guideline followed, GLP not specified), a key study [redacted] 1978);
iv. A 103 week carcinogenicity study via oral route (the Substance in diet) in rats (no test guideline followed, GLP not specified), a key study ([redacted] 1978);
v. A 52 week study via oral route (the Substance in diet) in male rats (no test guideline and GLP followed), a supporting study [redacted] 1991).

Moreover in your comments to the draft decision you indicate that the 90-day study is not required because *"carcinogenicity studies (exposure de 24 months) are available to evaluate the carcinogen potential of the registered substance"* and *"the DNELs were derived from the carcinogenicity studies (longer studies than sub-chronic)."*

Furthermore, in your comments on the draft decision, you provide an additional justification for Column 2 adaptation:

"a sub-chronic toxicity study (90 days) does not need to be conducted because a reliable short-term toxicity study (28 days) is available showing severe toxicity effects according to the relevant criteria or classifying the substance, for which the observed NOAEL-28 days, with the application of an appropriate uncertainty factor, allows the extrapolation towards he NOAEL-90 days for the same route of exposure".

We have assessed this information and identified the following issues:

A. The adaptation rule in Annex XI, Section 1.1.2 imposes a number of cumulative conditions for an adaptation to be valid, in particular:

1. Adequate and reliable coverage of the key parameters foreseen to be investigated in the corresponding test methods referred to in Article 13(3), in this case OECD TG 408. The key parameters of this test guideline include, among others: testing of at least three dose levels and a concurrent control in 10 animals/sex/group, and clinical observations, ophthalmological examination, sensory reactivity to various stimuli and functional observations of the animals, recording of body weight, hematology, clinical

biochemistry, and pathology of sexual (male and female) organs, Full detailed gross necropsy and subsequent histopathology of both types tissues;
2. Exposure duration comparable to or longer than the corresponding test methods referred to in Article 13(3);
3. Adequacy for the purpose of classification and labelling and/or risk assessment.

The above key parameters of an OECD TG 408 are not met by the provided studies (i) and (ii), because the number of animals examined per dose group (5 animals/sex/dose) is significantly lower than that required by *Sub-chronic toxicity 90-day study (10 animals/sex/dose)*. In addition, organ weights were not recorded at all. Therefore, the provided studies do not have a full detailed gross necropsy. Furthermore, opthalmological and haematological examinations, and sensory reactivity to various stimuli and functional observations, and also clinical biochemistry examination were not conducted as required by OECD TG 408. In addition, the exposure duration of the provided studies (i) and (ii) is less (49 days) than the required 90 days for a *Sub-chronic toxicity study*.

Based on the above, the provided information cannot be considered to be adequate for classification and labelling and/or risk assessment.

Therefore, your adaptation according to Annex XI, Section 1.1.2. is rejected and the information requirement is not fulfilled.

B. As provided in Annex IX, Section 8.6.2, Column 2, you may adapt the information requirement, provided you fulfil one of the following criteria:
   - a reliable chronic toxicity study conducted in an appropriate species and route of administration is available
   - a reliable short-term toxicity study (28-day) is available and shows severe toxicity effects leading to the classification of the Substance, and where the NOAEL-90 days can be extrapolated for the same route of exposure

Reliable chronic study

To be considered compliant and enable concluding whether the Substance has dangerous properties and supports the determination of the No-Observed Adverse Effect Level (NOAEL), a chronic study has to meet the requirements of OECD TG 452. The following key parameter(s) of this test guideline include, among others:

- testing of at least three dose levels and a concurrent control
- At least 20 female and 20 male animals should be used at each dose level (including control group)
- Clinical observations, ophthalmological examination, recording of body weight, hematology, clinical biochemistry, full detailed gross necropsy, recording of organ weights and histopathology

The studies (iii) – (v) you have provided were not performed according to the criteria of the OECD TG 452, since the following key parameters are missing:

- The studies were conducted with less than three dose levels, i.e. (iii) and (iv) had two dose levels and (v) had only one dose level.
- In (v), only male rats were used.
- In (iii) and (iv), organ weights were not recorded at all, and in (v) only weights of liver kidney and thyroid were recorded. Therefore, none of the studies has a full detailed gross necropsy.
- In (iii) – (v), opthalmological and haematological examinations were not

conducted.
- In (iii) and (iv), clinical biochemistry examination was not conducted. Also in (v), clinical biochemistry was deficient as it only included a measurement of thyroxine (T4).

