Danish Ministry of the Environment
Environmental Protection Agency

SUBSTANCE EVALUATION CONCLUSION and

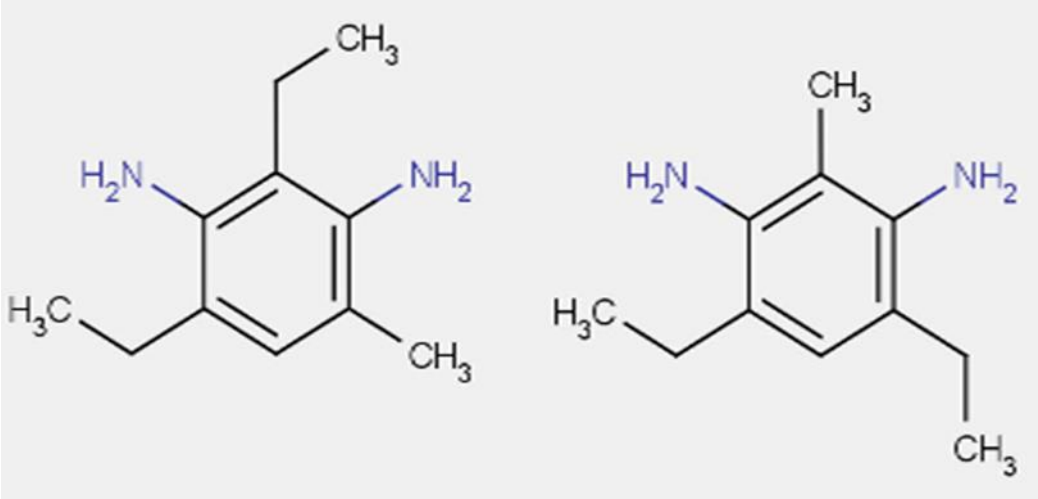
EVALUATION REPORT

for

Diethylmethylbenzenediamine (DETDA)

EC No 270-877-4

CAS RN 68479-98-1



Evaluating Member State:
Denmark

Dated: 11 December 2023

Evaluating Member State Competent Authority

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Year of evaluation in CoRAP: 2016

Further information on registered substances here:

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

Further information on the substance evaluation process here:

https://echa.europa.eu/regulations/reach/evaluation/substance-evaluation

Foreword

This Conclusion document, as required by Article 48 of the REACH Regulation, provides the outcome of the Substance Evaluation carried out by the evaluating MSCA. The document consists of two parts i.e. A) the conclusion and B) the evaluation report.

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 MSCA. In case the evaluating MSCA 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 MSCA, it does not preclude other MSCAs or the European Commission from initiating regulatory risk management measures which they deem appropriate.

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Part A. Conclusion

In the conclusion (part A), the evaluating MSCA 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.

Alternatively, the outcome of the evaluation may be that presently there is no need for regulatory follow-up at EU level if sufficient information on the potential hazards is available and all necessary measures for safe handling of the substance are in place.

1. Scope of the evaluation

Diethylmethylbenzenediamine (the Substance) was originally selected for substance evaluation in order to clarify concerns about:

Mutagenicity
Reproductive toxicity

During the evaluation the following additional concerns were identified:

Sensitisation (skin)
Specific target organ toxicity (repeated)
Endocrine disruption (human health)
Carcinogenicity

2. Overview of other processes / EU legislation

Table 2-1 Overview of other processes / EU legislation

No other processes	CCH	TPE	GMT	Previously on CoRAP	Annex VI (CLP)	Annex XVII (Restriction)	Candidate List/Annex XIV (Authorisation)
☐	☒	☒	☐	☐	☐	☐	☐

Other EU legislation	Previous legislation	Stockholm convention	Other
PPP/BPR	NONS/RAR	POP	(e.g., UNEP)
☐	☐	☐	☐

In 2012, a decision was issued requesting a pre-natal developmental toxicity study (OECD TG 414) according to REACH Annex IX, 8.7.2 following a testing proposal submitted by the Registrant.

In 2022, ECHA opened a comprehensive compliance check which is currently ongoing.

3. Conclusion and regulatory follow-up action

The evaluation of the available information on the Substance has led the evaluating MSCA to the following conclusions.

Table 3-1 Conclusion and regulatory follow-up action

Initial and additional concern	Conclusion on concern	Regulatory follow-up action
Mutagenicity	Inconclusive	RMOA

	Standard information is lacking in the registration dossier. This information should be requested under dossier evaluation.	
Reproductive toxicity	Inconclusive Crucial standard information is lacking in the registration dossier. This information should be requested under dossier evaluation.	RMOA
Sensitisation (skin)	Inconclusive Crucial standard information is lacking in the registration dossier. This information should be requested under dossier evaluation.	RMOA
Specific target organ toxicity (repeated)	Concern removed (clarification of hazard)	RMOA
Endocrine disruption (human health)	Inconclusive Available information is ambiguous but currently no further action is proposed. See Sections 15.2 and 15.3.	RMOA
Carcinogenicity	Inconclusive Available information is ambiguous and the evaluating MSCA suggests awaiting the outcome of compliance check before further action is considered.	RMOA

Table 3-2 Additional endpoint evaluated (outside scope of initial/additional concern)

Additional endpoint	Conclusion	Regulatory follow-up action
-	-	-

4. Regulatory follow-up actions at EU level

4.1 Harmonised Classification and Labelling

Not applicable

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

Not applicable

4.3 Restriction

Not applicable

4.4 Other EU-wide regulatory risk management measures

Not applicable

5. Currently no need for regulatory follow-up at EU level

5.1 No need for regulatory follow-up at EU level

Not applicable

5.2. Other actions

Further information is necessary to clarify the concerns identified by the evaluating MSCA. Based on the currently available information, no conclusion on most of the concerns is possible. However, compliance check has been identified by the evaluating MSCA as a more expedient process. Therefore, the substance evaluation is terminated. It is expected that the standard information will be sufficient to clarify the concerns for mutagenicity, skin sensitization and reproductive toxicity without need for further information requested under substance evaluation at present. However, should the standard information generated under a dossier evaluation not be sufficient to clarify the concerns, a new substance evaluation may be necessary. The evaluating MSCA will consider preparing an RMOA once follow-up evaluation of the information requested under compliance check has been completed..

6. Tentative plan for follow-up actions

As indicated in Tables 3-1/3-2, the following regulatory action(s) at EU level are proposed.

Indication of a tentative plan is not a formal commitment by the evaluating MSCA. A commitment to prepare a REACH Annex XV dossier (SVHC, restrictions) and/or CLP Annex VI dossier should be made via the Registry of Intentions.

Table 6-1 Follow-up actions

Follow-up action	Date for intention	Actor
RMOA The need for RMOA will be reassessed after CCH has been completed.	TBD	DK

Part B. Substance evaluation report

In the substance evaluation report (part B), the document provides explanation how the evaluating MSCA assessed and drew the conclusions from the information available.

7. Overview of the Substance Evaluation Process

In accordance with Article 45(4) of the REACH Regulation, the evaluating MSCA evaluated the Substance based on the information in the registration dossier(s) and on other relevant and available information. The evaluating Member State concluded the evaluation without any further need to ask more information from the registrants under an Article 46 decision.

On the basis of an opinion of the ECHA Member State Committee and due to initial grounds for concern for mutagenicity and toxicity to reproduction, the Substance (CAS No 68479-98-1; EC No 270-877-4) was included in the Community Rolling Action Plan (CoRAP) for substance evaluation to be evaluated in 2016. The Competent Authority of Denmark (hereafter called the evaluating MSCA) was appointed to carry out the evaluation.

Pursuant to Article 45(4) of the REACH Regulation, the evaluating MSCA carried out the evaluation of the Substance based on the information in the registration dossier and other relevant and available information.

In the course of the evaluation, the evaluating MSCA identified additional concerns regarding skin sensitisation, repeated dose toxicity (RDT) and endocrine disrupting properties. In addition, carcinogenicity was identified as an additional concern.

On 30 August 30 2016, the registrants updated the registration dossier. The update included: Robust Study Summary (RSS) for fish study, RSS for daphnia study, RSS for comet assay, updated PNEC values and an updated waiver for a developmental toxicity study in the second species.

In 2017, the evaluating MSCA submitted a draft decision requesting further information to clarify the identified concerns. The registrant submitted comments to the draft decision. Following an exceptional delay in decision-making, it was restarted. The evaluating MSCA identified data gaps for mutagenicity, reproductive toxicity and skin sensitization. Therefore, the evaluating MSCA recommended ECHA to open a compliance check to request the missing standard information. A substance evaluation draft decision pursuant to Article 46(1) of the REACH Regulation to address the remaining concern for endocrine-mediated induction of diabetes via severe effects on pancreas was submitted to the registrant in 2023. The registrant submitted comments. After careful scrutiny of the comments, the evaluating MSCA decided that the decision-making should be terminated.

8. Substance identity

The information on the Substance, including identifiers and structural formula, can be found on the cover page. For more details see ECHA: https://echa.europa.eu/home

Synonyms: DEDTA, DETDA 80, Diethyl toluenediamine, Ethacure 100, Ethacure 100-LC

8.1. Type of Substance

Multi-constituent.

8.2. Other relevant information

Table 8.2-1 Other information relevant to the composition of the Substance

Type	Identity*	Concentration range*
Constituent	2,4-diamino-3,5-diethyltoluene EC No 218-256-9	75.5-81.0%
Constituent	2,6-diamino-3,5-diethyltoluene EC No 218-255-3	18.0-20.0%
Impurity	Dialkylated m-phenylenediamines	0.5-3.0%
Impurity	Other trialkylated m-phenylenediamines	0.0-0.4%
Impurity	2,4,6-triethylbenzene, 1,3-diamine	0.0-0.1%

*Ethacure DETDA (Albemarle, 2017)

9. Physicochemical properties

Table 9-1 Overview of physicochemical properties

Property	Value
Molecular weight/weight range	178.3 g/mol
Physical state at 20°C and 101.3 kPa	Clear yellow liquid
Vapour pressure	The estimated vapour pressure is 0.000971 Pa and 0.00391 Pa at 25 ºC and 35 ºC, respectively
Water solubility	23 g/L at 30 ºC
Partition coefficient n-octanol/water (Log K_{ow})	1.38 at 25 °C
Partition coefficient organic carbon/water (Log K_{oc})	3.1 (EPISuite prediction)
Dissociation constant	4.6 (pKa at 20 ºC)

10. Manufacture and uses

10.1. Quantities

The aggregated tonnage (per year) of the Substance is 1,000 - 10,000 tonnes.

10.2. Overview of uses

Table 10.2-1 Overview of uses

Main uses	Key information

Formulation	Used in adhesives and sealants, coating products and polymers
Industrial	Used in adhesives and sealants, coating products and polymers
Professional	Used in adhesives and sealants, coating products and polymers
Consumer	Used in coating products

11. Classification and Labelling

Table 11-1 Classification of the Substance

Harmonised classification (Annex VI of CLP)	Self-classification in registrations	Self-classification in C&L notifications
Acute Tox. 4, H302, H312 Eye Irrit. 2, H319 STOT RE 2, H373 Aquatic Acute 1, H400 Aquatic Chronic 1, H410	Acute Tox. 4, H302, H312 Eye Irrit. 2, H319 STOT RE 2, H373 (pancreas) Aquatic Acute 1, H400 Aquatic Chronic 1, H410	STOT RE 2, H373 (other, liver, lung, spleen, endocrine system, body (dermal, oral, inhalation))

12. Environmental fate properties

Not evaluated in this substance evaluation.

13. Environmental hazard assessment

Not evaluated in this substance evaluation.

14. Human Health hazard assessment

14.1. Toxicokinetics

14.1.1. Non-testing methods

Estimates of bioavailability and uptake in the gastrointestinal (GI) tract were made using the Danish QSAR database. The Substance is predicted to be bioavailable according to Lipinski's rule-of-five and to be absorbed 95% at a dose of 1 mg.

14.1.2. *In vivo* toxicokinetic

Two toxicokinetic studies with the Substance have been submitted by the registrant. The first study (Unpublished study, 1982a) is a pre-guideline toxicokinetic study including a preliminary study exposing one male and one female rat to a single dose of 10 mg/kg and 3 males and 3 females to 50 mg/kg and a definitive study exposing 6 males and 6 females to 10 daily doses of 50 mg/kg and 6 males and 6 females exposed to 9 daily doses of vehicle and one tenth dose of 50 mg/kg of the Substance.

In addition, a non-guideline study (Unpublished study, 1996), is also included in the registration dossier where five SD rats were dose once with 179 µmol/kg and urine, faeces, blood and tissue were collected at 1, 4, 8, 24 and 48 hours after dose administration.

