BUREAU FOR CHEMICAL SUBSTANCES

SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

Substance name: 1,3-diethyl-2-thiourea (DETU)

EC No 203-308-5

CAS No 105-55-5

Evaluating Member State: Bureau for Chemical Substances, Poland

Dated: 27 November 2020

Template Version 2.1
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Evaluating Member State Competent Authority

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Year of evaluation in CoRAP: 2019

Member State concluded the evaluation without any further need to ask more information from the registrants under Article 46(1) decision.

Further information on registered substances here:

http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances

DISCLAIMER

This document has been prepared by the evaluating Member State as a part of the substance evaluation process under the REACH Regulation (EC) No 1907/2006. The information and views set out in this document are those of the author and do not necessarily reflect the position or opinion of the European Chemicals Agency or other Member States. The Agency does not guarantee the accuracy of the information included in the document. Neither the Agency nor the evaluating Member State nor any person acting on either of their behalves may be held liable for the use which may be made of the information contained therein. Statements made or information contained in the document are without prejudice to any further regulatory work that the Agency or Member States may initiate at a later stage.

Foreword

Substance evaluation is an evaluation process under REACH Regulation (EC) No. 1907/2006. Under this process the Member States perform the evaluation and ECHA secretariat coordinates the work. The Community rolling action plan (CoRAP) of substances subject to evaluation, is updated and published annually on the ECHA web site[1].

Substance evaluation is a concern driven process, which aims to clarify whether a substance constitutes a risk to human health or the environment. Member States evaluate assigned substances in the CoRAP with the objective to clarify the potential concern and, if necessary, to request further information from the registrant(s) concerning the substance. If the evaluating Member State concludes that no further information needs to be requested, the substance evaluation is completed. If additional information is required, this is sought by the evaluating Member State. The evaluating Member State then draws conclusions on how to use the existing and obtained information for the safe use of the substance.

This Conclusion document, as required by Article 48 of the REACH Regulation, provides the final outcome of the Substance Evaluation carried out by the evaluating Member State. The document consists of two parts i.e. A) the conclusion and B) the evaluation report. In the conclusion part A, the evaluating Member State considers how the information on the substance can be used for the purposes of regulatory risk management such as identification of substances of very high concern (SVHC), restriction and/or classification and labelling. In the evaluation report part B the document provides explanation how the evaluating Member State assessed and drew the conclusions from the information available.

With this Conclusion document the substance evaluation process is finished and the Commission, the Registrant(s) of the substance and the Competent Authorities of the other Member States are informed of the considerations of the evaluating Member State. In case the evaluating Member State proposes further regulatory risk management measures, this document shall not be considered initiating those other measures or processes. Further analyses may need to be performed which may change the proposed regulatory measures in this document. Since this document only reflects the views of the evaluating Member State, it does not preclude other Member States or the European Commission from initiating regulatory risk management measures which they deem appropriate.

[1] http://echa.europa.eu/regulations/reach/evaluation/substance-evaluation/community-rolling-action-plan

Contents

Part A. Conclusion

1. CONCERN(S) SUBJECT TO EVALUATION

1,3-diethyl-2-thiourea (DETU) was originally selected for substance evaluation in order to clarify concerns about:

- suspected carcinogen,
- suspected skin sensitiser,
- wide dispersive use,
- consumer use
- exposure of workers,
- exposure of environment.

During the evaluation also other concerns were identified. The additional concerns were:

- eye damage
- suspected Mutagenicity.

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

Two dossier evaluation decisions have been issued by ECHA:

One TPE: https://echa.europa.eu/documents/10162/30f50b08-f451-4d59-e100-32548d4a80bc

One CCH (ongoing): https://echa.europa.eu/pl/information-on-chemicals/dossier-evaluation-status/-/dislist/details/0b0236e183b7edc2

3. CONCLUSION OF SUBSTANCE EVALUATION

The evaluation of the available information on the substance has led the evaluating Member State to the following conclusions, as summarised in the table below.

Table 1

CONCLUSION OF SUBSTANCE EVALUATION Conclusions	Tick box
Need for follow-up regulatory action at EU level	X
Harmonised Classification and Labelling	X
Identification as SVHC (authorisation)	
Restrictions	
Other EU-wide measures	
No need for regulatory follow-up action at EU level	

Initial concern for consumer use was removed based on the data submitted in the updated registration dossier as no consumer uses identified. However, consumer exposure via use of articles is possible.

After finalising the substance evaluation, the evaluating Member State Competent Authority (eMSCA) concluded that provided data can be considered as limited evidence of

carcinogenicity in animal studies. Therefore DETU warrants classification as Carc. 2 with hazard statement H351: Suspected of causing cancer. In addition, the eMSCA considers that harmonised classification is also needed for some other hazards as explained under Section 4.1.1.
Regarding the exposure concern and wide dispersive use, the environmental exposure assessment cannot be concluded as complete as the safety assessment carried out does not cover all identified uses of the substance. Moreover, the available use information and the exposure data provided in the registration dossier suggested risk for the workers and the environment. Thus, the eMSCA recommends revision of the exposure assessment for workers and environment, as explained in Section 7.12.1 and Section 7.12.2, respectively. Although no consumer uses of DETU were identified, the eMSCA recommends that any measure to eliminate exposure via articles should be considered.

These conclusions were based on the originally available and updated registration dossiers and information from registrants, as well as the publicly available literature.
The available information is sufficient and reliable to clarify the initial concerns. For exposure-related concerns the eMSCA recommends registrants to revise the exposure assessment as explained above. The additional concern identified for eye damage was clarified. The mutagenicity concern has not been clarified, but further information was requested under dossier evaluation to clarify the concern.

4. FOLLOW-UP AT EU LEVEL

4.1. Need for follow-up regulatory action at EU level

4.1.1. Harmonised Classification and Labelling

DETU at present has no harmonised classification in accordance with Regulation (EC) 1272/2008. The proposal of classification presented below is based on currently available information. A further follow-up action cannot be excluded when new information requested in the compliance check decision (see Section 7.2) becomes available.

Table 2: Classification and labelling proposal of DETU

Classification and Labelling	Pictograms, Signal word
Acute Tox. 4 H302: Harmful if swallowed.	GHS07: exclamation mark GHS09: environment
Acute Tox. 4 H312: Harmful in contact with skin	
Eye Dam. 1 H318: Causes serious eye damage	
Skin Sens. 1 H317: May cause an allergic skin reaction	
Carc. 2, H351: Suspected of causing cancer	
Aquatic Chronic 3 H412: Harmful to aquatic life with long lasting effects	

The outcome of the evaluation performed by eMSCA leads to the conclusion that a new entry in CLP-Annex VI for DETU should be proposed.

Justification of classification

Acute oral toxicity: a reliable study with restrictions (no guideline followed) on mice is available for oral route. According to CLP Regulation for acute oral toxicity the preferred rodent species is the rat, although other rodent species may be used. The acute oral toxicity of DETU was studied in male mice. Groups of 10 -20 mice were treated with DETU by gavage. The doses used were : 500, 700, 1000, 1400, 2000, 2800 mg/kg body weight. A group of 10 non-treated mice was used as a control group. Animals were observed three hours after administration for clinical signs, and five days for mortality. Based on the experimental study on mice the LD50 value is 930 mg/kg and the substance fulfils CLP Regulation classification criteria (300 < LD50 ≤ 2000 mg/kg body weight) for Acute Toxicity category 4 with hazard statement H302: Harmful if swallowed.

Acute dermal toxicity: a reliable study according to OECD Test Guideline 402 (Acute Dermal Toxicity) is included in the registration dossier. The study was performed according GLP standards. DETU was applied onto the intact skin of 10 Sprague Dawley rats (5 males and 5 females) at the single dose of 2000 mg/kg body weight. Due to the mortalities observed at the dose of 2000 mg/kg in the 5 females rats, DETU was applied in the same experimental conditions in a group of 5 females at the single dose of 1000 mg/kg body weight. No mortality occurred during the study in the male treated at the dose of 2000 mg/kg body weight (0/5) or in the female treated at the dose of 1000 mg/kg body weight (0/5). In conclusion, the LD50 is 2000 mg/kg body weight by dermal route in the rat and the substance fulfils CLP Regulation classification criteria (1000 < LD50 ≤ 2000 mg/kg body weight) for Acute Toxicity category 4 with hazard statement H312: Harmful in contact with skin.

Eye Corrosion/Irritation: a reliable study performed in rabbits (New Zealand White rabbit) according to OECD Test Guideline 405 (Acute Eye Irritation/Corrosion) is included in the registration dossier. Based on the results of this test it can be concluded that irreversible effects on the eye (Category 1) is fulfilled (according to CLP Regulation classification criteria: if, when applied to the eye of an animal, a substance produces at least in one animal effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days, the substance causes irreversible effects on the eye). DETU produced in the tested animals effects on the cornea and conjunctiva that were not fully reversed within an observation period of 22 days. Based on the results of in vivo study DETU should be classified for serious eye damage (Category 1) with hazard statement H318: Causes serious eye damage.

Skin Sensitisation: the Dossier Submitters provided data from three available in vivo skin sensitisation studies. These data include results of a guinea pigs maximalisation test (GPMT) (publication, Nakamura 1994), a mouse local lymph node assay (LLNA) and a sensitive local node assay on mice (SLNA) (publication, Ikarashi 1994). The studies were not performed according GLP standards and no guideline were followed. Based on the GPMT (Nakamura, 1994) results and CLP Regulation criteria (≥30% to <60% animals responding at >0.1% to ≤1% intradermal induction dose and ≥30% responding at >1% intradermal induction dose) DETU should be classified as skin sensitiser category 1 with hazard statement H317: May cause an allergic skin reaction without subcategorisation taking into account that intradermal induction concentration ≤0.1% was not tested. Furthermore based on human data and according to CLP Regulation criteria, the eMSCA concludes that only a category 1 without subcategorisation can be reached based on the large variability in the frequency of skin sensitisation occurring after a relatively high exposure to DETU.

Carcinogenicity: three carcinogenicity studies are available in the CSR. The carcinogenicity studies in mice and rats (NCI 1978) did not follow the internationally recognised guideline and GLP principles, as they did not yet exist. The study was designed and carried out according to the sound scientific rules prevailing at that time. Hence, it is reliable with some limitations. The incidence of thyroid tumours increased in the male and female rats at the highest dose level (NCI 1978).The third carcinogenicity study (Hasegawa

et al. 1991) was not performed for regulatory purposes and under the internationally recognised guideline and GLP principles. The study in male F344 rats, conducted for 52 weeks, aimed to assess the effects of combined administration (52 weeks) of the three carcinogens, i.e. 2,4-diaminoanisole sulphate (DAAS), N,N'-Diethylthiourea (DETU) and 4,4'-thiodianiline (IDA).
Since the provided data can be considered as limited evidence of carcinogenicity in animal studies, DETU warrants classification as Carcinogen category 2 with hazard statement H351: Suspected of causing cancer.