As explained above, the chronic toxicity studies (iii) – (v) you provided are not considered compliant, in particular you failed to provide information that meets the requirements of OECD TG 452 to enable concluding whether the Substance has dangerous properties and the determination of the No-Observed Adverse Effect Level (NOAEL).

Reliable short-term toxicity study and classification of the Substance

To be considered compliant and enable concluding whether the Substance has dangerous properties and supports the determination of the NOAEL, a short-term toxicity study has to meet the requirements of OECD TG 407. The following key parameter(s) of this test guideline include, among others:

- Examination of haematology and clinical biochemistry parameters
- Gross necropsy and histopathology

The studies (i) and (ii) you have provided were not performed according to the criteria of the OECD TG 407, since the following key parameters are missing:

- Examination of haematology and clinical biochemistry parameters.
- A full detailed gross necropsy (i.e. organ weights were not recorded at all) and histopathology.

In addition, in your comments on the draft decision, you refer to studies (i) and (ii) and state that "*The objective of these studies in rats and mice was to establish the maximum tolerated concentrations for the long-term toxicity studies. However, the data are sufficient to identify thyroid as a target organ in rats*". Therefore, you apply self-classification as STOT RE 1 (H372, thyroid).

As explained above, the studies (i) and (ii) you provided are not considered compliant, in particular you failed to provide information that meets the requirements of OECD TG 407 to enable concluding whether the Substance has dangerous properties, the determination of the NOAEL, and extrapolation of a reliable NOAEL-90-days.

The self-classification as STOT RE 1 is based on the thyroid effects observed in carcinogenicity study in rats (iv). According to ECHA Guidance[6] (Section 3.9.2.), STOT RE classification based on severe toxic effects in experimental animals must have relevance to human health. The observed observed thyroid effects in rats (iv) are usually not considered relevant to humans (Bartsch et al. 2018[7]). In addition, as already explained above, the study (iv) is not compliant. Therefore, also the classification to STOT RE is not reliable and your adaptation is rejected.

Therefore, the provided information is not reliable and your adaptation is rejected.

Based on the above, the information you provided does not fulfil the information requirement.

[6] ECHA Guidance on the Application of the CLP criteria. Version 5.0 (July 2017).
[7] Bartsch et al. (2018) Human relevance of follicular thyroid tumors in rodents caused by non-genotoxic substances. Regul. Toxicol. Pharmacol. 98:199-208.

Information on the design of the study to be performed

Referring to the criteria provided in Annex IX, Section 8.6.2, Column 2, the oral route is the most appropriate route of administration to investigate repeated dose toxicity, because the Substance is reported to occur as a powder of low dustiness without a significant proportion (> 1% on weight basis) of particles of inhalable size (MMAD < 50 µm).

Therefore the sub-chronic toxicity study must be performed according to the OECD TG 408, in rats and with oral administration of the Substance

3. Pre-natal developmental toxicity study in one species

A Pre-natal developmental toxicity (PNDT) study (OECD TG 414) in one species is a standard information requirement under Annex IX to REACH.

You have provided an adaptation according to Annex XI, Section 1.5. and the following key study to support your adaptation:

i. A PNDT study similar to OECD TG 414 via oral route in rats with an analogue substance, 1,3-dimethyl-2-thiourea (EC No. 208-588-2) ([redacted] 1991);

In addition, you have provided the following supporting study. Although you do not explicitly claim an adaptation, ECHA understands that the provided supporting study was submitted in order to meet the required information by way of adaptation under Annex XI, Section 1.1.2.

ii. A developmental toxicity study via dermal route in rats with the Substance, no test guideline and GLP followed [redacted] 1973).

We have assessed this information and identified the following issues:

A. Annex XI, Section 1.5. specifies two conditions which must be fulfilled whenever a read-across approach is used. Firstly, there needs to be structural similarity between substances which results in a likelihood that the substances have similar physicochemical, toxicological and ecotoxicological properties so that the substances may be considered as a group or category. Secondly, it is required that the relevant properties of a substance within the group may be predicted from data for reference substance(s) within the group.