Absorption

Slow rates of appearance and disappearance from blood were demonstrated in all rats. Significantly higher blood levels of the Substance were observed in rats treated with 10 doses compared to rats treated with a single dose. However, the rates of appearance and disappearance were similar between the two groups at 24 hours while rats receiving 10 doses showed peak blood levels 1-2 hours after the last dose administration. Rats receiving a single dose showed peak blood levels 2-12 hours following administration (Unpublished study, 1982a).

Distribution

The Substance is distributed to various organs and tissues following absorption. Radioactivity recovered in tissues at 24 and 96 hours after dosing was highest in the thyroids followed by liver, kidneys and adrenals. Higher levels were found in skin compared to muscle or fat. In all tissues, the concentration of the Substance was significantly higher following 10 doses compared to 1 dose while disappearance from blood and tissue occurred at a faster rate in rats treated with a single dose compared to rats treated with 10 doses (unpublished study 1982a).

The distribution study (Unpublished study, 1996) revealed that high levels of radioactivity occurred in the GI tract and bladder during the first 8 hours. Liver and kidney also showed moderate levels. The radioactivity levels in the thyroid and pancreas were much higher than expected for non-excretory organs with thyroid levels which even exceeded that of liver tissue. Thyroid levels at 24 and 48 hours were the highest of all tissues examined at these time points (Unpublished study, 1996).

Metabolism

Although the study from 1996 did no analytical identification of the metabolites, they investigated the fraction of the excreted substance present in the organic soluble fraction, acid-labile conjugates or water-soluble fraction. 18 % of the administered dose was present in the organic fraction and 38% was present in the form of acid-labile conjugates. 44% was composed of a non-acid labile water-soluble fraction (Unpublished study, 1996).

Excretion

Rats receiving one dose of the Substance had eliminated 48% (males) and 56% (females) after 24 hours in urine. An additional 5-7 % was eliminated at 96 hours after administration. Faecal excretion happened at a slower rate and occurred primarily 12 to 48 hours after administration. The amount recovered in faeces was 42% (males) and 41% (females). According to the information in the registration dossier, this probably represents a balance between incomplete absorption and biliary elimination.

For rats receiving 10 doses of the Substance, excretion in urine following the last dose was similar or slightly higher than observed in rats receiving one single dose. After 24 hours, the urinary elimination was 52% (males) and 62% (females). An additional 7-8% was eliminated in urine after 96 hours.

96 hours after the last dose, excretion in faeces amounted to 58% and 45% in males and females, respectively.

8 hours after dosing, urinary excretion was the primary route of elimination. After 24, hours more than 60 % had been excreted. 6 days following treatment, approximately 4 % remained in the tissue (Unpublished study, 1996).

14.2. Acute toxicity and Corrosion/Irritation

Not evaluated in this substance evaluation.

14.3. Sensitisation

14.3.1. Non-testing methods for skin sensitisation

Skin sensitisation was predicted using the Danish QSAR database (Leadscope, SciQSAR and Case Ultra models). OECD Toolbox profilers were also applied.

Danish QSAR Database

In the Danish QSAR database, positive predictions (in domain) are obtained for the Substance in the Leadscope model for "Skin sensitisation GHS/CLP at least Cat 1, LLNA-based (open data and REACH registrations".

OECD Toolbox

The OECD Toolbox was used to predict the skin sensitisation potential of the Substance with and without skin metabolism.

Few alerts on skin sensitisation were found for the constituents of the Substance. In the DNA binding by OASIS v.1.4 profiler, alerts for all constituents were found based on "Single-Ring Substituted Primary Aromatic Amines". In the DNA binding by OECD profiler, no alerts were found for the constituents except one. It was not possible to classify any of the constituents according to the rules of neither the "DPRA Cysteine peptide depletion" nor "DPRA Lysine peptide depletion" profilers. Neither was it possible to classify according to the rules of the "Protein binding potency" and "Keratinocyte gene expression" profilers. In the Protein binding by OASIS v1.4 profiler, alerts for all constituents were found based on both "Single-Ring Substituted Primary Aromatic Amines" and "Substituted Anilines". No alerts were found in any of the constituents based on the "Protein binding by OECD" and "Protein binding alerts for skin sensitization by OASIS v1.4". Several alerts predicted constituents of the Substance and/or one or more skin metabolites as sensitizing including the DNA binding by OASIS v.1.4, DPRA Cysteine peptide depletion and Protein binding by OASIS v1.4 profilers.

14.3.2. *In vitro* skin sensitisation

No data on *in vitro* skin sensitisation with the Substance has been identified by the evaluating MSCA.

14.3.3. *In vivo* skin sensitisation

Two *in vivo* skin sensitisation studies have been submitted by the registrant. The results are summarised in the Table below.

Table 14.3.3-1: Overview *in vivo* studies on skin sensitisation

Reference and test method	Relevant results	Remarks
Unpublished study, 1979a Skin sensitisation: *in vivo* (non-LLNA). Non-guideline Guinea pig maximisation test (GPMT) conducted prior to	Skin irritation was observed in both groups during the induction.	Test material: The Substance (DETDA) EC Number: 270-877-4

adoption of OECD TG 406 and prior to GLP standards. 6 treated animals, 4 positive controls Dose: The first injections was 0.05 mL, while the remaining nine injections was 0.1 mL each. Ten weeks after the tenth injection, a challenge injection was made using 0.05 mL. Administration: Intracutaneous Reliability: Klimisch 3	Number of animals with positive reactions after the challenge: 1st reading at 24 hours: 0 out of 6 (test group); 4 out of 4 (positive control). 2nd reading at 48 hours: 0 out of 6 (test group); 4 out of 4 (positive control), higher degree of severity.	No information on purity or distribution between isomers Limitations: Dose, sex, vehicle and scoring system not stated, clinical observations not performed/reported, no negative controls
Unpublished study, 1977 Sensitisation: *in vivo* (non-LLNA). Non-guideline GPMT conducted prior to adoption of OECD TG 406 and prior to GLP standards. 15 males Dose: The test material was diluted to a 0.1 % solution in physiological saline. The first injection was 0.05 mL of a 0.1% solution. The remaining nine injections were 0.01 mL of the solution. The challenge dose was 0.05 mL of the solution. Administration: Intracutaneous Reliability: Klimisch 3	After 24 hours, three out of 15 guinea pigs had positive reactions.	Test material: The Substance (DETDA) EC Number: 270-877-4 No information on purity or distribution between isomers Limitations: No positive controls, clinical observations not performed/reported

14.3.4. Conclusion on skin sensitisation

Based on the available QSAR data, there is a concern for skin sensitisation for the Substance. Both the Danish QSAR database and several profilers of the OECD QSAR Toolbox found positive predictions for the possible sensitization properties of the Substance.

Several indications on sensitisation were predicted both for the parent compounds but especially for skin metabolites of the Substance.

The Danish QSAR Database is developed based on results from reports on human and animal studies and hence include skin metabolism implicitly (contrary to the OECD Toolbox). These predictions all indicate that the registered substance could be a skin sensitiser.

There are two available non-guideline *in vivo* skin sensitization studies in the registration dossier. They both have several shortcomings including low number of animals, no control group, lack of specification of test material, concentration and vehicle and poor reporting in general. These studies cannot be used to reject the concern for potential sensitizing properties of the Substance.

According to REACH Annex VII, Section 8.3, the information provided in the registration dossier must be sufficient to allow a conclusion as to whether the substance is a skin sensitizer and whether it can be presumed to have the potential to produce significant

sensitisation in humans (Cat. 1A), and — risk assessment, where required. If this is not the case, *in vitro* testing according to Section 8.3.1 should be performed unless reliable *in vivo* data is available. The evaluating MSCA considers that the available data are not sufficient to allow a conclusion on skin sensitisation potential of the Substance as the provided *in vivo* studies are not appropriate to clarify the concern. Hence, the evaluating MSCA considers that standard information requirement for skin sensitization is not fulfilled, and it should be addressed in the ongoing compliance check before concluding on potential follow-up actions.

14.4. Repeated dose toxicity

The registrant has submitted eight *in vivo* repeated dose toxicity (RDT) studies including one dermal 21-day study and seven studies using oral exposure.

14.4.1. Oral repeated dose toxicity

The results of the oral repeated-dose toxicity studies are summarised in the following table:

Table 14.4.1-2: Overview of oral *in vivo* studies on repeated dose toxicity

Reference and test method	Relevant results	Remarks
Unpublished study, 1986a 28-day RDT study equivalent or similar to OECD TG 407 GLP Species: Rat (SD); 5/sex/group Administration: Oral, feed Doses: 0, 40, 400, 800, 1200, 1600, 3200 ppm Reliability: Klimisch 1	Significantly decreased body weight in both males and females from the 400 ppm dose group upwards. Decreased food consumption was observed from 800 ppm. Clinical signs of toxicity (e.g. poor grooming, decreased activity). Increased relative liver and kidney weights in males in the 1200 ppm dose group upwards. In the 3200 ppm dose group, one female died. Clinical signs of toxicity included ataxia and morbidity. This group was sacrificed after 17 days. The NOAEL was 40 ppm based on decreased body weight and food consumption observed at higher dose levels.	Test material: The Substance (DETDA) (EC 270-877-4) Limitations: In comparison with the most recent OECD TG 407, deviations include that functional observation battery (FOB), ophthalmoscopy, haematology, clinical chemistry, and urinalysis were not performed, and organ weights and histopathology were only performed for liver and kidney
Unpublished study, 1989 28 day RDT study equivalent or similar to OECD TG 407 and including a 28 day recovery group GLP Species: Rat (SD), 15/sex/group Exposure: oral, feed	**Run 544** Body weight: Reduced body weight gain in all dose groups at several time periods. Decreased mean body weight and reduced food consumption at several time periods in high dose. Pancreatic effects	Test material: The Substance (DETDA) (EC 270-877-4) Limitations: In comparison with the most recent OECD TG 407 (adopted 3 October 2008), deviations include that FOB was not performed; reticulocyte count and a measure of blood clotting time/potential were not part of the haematological

Doses: 0, 50, 125, 320 ppm corresponding to 4/5, 10/12 and 24/28 mg/kg bw/day in males and females, respectively. Two commercial preparations (different lots) of the Substance was tested: "Run 544" and "Lot#2-87". Reliability: Klimisch 1 The Substance was administered daily for 14 or 28 days. 5 randomly selected rats pr. group/sex were necropsied. The remaining rats from each treatment group continued untreated on test for additional 28 days.	Moderate multifocal acinar degeneration and slight diffuse atrophy in the pancreas (1 male) at the 28-d sacrifice in low dose and mid dose. In mid dose, moderate multifocal acinar degeneration and slight diffuse atrophy in the pancreas (2 males) at the 56-d sacrifice In high dose, moderate to moderately severe multifocal acinar degeneration in the pancreas accompanied by a nominal to moderate diffuse atrophy of the acinar cells (all animals) at the 28-d sacrifice Moderate to moderately severe diffuse acinar atrophy in the pancreas (all males) at the 56-d sacrifice; concurrent in 4/5 male rats, there were bilateral cataractous changes in the eyes <u>Organ weight</u> Increased relative testes weight in males at the 14-d sacrifice in the mid dose group. Increased relative liver weight in high dose males at the 28-d sacrifice Increased relative kidney, liver and brain weight in high dose females at the 28-d sacrifice Increased relative kidney, brain and adrenal weight in high dose males at the 56-d sacrifice Increased relative liver weight in high dose females at the 56-d sacrifice <u>Other:</u> In the high dose males, clinical signs of toxicity and macroscopic changes was also observed. Cataracts were identified in two males. **<u>Lot#2-87</u>:** <u>Body weight</u>	examination; only liver, adrenals, kidneys, gonads and brain were weighed; fewer tissues and organs were examined histopathologically

	Decreased body weight gain at several intervals in all dose groups. In addition, decreased weight and food consumption in high dose at several time periods. <u>Pancreatic effects:</u> In mid dose, moderate multifocal acinar degeneration in the pancreas at the 28-d sacrifice (1 male and 1 female) and 56-d sacrifice (3/5 males). In high dose, moderate to moderately severe multifocal acinar degeneration in the pancreas at the 28-d sacrifice (all animals except 1 male) and 56-d sacrifice (2/5 males and 1/5 females) <u>Organ weight:</u> In high dose, increased relative brain and testes weight in males at the 14-d sacrifice Increased relative liver weight in males at the 28-d sacrifice Increased relative kidney weight in females at the 28-d and 56-d sacrifices The NOAEL was 50 ppm corresponding to 4/5 mg/kg bw/day for males/females, respectively, based on the pancreatic toxicity from 125 ppm.	
Unpublished study, 1987a 90-day RDT study equivalent or similar to OECD TG 408 Administration: oral, feed GLP Species: Rat (SD), 20/sex/group Doses: 0, 50, 125, 320 ppm corresponding to 8/10, 21/27 and 122/125 mg/kg bw in males and females, respectively. Reliability: Klimisch 1	<u>Body weight:</u> Decreased mean body weight, body weight gain and food consumption in mid and high dose. <u>Pancreas:</u> Minimal to moderate multifocal degeneration of the acinar cells of the pancreas in low dose (males only) and in mid dose. In high dose, diffuse atrophy of the acinar cells of the pancreas and vacuolation of the islet cells of the pancreas was observed. <u>Organ weight and histopathology</u>	Test material: The Substance (DETDA) (EC 270-877-4) Limitations: In comparison with the most recent OECD TG 408, deviations include that FOB was not performed, a measure of blood clotting time/potential was not part of the haematological examination, only liver, adrenals, kidneys, gonads and brain were weighed.