Aquatic environment: the OECD Test Guideline 301D "Ready biodegradability: Closed Bottle Test" was followed to assess the ready biodegradability of DETU. In the OECD TG 301D, the pass level for ready biodegradability is 60% of Theoretical Oxygen Demand (ThOD). Under the test conditions, the percentage of biodegradation of DETU reached 3% of the ThOD at the end of the test (28 days), which shows that DETU is not readily biodegradable.
Aquatic acute toxicity of DETU was evaluated with fish, Daphnia magna and algae. The most sensitive species was Daphnia magna. The value of EC50 (48 hours) for Daphnia, in a study performed following OECD Test Guideline 202 and GLP principles, was 56 mg/l. Taking into consideration the value of EC50 for Daphnia and information that DETU is not readily biodegradable, the substance should be classified as Aquatic Chronic toxicity category 3 with hazard statement H412: Harmful to aquatic life with long lasting effects[2].
Remark: the NOECs for algae and Daphnia magna are higher than 1 mg/L, but there is no information of NOEC for fish. Thus, according to eMSCA, the classification for chronic aquatic toxicity in category 3 is justified.

4.1.2. Identification as a substance of very high concern, SVHC (first step towards authorisation)

Not applicable

4.1.3. Restriction

Not applicable

4.1.4. Other EU-wide regulatory risk management measures

Not applicable

5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL

5.1. No need for regulatory follow-up at EU level

Not applicable

5.2. Other actions

Not applicable

[1] According to CLP Regulation classification criteria: the substance is classified for chronic aquatic toxicity in category if 96 hr LC50 (for fish) and/or 48 hr EC50 (for crustacea) and/or 72 or 96 hr ErC50 (for algae or other aquatic plants) > 10 to ≤ 100 mg/l and the substance is not rapidly degradable and/or the experimentally determined BCF ≥ 500 (or, if absent, the log Kow ≥ 4) unless the chronic toxicity NOECs are > 1 mg/l)

6. TENTATIVE PLAN FOR FOLLOW-UP ACTIONS (IF NECESSARY)

Table 3

FOLLOW-UP		
Follow-up action	Date for intention	Actor
CLH proposal	To be decided*	Poland

* Post-check[3] findings for CLH (according to the prioritisation criteria for substance flagged for CLH as the outcome of the post-check activity)

[3] 'Post-check' activity aims to ensure that information generated as a result of the dossier and substance evaluation decisions is used to initiate regulatory risk management follow-up (RRM FUP), where appropriate (ref. Art. 42(2) and 48 of REACH).

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

DETU was originally selected for substance evaluation in order to clarify concerns about:

- suspected carcinogen,
- suspected skin sensitiser,
- wide dispersive use,
- consumer use
- exposure of workers,
- exposure of environment.

During the evaluation also other concerns were identified. The additional concerns were:

- eye damage,
- suspected Mutagenicity.

Table 4

EVALUATED ENDPOINTS	
Endpoint evaluated	Outcome/conclusion
Suspected carcinogen	Carcinogenicity confirmed, harmonised C&L process to be initiated
Suspected mutagen	Concern is identified but it is unclarified. Further information has been requested under dossier evaluation
Suspected skin sensitiser	Skin sensitisation confirmed, harmonised C&L process to be initiated
Exposure of workers	The risk assessment based on standard parameters and the DNELs derived by the eMSCA shows particularly high RCR values (up to 2.76) for few exposure scenarios. Revision of the exposure assessment (see section 7.12.1) and the quantitative risk characterisation would need to be revised by the registrants in the CSRs.
Exposure of consumers	The provided information is not sufficient to perform a realistic quantitative exposure assessment. The eMSCA performed a qualitative risk assessment for the consumers. As a conservative approach, and since dermal exposure is likely, any level of dermal exposure is assumed to pose a risk for skin sensitisation/allergic reactions for consumer. Hence, any measure to eliminate exposure should be considered.
Exposure of environment	The eMSCA cannot conclude that the environmental exposure assessment is complete as the safety assessment carried out does not cover all identified uses of the substance. Thus, the eMSCA recommends revision of the existing exposure assessments and addition of missing exposure scenarios for all identified uses. The available information indicates a risk to aquatic organisms from the manufacturing process. The RCRs calculated from the measured concentration values exceed 1.

	The eMSCA strongly recommends that the registrants take further organizational and technical measures to reduce environmental exposure.
Eye damage	Eye damage confirmed, harmonised C&L process to be initiated

7.2. Procedure

The updated Community rolling action plan (CoRAP) was published on the ECHA website on 19 March 2019.

The substance evaluation was performed based on the updated registration dossier and the Chemical Safety Reports (CSRs) as well as on the basis of additional information available in scientific databases and publications.

The Lead Registrant updated the registration dossier on 21 September 2019. The update was taken into account during the evaluation.

All the information was assessed regarding reliability for evaluation of the main grounds of concern. The particular emphasis was placed on the suspected carcinogenicity and sensitizing properties of DETU.

The results of the evaluation are documented in this report. Available information is enough to clarify the initial concerns. For exposure-related concerns the eMSCA recommends registrants to revise the exposure assessment as explained under Section 3 above. No further information is requested under this substance evaluation but regarding the identified issues on exposure the eMSCA gives recommendations to the registrants.

Additional concerns were identified for eye damage (clarified) and mutagenicity. To clarify the concern for mutagenicity, further information was requested under compliance check.

7.3. Identity of the substance

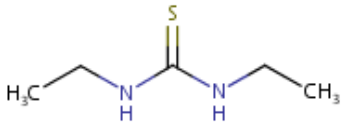
Table 5 SUBSTANCE IDENTITY

SUBSTANCE IDENTITY	
Public name:	1,3-Diethyl-2-thiourea 1,3-diethyl-thiourea 1,3-diethylthiourea DETU DIETHYLTHIOUREA N,N'-Diethylthioharnstoff N,N'-Diethylthiourea N,N`-Diethylthiourea Urea
EC number:	203-308-5
CAS number:	105-55-5
Index number in Annex VI of the CLP Regulation:	-
Molecular formula:	C5H12N2S
Molecular weight range:	132.23
Synonyms:	1,3-DIETHYL-2-THIOUREA

	1,3-DIETHYLTHIOUREA EKALAND DETU N'-Diethylthiourea N,N'-DIETHYLTHIOCARBAMIDE N,N'-Diethylthiourea NCI-C03816 PENNZONE E THIATE H THIOUREA, N,N'-DIETHYL- Urea, 1,3-diethyl-2-thio USAF EK-1803

Type of substance X Mono-constituent ☐ Multi-constituent ☐ UVCB

Structural formula:



7.4. Physico-chemical properties

Table 6 OVERVIEW OF PHYSICOCHEMICAL PROPERTIES

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES	
Property	Value
Vapour pressure	0.00065 Pa (25°C) P = 6.5 x 10-4 Pa
Water solubility	42 g/l Method: OECD TG 105 During the analysis,the temperature was 20 +/- 0.5°C and the mean value of pH was 6.26.
Partition coefficient n-octanol/water (Log Kow)	log Kow = 0.57 Method: OECD TG 107 (shake-flask method) The partition coefficient of DETU was determined in a study performed according to the principles of the shake-flask method (log Kow = 0.57) and was confirmed with the use of QSAR as the substance falls within the applicability domain of the model.

Flammability	DETU is not considered as highly flammable. Method: OECD TG A10 The test item has melted and formed a colourless limpid liquid that evaporates.
Explosive properties	Study does not need to be conducted because there are no chemical groups present in the molecule which are associated with explosive properties
Oxidising properties	DETU is an organic substance which does not contain oxygen, fluorine or chlorine . Therefore, negative results can be predicted and no testing for oxidising properties has been carried out.
Granulometry	Between 500 and 1000 µm. About 8% of particles are higher to 1000 µm = 1mm, and only some percents (ca 4%) are lower to 63%. DETU is not considerer as dusty (low dustisseness)
Melting / freezing point	76-80°C Method: OECD TG 102
Boiling point	200-230°C Method: OECD guideline 103
Relative density	0.77 g/cm^3 at 20°C Method: ASTM 1895 The bulk density of DETU is about 0.77 g/cm^3 whereas the crystal density is about 1.0 g/cm^3.

7.5. Manufacture and uses

7.5.1. Quantities

This substance has 2 active registrations under REACH (1 joint submission).

Table 7 AGGREGATED TONNAGE

AGGREGATED TONNAGE (PER YEAR)				
☐ 1 – 10 t	☐10 – 100 t	☒ 100 – 1000 t	☒ 1000- 10,000 t	☒ 10,000-50,000 t
☒ 50,000 – 100,000 t	☒ 100,000 – 500,000 t	☐ 500,000 – 1000,000 t	☒ > 1000,000 t	☐ Confidential

7.5.2. Overview of uses

Table 8 USES

USES	
	Use(s)
Uses as intermediate	use resulting in manufacture of another substance
Formulation	polymers and coating products
Uses at industrial sites	this substance is used in the following products: polymers and laboratory chemicals. this substance has an industrial use resulting in manufacture of another substance (use of intermediates). this substance is used in the following areas: formulation of mixtures and/or re-packaging. this substance is used for the manufacture of: rubber products (including tyres, wire/cables), metals and chemicals.
Uses by professional workers	laboratory chemicals
Consumer Uses	no consumer uses identified.
Article service life	outdoor use in long-life materials with low release rate (e.g. metal, wooden and plastic construction and building materials) and indoor use in long-life materials with low release rate (e.g. flooring, furniture, toys, construction materials, curtains, foot-wear, leather products, paper and cardboard products, electronic equipment). This substance can be found in complex articles, with no release intended: vehicles. This substance can be found in products with material based on: rubber (e.g. tyres, shoes, toys) and rubber used for large surface area articles (e.g. construction and building materials for flooring).

7.6. Classification and Labelling

7.6.1. Harmonised Classification (Annex VI of CLP)

No harmonised classification.

7.6.2. Self-classification

- In the registration:

Acute Tox. 4, H302: Harmful if swallowed.
Acute Tox. 4, H312: Harmful in contact with skin.

Eye Dam. 1, H318: Causes serious eye damage.
Skin Sens. 1B, H317: May cause an allergic skin reaction.
STOT RE 1, H372: Causes damage to organs (affected organ: thyroid)
Aquatic Chronic 3, H412: Harmful to aquatic life with long lasting effects.

- The following hazard classes are in addition notified among the aggregated self classifications in the C&L Inventory:

STOT SE 3, H335: May cause respiratory irritation.
Acute Tox. 3, H301: Toxic if swallowed.
Skin Irrit. 2, H315: Causes skin irritation.
Eye Irrit. 2, H319: Causes serious eye irritation.
Carc 2., H351: Suspected of causing cancer.

7.7. Environmental fate properties

Not assessed.