   Additional information on what is necessary when justifying a read-across approach can be found in the ECHA Guidance[8] and related documents[9, 10].

   For the the study (i), you have provided a read-across justification documents "Read-across justification between DETU and DMTU – A QSAR Toolbox category of chemicals" and "Justification of read-across" in IUCLID Section 7.8.2.

[8] Guidance on information requirements and chemical safety assessment Chapter R.6: QSARs and grouping of Chemicals. 2008 (May) ECHA, Helsinki. 134. pp. Available online: https://echa.europa.eu/documents/10162/13632/information_requirements_r6_en.pdf/77f49f81-b76d-40ab-8513-4f3a533b6ac9

[9] Read-Across Assessment Framework (RAAF). 2017 (March) ECHA, Helsinki. 60 pp. Available online: Read-Across Assessment Framework (https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across)

[10] Read-across assessment framework (RAAF) - considerations on multi-constituent substances and UVCBs. 2017 (March) ECHA, Helsinki. 40 pp. Available online: https://doi.org/10.2823/794394

You read-across between the structurally similar substances, 1,3-dimethyl-2-thiourea, EC No. 208-588-2 (CAS No. 534-13-4) as source substance and the Substance as target substance.

You have provided the following reasoning for the prediction of toxicological properties:

- *"The analogue approach is based on read-across to substances with the same chemical structure (thiourea), similar physicochemical properties and similar toxicological profiles."*
- *"Hypothesis is that a read-across between 1,3-diethyl-2-thiourea (DETU, 105-55-5) and 1,3-dimethyl-2-thiourea (DMTU, 534-13-4) is possible."*
- *"DETU has a molecular weight more higher than DMTU and therefore DETU is probably less toxic than DMTU."*

ECHA understands that you predict the properties of the Substance using a read-across hypothesis which assumes that different compounds have the same type of effects. The properties of your Substance are predicted based on a worst-case approach.

ECHA notes the following shortcomings with regards to prediction of toxicological properties.

Read-across hypothesis

A read-across hypothesis needs to be provided, establishing why a prediction for a toxicological or ecotoxicological property is reliable. This hypothesis should be based on recognition of the structural similarities and differences between the source substance(s) and your Substance[11]. It should explain why the differences in the chemical structures should not influence the toxicological/ ecotoxicological properties or should do so in a regular pattern.

Your read-across hypothesis is that the similarity in chemical structure and in some of the physicochemical and toxicological properties between the source substance and your Substance is a sufficient basis for predicting the properties of your Substance for other endpoints.

Similarity in chemical structure and similarity of some of the physicochemical and toxicological properties does not necessarily lead to predictable or similar human health properties in other endpoints. As described above, a well-founded hypothesis is needed to establish a reliable prediction for a toxicological property, based on recognition of the structural similarities and differences between the source substance and your Substance. You have not explained why the differences in the chemical structure should not influence the prediction of the properties of the Substance.

Supporting information

Annex XI, Section 1.5 of the REACH Regulation states that *"physicochemical properties, human health effects and environmental effects or environmental fate may be predicted from data for reference substance(s)"*. For this purpose *"it is important to provide supporting information to strengthen the rationale for the read-across"*[12].

[11] *Guidance on information requirements and chemical safety assessment*, Chapter R.6: QSARs and grouping of chemicals.
[12] Guidance on information requirements and chemical safety assessment Chapter R.6: QSARs and grouping of Chemicals, Section R.6.2.2.1.f

The set of supporting information should allow to verify the crucial aspects of the read-across hypothesis and establish that the properties of the Substance can be predicted from the data on the source substance(s).

Supporting information must include supporting information to compare properties of the Substance and source substance or information to confirm your claimed worst-case prediction.

As indicated above, your read-across hypothesis is based on the assumption that the source substance constitutes a worst-case for the prediction of the property under consideration of the Substance. In this context, relevant, reliable and adequate information allowing to compare the properties of the Substance and of the source substance(s) is necessary to confirm a conservative prediction of the properties of the Substance from the data on the source substance(s). Such information can be obtained, for example, from bridging studies of comparable design and duration for the Substance and of the source substance(s).