	Increased relative liver, kidney and brain (females only) weights in mid dose. Increased relative adrenal, kidney, liver (females only), testes (males) and brain weights in high dose. Increased splenic pigmentation in all dose groups. In high dose, bone marrow depletion, tubular vacuolation of the kidneys, atrophy of many organs, lymphoid depletion of the spleen, thymus and mesenteric lymph node, and increased pigmentation of the liver and spleen. <u>Ocular lesions:</u> Ocular lesions were observed in all dose groups (1, 4 and 13 in low, mid and high dose, respectively). High incidence of bilateral cataractous change in the eyes in high dose. <u>Blood chemistry:</u> Increased SGPT, SGOT, GGPT (females only) and BUN. <u>Systemic toxicity:</u> In high dose, several clinical signs of toxicity which in general resulted in either moribund sacrifice or death. 27 high dose animals died during the study. The LOAEL was 50 ppm (8/10 mg/kg bw/day in males and females, respectively) based on the pancreatic effects observed.	
Unpublished study, 1986b 90-day RDT study according to OECD TG 408 Administration: oral, feed Not GLP Reliability: Klimisch 3 Doses: 0, 100, 300, 900 ppm corresponding to approximately	In the 900 ppm dose group, one male died and poor general condition and weight loss was observed particularly in males. Increased food consumption was also observed. The NOAEL was 300 ppm based on the severe effects observed in high dose animals.	Test material: The Substance (DETDA) (EC 270-877-4) Specifications: Percentage of components: 78.2% 2,4-DETDA; 20.9% 2,6-DETDA Limitations: In comparison with the most recent OECD TG 408, deviations include that FOB, ophthalmoscopy, haematology, clinical chemistry, organ weights

15 and 45 mg/kg bw/day in mid and high dose Species: Rat (Wistar), 5/sex/group		and histopathology were not measured; only 5/sex/group, and age about 8 months at study start.
Unpublished study, 1986c Combined oral (feed) 2-year RDT and carcinogenicity study equivalent or similar to OECD TG 452 Administration: Oral, feed Not GLP Rat (Wistar), 50/sex/group + 10/sex/group for interim sacrifices) Doses: 0, 80, 240 ppm, halved from 456th day to 40 and 120 ppm. The initial dose corresponded to approximately 6 and 12 mg/kg bw/day Exposure: Lifetime, daily exposure Reliability: Klimisch 3	<u>80/40 ppm</u>: Survival only slightly different from control <u>240/120 ppm</u>: Survival markedly shorter Poor general condition Markedly decreased body weight – still clearly decreased towards the end of the study Markedly increased food consumption and drinking water consumption – less marked after the reduction of the dose to 120 ppm The NOAEL for non-neoplastic effects was 80/40 ppm based on the effects observed in high dose animals.	Test material: The Substance (DETDA) (EC 270-877-4) Specifications: Batch No. 74 used for the first 11.5 months of the study: 78.2% 2,4-DETDA; 20.9% 2,6-DETDA Batch No. 94 used from 11.5 months on until study completion: 64.3-68.7% 2,4-DETDA; 27.5-33.2% 2,6-DETDA In comparison with the most recent OECD TG, deviations include that only two dose levels were included and fewer tissues and organs were examined for histopathology.
Unpublished study, 1986d Combined 2-year repeated dose and carcinogenicity, equivalent or similar to OECD TG 452 Administration: oral, gavage Not GLP Species: Rat (Wistar), 20/sex/group Doses: 0, 4, 12 mg/kg bw/day, halved from 127th to 2 and 6 mg/kg bw/day, again halved from 256th day to 1 and 3 mg/kg bw/day. Exposure: 2 years (5 days per week). Reliability: Klimisch 3	<u>4/2/1 mg/kg bw/day</u>: Survival only slightly different from control <u>12/6/3 mg/kg bw/day</u>: Survival markedly shorter Poor general condition Markedly decreased body weight – still clearly decreased towards the end of the study in males – less clearly in females Increased drinking water consumption The NOAEL for non-neoplastic effects was 4/2/1 mg/kg bw/day based on the effects observed in high dose animals.	Test material: The Substance (DETDA) EC Number: 270-877-4 Specifications: Lot/batch No.: 94 (64.3-68.7% 2,4-DETDA and 27.5-33.2% 2,6-DETDA) Limitations: In comparison with the most recent OECD TG, deviations include that only two dose levels were included; only 20 animals/sex/group; fewer tissues and organs were examined for histopathology.
Unpublished study, 1992	<u>Survival:</u> Survival was comparable between treated and control animals of each	Test material: The Substance (DETDA) (EC 270-877-4)

Combined chronic repeated dose and carcinogenicity (2-year) study equivalent or similar to OECD TG 453 Administration: Oral, feed GLP Doses: 0, 10, 35, 70 ppm corresponding to 0.4, 1.4, and 3.2 mg/kg bw/day in males and 0.5, 1.8 and 3.8 mg/kg bw/day in females Exposure: Daily seven days per week for two years. Blood glucose evaluation was performed in selected animals sacrificed during the study. In addition, blood glucose evaluation was performed at 18 months and at terminal necropsy Ophthalmoscopy was performed on all animals prior to study initiation and at 6, 12, 18 and 24 months In addition to full histopathology on organs and tissues in the control and high-dose animals, and all animals sacrificed during the study, the adrenals, eyes, liver, pancreas, pituitary gland and thyroids were evaluated from the low- and mid-dose groups. The mammary glands and any tissue masses suspected of being a mammary gland tumour were also evaluated from the low- and mid-dose groups. Reliability: Klimisch 1	sex except in the low dose male rats which had significantly greater survival. <u>Body weight:</u> In mid dose, tendency for lower body weight in males. Body weight gain 12% and 10% reduced in males and females compared to control. In high dose, body weight gain was 25% and 13% reduced in males and females compared to control Statistically significantly decreased body weight in males <u>Effects on organs:</u> Increased incidence of proliferative lesions in the liver in all dose groups Increased incidence of thyroid follicular cell hypertrophy in high dose males and increased incidence of thyroid follicular cell hyperplasia / follicular cysts in mid and high dose Increased incidence in multifocal acinar atrophy in pancreas accompanied by interstitial fibrosis and fatty infiltration in males Increased incidence of cysts and focal areas of tubular hyperplasia in the kidney in males <u>Other:</u> Dense bilateral cataracts in 5 males Six males had blood glucose values >300 mg/dl The NOAEL for non-neoplastic effects was 10 ppm (0.4/0.5 mg/kg bw/day for males/females, respectively).	Pre-study specifications: 2,4-diamino-3,5-diethyltoluene (EC No 218-256-9): 75.8% 2,6-diamino-3,5-diethyltoluene (EC No 218-255-3): 20.1% Dialkylated phenylenediamines (3.1%) Concentrations were determined every 6 months and varied only slightly (<0.5%) throughout the study

14.4.2. Dermal repeated dose toxicity

The results of the experimental study on RDT after dermal administration is summarised in the following table:

Table 14.4.2-3: Overview of dermal *in vivo* study on repeated dose toxicity

Reference and test method	Results	Remarks
Unpublished study, 1981 21-day repeated dose toxicity study performed prior to the adoption of OECD TG 410 Administration: Dermal Species: Rabbit (New Zealand White), 10/sex/group Doses: 1, 10, 100 mg/kg bw/day Exposure: 5 days/week Rabbits were administered the test material on shaved intact or abraded skin. Following administration, the application site was occluded for 6 hours, at which time the test material was wiped off. Clinical signs, dermal irritation, mortality, body weights, haematology, clinical chemistry, organ weights (liver, kidneys, heart, ovary/testes, thyroid (with parathyroid), adrenals, lungs, brain, pituitary), gross pathology and histopathology (liver, kidneys, heart, ovary/testes, thyroid (with parathyroid), adrenals, lungs, brain, pituitary) were evaluated for all animals. Reliability: Klimisch 2 GLP	Very slight irritation in low dose animals and mild to moderate local irritant effects in mid and high dose Chronic dermatitis was observed in treated skin of control and treated rabbits of either sex. The incidence and severity were greater in treated rabbits than in controls and the incidence increased with increasing dose Cysts were observed in thyroid glands of rabbits of either sex. The incidence was dose-related in males but not in females. The NOAEL for systemic toxicity was ≥ 100 mg/kg bw/day. Local irritant effects in form of skin irritation and chronic dermatitis were observed at all dose levels, i.e. the LOAEL for local toxicity is 1 mg/kg bw/day.	Test material: The Substance (DETDA) (EC 270-877-4) Specifications: 80:20 ratio of 2,4- and 2,6-diethyltoluenediamine Analytical purity: 95.78%

14.4.3. Summary and discussion of repeated dose toxicity

Body weight, body weight gain and food and water consumption

In all seven oral RDT/carcinogenicity studies, body weight gain and body weight were reduced in a dose-dependent manner compared to controls often reaching statistical significance in the high dose animals. In the 28+28 progressive/reversibility study (Unpublished study, 1989), the high dose males exposed to "Run 544" continued to lose weight during the 28 day recovery period, while animals treated with "Lot 2-87" at the same dose gained weight and became comparable to controls. In the two carcinogenicity studies from 1986 (Unpublished study, 1986c and 1986d), body weight was reported to be markedly decreased. However, statistical significance was not reported. The reduced body weight and weight gain was observed to a lesser extent in females.

Decreased food consumption was reported in some studies (Unpublished study, 1986a, 1987a, 1989). However, no effects on food consumption were observed in one of the two-year studies (Unpublished study, 1992) whereas significant increases were also observed

in another two-year study (Unpublished study, 1986c). Increased water consumption was observed in two two-year studies (Unpublished study, 1986c; 1986d).

Ocular examinations

Ocular examinations were performed in three of the oral RDT/carcinogenicity studies (Unpublished study, 1987a, 1989, 1992). In the 28+28 day study (Unpublished study, 1989), cataracts was observed in two high dose males treated with "Run 544". This finding could indicate a possible ocular toxic effect of the test substance. It was reported to occur after 56 days from the start of dosing including 28 days recovery period. In one 90-day RDT study (Unpublished study, 1987a) different ocular lesions were observed. The study authors considered these to be a direct or indirect effect of the treatment. In one two-year study (Unpublished study, 1992) dense bilateral cataracts in six of 43 high dose males was observed after 18 months of exposure.

Clinical chemistry

Clinical chemistry was investigated in three of the oral RDT/carcinogenicity studies. The unpublished 90-day study (1987a) found increases in SGPT, SGOT, GGPT (females) and BUN in high dose animals. This indicates occurrence of liver toxicity in the highest dosed rats. Mean blood glucose values were comparable between all groups. However, inspection of individual data for high dose rats showed a very wide variation in glucose levels ranging from 16 to 436 mg glucose/dl blood. Control glucose levels were 127±20.8 mg/dl and 139±25.8 mg/dl for male and female rats, respectively. The 28+28 day study (Unpublished study, 1989) measured blood glucose and insulin levels in fasting animals after exposure of 14 and 28 days and after additional 28 days recovery. No consistent pattern was observed but several males in the high dose group seemed to have higher glucose values on day 56 compared to the control group. The unpublished two-year study (1992) also observed no statistically significant differences in mean blood glucose values between groups even though the mean value for high dose males seemed higher (150±95) compared to the controls and other treated groups (117±23, 125±20, and 124±26, control, low dose, mid dose, respectively). The standard deviation in blood glucose values was much higher in the high dose group and thus the effect was not statistically significantly different. However, after 18 months treatment six high dose males showed hyperglycaemia (i.e. blood glucose level >300 mg/dl), whereas no animals in the other dose groups showed such high glucose levels.