7.8. Environmental hazard assessment

7.8.1. Aquatic compartment (including sediment)

7.8.1.1. Fish

Short-term toxicity to fish

The key study presented in the registration dossier (disseminated registration dossier, study report, 2012; Klimisch 1 - reliable without restriction), was performed on DETU, according to OECD TG 203 and adhering to principles of GLP, see Table 9.
In a 96-hour acute toxicity study, Danio rerio (previous name: *Brachydanio rerio*) were exposed to DETU under static conditions. *Danio rerio* were exposed to test item at nominal concentration of 0, 390.5, 546.6, 765.3, 1071.4, and 1500 mg/L.
The fish were observed for mortality at 24, 48, 72 and 96 hours. The biological results were based on nominal concentrations.

Table 9 Test results

Method	Results	Remarks
Danio rerio (previous name: *Brachydanio rerio*) freshwater static short-term toxicity to fish according to OECD Guideline 203 (Fish, Acute Toxicity Test)	LC50 (24h): 920 mg/L (nominal) based on: mortality (95% CI 860 - 990 mg/L) LC50 (48h): 920 mg/L (nominal) based on: mortality (95% CI 860 - 990 mg/L) LC50 (72h): 910 mg/L (nominal) based on: mortality (95% CI 880 - 930 mg/L) LC50 (96h): 910 mg/L (nominal) based on: mortality (95% CI 880 - 930 mg/L)	1 (reliable without restriction) -key study experimental study Test material 1,3-diethylthiourea / CAS RN 105-55-5 / EC number 203-308-5 Reference Registration dossier (study report, 2012) GLP

Long-term toxicity to fish

No relevant information available. One fish early-life stage toxicity study disregarded due to major methodological deficiencies.

7.8.1.2. Aquatic invertebrates

Short-term toxicity to aquatic invertebrates

The key study presented in the registration dossier (disseminated registration dossier, study report, 2012; Klimisch 1 - reliable without restriction), was performed on DETU, according to OECD TG 202 and adhering to principles of GLP, see Table 10.
In a 48-hour acute toxicity study, young daphnids (*Daphnia magna*) were exposed to DETU under static conditions.

Daphnids were exposed test item at nominal concentration of 0, 10.2, 18, 30, 51, 87.6 and 150 mg/L. Since mean measured concentrations were maintained between 80 and 120% of nominal, biological results were based on nominal concentrations. The daphnids were observed for immobilisation at 24 and 48 hours.

Table 10 Test results of short-term toxicity to aquatic invertebrates

Method	Results	Remarks	Reference
Daphnia magna freshwater static according to OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test)	EC50 (48h): 56 mg/L test mat. (nominal) based on: mobility (95% CI 42 -78 mg/L) NOEC (48h): 30 mg/L test mat. (nominal) based on: mobility EC10 (48h): 30.2 mg/L test mat. (nominal) based on: mobility (value graphically estimated)	1 (reliable without restriction) key study experimental study Test material 1,3-diethylthiourea/ CAS RN 105-55-5/ EC number 203-308-5	Registration dossier (study report, 2012)

Long-term toxicity to aquatic invertebrates

The key study presented in the registration dossier (disseminated registration dossier, unpublished study report, 2016; Klimisch 1 - reliable without restriction), was performed on DETU, according to OECD TG 211 and adhering to principles of GLP, see Table 11.

The study was carried out to determine the effects of test item on *Daphnia magna* reproduction and survival in 21 days test under semi-static conditions.Daphnids were exposed to test item at nominal concentration of 0, 0.05, 0.15, 0.49, 1.56 and 5 mg/L. Since mean measured concentrations were maintained between 80 and 120% of nominal, biological results were based on nominal concentrations. The daphnids were observed for immobilisation at 24 and 48 hours.

Table 11 Test results of long-term toxicity to aquatic invertebrates

Method	Results	Remarks	Reference
Daphnia magna freshwater long-term toxicity to aquatic invertebrates according to OECD Guideline 211 (Daphnia magna Reproduction Test)	EC10 (21d): 1.67 mg/L act. ingr. (nominal) based on: reproduction NOEC (21d): >5 mg/L act. ingr. (nominal) based on: mortality - Parental mortality NOEC (21d): >5 mg/L act. ingr. (nominal) based on: Age at first brood	1 (reliable without restriction) key study experimental study Test material 1,3-diethylthiourea / 105-55-5 / 203-308-5	Registration dossier (unpublished study report, 2016)

7.8.1.3. Algae

The key study presented in the registration dossier (disseminated registration dossier, study report, 2011) was performed on DETU, according to OECD TG 201 and adhering to principles of GLP with reliability 1, see Table 12.

The influence of DETU on the growth of the freshwater green algal species Pseudokirchneriella subcapitata was investigated in a 72 -hour static test. Algae were exposed test item at nominal concentration of 0, 50, 73, 107, 157, 231, 340 and 500 mg/L. Since mean measured concentrations were maintained between 80 and 120% of nominal, biological results were based on nominal concentrations. The toxic effect measured during the assay was the inhibition of cellular multiplication over a time period of 72 hours. The concentrations of test item causing a 50 % reduction in biomass (EbC50) and growth rate (ErC50) were estimated. It was possible to determine No Observed Effect Concentration (NOEC).

Table 12 Test results of toxicity to algae

Method	Results	Remarks	Reference
Pseudokirchneriella subcapitata (previous names: *Raphidocelis subcapitata, Selenastrum capricornutum*) Freshwater toxicity to aquatic algae and cyanobacteria according to OECD TG 201 (Alga, Growth Inhibition Test) [before 23 March 2006]	EC50 (72h): 310 mg/L test mat. (meas. (initial)) based on: growth rate (95% CI 230 - 470 mg/L) EC50 (72h): 200 mg/L test mat. (meas. (initial)) based on: biomass (95% CI 150 - 270 mg/L) NOEC (72h): 73 mg/L test mat. (meas. (initial))	1 (reliable without restriction) key study experimental study Test material 1,3-diethylthiourea/ CAS RN 105-55-5 / EC number 203-308-5	Registration dossier (unpublished study report, 2011)

7.8.1.4 Toxicity to microorganisms

In the key study presented in the registration dossier , the toxicity of DETU was assessed in a ready biodegradability study performed according to OECD 301D guideline (disseminated registration dossier, study report, 2011) and adhering to principles of GLP with reliability 1, see Table 13.

DETU did not inhibit the micro-organisms as shown by the toxicity control flask where biodegradation percentage is already 47 %, higher than 25 % after 14 days. After reviewing the study report of the ready biodegradation test, the value of the NOEC = 2 mg/L was incorrect in the registration dossier.

Indeed the concentration tested in the inhibition control vessel during the ready biodegradation study was 1mg/L of substance, therefore this study indicates that the substance is not toxic to micro-organisms at the 1 mg/L concentration (NOEC = 1 mg/L)

Table 13. Effects on micro-organisms

Method	Results	Remarks	
activated sludge, domestic freshwater static OECD Guideline 301 D (Ready Biodegradability: Closed Bottle Test)	NOEC (28d): 1 mg/L test mat. (nominal) based on: inhibition of the biodegradation of the reference test item during a ready biodegradability test	1 (reliable without restriction) key study experimental study Test material 1,3-diethylthiourea/ CAS RN 105-55-5/ EC number 203-308-5	Registration dossier (study report, 2011)

7.8.1.5 Sediment organisms

No relevant information available.

7.8.1.6 Other aquatic organisms

No relevant information available.

7.8.2. Terrestrial compartment

No relevant information available.

7.8.3. PNEC derivation and other hazard conclusions

Table 14. Derived PNEC values

PNEC DERIVATION AND OTHER HAZARD CONCLUSIONS		
Hazard assessment conclusion for the environment compartment	Hazard conclusion	Remarks/Justification
Freshwater	PNEC aqua (freshwater): 33.4 µg/L Intermittent releases: 560 µg/L	21d EC10 = 1.67 mg/L Assessment factor: 50 48h EC50 = 56 mg/L Assessment factor: 100
Marine water	PNEC aqua (marine water): 3.3 µg/L	21d EC10 = 1.67 mg/L Assessment factor: 500
Sediments (freshwater)	PNEC sediment (freshwater): 0.248 mg/kg sediment dw	Extrapolation method: equilibrium partitioning method
Sediments (marine water)	PNEC sediment (marine water): 0.0248 mg/kg sediment dw	Extrapolation method: equilibrium partitioning method
Sewage treatment plant	PNEC STP: 0.1 mg/L	NOEC-28d = 1 mg/L Assessment factor: 10
Soil	PNEC soil: 0.03 mg/kg soil dw	Extrapolation method: equilibrium partitioning method
Air	No hazard identified.	Due to the low vapour pressure of substance no adverse effects are expected.
Secondary poisoning	No potential for bioaccumulation.	No study on bioaccumulation is available. Due to a low log kow value substance is considered to have a low potential for bioaccumulation in organisms.

7.8.4. Conclusions for classification and labelling

Aquatic acute toxicity of DETU was evaluated with fish, Daphnia magna and algae. The most sensitive species was Daphnia magna. The value of EC50 (48 hours) for Daphnia, in a study performed following OECD TG 202 and GLP principles, was 56 mg/l. Considering the value of EC50 for daphnia and information that DETU is not readily biodegradable, the substance should be classified as Aquatic Chronic toxicity category 3 with hazard statement H412: Harmful to aquatic life with long lasting effects (according to CLP Regulation classification criteria).

Remark: the NOECs for algae and daphnia magna are higher than 1 mg/L, but there is no information of NOEC for fish. Therefore, the eMSCA considers that the classification for chronic aquatic toxicity in category 3 is justified.

7.9. Human Health hazard assessment

DETU was selected for substance evaluation in order to clarify concerns about potential carcinogenicity and skin sensitisation. The other toxicological data are described briefly with exception of genetic toxicity due to possible additional concern for gene mutation.

7.9.1. Toxicokinetics

Experimental toxicokinetic studies were not available. The information on toxicokinetics may be partly deduced from the physicochemical properties, including:

-Molecular weight: 132.23 g/mol

-Water solubility: 42 g/L (20°C), DETU is highly soluble in water.

-Partition coefficient Log Kow: 0.57

-Vapour pressure: 0.00065 Pa (25°C)

DETU can be absorbed from the gastro-intestinal tract and through the skin. As a small molecule a wide distribution of DETU is expected. No specific information was found on metabolism of DETU. The substance is probably excreted in the urine as it is a water-soluble substance with a low molecular weight (Registration Dossier, 2019).

7.9.2. Acute toxicity and Corrosion/Irritation

All information has been taken from the Registration Dossier (2019).

Oral

Based on the acute oral experimental study in mice the LD50 value is 930 mg/kg. The eMSCA concludes that the Substance fulfils classification criteria as Acute Tox. 4, H302 (Harmful if swallowed).

Inhalation

Study not required. Acute studies are available for oral and dermal exposure to DETU.