Based on your statement "*DETU has a molecular weight more higher than DMTU and therefore DETU is probably less toxic than DMTU*" ECHA understands that you consider that the source substance constitutes a worst-case for the prediction of the property.

You have provided the following information to compare toxicological properties of the Substance and the source substance:

- *"For local effects, DETU is not irritating for skin and eyes, however DMTU is highly irritating for skin but not irritating for eyes."*
- *"DETU is a skin sensitizer, but no data on skin sensitisation are available on DMTU."*
- *"For systemic effects, both substances are harmful by oral route, with a LD50 of 930 mg/kg bw for DETU and 1300-1600 mg/kg bw for DMTU."*
- *"DETU is harmful by dermal route (LD50 = 2000 mg/kg bw) and induces toxicity on thyroid after a repeated exposure by oral route."*
- *"In the oral 17-week study, male and female rats were exposed in the diet to DETU and showed thyroid toxicity at 125 ppm (=6.25 mg/kg bw)."*
- *"No data are available on DMTU on dermal acute toxicity, and on repeated toxicity."*
- *"Concerning the reproduction endpoint, no reliable data are available on DETU; however a developmental study on rats is available on DMTU."*

You have provided a very limited information to compare the toxicological properties of the source substance and the Substance. No bridging studies of comparable design and duration on repeated dose and reproductive toxicity are available. In addition, the LD50 values from acute oral toxicity studies do not support the assumption that the source substance constitutes a worst-case for the prediction of toxicological properties of the Substance.

Therefore, the data set reported in the technical dossier does not include such relevant, reliable and adequate information for the Substance and of the source substance to support your read-across hypothesis.

In the absence of such information, you have not established that the source substance constitutes a worst-case for the prediction of the property under consideration of the

Substance. Therefore you have not provided sufficient supporting information to strengthen the rationale for the read-across.

As explained above, you have not established that relevant properties of the Substance can be predicted from data on the analogue substance. Therefore, your adaptation does not comply with the general rules of adaptation as set out in Annex XI, Section 1.5. and your grouping and read-across approach is rejected.

B. The adaptation rule in Annex XI, Section 1.1.2 imposes a number of cumulative conditions for an adaptation to be valid, in particular:

1. Adequate and reliable coverage of the key parameters foreseen to be investigated in the corresponding test methods referred to in Article 13(3), in this case OECD TG 414. The key parameters of this test guideline include, among others: testing 20 female animals with implantation sites for each test and control group, and dosing of the Substance from implantation until the day prior to scheduled caesarean section;
2. Exposure duration comparable to or longer than the corresponding test methods referred to in Article 13(3);
3. Adequacy for the purpose of classification and labelling and/or risk assessment.

The above key parameters of an OECD TG 414 are not met by the provided study (ii), because it was conducted with only 4 pregnant females for each test group. The statistical power of the information is not sufficient because it does not fulfil the criterion of 20 pregnant females for each test group set in OECD TG 414. In addition, the animals were exposed only on GD 12 (single exposure). Therefore, the study (ii) does not have a required exposure duration because the exposure is not from implantation until the day prior to scheduled caesarean section as required in OECD TG 414.

Based on the above, the provided information cannot be considered to be adequate for classification and labelling and/or risk assessment.

Therefore, your adaptation according to Annex XI, Section 1.2.1. is rejected.

Based on the above, the information you provided do not fulfil the information requirement.

A PNDT study according to the test method OECD TG 414 must be performed in rat or rabbit as preferred species with oral[13] administration of the Substance.

In your comments on the draft decision, you agree to perform the requested study if no classification as Repr. 1B (H360D) is applied based on reproduction screening test.

[13] ECHA Guidance R.7a, Section R.7.6.2.3.2.

Appendix C: Requirements to fulfil when conducting and reporting new tests for REACH purposes

A. Test methods, GLP requirements and reporting

1. Under Article 13(3) of REACH, all new data generated as a result of this decision must be conducted according to the test methods laid down in a European Commission Regulation or to international test methods recognised by the Commission or ECHA as being appropriate.

2. Under Article 13(4) of REACH, ecotoxicological and toxicological tests and analyses must be carried out according to the GLP principles (Directive 2004/10/EC) or other international standards recognised by the Commission or ECHA.