Organs

All studies investigating pathology on the pancreas found effects on this organ. In the 28+28 day study (Unpublished study, 1989), histopathological evaluation of the tissues from the rats exposed to one of the 2 lots of the Substance, i.e. "lot #2-87" or "Run 544", for 28 days revealed treatment-related degenerative acinar changes in the pancreas in mid- and high dose males as well as high dose females. A similar pancreatic acinar degeneration was not present in the low dose groups. Male rats were more severely affected and at an earlier time than the female rats. The pancreatic effects of "lot #2-87" almost recovered by the end of the 28-day recovery period. However, the changes in males treated with "Run 544" included degeneration of the islet cells of the pancreas. In one of the 90-days studies (Unpublished study, 1987a), a minimal to moderate multifocal degeneration of the acinar cells of the pancreas was observed in males in the low and mid dose groups. Animals in the high dose groups showed diffuse atrophy of the acinar cells and vacuolation of the islet cells of the pancreas. It should be taken into account that 27/40 of the high dose animals died and several clinical signs of toxicity were observed in this high dose group. In the two-year study (Unpublished study, 1992), an increased incidence in multifocal acinar atrophy accompanied by interstitial fibrosis and fatty infiltration was observed in high dose males. No effects were observed in islet cells in this study. However, the authors stated that the islet cells were difficult to investigate due to the destruction of acinar cells. Pathological investigations on pancreas were not performed in the other 90-day study (Unpublished study, 1986b) or in the 28-day study (Unpublished study, 1986a). As stated earlier, it is not possible to assess the observation on pancreas in the two life-time studies (Unpublished study, 1986c, 1986d) due to the study design. In the 90-day

RDT study where effects on islet cells were observed (Unpublished study, 1987a), it was not specified if the effects were observed on (glucagon producing) alpha or (insulin producing) beta islet cells.

Various scarce effects on other organs were observed in the available studies including increased pigmentation of the liver in one of the 90-day studies (Unpublished study, 1987a) and gross changes in liver in the 2-year study (Unpublished study, 1992).

Scarce effects on kidneys were also observed in some of the available studies referred to above. In the 28+28-day study (Unpublished study, 1989), pale and/or spotted kidneys were observed and tubular vacuolation of the kidneys was observed in one of the 90-day studies (Unpublished study, 1987a).

An increased incidence of thyroid follicular cell hypertrophy in high dose males, increased incidence of follicular cell hyperplasia/follicular cysts in mid- and high dose males and in high dose females were observed in the two-year study (Unpublished study, 1992). In the 21-day dermal study (Unpublished study, 1981), cysts were observed in thyroids and incidence was dose-related in males but not in females. According to the author, these lesions are common histopathologic findings in rabbits of this age and strain and the cysts observed were probably not a consequence of the treatment.

Potential mechanisms of toxicity

The 28+28-day study (Unpublished study, 1989) and one sub-chronic 90-day study (Unpublished study, 1987a) identified the pancreas as the target organ. This was investigated further in the two-year carcinogenicity study (Unpublished study, 1992). Histopathology results of selected tissues in treatment groups indicated that the primary or most sensitive histopathologically observable effect of the Substance at low doses was that on the pancreatic acinar cells. The acinar cells, one of two major tissue types in the pancreas, are exocrine cells producing enzymes, which are excreted into the GI tract and aid in the digestion of food. The decrease in body weight observed during the latter phase of the studies may be related to a deficiency of these enzymes and a resultant inefficiency in digestion.

In addition to the acinar cell atrophy detected in the low (5 mg/kg bw) and mid dose (12.5 mg/kg bw) groups in the 90-day study (Unpublished study, 1987a), vacuolation and a reduction in the number of islet cells was also observed in high dose rats. The major product of pancreatic islet cells is the hormone insulin. If the islet cell lesions led to an insulin deficiency, a resultant increase in blood glucose levels could occur. Elevated blood levels of various sugars could produce lesions similar to the ones observed in the kidneys and eyes (i.e. bilateral cataracts) in the same study. Similar findings of bilateral cataract in the eyes were also observed in two other RDT studies. However, in the three studies which measured blood glucose levels, there was no statistically significant increase in the mean blood glucose levels of the high dose groups compared to controls. However, the test designs had several shortcomings in this regard which would make mean blood glucose measurements insensitive to such changes. Nevertheless, the two-year study (Unpublished study, 1992) did observe six high dose males with abnormal high blood glucose values (>300 mg/dl) of which five had bilateral cataracts after 18 months exposure and all six had histopathological changes in pancreas. This indicates a possible correlation between cataracts and effect of the Substance on the pancreas. In this study, no effects were observed on islet cells but the authors stated that it was difficult to evaluate the islet cells due to the destruction of the acinar cells. The standard deviation in the high dose males was quite high. It is possible that the more sensitive individuals reacted with elevated glucose levels, which was followed by the observed adverse effects, whereas less sensitive individuals could better tolerate the exposure to the Substance.

It is the opinion of the evaluating MSCA that the observed toxicity of the Substance in these animals could be caused by the effects observed in the pancreas. The depressed body weight gain observed in mid- and high dose animals may be related to a functional pancreatic defect and result in inanition. Beta islet cells are responsible for release of the necessary amount of insulin to maintain normal blood sugar levels and glucose cannot be

utilized by the majority of organs without the aid of insulin. As glucose is the primary energy source for the body, an effect on beta islet cells means that other energy sources must be utilized i.e. fat from body stores and protein supplied from organs not critically essential for life are broken down to supply the body with the needed energy. This process, coupled with inadequate digestion due to an insufficient release of pancreatic enzymes from the exocrine acinar cells, may explain the severe body weight loss and atrophy observed in several organs, especially in the high dose groups of most of the available RDT studies. Thus, the reduction in body weight and body weight gain could, in the opinion of the evaluating MSCA, be considered as a secondary effect of the toxicity to pancreas.

Lack of insulin and the corresponding elevation of glucagon leads to increased release of glucose by the liver (a process that is normally suppressed by insulin) from glycogen via glycogenolysis and also through gluconeogenesis. High glucose levels spill over into the urine, taking water and solutes (such as sodium and potassium) along with it in a process known as osmotic diuresis. This leads to polyuria, dehydration, and compensatory thirst and polydipsia. Several of the studies described animals as appearing dehydrated and some studies reported increased water consumption.

Alterations in absolute and relative organ weights and certain hematologic and serum chemistry values may also be consistent with the emaciated condition of the high dose animals. Increases in serum SGPT, SGOT, GGTP and BUN levels may be related to the catabolism of body protein for use as an energy source.

The absence of insulin also leads to the release of free fatty acids from adipose tissue (lipolysis), which are converted through beta oxidation in the liver into ketone bodies. The ketone bodies have a low pKa and therefore turn the blood acidic. The body initially buffers a pH-change with the bicarbonate buffering system, but this system may quickly become overwhelmed and other mechanisms must then take over to compensate for the acidosis. One such mechanism is hyperventilation to lower the blood carbon dioxide levels (a form of compensatory respiratory alkalosis). This could explain the dyspnea observed.

14.4.4. Conclusion on repeated dose toxicity

Based on the observed effects on pancreas, blood glucose levels and eyes in multiple RDT/carcinogenicity studies, a concern for a substance related induction of diabetes cannot be excluded. Based on the results from the ADME, RDT and carcinogenicity studies, it is clear that the pancreas appears to be the target organ. The evaluating MSCA is of the opinion that some or all of the effects observed in several studies (in particular the elevated blood glucose levels and ocular effects) could be secondary effects of the specific toxicity towards the pancreas and these effects are not considered to be secondary to general systemic toxicity. Moreover, elevated blood glucose levels and adverse ocular effects are well known secondary effects of diabetes. The exocrine acinar cells in the pancreas may be affected first and that this leads to dysfunction of the endocrine islet beta cells. Another possible explanation could be that effects on the exocrine acinar cells are easier to detect than effects on islet beta cells or on insulin/glucagon production. The Substance already has a harmonised classification as STOT RE 2 (H373) based on the effects on pancreas. The evaluating MSCA considers it disproportionate to request further studies under substance evaluation to elucidate the exact mechanism considering the amount of data already available.

14.5. Mutagenicity

14.5.1. Non-human information

In the Danish QSAR database, predictions for the two constituents of the Substance, i.e. 2,4-DETDA and 2,6-DETDA were positive for Ashby Structural Alerts.

Predictions for the Bacterial Reverse Mutation Test (Ames test) were positive for Frameshift Ames Mutagens in the commercial models Leadscope and SciQSAR for both constituents. The CASE Ultra model was inconclusive. Basepair mutagenicity was predicted to be negative in all three models (SciQSAR, Leadscope and Case Ultra).

The predicted mutagenicity in thymidine kinase Locus in mouse lymphoma cells is positive in the Leadscope model for both main constituents but out of applicability domain for the other two models. Predictions for mutations in Chinese hamster ovary cells were negative in all three models. Predictions for chromosomal aberrations in Chinese hamster ovary cells were positive in two out of three models for both main constituents. Predictions for chromosomal aberrations in Chinese hamster lung cells were inconclusive and out of domain for both main constituents.

Predictions for unscheduled DNA Synthesis in rat hepatocytes were positive in 2 out of 3 models. Predictions for Syrian hamster embryo cell transformation were inconclusive.

14.5.2. *In vitro* mutagenicity

The results of *in vitro* mutagenicity studies with the Substance are summarised in the following table. All studies are available in the registration dossier.

Table 14.5.2-4: Overview of *in vitro* genotoxicity studies.

Test method	Results	Remarks
OECD TG 473 *In vitro* mammalian chromosome aberration test EU Method B.10 (Mutagenicity - *In Vitro* Mammalian Chromosome Aberration Test) With and without metabolic activation Doses: 112.5 to 1800 μg/ml Reliability: Klimisch 1	Ambiguous Specifications: Negative (with S_9 (1 %) or without) for lymphocytes: Chromosome Aberration Test - Experiment 1 Positive (Significant increase in polyploid cells in the absence of S_9 at 1800 μg/ml and in the presence of S_9 (1 %) at 900 μg/ml) for lymphocytes: Chromosome Aberration Test - Experiment 1 Positive (in the presence of S_9 (2 %)) for lymphocytes: Chromosome Aberration Test - Experiment 2 Positive (in the absence of S_9 at 1800 μg/ml and in the presence of S_9 (2 %) at 900 μg/ml) for lymphocytes: Chromosome Aberration Test - Experiment 2 Negative (with S_9 (2 % or 5 %)) for lymphocytes: Chromosome Aberration Test - Experiment 3 Positive (in the presence of S_9 (2 %) at 1800 μg/ml) for lymphocytes: Chromosome Aberration Test - Experiment 3 Cytotoxicity: No	Key Study (Unpublished study, 2000a) Test material: The Substance Purity 98.4%

OECD Guideline 476 (*In vitro* Mammalian Cell Gene Mutation Test) Mouse lymphoma L5178Y cells With and without metabolic activation Doses: Preliminary toxicity test: 28.1 to 1800 μg/ml. Experiment 1: 28.1 to 600 μg/ml (without S_9); 225 to 1800 μg/ml (with S_9). Experiment 2: 25 to 400 μg/ml (without S_9); 200 to 1100 μg/ml (with S_9). Experiment 3: 300 to 1300 μg/ml (with S_9). Reliability: Klimisch 1	Experiment 1: Positive (statistically significant dose related (linear trend) increase in mutant frequency at and above 900 μg/ml) with metabolic activity and negative without metabolic activation. Cytotoxicity: yes Experiment 2: Positive (statistically significant dose-related increase in the mutant frequency) with metabolic activation. Cytotoxicity: no (also positive without activation accompanied with excessive cytotoxicity). Experiment 3: Positive (statistically significant dose-related increase in mutant frequency) for mouse lymphoma with metabolic activation.	Key Study (Unpublished study, 2000b) Test material: The Substance Purity: 98.4%
DNA damage and repair assay, unscheduled DNA synthesis in mammalian cells *in vitro* (DNA damage and/or repair) Hepatocytes obtained from adult male Fischer-344 rats (met. act.: not applicable) Doses: 100, 500, or 1000 μg/well in 2 ml of media Reliability: Klimisch 2	Negative Cytotoxicity: no (up to 1000 μg/well in 2 ml of media)	Supporting study (Unpublished study, 1984) Test material: The Substance Purity not stated
In vitro gene mutation study in mammalian cells Mammalian cell line: BALB/3T3 Cl. A31 With and without metabolic activation Reliability: Klimisch 2	Negative for mammalian cell line, other: BALB/3T3 Cl. A31 (all strains/cell types tested) with metabolic activation Cytotoxicity: yes (at the highest concentration tested)	Supporting study (Unpublished study, 1979b) Test material: The Substance Purity not stated
In vitro gene mutation study in bacteria (Bacterial reverse mutation assay) *S. typhimurium* TA 1535	Negative with and without metabolic activation Details:	Supporting study (Unpublished study, 1986e)