Dermal

Based on the acute dermal experimental study in rats the LD50 value is 2000 mg/kg. The eMSCA concludes that the Substance fulfils classification criteria as Acute Tox. 4, H312 (Harmful if contact with skin).

Skin irritation/corrosion

The results of study according to OECD TG 404 in rabbits showed that DETU is not a skin irritating. No cutaneous reactions were observed during the study. Mean scores over 24, 48 and 72 hours for each animal were 0.0 for erythema and oedema.

Eye irritation/corrosion

In 3 tested rabbits DETU produced effects on the cornea and conjunctiva that were not fully reversed within an observation period of 22 days. The results of study on rabbits are presented in Table 15.

Table 15. Eye irritation – results

Animal No	Chemosis 24/48/72 h	Conjunctivae redness 24/48/72 h	Iris 24/48/72 h	Corneal opacity – intensity 24/48/72 h	Corneal opacity – area 24/48/72 h
1	3/2/2 mean:2.3	3/3/3 mean:3.0	1/1/1 mean: 1.0	2/2/2 mean: 2.0	2/2/2 mean: 2.0
2	3/3/3 mean:3.0	3/3/3 mean:3.0	1/1/1 mean: 1.0	2/2/2 mean: 2.0	2/2/2 mean: 2.0
3	3/2/2 mean:2.3	3/3/3 mean:3.0	1/1/1 mean: 1.0	2/2/2 mean: 2.0	2/2/2 mean: 2.0

The eMSCA concludes that DETU fulfils classification criteria as Eye Dam.1, H318 (Causes serious eye damage).

7.9.3. Sensitisation

7.9.3.1 Skin

7.9.3.1.1 Non-human information

The Registrants provided data from three available *in vivo* skin sensitisation studies. These data include results of a guinea pigs maximisation test (GPMT, Nakamura, 1994), a mouse local lymph node assay (LLNA) and a sensitive local node assay on mice (SLNA) (Ikarashi, 1994). The studies were not GLP-compliant, and no guideline followed.

In the GMPT study (Nakamura 1994) performed according to the Magnuson and Kligman method, Hartley guinea pigs (males) were exposed in the induction phase by intradermal injection (0,002, 0.02, 0.2, 2 % of DETU in olive oil as a vehicle) and by topical administration (0 or 25 % of DETU in petrolatum). In the challenge phase, animals were exposed by topical administration at 0,002, 0.02, 0.2 % of DETU in acetone. There is no justification for using three different vehicles during the study. Only 7 animals were used in each of the treatment groups when a minimum of 10 guinea pigs should be tested according to GPMT protocol (OECD TG 406).

In the animals induced by intradermal injection of 0.2% of DETU, 3/7 (43%) of those challenged by 0.002%, and 4/7 (57%) of those challenged by 0.02% and 0.2% displayed a positive reaction. In animals induced by intradermal injection of 2% of tested substance, 2/7 (29%) of those challenged by 0.002%, 4/7 (57%) of those challenged by 0.002%, and 7/7 (100%) of those challenged by 0.02 and 0.2% had a positive reaction.

Below 0.2% (intradermal induction) no cutaneous reactions were observed after the challenge application. However, the highest tested challenge dose was 0.2% while the concentration used for the challenge exposure should be the highest non-irritant dose, based on GPMT protocol. Taking into account that undiluted DETU is non-irritant (unpublished study report, 2001), the challenge concentrations used in GPMT are too low to consider the potency of skin sensitisation properties of DETU. Based on these GPMT

results (Nakamura, 1994) and CLP criteria[4] DETU should be classified as skin sensitiser category 1 without subcategorisation, taking into account that intradermal induction concentration ≤0.1% was not tested.

In a Local Lymph Node assay (LLNA according to the method of Kimber and Weisenberger, 1989), sensitization of DETU in dimethyl sulfoxide (DMSO) and acetone-olive oil 4:1 (AOO) 6-8 week BALB/c mice (3 females per dose group) was tested, at the concentrations of 0%, 10%, 25% and 50% (publication, Ikarashi 1994). Mice were exposed to 25µl of various concentrations of DETU in AOO or AOO alone (control) on each ear for three consecutive days.

On the fourth day, auricular lymph nodes were excised and pooled for each group. A suspension of lymph node cells (LNC) was then prepared by mechanical disaggregation. After having been washed once with Hank's balanced salt solution, LNC were counted. 200 µl of LNC suspension were seeded in 96-well culture plates (5 wells/group) and cultured with 0.5 µCi of tritiated methylthymidine (3HTdR) for 24h at 37°C. 3HTdR incorporation was measured by liquid scintillation counting and stimulation index (SI) calculated (Table 16).

Table 16. Results of the murine local lymph node assay (LLNA) (Ikarashi 1994)

Concentration of DETU(%)	Vehicle	SI	Vehicle	SI
0	AOO	-	DMSO	-
10		0.8		1.2
25		0.9		0.8
50		1.8		1.1

Based on the LLNA performed with DETU in two vehicles, no potential of skin sensitisation was shown in mice (Stimulation Index <3 at all tested concentrations of DETU).

However, many deficiencies can be noted in this study in comparison with LLNA method protocol (OECD TG 429):

- only 3 animals are used in each of the treatment groups when a minimum of 4 mice should be tested according to LLNA method;
- lymphocyte proliferation must be measured during the induction phase of skin sensitization. Usually, the induction phase lasts five to seven days in the mouse (Saint-Mezard *et al.*, 2003). However, in Ikarshi 1994 study measurement of lymphocyte proliferation was carried out on the 4th day after the first application of DETU, thus before the induction phase;

Furthermore, no data on the positive control group were provided in the Ikarashi 1994 study. Based on the above deficiencies the eMSCA considers the study as inconclusive.

In the second study by Ikarashi 1994, named ‘sensitive mouse lymph node assay’ (SLNA), BALB/c mice (3 females per dose group, 5 females per control group) were tested, at the concentrations 0.2% and 2% of DETU in intradermal injections. Two 25 µl aliquots of DETU-FCA emulsion (water-in-oil emulsion was prepared from a mixture of DMSO containing the chemicals, FCA and saline at a ratio of 1:4:5) were injected intradermally into two sites of the abdominal skin located at both sites of the ventral midline. Five days after injection, 25 µl DETU in acetone-olive oil (4:1) (AOO) was applied to both sites of each ear daily for 3 consecutive days. The day after the final topical application, auricular lymph nodes were excised and pooled for each experimental group.

Preparation of cell suspensions and determination of cellular proliferation was carried out in the same way as in LLNA study above (Ikarashi 1994) by determination of SI (Table 17).

[4] ≥30% to <60% animals responding at >0.1% to ≤1% intradermal induction dose and ≥30% responding at >1% intradermal induction dose

Table 17. Results of the sensitive mouse lymph node assay (SLNA) (Ikarashi 1994)

Intradermal injection - Concentration of DETU(%) in DMSO as vehicle	Topical application – Concentration of DETU(%) in AOO as vehicle	SI
0	0	-
0.2	5	2.1
2	5	3.0

Based on the SLNA results (EC3 value= Stimulation index (SI) 3 after intradermal injection of 2% of DETU), DETU should be regarded as a skin sensitiser. However, SLNA method does not comply with recognised guidelines, thus the results published by Ikarashi, 1994 can be used only as supportive data.

7.9.3.1.2 Human information

Thioureas are widely-used accelerators in the manufacture of synthetic rubber products. Allergic contact dermatitis has been reported because of thiourea sensitisation from orthopaedic joint braces, protective clothing, swimsuits and various sports equipment.

In 2012, Dall et al. analysed patch test data from 239 patients who had been tested with DETU 1% in petrolatum obtained from the Allergen Bank database was published. 15% (37/239) of the patients tested with DETU had positive patch test reactions, all with current clinical relevance and all strong. Therefore, DETU 1% in petrolatum is a contact allergen giving a high frequency of strongly positive clinically relevant patch test reactions following targeted testing in patients with a history of neoprene rubber dermatitis (Dall *et al.*, 2012).

In a 2010 published study (Liippo *et al.*) patients were patch tested with a mixture of three thiourea chemicals consisted of 0.5% (w/w) DETU, 0.5% DBTU, and 0.5% DPTU in petrolatum. Thiourea mix yielded positive patch test reactions in 59 of 15100 patients (0.39%). 33/59 patients were also tested with individual rubber chemicals. DETU was positive in 24/33 (73%) patients who had positive patch test reactions after exposure to thiourea mix. Several cases of allergic contact dermatitis due to DETU were observed and described in numerous publications (Friis *et al.*, 2010; Alomar *et al.*, 1985; Adams, 1981; Andersen, 1983).

Based on human data and according to CLP criteria, the eMSCA concludes that only a category 1 without subcategorisation can be reached based on the large variability in the frequency of skin sensitisation occurring after relatively high exposure to DETU.

In conclusion, based on the data available, the classification of DETU as Skin Sensitiser Category 1, H317 (May cause an allergic skin reaction) is justified. In the opinion of the eMSCA, the available animal and human data do not allow subcategorisation of skin sensitisation according to the CLP regulation.

7.9.4. Repeated dose toxicity

<u>Oral</u>

Two oral studies of 7 weeks in rats and mice are available in the Registration Dossier (2019). The target organ of DETU is the thyroid in rats. No target organ is showed in mice. Based on the results the LOAEL in rats is 6.25 mg/kg bw/d (125 ppm). For further information, see section 7.9.6.

No repeated dose toxicity studies following inhalation and dermal exposure are available.

7.9.5. Mutagenicity

Mutagenicity was identified as an additional concern during the evaluationand due to possible carcinogenic potential the genotoxic effect cannot be excluded.

Several studies were available to evaluate the genotoxic potential of DETU. The registration dossier incudes an *in vitro* gene mutation study in mammalian cells with positive result, which raises a concern for gene mutation.

Genotoxicity in vivo

In the mammalian erythrocyte micronucleus test (2011) performed in male and female rats according to OECD TG 474 and in GLP conditions (reliability 1), 1,3-Diethyl-2-thiourea given by gavage in 2 successive administrations at 24 hour interval at doses 80 mg/kg bw, 160 mg/kg bw and 320 mg/kg bw did not induce an increase in frequency of micronucleated polychromatic erythrocytes in bone marrow of rat femur. At higher doses of 500 mg/kg bw (2x) and 800 mg/kg bw a substance caused mortality and/or excessive toxicity leading to euthanasia of animals due to ethical reasons. The results indicate that 1,3-Diethyl-2-thiourea is not clastogenic in bone marrow of rats exposed by gavage at sublethal doses.