3. Under Article 10(a)(vi) and (vii) of REACH, all new data generated as a result of this decision must be reported as study summaries, or as robust study summaries, if required under Annex I of REACH. See ECHA Practical Guide on How to report robust study summaries[14].

B. Test material

Before generating new data, you must agree within the joint submission on the chemical composition of the material to be tested (Test Material) which must be relevant for all the registrants of the Substance.

1. Selection of the Test material(s)

   The Test Material used to generate the new data must be selected taking into account the following:
   - the variation in compositions reported by all members of the joint submission,
   - the boundary composition(s) of the Substance,
   - the impact of each constituent/ impurity on the test results for the endpoint to be assessed. For example, if a constituent/ impurity of the Substance is known to have an impact on (eco)toxicity, the selected Test Material must contain that constituent/ impurity.

2. Information on the Test Material needed in the updated dossier
   - You must report the composition of the Test Material selected for each study, under the "Test material information" section, for each respective endpoint study record in IUCLID.
   - The reported composition must include all constituents of each Test Material and their concentration values and other parameters relevant for the property to be tested.

This information is needed to assess whether the Test Material is relevant for the Substance and whether it is suitable for use by all members of the joint submission.

Technical instructions on how to report the above is available in the manual on How to prepare registration and PPORD dossiers[15].

[14] https://echa.europa.eu/practical-guides
[15] https://echa.europa.eu/manuals

Appendix D: Procedure

The Substance is listed in the Community rolling action plan (CoRAP) for the start of substance evaluation in 2019.

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

ECHA followed the procedure detailed in Articles 50 and 51 of REACH.

The compliance check was initiated on 23 August 2019.

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

ECHA invited you to comment on the proposed amendment(s) and referred the modified draft decision to the Member State Committee.

You informed that you had no comments on the proposed amendment(s).

The Member State Committee reached a unanimous agreement on the draft decision in its MSC-73 written procedure and ECHA took the decision according to Article 51(6) of the REACH Regulation.

Appendix E: List of references - ECHA Guidance[16] and other supporting documents

Evaluation of available information
Guidance on information requirements and chemical safety assessment, Chapter R.4 (version 1.1., December 2011), referred to as ECHA Guidance R.4 where relevant.

QSARs, read-across and grouping
Guidance on information requirements and chemical safety assessment, Chapter R.6 (version 1.0, May 2008), referred to as ECHA Guidance R.6 where relevant.

Read-across assessment framework (RAAF, March 2017)[17]

RAAF - considerations on multiconstituent substances and UVCBs (RAAF UVCB, March 2017)[17]

Physical-chemical properties
Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Toxicology
Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7c (version 3.0, June 2017), referred to as ECHA Guidance R.7c in this decision.

Environmental toxicology and fate
Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7b (version 4.0, June 2017), referred to as ECHA Guidance R.7b in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7c (version 3.0, June 2017), referred to as ECHA Guidance R.7c in this decision.

PBT assessment
Guidance on information requirements and chemical safety assessment, Chapter R.11 (version 3.0, June 2017), referred to as ECHA Guidance R.11 in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.16 (version 3.0, February 2016), referred to as ECHA Guidance R.16 in this decision.

Data sharing
Guidance on data-sharing (version 3.1, January 2017), referred to as ECHA Guidance on data sharing in this decision.

[16] https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment
[17] https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across

OECD Guidance documents[18]
Guidance Document on aqueous-phase aquatic toxicity testing of difficult test chemicals – No 23, referred to as OECD GD 23.

Guidance document on transformation/dissolution of metals and metal compounds in aqueous media – No 29, referred to as OECD GD 29.

Guidance Document on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption – No 150, referred to as OECD GD 150.

Guidance Document supporting OECD test guideline 443 on the extended one-generation reproductive toxicity test – No 151, referred to as OECD GD 151.

[18] http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm

Appendix F: Addressees of this decision and the corresponding information requirements applicable to them

You must provide the information requested in this decision for all REACH Annexes applicable to you.

Registrant Name	Registration number	Highest REACH Annex applicable to you
[redacted]	[redacted]	[redacted]

Where applicable, the name of a third party representative (TPR) may be displayed in the list of recipients whereas ECHA will send the decision to the actual registrant.