S. typhimurium TA 1537 *S. typhimurium* TA 1538 *S. typhimurium* TA 98 *S. typhimurium* TA 100 With and without metabolic activation Doses: 60, 200, 600, 2000, and 6000 μg/plate OECD Guidelines for Testing Chemicals, ISBN 92-64-12221-4, adopted by the council at its 535th meeting on May 12th, 1981 U.S. EPA Health Effects Test Guidelines, EPA-560/6/82-001, or where appropriate its revision, EPA 560/6-84-001 Reliability: Klimisch 1	Negative (up to 6000 μg/plate) for *S. typhimurium* TA 1535, TA 1537, TA 98 and TA 100 (all strains/cell types tested) with and without metabolic activation Cytotoxicity: yes (Limit dose of 6000 μg/plate caused a reduction in the mean number of revertant colonies relative to the solvent control values)	Test material: The Substance Purity not stated
In vitro gene mutation study in bacteria (Bacterial reverse mutation assay) *S. typhimurium* TA 1538 *S. typhimurium* TA 98 *S. typhimurium* TA 100 With metabolic activation Assay 1: 500, 750, 1000, 3750, or 7500 μg/plate Assay 2: 1000, 3750, 7500, 10000, or 12500 μg/plate OECD Guidelines for Testing Chemicals, ISBN 92-64-12221-4, adopted by the council at its 535th meeting on May 12th, 1981 U.S. EPA Health Effects Test Guidelines EPA-560/6/82-001, or where appropriate its revision EPA 560/6-84-001 Reliability: Klimisch 1	Negative with metabolic activation Details: Negative (up to 12500 μg/plate) for *S. typhimurium* TA 1538, TA 98 and TA 100 Cytotoxicity: yes (observed at 12500 μg/plate)	Supporting study (Unpublished study, 1987b) Test material: The Substance Purity not stated
In vitro gene mutation study in bacteria (Bacterial reverse mutation assay) *S. typhimurium* TA 1535, TA 1537, TA 1538, TA 98 and TA 100	Negative without metabolic activation (TA 1538, TA 100, and TA 98) Positive without metabolic activation (TA 1535 and TA 1537)	Supporting study (Unpublished study, 1978a) experimental result

With and without metabolic activation Doses: 0.05, 0.1, 1.0, or 5.0 μl of a 5.0 % solution (v) of DETDA in DMSO. Reliability: Klimisch 2	Negative with metabolic activation (TA 1535, TA 1537, TA 1538, TA 100, and TA 98). Cytotoxicity: no	Test material: The Substance Purity not stated
In vitro DNA damage and/or repair assay, unscheduled DNA synthesis in mammalian cells *in vitro* Hepatocytes obtained from adult male Fischer-344 rats (met. act.: not applicable) Doses: 0.05, 0.1, 0.5, 1, 5, 10, 50, 100, 500, or 1000 μg in 2 ml/well Reliability: Klimisch 2	Negative Cytotoxicity: Yes (produced at 10 μg/well in 2 ml of media and higher)	Supporting study (Unpublished study, 1986f) Test material: The Substance Purity not stated
In vitro gene mutation study in bacteria (Bacterial reverse mutation assay) OECD Guidelines for Testing Chemicals, ISBN 92-64-12221-4, adopted May 12th, 1981 U.S. EPA Health Effects Test Guidelines EPA-560/6/82-001, or where appropriate its revision, EPA 560/6-84-001 *S. typhimurium* TA 1538 *S. typhimurium* TA 98 *S. typhimurium* TA 100 Metabolic activation: With and without Doses: 625, 1250, 2500, 5000, and 7500 μg/plate Reliability: Klimisch 1	Negative without metabolic activation (TA 1535, TA 1537, TA 1538, TA 98, and TA 100) Negative with metabolic activation (TA 1535) Positive with metabolic activation (TA 1537, TA 1538, TA 98, and TA 100) Cytotoxicity: No	Supporting study (Unpublished study, 1985a) Test material: The Substance Purity not stated
In vitro DNA damage and/or repair study *E. coli*, DNA damage/DNA repair (DNA damage and/or repair) *E. coli*, other: W3110/polA$^+$ (ATCC No. 27325) and P3478/polA$^-$ (ATCC No. 25947)	Negative (with/without metabolic activation) Cytotoxicity: no	Supporting study (Unpublished study, 1978b) Test material: The Substance Purity not stated

Metabolic activation: With and without Doses: 2, 10, or 20 μl of undiluted test material Reliability: Klimisch 2		
In vitro gene mutation study in bacteria (Bacterial reverse mutation assay) *S. typhimurium* TA 1538 *S. typhimurium* TA 98 *S. typhimurium* TA 100 Metabolic activation: With Doses: 100, 333, 1000, 1000, 3333, and 10000 μg/plate OECD Guidelines for Testing Chemicals, ISBN 92-64-12221-4, adopted by the council at its 535th meeting on May 12th, 1981 U.S. EPA Health Effects Test Guidelines EPA-560/6/82-001, or where appropriate its revision, EPA 560/6-84-001 Reliability: Klimisch 1	Positive for TA 1538 and TA 98 with metabolic activation Negative for 100 with metabolic activation Cytotoxicity: No	Supporting study (Unpublished study, 1985b) Test material: The substance Purity not stated
In vitro gene mutation study in mammalian cells No guideline followed Cell line: BALB/3T3 Clone A31-1 mouse embryo cells Metabolic activation: Without Doses: 0.5, 0.17, and 0.05 μl/ml Reliability: Klimisch 1	Negative without metabolic activation Cytotoxicity: No	Supporting study (Unpublished study, 1982b) Test material: The Substance Purity not stated
In vitro gene mutation study in bacteria (Bacterial reverse mutation assay) *S. typhimurium* TA 1535, TA 1537, TA 98 and TA 100 Metabolic activation: With and without Doses: Preliminary toxicity screen performed at: 100, 330, 1000, 3300, and 10000 μg/plate	Positive in all strains with metabolic activation. Negative in all strains without metabolic activation. Cytotoxicity: No	Supporting study (Unpublished study 1986g) Test material: The Substance Purity not stated

Definitive evaluation performed at: 60, 200, 600, 2000, and 6000 μg/plate Reliability: Klimisch 1		
In vitro gene mutation study in bacteria (Bacterial reverse mutation assay) *S. typhimurium* TA 1535 *S. typhimurium* TA 1537 *S. typhimurium* TA 1538 *S. typhimurium* TA 98 *S. typhimurium* TA 100 Metabolic activation: With and without Doses: 625, 1250, 2500, 5000, and 7500 μg/plate OECD Guidelines for Testing Chemicals, ISBN 92-64-12221-4, adopted by the council at its 535th meeting on May 12th, 1981 U.S. EPA Health Effects Test Guidelines, EPA-560/6/82-001, or where appropriate its revision, EPA 560/6-84-001 Reliability: Klimisch 1	Negative with and without metabolic activation Cytotoxicity: no	Supporting study (Unpublished study, 1985c) Test material: The Substance Purity not stated
In vitro gene mutation study in bacteria (Bacterial reverse mutation assay) *S. typhimurium* TA 1538 *S. typhimurium* TA 98 *S. typhimurium* TA 100 Metabolic activation: With Doses: 100, 333, 1000, 3333, and 10000 μg/plate OECD Guidelines for Testing Chemicals, ISBN 92-64-12221-4, adopted by the council at its 535th meeting on May 12th, 1981 U.S. EPA Health Effects Test Guidelines EPA-560/6/82-001, or where appropriate its revision EPA 560/6-84-001	Positive in all three strains tested with metabolic activation Cytotoxicity: No	Supporting study (Unpublished study, 1985d) Test material: The Substance Purity not stated

Reliability: Klimisch 1		
In vitro mammalian cell transformation assay (gene mutation) Mammalian cell line, other: BALB/3T3 Cl. A31 Metabolic activation: With and without Doses: preliminary cytotoxicity dilutions: 1:10, 1:100, 1:250, 1:500, and 1:1000 1:1 × 10^3, 1:2 × 10^3, 1:3 × 10^3, and 1:5 × 10^3 1:1 × 10^4, 1:1.5 × 10^4, and 1.2 × 10^4 cytotoxicity with metabolic activation dilutions 1:1 × 10^4, 1:1.5 × 10^4, and 1.2 × 10^4 cell transformation with metabolic activation dilutions 1:1 × 10^4, 1:1.5 × 10^4, and 1.2 × 10^4 Reliability: Klimisch 3	Negative (cell transformation, with metabolic activation) Cytotoxicity: Yes	Supporting study (Unpublished study, 1979c) Test material: The Substance Purity not stated
Mammalian cell gene mutation assay (gene mutation) Mammalian cell line, other: Mouse BALB/3T3 Clone A31 Metabolic activation: Without Doses: preliminary cytotoxicity dilutions: 1:10, 1:100, 1:250, 1:500, and 1:1000 cytotoxicity dilutions: 1:1000, 1:2000, 1:3000, and 1:5000 1:10000, 1:15000, 1:20000, and 1:30000 mutagenicity dilutions:	Positive without metabolic activation Cytotoxicity: Yes	Supporting study (Unpublished study, 1978c) Test material: The Substance Purity not stated

1:10000, 1:15000, 1:20000, and 1:30000 Mammalian cell mutagenesis tests with BALB/3T3 Clone A31 mouse cells were performed according to the methods of Schechtman and Kouri (1977). Reliability: Klimisch 2		
In vitro gene mutation study in bacteria (Bacterial reverse mutation assay) *S. typhimurium* TA 1535, TA 1537, TA 98 and TA 100 *S. typhimurium* TA 1538 Metabolic activation: With and without Doses: Preliminary toxicity screen: 5000, 1000, 200, 40, and 8 μg/ plate. Definitive study: 1000, 333, 100, 33, and 10 μg/plate. Reliability: Klimisch 2	Negative in TA 1535, TA 1537, TA 98 and TA 100 with and without metabolic activation. Cytotoxicity: No	Supporting study (Unpublished study, 1979d) Test material: The Substance Purity not stated
DNA damage and repair assay, unscheduled DNA synthesis in mammalian cells *in vitro* (DNA damage and/or repair) Hepatocytes obtained from adult male Fischer-344 rats (met. act.: not applicable) Doses: 0.05, 0.1, 0.5, 1, 5, 10, 50, 100, 500, or 1000 μg in 2 ml of media per well Reliability: Klimisch 2	Negative Cytotoxicity: Yes (produced at 10 μg/well of media and higher)	Supporting study (Unpublished study, 1986h) Test material: The Substance Purity not stated
In vitro gene mutation study in bacteria (Bacterial reverse mutation assay) Metabolic activation: With Positive and negative controls *S. typhimurium* TA 1538 (met. act.: with) *S. typhimurium* TA 98 (met. act.: with)	Negative with metabolic activation in TA 1538 and TA 98. Positive with metabolic activation in strain TA 100.	Supporting study (Unpublished study, 1987c) Test material: The Substance Purity not stated

S. typhimurium TA 100 (met. act.: with) Doses: 1000, 3750, 7500, 10000, and 12500 μg/plate Reliability: Klimisch 1		
In vitro mammalian cell transformation assay (gene mutation) mammalian cell line, other: BALB/3T3 Clone A31 Cells Metabolic activation: Without Doses: The following dilutions of the test material were used: 1:1000, 1:2000, 1:3000, 1:5000, 1:10000, 1:15000, 1:20000, and 1:30000. Reliability: Klimisch 3	Negative without metabolic activation Cytotoxicity: yes (virtually 100 % cytotoxic at 1:10000 dilution; the approximate LD_{40} was at the 1:30000 dilution of the test material.)	Supporting study (Unpublished study, 1978d) Test material: The Substance Purity not stated

Gene mutations in bacteria

A number of *Salmonella* Typhimurium assays have been performed with the Substance. Without metabolic activation, four out of five studies were negative in inducing base-pair and frameshift mutations. One study was positive in the strain TA 1535 (base-pair substitutions) and weakly positive in strain 1537 (frameshift mutations). However, the statistically significantly induced increase in the number of revertants was within the laboratory's historical control distribution. In the presence of metabolic activation, four out of 10 studies were positive, and one was weakly positive inducing frameshift and base-pair substitutions. All 10 studies were given a Klimisch score of 1 or 2 by the evaluating MSCA. All taken together, there is evidence that the Substance induces gene mutations (both base-pair substitutions and frameshift mutations) in bacteria in the presence of metabolic activation. In the view of the evaluating MSCA, the overall conclusion is that the Substance is positive in Ames test with metabolic activation.