In the *in vivo* Comet assay (Mattioli *et al.*, 2006) was performed according to method described by Singh et. 1988 (N.P. Singh, M.T. McCoy, R.R. Tice, E.L. Schneider, A simple technique for quantitation of low levels of DNA damage in individual cells, Exp. Cell Res. 175 (1988) 184–191.), therefore not according to current OECD TG 489 *In Vivo* Mammalian Alkaline Comet Assay (adopted on 29 July 2016) and not in GLP conditions, thus its reliability is limited.
1,3-Diethyl-2-thiourea dissolved in DMSO was given by gavage to three rats (gender of animals not reported) at dose 158 mg/kg bw/d (1/2 LD50 of test compound). Negative control animals were given DMSO. No positive control was used. Rats were sacrificed 16 hours after dosage for evaluation of DNA fragmentation in thyroid, liver and kidneys (Table 18.).

Table 18. The results (mean±SD) of in vivo Comet assay of cells from thyroid, liver and kidney of 3 rats.

Treatment	Thyroid		Liver		kidney	
	Tail length (µm)	Tail moment	Tail length (µm)	Tail moment	Tail length (µm)	Tail moment
Control (DMSO)	1.9±0.4	167±25	1.8±0.4	155±28	1.9±0.4	161±28
Diethyl-2-thiourea	16.3±0.6[a]	1398±46[a]	1.5±0.5	138±35	4.3±0.9	338±55[a]

[a] statistically different from control group by ANOVA ($p < 0.05$)

The results suggest that Diethyl-2-thiourea induced DNA damage in thyroid cells of rats as indicated by the increased tail length and tail moment in the comet assay, thus demonstrating its genotoxicity in *in vivo* conditions. It is noted that fragmentation of DNA was not detected in the liver, but some indications of DNA damage in kidney cells of rats treated the same way were observed. However , the above results provide very limited evidence on genotoxicity *in vivo* of Diethyl-2-thiourea due to severe deviations from currently recommended methodology described in OECD TG 489:

1. historical negative control data with ranges, means/medians and standard deviations for each tissue evaluated; and concurrent and historical positive control data were not provided;
2. Low number of animals was used: minimum of 5 analysable animals of one sex, or of each sex if both are used per group should be used

3. Three dose groups and concurrent negative and positive controls (each group composed of five animals of a single sex) were not used
4. Animals should be given daily treatments over a duration of 2 or more days (i.e. two or more treatments at approximately 24 hour intervals), and samples should be collected once at 2-6 h (or at the Tmax) after the last treatment
5. Comets should be scored quantitatively using an automated or semi-automated image-analysis system.
6. Only scorable cells (clearly defined head and tail with no interference with neighbouring cells) should be scored for % tail DNA to avoid artefacts. No data provided on this topic in study description.
7. For each sample (per tissue per animal), at least 150 cells (excluding hedgehogs) should be analysed. No data provided in study description how many cells were analysed
8. DNA strand breaks in the comet assay can be measured by independent endpoints such as % tail DNA, tail length and tail moment. All three measurements can be made if the appropriate image software analyser system is used. However, the % tail DNA (also known as % tail intensity) is recommended for the evaluation and interpretation of results and is determined by the DNA fragment intensity in the tail expressed as a percentage of the cell's total intensity. No data on this topic were provided in the study description.
9. Positive findings in the comet assay may not be solely due to genotoxicity, target tissue toxicity may also result in increases in DNA migration, thus test compound cytotoxicity based on one or more indicators of cytotoxicity in target tissue should be assessed

Due to the above described deviations the study should not be considered as sufficiently reliable for regulatory purposes, although it is useful for formulation of scientific hypothesis.

At present with one clearly negative mammalian erythrocyte micronucleus test and one positive *in vivo* Comet assay with limited reliability the existing data are inconclusive for classification of Diethyl-2-thiourea to germ cell mutagenicity hazard class.

7.9.6. Carcinogenicity

Three carcinogenicity studies are provided in the registration dossier: Two studies in rats and mice (National Cancer Institute Technical Report Series No. 149, NCI 1979), and a third study in male rats (Hasegawa *et al.*, 1991).

The carcinogenicity studies in mice and rats were performed in 1977-1978, and were designed and carried out according to the sound scientific rules prevailing at that time (NCI, 1979). It did not follow the internationally recognised guideline and GLP principles as they did not exist yet. Thus, the study is considered to be reliable with some limitations.

Mice

Preliminary study

The concentrations used in the study were determined based on the results of range-finding toxicity tests in which groups of mice, each consisting of five males and five females, were given DETU in diet for 7 weeks at concentrations of 680, 1000, 1470, 2160 and 3150 ppm corresponding roughly to 102, 150, 220, 324 and 472 mg/kg bw/d . The control group of mice received the basal laboratory diet.

The mean body weight gain among male mice dosed with 680 ppm was 10 percent less than in controls, while female mice receiving the same concentration had a mean body weight gain 8 percent less than that of their controls. The study report does not provide any information on toxicity symptoms or body weight gain of animals exposed at 1000,

1470, 2160 and 3150 ppm. The concentration of 500 ppm was chosen for the main study as the highest concentration aiming to ensure the survival of animals for two years in the carcinogenicity study.

Main study

In the carcinogenicity study in B6C3F1 mice, DETU was administered in the diet at concentrations of 250 and 500 ppm (corresponding[1] roughly 37.5 and 75 mg/kg bw/d) to groups of 50 males and 50 females for 103 weeks, followed by one week observation period. Twenty female mice and nineteen male mice were used as negative controls.

The body weight of male and female was reduced in a dose-dependent manner in mice exposed at 250 and 500 ppm and was observed after 30 weeks of exposure. Detailed numerical data are not provided. The survival of mice was not significantly affected by exposure being in female mice in the range of 70% in control and low dose groups and 60% in high dose group, while in male mice ca. 50% in control group and 80% in low and high dose group at the end of the 103 week exposure period.

The dietary exposure of male and female mice to DETU at concentrations of 250 and 500 ppm during 103 weeks did not affect an incidence of any neoplasm at any site in neither sex. There was no increase in an incidence of any non-neoplastic pathological findings in any organs of animals examined histopathologically from both exposed groups in comparisons with control animals.

Rats

Preliminary study

The concentrations used in the main study were determined based on the results of range-finding toxicity tests in which groups of rats, each consisting of five males and five females, were given DETU in diet for 7 weeks at concentrations of 147, 215, 316 and 464 ppm. The control group of rats received the basal laboratory diet. One female receiving DETU at 316 ppm died, while another one had an arched back and rough coat. Mean body weight gain in males exposed at 316 ppm was 3% greater, while those exposed at 215 ppm was 10% less than in controls. In females exposed at 316 ppm, the mean body weight gain was 11% less than in controls, while females at 215 ppm were 1% less than in controls. The study report does not provide any information on toxicity symptoms or body weight gain of animals exposed at 464 ppm. The concentration of 250 ppm was chosen for the main study as the highest concentration aiming to ensure the survival of animals for two years in the carcinogenicity study.

Main study

In the carcinogenicity study in Fischer 344 rats, DETU was administered in the diet at concentrations of 125 and 250 ppm to groups of 50 males and 50 females for 103 weeks, followed by one week observation period. Twenty animals of each sex were used as negative controls.

The body weight and the survival till the end of the study of male and female rats were not affected by the treatment. The incidence of thyroid tumours increased in the male and female rats at the highest dose level (Table 19).

Table 19. The incidence of thyroid tumours in rats exposed to DETU. Data are expressed as number of animal with tumour/number of animal examined histopathologically.

	Males rats			Female rats		
Type of tumor	Control group	Low dose group 125 ppm (6.25 mg/kg bw/day	High dose group 250 ppm (13 mg/kg bw/day	Control group	Low dose group 125 ppm (ca. 6.25 mg/kg bw/day	High dose group 250 ppm (ca. 12.5 mg/kg bw/day
C-cell	0/18	0/45	2/48	0/18	1/46	1/46

Adenoma						
C-cell carcinoma	1/18	0/45	1/48	0/18	0/46	1/46
Follicular cell adenoma	0/18	0/45	6/48	0/18	4/46	9/46
Follicular cell carcinoma	0/18	1/45	11/48[a]	0/18	1/46	8/46
Combined follicular adenoma and carcinoma	0/18	1/45	15/48[b]	0/18	5/46	17/46[b]

[a] significant statistical difference in comparison with control group (p< 0.05) with Fisher exact test
[b] significant statistical difference in comparison with control group (p< 0.005) with Fisher exact test

The dietary exposure to DETU at the highest concentration induced a statistically significant increase in an incidence of follicular cell carcinoma, combined follicular cell adenoma and carcinoma in male rats, and of combined follicular cell adenoma and carcinoma in female rats, in the thyroid. There were no other statistically significant changes in the incidence of any neoplasm at any site in either male or female rats. There was no increase in the incidence of any non-neoplastic pathological findings in any organs of animals exposed and examined histopathologically.

In the tables of the study report presenting results of histopathological examinations, no increase in the incidence of hyperplasia of thyroid c-cells or thyroid follicular cells was noted in female and male rats at any dose of the tested substance, despite mentioning such finding in a description of the results. No hypertrophy of the liver was reported in male and female rats exposed to DETU.

Discussion

The study has several deficiencies such as:

1. The small number of animals in concurrent control (20 instead of 50);
2. Top doses, particularly in the rat study, were relatively low, although chosen based on results of preliminary short-term toxicity study. Top doses for both species were well below MTD exerting very slight systemic toxicity;
3. Only two, instead of three, dose-level were used, so chances to demonstrate a dose-response relationship was lowered;
4. Food consumption was checked in both species, but not reported therefore a dose-level in mg/kg bw/day cannot be calculated, although it can be estimated;
5. No data on the absolute and relative weight of organs were provided;
6. Haematology and clinical biochemistry measurement were not done, therefore results do not contribute to evaluation of repeated-dose toxicity;
7. No historical control data on the incidence of thyroid tumours in rats and mice in the study performing laboratory was provided;
8. The animals under this study were kept in the same room in which other substances were under toxicity tests.
9. Despite these deficiencies, the study is considered valid and results may be used for assessment of the carcinogenicity of DETU.

The third carcinogenicity study (Hasegawa et al. 1991) was not performed for regulatory purpose and not according to GLP principles and internationally recognised guideline. The study was performed on male F344 rats to assess the effects of combined administration of the three carcinogens (2,4-diaminoanisole sulphate (DAAS), N,N'-Diethylthiourea (DETU) and 4,4'-thiodianiline (IDA) for 52 weeks (Table 20 and 21.).

DAAS, DETU or TDA alone were given in the diet to male rats at the following concentrations: 610, 200 and 46 ppm, respectively (corresponding[5] to 30, 10 and 2.3 mg/kg bw/d); i.e. only one concentration per tested substance. For the combined treatment, DAAS, DETU and IDA were incorporated into the diet at the concentrations: 610, 200 and 46 ppm, respectively. The negative control group received a basal laboratory diet.