Gene mutations in mammalian cells

Four assays were performed to investigate gene mutations in mammalian cells. Only one study tested the Substance both in the presence and absence of metabolic activation (Unpublished study, 2000b). This study was performed according to the OECD TG 476 (version 1997) and is considered a key study by the evaluating MSCA. The Substance induced statistically significant dose-related increases in mutant frequency at the TK +/- locus in the presence of metabolic activation at and above 900 μg/ml. In the absence of metabolic activation, no increase in mutant frequency was observed. The three highest doses (1200 to 1800 μg/ml) were excluded from the data due to excessive cytotoxicity. The increase in mutant frequency was partly due to small colony formation indicating a clastogenic response.

A non-guideline study in in BALB/3T3 Clone A31-1 mouse embryo cells also found a positive result (Unpublished study, 1978c). The Substance was only tested without metabolic activation and an increase in mutant frequency at the ouabain membrane receptor locus was observed. This study is considered a supporting study and has been given a Klimisch score of 2 by the evaluating MSCA. Two other non-guideline studies also performed in BALB/3T3 Clone A31-1 mouse embryo cells did not yield positive results for point mutations

at the ouabain membrane receptor locus (one performed with and one without metabolic activation). All taken together, the evaluating MSCA considers that there is evidence that the Substance is mutagenic in mammalian cells in the presence of metabolic activation based primarily on the key study. The mechanism of mutagenicity seems to be due to both gene mutations and chromosomal aberrations.

Chromosomal aberrations *in vitro*

An *in vitro* chromosomal aberration test has been performed according to the OECD guideline 473 (Unpublished study, 2000a). The study is considered a key study and has been given a Klimisch score of 1 by the evaluating MSCA. Initially, two independent experiments were performed. In the first experiment, no effect was observed. In the second experiment, an increase in aberrant cells without gaps was observed with metabolic activation. However, a clear dose-response relationship was absent. Therefore, a third experiment was performed and a small statistically significant increase in the frequency of cells with aberrations including gaps was observed with metabolic activation. Furthermore, small increases in polyploid cell frequency (not dose-dependent) both with and without metabolic activation was observed in all three experiments which may indicate the potential to induce numerical aberrations. According to the registrant, the results are ambiguous.

Small colonies were formed in the *in vitro* Mammalian Cell Gene Mutation Test mentioned above indicating that the positive result seen in this study may in part be due to chromosomal aberrations.

As a conclusion, the evaluating MSCA considers that the observed effects suggest that the Substance may cause chromosomal aberrations *in vitro*.

Other genotoxicity studies *in vitro*

Three non-guideline unscheduled DNA synthesis (UDS) studies (Unpublished study, 1984; 1986f; 1986h) were performed to investigate DNA damage and repair in liver cells extracted from rats. All three studies were given a Klimisch score of 2 by the evaluating MSCA. All three tests were negative. The OECD guideline for this test was adopted in 1986 and archived/deleted in 2014.

Furthermore, a non-guideline E. coli DNA damage and/or repair test was performed in 1978, which yielded a negative result with and without metabolic activation (Unpublished study, 1978b). The evaluating MSCA has evaluated this study as Klimisch 2.

Two *in vitro* mammalian cell transformation assays have been performed with BALB/3T3 - Clone A31 mouse cells. The first from 1978 was performed without metabolic activation (Unpublished study, 1978d) and the other one from 1979 was performed with metabolic activation (Unpublished study, 1979c). Both studies yielded a negative result.

The test method used in the two studies was from before the SHE and Bhas cell transformation assays were drafted for consideration as OECD Guidance Documents. Hence, the validity of the performance of the BALB/3T3 for rodent carcinogenicity is unknown (e.g. as regards number of rodent carcinogens and non-carcinogens included in a validation exercise, its inter- and intra-laboratory variability and its sensitivity, specificity, positive and negative predictive values). According to the ECVAM pre-validation study on *in vitro* cell transformation assays, some modifications to the protocol are needed to obtain reproducible results for the BALB/c 3T3 method between laboratories. Based on the issues mentioned above the evaluating MSCA has evaluated these studies as Klimisch 3.

Conclusion of *in vitro* mutagenicity

Based on all available *in vitro* genotoxicity studies, the evaluating MSCA concludes that there is a concern for gene mutations and chromosomal aberrations.

14.5.3. *In vivo* genotoxicity studies data

The results of *in vivo* genotoxicity studies are summarised in the following table.

Table 14.5.3-5: Overview of *in vivo* genotoxicity studies

Test method	Results	Remarks
Pre-guideline Dominant lethal assay (gene mutation) Species: Rat (SD) male/female Administration: Oral gavage Doses: 0.7, 7, 70 mg/kg bw (nominal conc.) Reliability: Klimisch 2	The total number of implantations per pregnant female was significantly lower in the 7 mg/kg dose level group at sacrifice week 3. In all other sacrifice weeks, there was no significant difference. The numbers of corpora lutea were significantly reduced in the 7 mg/kg dose level group at sacrifice weeks 2 and 4 and in the 0.7 mg/kg dose level group at week 6. There were no significant differences at sacrifice weeks 1, 3, 5, and 7 or at other dose levels. The numbers of preimplantation losses per pregnant female were significantly increased at 70 mg/kg, 7 mg/kg and 0.7 mg/kg dose levels at sacrifice week 5. All other sacrifice weeks showed no increase above the control. The number of dead implants per total implants was significantly increased in the 0.7 mg/kg dose level group at sacrifice week 2. The number of live implantations per pregnant female was significantly lower in the 7 mg/kg dose level group at sacrifice week 2. No statistically significant differences observed in fertility index, number of dead implants per pregnant female, proportion of pregnant females with dead implants.	Supporting study (Unpublished study, 1979e) Test material: the Substance
OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test) Species: Mouse (ICR) male/female Administration: Oral gavage Doses: 125, 250, 500 125 mg/kg bw (nominal conc.) Basic Mutagenicity Tests: UKEMS recommended procedures, 1990	Genotoxicity: negative (up to the limit dose of 500 mg/kg bw) (male); toxicity: yes (observed at 125 mg/kg bw and higher)	Key study (Unpublished study, 2000c) Test material: the Substance

Reliability: Klimisch 2		
Non-guideline Mammalian Erythrocyte Micronucleus Test Species: Mouse (CF-1) male/female Administration: Intraperitoneal Doses: 50, 100, 150, 250, 500 mg/kg Reliability: Klimisch 2	Genotoxicity: negative (100 mg/kg-bw) (male/female); toxicity: yes (Body drop, loss of body tone, ptosis, depression, abnormal gait, and decreased spontaneous activity)	Supporting study (Unpublished study, 1979f) Test material: the Substance
Comet assay Oral gavage Male Wistar rats Doses: 300, 400 and 500 mg/kg bw in range finding study and 100, 200, 400 mg/kg bw in main study Animals were administered vehicle (negative control: Methylcellulose), N,N'-dimethylhydrazine dihydrochloride (DMH) as positive control. Test material was administered twice, 24 h apart. 3 to 6 hours after the second treatment the animals were sacrificed and target organs isolated (bladder and liver). Comet assay slides were prepared on fresh cells. Three slides for each dose and each animal and 50 cells were scored for each slide. % tail DNA was used to evaluate the DNA strand breaks. Reliability: Klimisch 1	Negative Range finding study: 300, 400 and 500 mg/kg bw. Toxicity: yes at 500 mg/kg bw (reduced activity, rapid breathing, small palpebral fissure, bleeding of nose, coloured urine). Main study: No difference in DNA strand breaks between the treated dose groups and the negative control dose group. The positive control gave a clear significant response in both bladder and liver cells.	Key study (Unpublished study, 2013) Test material: the Substance

Conclusion on *in vivo* mutagenicity

Gene mutations

A pre-guideline *in vivo* comet assay was performed in 2013 in accordance with the later adopted OECD guideline. No increase in strand breaks were observed in the liver or bladder cells. Contrary to the experimental result, the *in vivo* Leadscope DTU QSAR model predicted positive outcomes for all constituents in a model for comet assay except one, which is inconclusive. It is not clear to the evaluating MSCA why liver and bladder were the only chosen test organs as no information regarding this has been available. In the three carcinogenic studies with the Substance (Unpublished study, 1986c; 1986d and 1992), no effects in the bladder was observed (See Section 14.4.1). Based on the systemic distribution of the Substance, it is distributed to a higher extent to pancreas and thyroid compared to liver, which may make these tissues more sensitive to the effects of the Substance. Therefore, the evaluating MSCA considers that the comet assay would possibly yield a positive result in tissues other than the liver even if the liver yields a negative result. Several examples of this exist in the literature e.g. o-Anidisine, Potassium chromate (IV), 2-Nitrofluorene, 2,6-Dimethylaniline, Benzyl acetate and the Benzo[a]pyrene (harm. Class. Carc 1B) (cited in Sasaki *et al*., 2000). All these substances yielded positive results in other

tissues for example the stomach, colon, lung and/or brain, but not in the liver. However, *in vitro* genotoxicity studies are mainly positive with metabolic activation.

Because not all relevant tissues in terms of indications of carcinogenicity have been targeted in the available comet assay, the evaluating MSCA does not consider the concern for *in vivo* gene mutagenicity to be fully clarified.

Chromosomal aberrations:

Two micronucleus assays with the Substance in mice yielded negative results. However, it was not demonstrated if bone marrow exposure to the Substance and/or its metabolites occurred. According to the current version of TG 474 (2016) "*ADME data, obtained in an independent study using the same route and same species can be used to demonstrate bone marrow exposure.*" (paragraph 48). Unfortunately, toxicokinetic information for the Substance is only available in rats.

In a study on toxicokinetics (Unpublished study, 1982a), F344 rats received 10 mg/kg bw of DETDA by oral gavage. >60% of this dose was excreted in the urine and ~1% of the absorbed dose of the radiolabelled Substance was recovered from blood during peak concentration.

In an oral repeated dose toxicity study in SD rats (Unpublished study, 1987a), decreased leucocyte and platelet counts as well as decreased calcium and increased erythrocytes and haemoglobin in blood was observed in the high dose group (320 ppm). Bone marrow depletion was also observed in the high dose group, which could indicate that the Substance is able to reach the bone marrow under the conditions of this study. However, it is possible that the bone marrow depletion is not caused by the Substance directly but is a secondary effect caused by changes in the blood calcium levels.

The evaluating MSCA considers that bone marrow exposure has not been conclusively demonstrated in mice. However, the clear systemic toxicity observed in other studies is an indication of systemic absorption, i.e. the substance and/or it metabolites have entered blood and also bone marrow is exposed.

14.5.4. Conclusion on mutagenicity

In the opinion of the evaluating MSCA, the concern for gene mutations have not been clarified because relevant tissues, where indications of carcinogenicity have been observed, have not been targeted in the available comet assay. It is noted that the observed carcinogenicity is not conclusive and that the mechanism behind possible carcinogenic effects is unclear as to the extent this may be caused by genotoxicity. Furthermore, the examination of thyroid should be included as a significant increase in follicular cell adenomas were observed in a carcinogenicity study. According to REACH, appropriate *in vivo* mutagenicity studies shall be considered in case of a positive result in any of the *in vitro* genotoxicity studies in Annex VII or VIII. In the opinion of the evaluating MSCA, the *in vivo* comet assay is appropriate to address the concern for gene mutations. However, the evaluating MSCA considers that available study is not adequate and there is an information gap in the standard information requirements of REACH, and it should be addressed under compliance check. The evaluating MSCA will consider the need for further actions once the compliance check has been completed.

14.6. Carcinogenicity

14.6.1. Non-testing methods

The carcinogenic potential of the Substance was predicted using the Danish QSAR database. For the constituent 2,4-diamino-3,5-diethyltoluene (EC No 218-256-9), Leadscope had several positive predictions whereas most models in CASE Ultra were out

of domain. For the constituent 2,6-diamino-3,5-diethyltoluene (EC No 218-255-3), Leadscope had several positive predictions for carcinogenicity whereas most models in CASE Ultra were either negative or out of domain. For the Substance, predictions for carcinogenicity in Leadscope are positive while CASE Ultra predictions are negative.

14.6.2. *In vivo* carcinogenicity

The registrant has submitted two oral dietary studies (Unpublished study, 1986c; 1992) and one oral gavage study (Unpublished study, 1986d) investigating carcinogenic effects of the Substance. The results are summarised in the following table:

Table 14.6.2-6: Overview of oral *in vivo* carcinogenicity studies

Reference and test method	Results	Remarks
Unpublished study, 1986c Combined oral (feed) 2-year RDT and carcinogenicity study equivalent or similar to OECD TG 452 Administration: Oral, feed Not GLP Rat (Wistar), 50/sex/group + 10/sex/group for interim sacrifices) Doses: 0, 80, 240 ppm, halved from 456th day to 40 and 120 ppm. The initial dose corresponded to approximately 6 and 12 mg/kg bw/day Exposure: Lifetime, daily exposure Reliability: Klimisch 3 Significance of neoplastic findings were investigated using the prevalence analysis method.	Poor general condition in high dose groups Markedly decreased body weight in high dose males – still clearly decreased towards the end of the study Markedly increased food consumption and drinking water consumption in high dose males – less marked after the reduction of the dose to 120 ppm <u>Neoplastic findings</u> Total number of tumours was elevated in the dosed females. The increase did not show any clear dependence on the dose and the higher rates of tumors of the uterus and of the mammary gland in the treatment groups were within the range of the historical controls.	Test material: The Substance (DETDA) (EC 270-877-4) Specifications: Batch No. 74 used for the first 11.5 months of the study: 78.2% 2,4-DETDA; 20.9% 2,6-DETDA Batch No. 94 used from 11.5 months on until study completion: 64.3-68.7% 2,4-DETDA; 27.5-33.2% 2,6-DETDA Limitations: This study included major flaws such as both the method and reporting. In comparison with the most recent OECD TG 451 (adopted 7 September 2009), deviations include that only two dose levels were included; life-time study, doses halved during test period, fewer tissues and organs were examined histopathologically

Unpublished study, 1986d Combined 2-year repeated dose and carcinogenicity, equivalent or similar to OECD TG 452 Administration: oral, gavage Not GLP Species: Rat (Wistar), 20/sex/group Doses: 0, 4, 12 mg/kg bw/day, halved from 127th day to 2 and 6 mg/kg bw/day, again halved from 256th day to 1 and 3 mg/kg bw/day. Exposure: 2 years (5 days per week). Reliability: Klimisch 3	Poor general condition of the high dose rats. Body weight was lower in the high dose group. This became very marked as the study progressed. First dose reduction did not result in any improvement in condition. Second reduction resulted in gradual return to control values for females while male body weights remained below control values. Survival time markedly shorter in high dose males and slightly shorter in females Increased drinking water consumption <u>Neoplastic findings</u> No dose-dependent increase in tumor incidence was observed.	Test material: The Substance (DETDA) EC Number: 270-877-4 Specifications: Lot/batch No.: 94 (64.3-68.7% 2,4-DETDA and 27.5-33.2% 2,6-DETDA) Limitations: In comparison with the most recent OECD TG 451 (adopted 7 September 2009), deviations include that only two dose levels were included; only 20 animals/sex/group; fewer tissues and organs were examined histopathologically
Unpublished study, 1992 Combined repeated dose and carcinogenicity (2-year) study equivalent or similar to OECD TG 453 Administration: Oral, feed GLP Species: Rats (SD), 50/sex/dose group Doses: 0, 10, 35, 70 ppm corresponding to 0.4, 1.4, and 3.2 mg/kg bw/day in males and 0.5, 1.8 and 3.8 mg/kg bw/day in females Exposure: Daily seven days per week for two years. Blood glucose evaluation was performed in selected animals sacrificed during the study. In addition, blood glucose evaluation was performed at 18 months and at terminal necropsy Ophthalmoscopy was performed on all animals prior	<u>Clinical signs and survival:</u> Eye opacity was noted in a few high dose males. Low dose male survival was significantly higher than the control group. Survival in the other treated groups was comparable to the control group. <u>Body weight:</u> Mean body weight of the male high dose group was statistically significantly decreased from the control male body weight beginning on week 43 and continuing throughout the majority of the study. Control and mid dose mean body weights were comparable throughout most of the study. However, a tendency for lower mean body weight in the mid dose males was observed from month 15 onwards. Statistically significant lower body weight was observed in the high dose group on weeks 80 through 83. A tendency for a lower body weight in the high dose females compared to the control mean was also seen in the last 3 months of the study. A tendency for a lower body weight in the mid dose was seen during the last four weeks of the	Test substance: diethyl toluene diamine (The Substance) Pre-study specifications: 2,4-diamino-3,5-diethyltoluene (EC No 218-256-9): 75.8% 2,6-diamino-3,5-diethyltoluene (EC No 218-255-3): 20.1% Dialkylated phenylenediamines (3.1%) Concentrations were determined every 6 months and varied only slightly (<0.5%) throughout the study

to study initiation and at 6, 12, 18 and 24 months In addition to full histopathology on organs and tissues in the control and high-dose animals, and all animals sacrificed during the study, the adrenals, eyes, liver, pancreas, pituitary gland and thyroids were evaluated from the low- and mid-dose groups. The mammary glands and any tissue masses suspected of being a mammary gland tumour were also evaluated from the low- and mid-dose groups. Reliability: Klimisch 1	study. However, no statistical differences were found in this group. <u>Carcinogenicity:</u> *Liver:* The livers of the high dose males had a significant increase in hepatocellular carcinomas (nine). High dose females had a significant increase in hepatocellular adenomas and an increased incidence of basophilic foci. Hepatocellular carcinomas were present in one high dose female and two mid dose females. *Thyroid:* The high dose males had a significant increase in follicular cell adenomas (five) and an increased incidence of follicular cell hypertrophy (seven). Follicular cell hypertrophy was significantly increased over the mid dose male rats. Follicular cell carcinomas were present in two low dose males. Follicular cell adenomas were present in three low dose males and in four mid dose males. No follicular cell adenomas or carcinomas were present in the control males. Follicular cell hyperplasia/follicular cysts were increased in the thyroids of the mid and high dose males. *Mammary gland:* The mammary glands of the mid and high dose females had a significant increase in the incidence of fibroadenomas. However, the incidence of mammary gland adenocarcinomas was higher in the control females than in the dose groups of DETDA treated females. *Kidneys:* The incidences of cysts and focal areas of tubular hyperplasia of the kidneys were increased in high dose male rats when compared to the male controls.	

14.6.3. Discussion of carcinogenicity

Liver

In the unpublished study from 1992, a statistically significantly increased incidence of hepatocellular carcinomas and proliferative lesions in high dose males and an increased incidence of hepatocellular adenomas in high dose females were observed. An increased incidence of proliferative lesions in the liver in all treated dose groups is reported; nodular regeneration usually occurred in liver lobules with a marked amount of hepatocellular damage. No effects in liver were observed in the other long-term RDT studies (Unpublished study, 1986c; 1986d). Radioactivity recovered in tissues was highest in the thyroids and pancreas followed by liver (See section 14.1).

Kidney

The 3rd highest distribution of the Substance was observed in the kidney (Unpublished study, 1982). In the unpublished carcinogenicity study from 1992, increased incidence of cysts and focal areas of tubular hyperplasia were observed in the kidney of high dose males. Because some degree of age-dependent chronic nephropathy (cysts and tubular hyperplasia) was present in nearly all kidneys across the groups, it is unclear if the observed effect in high dose males is treatment related.

Pancreas

No relevant neoplastic findings were reported in any of the three carcinogenicity studies, which investigated the pancreas.

Thyroid

In the unpublished study from 1992, a statistically significant increase in follicular cell adenomas and hypertrophy was observed in the high dose males. Also, a statistically significant increase in follicular cell hyperplasia/follicular cysts was observed in mid and high dose males and in high dose females. No effects in the thyroid were reported in the other 2-year studies. The ADME study (Unpublished study, 1982) showed that the amount of the Substance was highest in the thyroid (and pancreas). This, in combination with the potentially carcinogenic effects observed in one 2-year study (Unpublished study, 1992), indicates that the thyroid is a target of toxicity including potentially carcinogenic effects of the Substance.

Bladder

Bladder was investigated in all three carcinogenicity studies. None of the studies found any effects on bladder.

Mammary glands

Two carcinogenicity studies observed neoplastic findings in the mammary gland. The change in dose in the two unpublished studies from 1986 and the fact that these are life-time studies with different exposure time, i.e. life-time exposure (Unpublished study 1986c) and 2-year exposure (Unpublished study 1986d) make it difficult to compare the results of these two studies. As the doses used in the 1992-study are within the same range after the reduction of doses in the two earlier studies, it could be expected that similar but less severe effects would be observed.

The findings include an increase in mammary gland tumours (Unpublished study 1986c) while the study from 1992 found an increased incidence of fibroadenomas in mid- and high dose females. However, the incidence of mammary gland adenocarcinomas was higher in the control females than in the three dose groups.

The evaluating MSCA is of the opinion that a treatment-related increase in mammary gland tumours was observed in one of two studies from 1986. The incidence of malignant

mammary gland tumours (adenocarcinomas) was higher in the control females than in any of the treatment groups thus a dose-response relationship was not observed for malignant mammary gland tumours in one study (Unpublished study, 1992). However, as this study had lower doses compared with the two studies from 1986, this could explain why only a statistically significant increase in benign mammary gland tumours (fibroadenomas) was identified. Total and benign tumour incidence in mammary gland was not assessed in this statistical analysis.

Overall, there are indications of an increase in total and benign mammary tumours in groups exposed to the Substance.

Uterus

No statistically significant changes were observed in one of the carcinogenicity studies (Unpublished study, 1992). Benign effects such as endometrial stromal polyps were present in the uterus of four high dose females and a leiomyoma was present in the uterus of another. No primary uterine neoplasms were diagnosed in the control, low dose, or mid dose groups. In another study, an increase in tumour rate was observed in the uterus (Unpublished study, 1986c). Overall, there are indications of a dose-related increase in uterine tumours.

14.6.4. Conclusion on carcinogenicity

Three studies investigated the carcinogenic potential of the Substance. It is clear from the studies that the exposure to the Substance resulted in systemic toxicity (i.e. due to effects on pancreas see section 14.4) even at relatively low doses. In all studies, only the low dose group did not show excessive toxicity. The toxicity was most severe in males and survival was generally higher for female rats compared with male rats in all three studies. This is probably why higher incidence of neoplastic changes was observed in females.

The two two-year studies from 1986 (Unpublished study report, 1986c; 1986d) have several shortcomings including the life-time duration of the studies yielding a higher incidence of spontaneous tumours in controls thus making the studies less sensitive. Only two treated groups were included which makes it difficult to observe dose-response trends. In the gavage study, only 20 animals/sex/dose were used resulting in a less sensitive study. Furthermore, the dose reductions complicate the evaluation of these studies. In the view of the evaluating MSCA, these shortcomings reduce the sensitivity of the studies. However, these shortcomings cannot be used to reject the potential carcinogenic effects of the Substance. On the contrary, positive findings should be included in the assessment of overall carcinogenicity potential of the Substance in a weight of evidence approach.

Tumours were observed in different organs and tissues. In two studies, an increase in mammary gland tumours was observed. In liver, statistically significant increase in adenocarcinomas (males) and fribroadenomas (females) was observed. Potential carcinogenic effects were also observed in thyroid, uterus and potentially – but with higher uncertainty – also in the kidney.

In the opinion of the evaluating MSCA, the available information raises some concern for carcinogenicity. However, it is not possible to conclude on the carcinogenic potential of Substance based on the currently available data. The evaluating MSCA will revisit the topic once the compliance check has been performed.

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

14.7.1. Non-human information

In the QSAR predictions performed by the evaluating MSCA, negative or inconclusive predictions were obtained in models in the commercial MultiCASE CASE Ultra suite for mammalian reproductive toxicity ("Mammalian ReproTox Models"). Leadscope commercial 'Non-human Developmental and Reproductive Toxicity Suites models' generally showed negative or inconclusive predictions in all models except for positive predictions for foetal death, sperm effects and post implantation loss.

14.7.2. *In vivo* reproductive toxicity

Results from a pre-natal developmental toxicity (PNDT) study conducted in accordance with OECD TG 414 are included in the registration dossier.