Table 20. Body and organ weights at the end of the experiment and average food consumption throughout the experiment

Treatment	No. of rats	Final weight (g)			
		Body	Liver	Thyroid	Food consumption (g/rat/day
Combined	17	371 ± 24a	14.9 ± 3.4[a]	0.55 ± 0.50[a]	12.6 ± 1.4
DAAS	21	415 ± 25	11.4 ± 1.2	0.13 ± 0.01	14.5 ± 1.0
DETU	20	424 ± 18	11.2 ± 0.9	0.16 ± 0.01[a]	13.4 ± 1.3
TDA	20	390 ± 22 a	13.9 ± 1.2[a]	0.14 ± 0.01	14.0 ± 1.2
No treatment	20	462 ± 21	12.3 ± 0.8	0.13 ± 0.01	14.4 ± 0.4

[a] – statistically significantly different from non-treatment group

DETU at concentration of 200 ppm (ca. 10 mg/kg bw/day) in the diet caused mild, but statistically significant, increase in absolute weight of thyroid.

Table 21. Incidences of thyroid tumours

Treatment	No. of rats	No. of Follicular cell carcinoma (%)	No. of C- cell carcinoma (%)
Combined	18	18 (100%)[a]	0
DAAS	21	0	0
DETU	21	1 (5%)	2(10%)
TDA	20	2 (10%)	0
No treatment	20	0	0

[a] – statistically significantly different from non-treatment group

The incidence of thyroid follicular cell carcinoma (5%) and C-cell carcinoma (10%) in rats treated with DETU alone was not statistically significant in comparison with the concurrent control. It was not reported whether follicular cell carcinoma and C-cell carcinomas occurred in the same or different rats. The incidence of thyroid follicular cell carcinoma was higher than the historical control incidence in rats from the same laboratory in the comparable period. The incidence of follicular cell tumours observed in 2-year experiments was: 0.8-1.0% for carcinomas and 0.7-1.0% for adenomas in males (Hasegawa et al., 1991). No increase in the incidence of lung carcinoma or adenoma was observed in male rats treated with DETU alone.

The level of serum thyroxine (T4 levels) at the end of the experiment (3.67± 0.58 µg/dl) was significantly increased in male rats treated with DETU alone in comparison with the

[5] 1 ppm in feed for older rats = 0.050 mg/kg bw/d according to Guidelines for the preparation of toxicological working papers for the Joint FAO/WHO Expert Committee on Food Additives, Geneva, December 2000

control males (2.93±0.55 µg/dl). The thyroid weight was also significantly increased in rats treated with DETU: from 0.13±0.01g in control group to 0.16±0.01g in DETU treated rats.

Discussion

The results show that combined exposure induced significantly more follicular cell carcinomas in the thyroid of male rats than exposure to any of the substance alone. It indicates a strong synergistic carcinogenic effect of DAAS, DETU and IDA. DETU alone did not induce a significant increase in the incidence of follicular cell carcinoma or c-cell carcinoma in comparison with concurrent control. However, the incidence in that group was above the upper limit of historical control data, which indicates its carcinogenic potential in the thyroid of rats.

Comparison with classification criteria

There is no data on humans, therefore DETU does not warrant classification to category 1A.The studies of DETU regarding carcinogenicity in animals were not performed according to internationally recognised guidelines and GLP principles.

The key study in the rats (NCI 1979), has several deficiencies and is not considered as a well-conducted study. Therefore, the available data does not provide sufficient evidence of carcinogenicity in animals, thus classification of DETU to category Carc. 1B is not appropriate.

However, the animal studies on the carcinogenic potential of DETU give some evidence that DETU is carcinogenic to animals. In the 103-week carcinogenicity key study (NCI 1979) in rats, DETU at the highest concentration induced a statistically significant increase in the incidence of follicular cell carcinoma and combined follicular cell adenoma and carcinoma in male rats, and in an incidence of combined follicular cell adenoma and carcinoma in female rats. There was no increase in the incidence of neoplasms in mice exposed in the diet to DETU for 103 weeks, even at concentrations higher than used in rats study. In another carcinogenicity study in male rats (Hasegawa et al., 1991) DETU alone did not induce a significant increase in the incidence of follicular cell carcinoma or c-cell carcinoma in male rats in comparison with concurrent control, even if the incidence in that group was above the upper limit of historical control data.

The potential mode of action of DETU is not known. Thus, it cannot be excluded that it is not relevant for humans. There is not sufficient data to conclude on mutagenicity of DETU. The substance has been shown to be toxic to thyroid as shown in the carcinogenicity study in male rats (Hasewaga et al. 1991) as demonstrated by the increased weight of thyroid and increased level of serum thyroxine (T4 levels) in male rats treated with DETU.

Since provided data can be considered as limited evidence of carcinogenicity in animal studies DETU warrants classification as Carc. 2 with hazard statement H351: Suspected of causing cancer.

Specific Target Organ Toxicity: Repeated Exposure

The first two repeated dose oral toxicity studies in mice and rats (NCI, 1979) were performed in the late seventies of the twentieth century as a range-finding studies before 2-years carcinogenicity study of DETU, and they do not meet criteria required neither by OECD 407 for Repeated Dose 28-Day Oral Toxicity Study and nor OECD 408 Repeated dose 90 day oral toxicity study. These studies are not adequate to draw a conclusion on the need for STOT RE classification

1. A repeated Dose Oral Toxicity Study in mice was performed as a range-finding study before 2-years carcinogenicity study of DETU (NCI 1979). In the study groups of mice, each consisting of five males and five females (according to OECD TG 408 at least 20 animals (ten female and ten male should be used at each dose level) were given DETU in

the diet at concentrations of 680, 1000, 1470, 2160 and 3150 ppm, corresponding[6] roughly to 102, 150, 220, 324 and 472 mg/kg bw/d, for seven weeks (thus only for 49 days instead 90 days required by OECD TG 408). The control group of mice received a basal laboratory diet.

The mean body weight gain among male mice dosed with 680 ppm (102 mg/kg bw/d) was 10 percent less than in controls, while female mice receiving the same concentration had a mean body weight gain 8 percent less than that of their controls. The study report does not provide any information on toxicity symptoms or body weight gain of animals exposed at 1000, 1470, 2160 and 3150 ppm (220, 324 and 472 mg/kg bw/d).

2. A repeated Dose Oral Toxicity Study in rats was performed as a range-finding before 2-years carcinogencity study of DETU (NCI 1979). In the study, groups of rats, each consisting of five males and five females (according to OECD TG 408 at least 20 animals (ten female and ten male should be used at each dose level), were given DETU in the diet at concentrations of 147, 215, 316 and 464 ppm, corresponding roughly to 14.7, 21.5, 31.6 and 46.4 mg/kg bw/d for seven weeks. The control group of rats received a basal laboratory diet. One female receiving DETU at 316 ppm (31.6 mg/kg bw/d) died, while another had an arched back and rough coat. Mean body weight gain in males exposed at 316 ppm (31.6 mg/kg bw/d) was 3% greater, while those exposed at 215 ppm (21.5mg/kg bw/d) was 10% less than in controls. In females exposed at 316 ppm (31.6 mg/kg bw/d) the mean body weight gain was 11% less than in controls, while females at 215 ppm (21.5mg/kg bw/d) was 1% less than in controls. The study report does not provide any information on toxicity symptoms or body weight gain of animals exposed at 464 ppm (46.4 mg/kg bw/d).

The guidance value to assist in classification to Category 2 based on results of 90-day repeated dose oral toxicity study is $10 < C \leq 100$ mg/kg body weight/day. Since in both range-finding studies (49-day studies) no adverse effects were reported at doses lower than 100 mg/kg bw/d or 183 mg/kg (Haber's rule) the results of this tests do not justify classification of DETU to category STOT RE 2. It is noted that the scope of investigations was limited to clinical observation, twice a week measurement of food consumption and body weight and necropsy at study termination do not follow the requirements of the corresponding OECD TGs, i.e. OECD 407 for short-term toxicity (28-d) or OECD 408 for subchronic toxicity (90-d). In comparison with OECD TG 408 requirements the study reports do not provide data on the following parameters: nature, severity and duration of clinical observations, results of ophthalmological examination, haematological tests, clinical biochemistry tests, circulating thyroid hormones (T4, T3, TSH), terminal vaginal cytology, a detailed description of all histopathological findings, total cauda epididymal sperm number, percent progressively motile sperm, percent morphologically normal sperm. Taking that into account both these studies (NCI, 1979) are considered as inconclusive regarding repeated dose toxicity.

Additional repeated Dose Oral Toxicity Studies were performed as the 103- week carcinogenicity study in mice and rats (NCI 1978) and 52-weeks study in male rats (Hasegawa *et al.*, 1991).

In the carcinogenicity study in Fischer 344 rats, DETU was administered in the diet at concentrations of 125 and 250 ppm, corresponding roughly to 6.25 and 12.5 mg/kg bw/d[7]. DETU did not induce in rats increased mortality or reduction of body weight during a course of this study.

In the carcinogenicity study in B6C3F1 mice DETU was administered in the diet for 103 weeks, followed by one week observation period, at concentrations of 250 and 500 ppm,

[6] 1 ppm in feed for older rats = 0.050 mg/kg bw/d according to Guidelines for the preparation of toxicological working papers for the Joint FAO/WHO Expert Committee on Food Additives, Geneva, December 2000

[7] 1 ppm=0.05 mg/kg bw/d for older rat according to WHO recommendations

corresponding roughly to 37.5 and 70 mg/kg bw/d[8]. At both doses, mean body weight was reduced in male and female mice. In the histopathological examinations of rats and mice thyroid hyperplasia (cystic and follicular-cell) was commonly recognized and appeared to be related to dietary administration and dosage of the compound. In other internal organs no significant increase in the incidence of any pathological findings in comparison with concurrent control was observed. The eMSCA considers that thyroid hyperplasia alone is not such an adverse effect justifying classification for repeated dose toxicity. Thus, the conclusion by the eMSCA based on results of this NCI study is that DETU should not be classified to a hazard class of specific target organ toxicity: repeated exposure.

The guidance value for classification to category 2 of STOT RE for 103-week repeated toxicity study is 12.6 mg/kg bw/d following Haber's rule (100 mg/kg bw/d x 13 weeks/103 weeks). Since the adverse, non-neoplastic effects were not found, even at higher doses, the results of the study do not justify the classification of DETU to a hazard class of STOT RE.

A long-term repeated dose toxicity study was performed in male rats given DETU at a concentration of 200 ppm (10 mg/kg bw/day) for 52 weeks (Hasegawa *et al.*, 1991). The guidance values for classification to category 2 of STOT RE for 52-week repeated toxicity study would be 2.5 - 25 mg/kg bw/d following Haber's rule (10-100 mg/kg bw/d x 13 weeks/52 weeks). At the dose level of 10 mg/kg bw/d DETU induced an increase in thyroid weight by 23% and in thyroxine concentration by 25 % in comparison with the control values, however no histopathological changes other than few rats with follicular cell carcinoma or c-cell adenoma (not statistically significant) were reported in thyroid of DETU exposed rats. Taking into account that observed moderate increase in thyroid weight and thyroxine concentration can be related to tumour formation in the thyroid, it is considered that this finding not supported by other adverse non-neoplastic effects in this or other organs is not sufficient for classification to STOT RE 2.