Table 14-7: Developmental toxicity

Reference and test method	Results	Remarks
Unpublished study, 2014 OECD Test Guideline 414 Species: Rat (Wistar), n=24 Administration: Oral feed Doses: 0, 50, 150 and 500 mg/kg bw/day (nominal in diet) corresponding to actual ingested doses of: 0, 2.63, 7.83 and 20.45 mg/kg bw/day Reliability: Klimisch 1	The NOAEL for maternal toxicity was 2.63 mg/kg bw/day based on minor histopathological effects in the pancreas in the mid dose group. 20.45 mg/kg bw/day induced decreased body weight gain, decreased food consumption, decreased mean ovary weight, and significant histopathological findings in the pancreas in dams. The NOAEL for fetotoxicity was set to be 7.83 mg/kg bw/day, based on increased pre-implantation loss, decreased mean number of implantation sites and live fetuses, decreased mean fetal weight and increased incidence of small foetuses observed in the high dose group. No treatment-related adverse effects were observed in visceral and skeletal examination of the fetuses, except for a slightly retarded ossification in the high dose group.	Key study Test material: The Substance

14.7.3. Conclusion on reproductive toxicity

Based on the available PNDT study, the evaluating MSCA concludes that the observed developmental effects can plausibly be regarded as secondary to maternal toxicity. This is supported by other repeated dose toxicity studies in rodents, which have shown that the Substance results in systemic toxicity at dose level of 20 mg/kg bw/day, which is likely to explain the observed fetotoxicity effects.

Some of the performed repeated dose toxicity and carcinogenicity studies show indications of adverse effects on the reproductive system. In male rats exposed to the Substance at

dose of 32 mg/kg bw/day for 28 (Unpublished study, 1989) and 90 days (Unpublished study, 1987), decreased absolute weights of testes were observed. However, signs of general toxicity, including decreased body weight and decreased weight of several other organs, were also observed at this dose level. In female rats, carcinogenicity studies have revealed dose-related increases in uterine tumours and mammary gland tumours (Unpublished study, 1986c; 1986d) as well as increased incidence of fibroadenomas in mammary tissue (Unpublished study, 1992).

The evaluating MSCA notes that no *in vivo* studies have been performed where the effect of the Substance on sperm quality effects has been investigated. No *in vivo* reproductive toxicity studies investigating the effects of the Substance on fertility are available. The QSAR results lead to a concern regarding the effects of the Substance on male fertility, which cannot be clarified based on the presently existing experimental data. An Extended One-Generation Reproductive Toxicity (EOGRT) study (OECD TG 443) is a standard information requirement for substances registered at Annex X with a tonnage above 1000 tons/year. Therefore, in the opinion of the evaluating MSCA, an EOGRT study should be requested under the ongoing compliance check. Furthermore, a pre-natal developmental toxicity study in a second species is also a requirement of REACH Annex X and therefore, the evaluating MSCA considers that this study should also be requested under the compliance check. Following submission of the relevant data, the evaluating MSCA will revisit the need for further action.

14.8. Hazard assessment of physico-chemical properties

Not evaluated in this substance evaluation.

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

Not evaluated in this substance evaluation.

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

Further information is necessary to clarify the concerns identified by the evaluating MSCA. Based on the currently available information, no conclusion on most of the concerns is possible. However, compliance check has been identified as a more expedient process. It is expected that the standard information will be sufficient to clarify the concerns for mutagenicity, skin sensitization and reproductive toxicity without need for further information requested under substance evaluation at present.

Based on currently available data, the evaluating MSCA supports the present harmonised classifications for human health hazards, i.e. Acute Tox. 4 (H302, H312), Eye Irrit. 2 (H319) and STOT RE 2 (H373). Once new data on critical endpoints are available following a compliance check, the evaluating MSCA will revisit the topic.

15. Endocrine disrupting (ED) properties assessment

15.1. Endocrine disruption – Environment

Not evaluated in this substance evaluation.

15.2. Endocrine disruption - Human health

Thyroid

Positive QSAR predictions were obtained in a Leadscope DTU model for Thyroid Peroxidase (TPO) inhibition (*in vitro*) for all constituents of the Substance. No experimental *in vitro* studies for TPO inhibition or for any other mechanisms of thyroid disruption have been identified by the evaluating MSCA.

Increased incidence of thyroid follicular cell adenomas and follicular cell hypertrophy was seen in high dose males and an increased incidence of follicular cell hyperplasia/follicular cysts was seen in mid- and high dose males and in high dose females after a two-year exposure (Unpublished study, 1992).

In contrast, no gross lesions were reported in the thyroid in studies with a shorter exposure period, i.e. neither in a 28-day study (Unpublished study, 1989) nor in a 90-day study (Unpublished study, 1987a). Histopathological examinations of the thyroid in the control and high dose animals as well as the animals which died or were sacrificed during the study were performed in the 90-day study without reporting any findings and it is not clear to the evaluating MSCA if the thyroid weight was measured. No histopathological examinations were performed on the thyroid in the 28-day study and the thyroid weight was not measured. The length of the exposure period could explain that effects on the thyroid are not consistently seen in all available studies.

A dermal 21-day study performed in rabbits (Unpublished study, 1981) showed no effect on thyroid weight. Following a histopathological examination of the thyroid, cysts were observed. The incidence was dose-related in males but not in females. However, since thyroid cyst are not a typical sign of thyroid disruption and since the no dose-related effects were observed in females, this could be a chance finding because thyroid cysts may arise spontaneously in rabbits.

In conclusion, *in vivo* indications of adverse effects on the thyroid glands have been observed in some chronic studies while studies with shorter exposure time are without clear effects. On this basis, the evaluating MSCA considers that it is difficult to pursue a concern for endocrine disruption based on the adverse thyroid effects of the Substance caused solely in chronic toxicity studies.

Pancreas: Islet cells

In a 90-day RDT study, vacuolation of the islet cells in the high dose rats was observed. This was accompanied by a high incidence of diffuse atrophy of the acinar cells of the pancreas. In addition, there were several severe clinical signs of toxicity observed in this dose group resulting in death or sacrifice of 27/40 animals (Unpublished study, 1987a).

In a 28+28 day study (Unpublished study, 1989), one male had a moderate vacuolation of islet cells and atrophy of the adipose tissue in the pancreas of the rats exposed to Lot 2-87 and sacrificed at day 56. Moderate multifocal acinar degeneration and minimal diffuse atrophy of acinar cells was also observed. In animals exposed to Run 544, a moderate to moderately severe diffuse acinar atrophy was present in four of the five high dose males. This was accompanied by a slight to moderate diffuse basophilia of the acinar cells and a slight to moderately severe vacuolation of the islet cells. In the fifth high dose male rat,

the pancreas had a moderate multifocal acinar cell degeneration, a slight diffuse atrophy of the acinar cells and a minimal vacuolation of islet cells (Unpublished study, 1989).

In the two year study from 1992, an increased incidence in multifocal acinar atrophy accompanied by interstitial fibrosis and fatty infiltration was observed in high dose males. No effects were observed in islet cells in this study. However, the authors stated that the islet cells were difficult to investigate due to the destruction of acinar cells (Unpublished study, 1992).

The islet cells are endocrine cells involved in production and release of glucagon and insulin hormones, which are part of a feedback system that keeps blood glucose levels at a stable level while the acinar cells are exocrine cells producing enzymes needed for digestion.

15.3. Conclusion on endocrine disrupting properties for human health

The QSAR predictions of thyroid peroxidase (TPO) inhibition could raise some concern for potential thyroid disrupting properties of the Substance. The concern for thyroid effects could be further substantiated by ADME studies showing that the Substance is distributed to the thyroid. Also, the carcinogenicity study (Unpublished study, 1992) indicated thyroid as a potential target organ by showing increased incidence of thyroid follicular cell adenomas and follicular cell hypertrophy at rather low dose levels. On the other hand, repeated dose studies investigating much higher doses in rats and in rabbits showed no relevant dose-related adverse effects on thyroid histopathology. This pattern of effects is not similar to what would be expected from an *in vivo* thyroid disrupting chemical with TPO inhibition as its principal mechanism of action. Exposure to high doses of thyroid disrupting chemicals, which inhibit TPO *in vivo,* would be expected to cause decreased levels of circulating T4 levels after a relative short exposure period. Unfortunately, this has not been investigated in any of the performed animal studies with the Substance. Such a decrease would be expected to cause increased levels of thyroid-stimulating hormone (TSH) and thyroid gland activity and would be seen as increased thyroid weight and altered thyroid histopathology (hypertrophy in thyroid follicles) in the 28-day and especially in the 90-day studies. In the present case, the histopathology of the thyroid was only affected after two years of exposure. This could indicate that some other mode of action than TPO inhibition was predominant in causing the follicular cell adenomas/hyperplasia and follicular cysts in the two-year study.

Damage to the endocrine islet cells of the pancreas was observed in some studies. However, these observations were accompanied by severe clinical signs of toxicity and/or damage to the pancreatic acinar cells. Based on the available evidence, it could be that the exocrine acinar cells in the pancreas may be affected first and that this leads to dysfunction of the endocrine islet beta cells. However, it is also possible that that effects on the exocrine acinar cells are easier to observe compared to the effects on endocrine islet. Based on the available data, it is difficult to elucidate the exact mode of action. The Substance already has a harmonised classification for STOT RE 2 (H373) based on the effects on the pancreas.

In conclusion, the evaluating MSCA considers that currently there is no need to further clarify the concern for potential human health endocrine disrupting properties of the Substance under substance evaluation but it will be revisited after CCH.

16. PBT/vPvB and PMT/vPvM assessment

Not evaluated in this substance evaluation.

17. Exposure assessment

Not evaluated in this substance evaluation.

18. Risk characterisation

Not evaluated in this substance evaluation.

19. References

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Unpublished study (1986g): DNA damage and repair assay, unscheduled DNA synthesis in mammalian cells *in vitro*

Unpublished study (1986h): *In vitro* gene mutation study in bacteria

Unpublished study (1987a): 90-day RDT study equivalent or similar to OECD TG 408

Unpublished study (1987b): *In vitro* gene mutation study in bacteria

Unpublished study (1987c): *In vitro* gene mutation study in bacteria

Unpublished study (1989): 28-day RDT study equivalent or similar to OECD TG 407 and including a 28 day recovery group

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Unpublished study (2000a): *In vitro* mammalian chromosome aberration test

Unpublished study (2000b): *In vitro* mammalian cell gene mutation test

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Unpublished study (2013): Rodent Alkaline Single Cell Gel Electrophoresis (*in vivo* Comet Assay) on the Rat Liver and Bladder with DETDA

Unpublished study (2014): Prenatal Developmental Toxicity Study according to OECD TG 414

20. Abbreviations

ADME	Absorption, distribution, metabolism and excretion
BPR	Biocidal products regulation (EU) 528/2012
BUN	Blood urea nitrogen
BW	Body weight
CAS RN	CAS registry number
CCH	Compliance check
CLP	Classification, labelling and packaging
CoRAP	Community rolling action plan
DETDA	Diethylmethylbenzenediamine
DMEL	Derived minimal effect level

DNEL	Derived no-effect level
DTU	Technical university of Denmark
EC	European community
ECHA	European chemicals agency
ED	Endocrine disruption
EFSA	European food safety authority
EU	European union
FOB	Functional observation battery
GGPT	Gamma-glutamyl transpeptidase
GI	Gastrointestinal
GLP	Good laboratory practice
GMT	Group management team
GPMT	Guinea pig maximization test
MSCA	Member state competent authority
LLNA	Local lymph node assay
LOAEL	Lowest observed adverse effect level
NCE	Normochromatic erythrocytes
NOAEC	No observed adverse effect concentration
NOAEL	No observed adverse effect level
NONs	Notification of new substances
OECD	Organisation for economic co-operation and development
PBT	Persistent, bioaccumulative and toxic
PMT	Persistent, mobile and toxic
PCE	Polychromatic erythrocytes
PNDT	Prenatal developmental toxicity
PNEC	Predicted no effect level
POP	Persistent organic pollutants
PPP	Plant protection products regulation EC 1107/2009
QSAR	Quantitative structure activity relationship
RAR	Risk assessment report
RDT	Repeated dose toxicity
REACH	Regulation No 1907/2006 concerning registration, evaluation, authorization, and restriction of chemicals
RMOA	Regulatory management option analysis

RSS	Robust study summary
SD	Sprague-dawley (rat)
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
STOT RE	Specific target organ toxicity – repeated exposure
SVHC	Substance of very high concern
TBD	To be decided
TPE	Testing proposal examination
TPO	Thyroid peroxidase
TSH	Thyroid-stimulating hormone
UNEP	United nations environment program
UVCB	Unknown or variable composition, complex reaction products or of biological origin materials
vPvB	Very persistent and very bioaccumulative
vPvM	Very persistent and very mobile