In conclusion, the results of several non-guidance repeated dose toxicity studies, not performed according to internationally recognised guidelines and GLP principles, do not provide sufficient evidence to classify DETU as STOT RE 2.

7.9.7. Toxicity to reproduction (effects on fertility and developmental toxicity)

No specific study on fertility is available, but the two developmental studies (oral and dermal) give data on prenatal developmental toxicity.
The LOAEL for developmental toxicity was 15 mg/kg/day based on the decrease of fetal body weight gain based on a study performed with an analogue substance dimethylthiourea. No teratogenic effects were observed in this study. Further information on reproduction has been requested under compliance check to complete the information on fertility and prenatal developmental toxicity.

7.9.8. Hazard assessment of physico-chemical properties

Not assessed.

7.9.9. Selection of the critical DNEL(s)/DMEL(s) and/or qualitative/semi-quantitative descriptors for critical health effects

DNELs derived by the eMSCA are lower than DNELs derived by the registrants.

[8] 1 ppm=0.150 mg/kg bw/d for mice according to WHO recommendations

The eMSCA performed DNEL derivation for systemic effects, following the method proposed in the ECHA Guidance on information requirements and chemical safety assessment, Chapter R.8.

Considering that the dossier is incomplete for many endpoints, which is the subject of dossier evaluation and compliance check decision by ECHA, the eMSCA notes that the assessment factor for the quality of a whole database should be 2, bearing in mind completness.

DNEL worker inhalation

The eMSCA derived the human DNEL for inhalation based on the oral study on rats. The 103-weeks rat dietary toxicity study (NCI 1978), where LOAEL was 125 ppm (equivalent to 6.25 mg/kg bw), was selected by eMSCA for DNEL derivation. The target organ of DETU was the thyroid of rat (carcinogenicity and toxicity).

The starting point was converted using an oral-to-inhalation route extrapolation.

The eMSCA assumed in the extrapolation, the correction of respiratory volume considering light activity for a worker (8h exposure) and absorption.

LOAECworker = 6.25 x (1/0.38) x (6.7/10) x 0.5 = 5.51

LOAECworker derived by eMSCA is 5.51 mg/m3.

The same LOAEC from the same study was selected by the registrant for DNEL derivation. But overall assessment factor obtained by the eMSCA is twice higher than overall AF derived by the registrant.

Overall Assessment Factor obtained by eMSCA: 75

AF for dose response relationship: 3 (starting point is a LOAEC.)

AF for difference in duration of exposure: 1 (chronic study)

AF for interspecies differences (allometric scaling): 1

AF for other interspecies differences: 2.5 (for remaining difference.)

AF for intraspecies differences: 5 (for worker DNELs)

AF for the quality of the whole database: 2 (taking into account completeness of database - the assessment of genotoxicity and systemic toxicity cannot be completed based on information provided by the registrant)

DNELworker 5.51/75 =0.0735 mg/m3

DNELworker for inhalation route derived by the eMSCA is 0.0735 mg/m3 and is two times lower than DNELworker derived by the registrant.

DNEL worker dermal

The eMSCA derived DNEL for the dermal route based on the oral study on rats. The 103-weeks rat dietary toxicity study (NCI 1978), where LOAEL was 125 ppm (equivalent to 6.25 mg/kg bw), was selected by the eMSCA for DNEL derivation. The same LOAEL from the same study was selected by the registrant for DNEL derivation. The target organ of DETU was the thyroid of rat (carcinogenicity and toxicity).

The starting point was converted using an oral-to-dermal route extrapolation.

The eMSCA took into account in the extrapolation correction for absorption difference between the dermal and oral absorption. Considering the human dermal absorption of thiourea, an analogue substance to DETU, dermal absorption of 4% is used as the default

value for DETU. Since exposure to solid and liquid DETU is expected, as a worst case, absorption for liquid thiourea was used by the eMSCA in DNEL derivation.

Therefore, the corresponding dermal LOAEL is 78 mg/kg bw/day

LOAEL worker dermal 6.25 mg/kg bw/day x 50/4 = 78 mg/kg bw/day

Overall Assessment Factor: 300

AF for dose response relationship: 3 (starting point is a LOAEL)

AF for difference in duration of exposure: 1 (chronic study)

AF for interspecies differences (allometric scaling): 4 (key study was performed on rats.)

AF for other interspecies differences: 2.5 (for remaining difference.)

AF for intraspecies differences: 5 (worker DNEL)

AF for the quality of the whole database: 2 (taking into account completeness of database - the assessment of genotoxicity and systemic toxicity can not be completed based on information provided by the registrant)

DNELworker for the dermal route derived by eMSCA is 0.26 mg/kg bw/day and is eight times lower than DNELworker derived by the registrant.

DNEL general population inhalation

The eMSCA derived the human DNEL for general population for the inhalation route of exposure based on the oral study on rats. The 103-weeks rat dietary toxicity study (NCI 1978), where LOAEL was 125 ppm (equivalent to 6.25 mg/kg bw), was selected by the eMSCA for DNEL derivation. The target organ of DETU was the thyroid of rat (carcinogenicity and toxicity).

The starting point was converted using an oral-to-inhalation route extrapolation. The eMSCA has assumed in the extrapolation correction of respiratory volume for general population and correction for absorption.

LOAEC 6.25 x 1/1.15 x 0.5 = 2.71 mg/m3

The same LOAEC from the same study was selected by the registrant for DNEL derivation. But overall assessment factor obtained by the eMSCA is twice higher than overall AF derived by the registrant.

Overall Assessment Factor obtained by the eMSCA: 150

AF for dose response relationship: 3 (starting point is a LOAEC)

AF for difference in duration of exposure: 1 (chronic study)

AF for interspecies differences (allometric scaling): 1

AF for other interspecies differences: 2.5 (for remaining difference.)

AF for intraspecies differences: 10 (general population DNEL)

AF for the quality of the whole database: 2 (taking into account completeness of database - the assessment of genotoxicity and systemic toxicity can not be completed based on information provided by the registrant)

DNELgeneral population for the inhalation route derived by the eMSCA is 0.018 mg/m3 and is two times lower than DNEL derived by the registrant.

DNEL general population dermal

The eMSCA derived the human DNEL for the dermal route based on the oral study on rats. The 103-weeks rat dietary toxicity study (NCI 1978), where LOAEL was 125 ppm (equivalent to 6.25 mg/kg bw), was selected by the eMSCA for DNEL derivation. The same LOAEL from the same study was selected by the registrant for DNEL derivation. The target organ of DETU was the thyroid of rat (carcinogenicity and toxicity).

The starting point was converted using an oral-to-dermal route extrapolation.

The eMSCA has assumed in the extrapolation correction for absorption difference between the dermal and oral absorption. Considering the human dermal absorption of thiourea, an analogue substance to DETU, dermal absorption of 4% is used as the default value for DETU. Since exposure to solid and liquid DETU is expected, as a worst case, absorption for liquid thiourea was used by the eMSCA in DNEL derivation.

Therefore, the corresponding dermal LOAEL is 78 mg/kg bw/day

LOAEL worker dermal 6.25 mg/kg bw/day x 50/4 = 78 mg/kg bw/day

Overall Assessment Factor: 600

AF for dose response relationship: 3 (starting point is a LOAEL)

AF for difference in duration of exposure: 1 (chronic study)

AF for interspecies differences (allometric scaling): 4 (the key study was performed on Rats)

AF for other interspecies differences: 2.5 (for remaining difference)

AF for intraspecies differences: 10 (for general population DNEL)

AF for the quality of the whole database: 2 (taking into account completeness of database - the assessment of genotoxicity and systemic toxicity can not be completed based on information provided by the registrant)

DNELgeneral population for the dermal route derived by eMSCA is 0.13 mg/kg bw/day and is eight times lower than DNEL derived by the registrant.

DNEL general population oral

The eMSCA derived the human DNEL for the oral route based on the oral study on rats. The 103-weeks rat dietary toxicity study (NCI 1978), where LOAEL was 125 ppm (equivalent to 6.25 mg/kg bw), was selected by the eMSCA for DNEL derivation. The target organ of DETU was the thyroid of rat (carcinogenicity and toxicity).

No difference in oral absorption is expected between rat and human.

The same LOAEL from the same study was selected by the registrant for DNEL derivation. But overall assessment factor obtained by the eMSCA is two times higher than overall AF derived by the registrant.

Overall Assessment Factor obtained by the eMSCA: 600

AF for dose response relationship: 3 (starting point is a LOAEL)

AF for difference in duration of exposure: 1 (chronic study)

AF for interspecies differences (allometric scaling): 4 (the key study was performed on rats)

AF for other interspecies differences: 2.5 (for remaining difference)

AF for intraspecies differences: 10 (for general population DNELs)

AF for the quality of the whole database: 2 (taking into account completeness of database - the assessment of genotoxicity and systemic toxicity can not be completed based on information provided by the registrant)

DNELgeneral population for the oral route derived by eMSCA is 0.01 mg/kg bw/day and is two times lower than DNEL derived by the registrant.

7.9.10. Conclusions of the human health hazard assessment and related classification and labelling

Acute oral toxicity: a reliable study with restrictions (no guideline followed) in mice is available for the oral route. According to the CLP Regulation, the preferred rodent species for acute oral toxicity is a rat, although other rodent species may be used. The acute oral toxicity of DETU was studied in male mice. Groups of 10-20 mice were treated with DETU by gavage with doses: 500, 700, 1000, 1400, 2000, 2800 mg/kg body weight. A group of 10 mice was a control group. Animals were observed for clinical signs and mortality, respectively, three hours and five days after administration. Based on the experimental study on mice, the LD50 value is 930 mg/kg, and the substance fulfils CLP Regulation classification criteria (300 < LD50 ≤ 2000 mg/kg body weight) for Acute Toxicity category 4 with hazard statement H302: Harmful if swallowed.

Acute dermal toxicity: a reliable study according to OECD Test Guideline 402 (Acute Dermal Toxicity) in rats was included in the dossier. The study was performed according GLP standards. DETU was applied onto the intact skin of 10 Sprague Dawley rats (5 males and 5 females) at the single dose of 2000 mg/kg body weight. Due to the mortalities observed at the dose of 2000 mg/kg in the 5 females rats, DETU was applied in the same experimental conditions in a group of 5 females at the single dose of 1000 mg/kg body weight. No mortality occurred during the study in the male treated at the dose of 2000 mg/kg body weight (0/5) or in the female treated at the dose of 1000 mg/kg body weight (0/5). In conclusion, the LD50 is 2000 mg/kg body weight by dermal route in the rat and the substance fulfils CLP Regulation classification criteria (1000 < LD50 ≤ 2000 mg/kg body weight) for Acute Toxicity category 4 with hazard statement H312: Harmful in contact with skin.

Eye Corrosion/Irritation: a reliable study performed in rabbits (New Zealand White rabbit) according to OECD Test Guideline 405 (Acute Eye Irritation/Corrosion) was included in the dossier. Based on the results it can be concluded that DETU causes irreversible effects on the eye and Eye Damage 1 is warranted (according to CLP Regulation classification criteria: if, when applied to the eye of an animal, a substance produces at least in one animal effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days, the substance causes irreversible effects on the eye). DETU produced in the tested animals effects on the cornea and conjunctiva that were not fully reversed within an observation period of 22 days. Based on the results of in vivo study DETU should be classified for serious eye damage (Category 1) with hazard statement H318: Causes serious eye damage.

Skin Sensitisation: registrants provided data from three available in vivo skin sensitisation studies. These data include results of a guinea pigs maximalisation test (GPMT) (publication, Nakamura 1994), a mouse local lymph node assay (LLNA) and a sensitive local node assay on mice (SLNA) (publication, Ikarashi 1994). The studies were not performed according GLP standards and no guideline were followed. Based on the GPMT results (Nakamura, 1994) and CLP Regulation criteria (≥30% to <60% animals responding at >0.1% to ≤1% intradermal induction dose and ≥30% responding at >1% intradermal induction dose) DETU should be classified as skin sensitiser category 1 (Skin Sens 1) with hazard statement H317: “May cause an allergic skin reaction”. No subcategorisation is possible taking into account that intradermal induction concentration ≤0.1% was not tested. Furthermore, based on human data and according to CLP Regulation criteria, the eMSCA concludes that only a category 1 without subcategorisation can be reached based

on the large variability in the frequency of skin sensitisation occurring after a relatively high exposure to DETU.

Carcinogenicity: The carcinogenicity studies in mice and rats (NCI 1978) did not follow the internationally recognised guideline and GLP principles, as they did not yet exist. The study was designed and carried out according to the sound scientific rules prevailing at that time. Hence, the eMSCA considers that these studies are reliable with some limitations. The incidence of thyroid tumours increased in the male and female rats at the highest dose level (NCI 1978). Another carcinogenicity study in rats (Hasegawa et al. 1991) was not performed for regulatory purpose and under the internationally recognised guideline and GLP principles. The study in male F344 rats, conducted for 52 weeks, aimed to assess the effects of combined administration (52 weeks) of the three assumed carcinogens, i.e. 2,4-diaminoanisole sulphate (DAAS), N,N'-Diethylthiourea (DETU) and 4,4'-thiodianiline (IDA).
Since the provided data can be considered as limited evidence of carcinogenicity in animal studies, DETU warrants classification as Carcinogen category 2 with hazard statement H351: Suspected of causing cancer.

7.10. Assessment of endocrine disrupting (ED) properties

Not assessed.

7.11. PBT and VPVB assessment

Not assessed.

7.12. Exposure assessment

The eMSCA notes that based on the information gathered during evaluation, some of the exposure scenarios that result from the uses identified during the assessment are missing. In the registration dossier, there are no specific exposure scenarios for the use of the DETU in the manufacture of rubber products such as tyres, wire/cables, etc. by both professional and industrial users and exposure scenarios during the service life of these articles for professional and consumers are missing.

Thus each category of products with similar characteristics should be analysed in terms of human and environmental exposure.

Therefore, the documentation presented does not allow an overall assessment of the exposure of all human groups and the environment to the substance under consideration.

The registration dossier and in particular the chemical safety report should therefore be updated to cover all identified uses in detail.

Nevertheless, the eMSCA also has comments on the scenarios presented by the registrant.

7.12.1. Human health

7.12.1.1. Worker

The eMSCA has several reservations regarding the assessment of inhalation exposure for workers (quantitative), provided by the registrants.

In majority of exposure scenarios provided by the registrats, there are no estimations for a full work shift. There is no RCR calculated for the whole exposure scenarios but only for each task separately. The eMSCA doubts that every task is performed by different workers. The eMSCA notes that an 8-hour time-weighted average will be higher than the value estimated for one task and it may lead to an unacceptable level of exposure.

Therefore, the eMSCA recommends that the registrants should revise their exposure assessment.

Moreover, it is not clear if expected exposure related to the cleaning of equipment is already covered in the provided contributing scenarios.

In three contributing scenarios, the process temperature, which goes above the melting point, must be used for exposure estimation, instead of the temperature in the vicinity of the system. Besides that, the exposure estimation should be performed for the liquid instead of dust.

In other four contributing scenarios, where process temperature was above the melting point, exposure estimation for the liquid should be performed instead of dust.

The main recommended correction includes equipment cleaning task, if appropriate, consideration of exposure to liquid when process temperature is above the melting point and estimation for a full work shift. The eMSCA notes that revised exposure assessment may lead to higher estimated values, and in consequence to an unacceptable level of exposure. In such a case, further risk management measures shall be considered by the registrant.

7.12.1.2. Consumer

All parameters used in an exposure assessment must be included in the Chemical Safety Report. In the case of using non-standard parameters, such an approach must be justified. Furthermore, since not all General Rubber Goods are used in water, direct contact should be assumed. Therefore, the eMSCA proposes to use the precautionary principle while estimating consumer exposure. In ECETOC TRA default value (1) should be used as dermal transfer factor.

Dermal exposure derived by the eMSCA for children is 1.526 mg/kg bw/day.

Because DETU should be classified as Category 1 skin sensitizer, the eMSCA performed a qualitative risk assessment for the consumer.

Consumer exposure to DETU is possible during the service life of articles. It depends on the type of article containing DETU and the rate of migrating the substance out of it. The CSRs do not provide sufficient information on either of these aspects. Therefore, the provided information does not enable to perform a realistic quantitative exposure assessment. The information on the use and the exposure data available in the registration dossier suggest that DETU has wide-dispersive use and dermal exposure is likely to occur. Besides, DETU transfer from articles cannot be excluded. According to literature reviewed by the eMSCA, several cases of allergic contact dermatitis were observed and described in humans wearing general rubber goods.

7.12.2. Environment

The highest release of DETU into the environment may occur during manufacture of the substance, formulation and manufacture of rubber goods (including tyres). Due to trace amounts of DETU in cured rubber products, the substance may also be released into the environment during the service life of the products.

The eMSCA has assessed the exposure scenarios provided by the registrants. The characterisation of the general exposure scenarios, as well as the description of the activities and technical processes covered in the exposure scenarios were found plausible and acceptable. Furthermore, if other than default ERC release factors were used for emission estimation, this should be clearly explained in the chemical safety assessment and these release factors have to be well justified.

However, similarly as for the worker and consumer exposure, the safety assessment carried out does not cover all identified uses of the substance, in particular in tyres (whole article life cycle) and rubber products (cables, wires, etc.) by industrial and professional users.
Therefore, the eMSCA cannot conclude that the environmental exposure assessment is complete.

7.13. Risk characterisation

Workers

In consequence of the recommended revision of the exposure assessment (see section 7.12), the quantitative risk characterisation needs to be revised by the registrants in the CSRs.
Moreover, DNELs for workers and general population derived by eMSCA are lower than DNELs derived by the registrant. The risk assessment on the basis of standard parameters and the DNELs derived by eMSCA shows particularly high RCR values (up to 2.76) for few contributing scenarios.

The eMSCA identified the need for a harmonised classification of DETU as carcinogenic: Carc. 2, H351: Suspected of causing cancer.

DMELs to describe the likelihood of risks to workers concerning the carcinogenic potential of DETU were not derived. This effect is predicted to be the most critical one leading to the lowest hazard reference levels for risk characterisation, that need to be taken into account.

Therefore, further risk management measures shall be considered by the registrants, if the carcinogenicity of the substance is confirmed by the harmonised classification and labelling proposed by the eMSCA.

Consumers

The eMSCA, based on the available human and experimental data, considers that DETU should be classified as a Category 1 skin sensitiser. It was not possible to derive a threshold and set a DNEL as the data were insufficient. Therefore, following the REACH Guidance on information requirements and chemical safety assessment Part E: Risk Characterisation, the qualitative approach for risk characterisation according to the section E.3.4.3 needs to be performed.

The uses identified by the registrants take into account that consumers use articles, made of rubber produced with DETU as a vulcanisation agent, during their subsequent service life.
Therefore, as a conservative approach, and since dermal exposure is likely, any level of dermal exposure is assumed to pose a risk for skin sensitisation/allergic reactions for consumer and any measure to eliminate exposure should be considered.

Due to the lower long-term DNEL for the dermal route derived by the eMSCA in comparison to the DNEL derived by the registrant, and higher estimated exposure for consumers, the estimated risk is unacceptable for children (RCR over 10 for gloves).

Further risk management measures shall be considered by the registrants, if the carcinogenicity of the substance is confirmed by the harmonised classification and labelling proposed by the eMSCA.

Environment

Based on PECs and PNECs provided by the registrant and assessed in Sections 7.12.2 and 7.8, RCRs (PEC/PNEC) were found to be below 1, indicating no unacceptable risk for the local as well as the regional environment.

Nevertheless, exposure scenario for manufacture of substance indicate that the RCR values for the fresh water (including sediment) based on the measured values indicate high variability of RCR values during a year due to changes of release rates and composition of effluents. Several times during the year the RCRs are above 1. However, the registrant declares to have taken action to achieve RCR lower to 1 all over the year.

Therefore, the eMSCA cannot conclude that the environmental exposure assessment is complete and recommend revision of the existing exposure assessments and addition of exposure scenarios for all missing identified uses.

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7.15. Abbreviations

AOO – acetone olive oil

C&L - Classification and labelling

CLP – Classification, Labelling and Packaging

CoRAP – Community Rolling Action Plan

CSR – Chemical Safety Report

DMEL - Derived Minimal Effect Level

DMSO – dimethyl-sulpho oxide

DNEL – Derived No Effect Level

ECETOC - European Centre for Ecotoxicology and Toxicology of Chemicals

ECHA – European Chemical AgencyDMEL

EC3 - the concentration of test chemical required to induce a 3-fold increase in lymph node cell proliferation

EU – European Union

eMSCA – Evaluating Member State

GLP – Good Laboratory Practice

GPMT -Magnuson and Kligman

LD – Lethal Dose

LC – Lethal Concentration

LLNA - Local lymph node assay

LNC – lymph node celle

LOAEL – Lowest Adverse Observed Effect Level

LOAEC - Lowest Adverse Observed Effect Concentration

NOAEC - No Observed Adverse Effect Concentration

NOAEL – No Observed Adverse Effect Level

PBT – Persistent, Bioaccumulative, Toxic

(Q)SAR - Quantitative structure–activity relationship

RCR – Risk Characterisation Ratio

SLNA - sensitive mouse lymph node assay

SVHC – Substance of Very High Concern

TG – Technical Guideline

ThOD - Theoretical Oxygen Demand

US NICI – United States National Cancer Institutr

vPvB – very Persistent, very Bioaccumulative