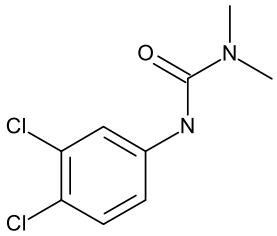
SUBSTANCE EVALUATION CONCLUSION and EVALUATION REPORT

for

Diuron

EC No 206-354-4

CAS RN 330-54-1



Evaluating Member State Competent Authority: Finland

Dated: 18 June 2024

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Year of evaluation in CoRAP: 2014

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

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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

1. Scope of the evaluation

Diuron ("the Substance") was originally selected for substance evaluation to clarify concerns about:

Endocrine disruption (human health)
Exposure of environment
Wide dispersive use
Ground and surface water pollutant

During the evaluation the following additional concern was identified:

Endocrine disruption (environment)

2. Overview of other processes / EU legislation

Table 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	
☒	☐	☐	☐

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 2 Conclusion and regulatory follow-up action

Initial and additional concern	Conclusion on concern	Regulatory follow-up action
Endocrine disruption (human health)	Inconclusive	No need for regulatory follow-up at EU level
Endocrine disruption (environment)	Concern confirmed	Harmonised classification and labelling
Exposure of environment/Wide dispersive use	Concern confirmed	Need for follow-up regulatory action at EU level
Ground and surface water pollutant	Concern confirmed	Need for follow-up regulatory action at EU level

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

Additional endpoint	Conclusion	Regulatory follow-up action
PMT/vPvM	Inconclusive	Further assessment on PMT/vPvM properties
PBT/vPvB	Inconclusive	Further assessment on PBT/vPvB properties

Based on the available information the concern for *endocrine disruption (environment)* is confirmed for the Substance.

The concern for *endocrine disruption (human health)* can be considered inconclusive as the information available is neither sufficient for identifying the Substance as an endocrine disruptor for human health nor for excluding the concern.

The concerns *exposure of environment* and *wide dispersive use* are confirmed. The exposure of environment is of concern due to the identified hazards of the Substance (including e.g. endocrine disruption), uses, and the information available on the persistence, bioaccumulation, mobility, and environmental presence of the Substance. Due to the same reasons, as well as the predicted environmental distribution and the reported presence of the Substance in wastewater treatment plant effluents, surface water, groundwater, and biota, the initial concern *ground and surface water pollutant* is also confirmed.

The assessment of the PMT/vPvM/PBT/vPvB properties was not in the scope of the evaluation[1]. However, environmental fate and hazard properties, including those relevant for PMT/vPvM/PBT/vPvB, were included in the assessment. The reason to this was, rather than concluding on the PMT/vPvM/PBT/vPvB properties of the Substance, to obtain relevant background information and a general understanding of the properties of the Substance to support the assessment of the concerns belonging to the scope of the evaluation (Section 1). Information on degradation and biotransformation was needed for the assessment of endocrine disruption, which should also cover transformation/degradation products. In addition, some of these properties were considered by the evaluating MSCA in the context of "equivalent level of concern" (ELoC) assessment which is relevant to SVHC identification under REACH Article 57 (f)[2].

The evaluating MSCA will continue the assessment of the PMT/vPvM and PBT/vPvB properties outside of this substance evaluation to conclude whether the Substance has these properties.

[1] Regarding the combinations of persistent, mobile, and toxic (PMT) properties and very persistent and very mobile (vPvM) properties, it should be noted that these were introduced in the European Union legislation as hazard properties only in 2023 (Commission delegated regulation (EU) 2023/707), i.e., in the final stages of this substance evaluation.

[2] During the initial stages of the assesssment, the evaluating MSCA considered SVHC identification due to endocrine disrupting properties under REACH Article 57 (f) as the primary risk management measure for the Substance in case that endocrine disrupting properties are confirmed. CLP classification for endocrine disruption was not yet a possibility during the initial stages of this substance evaluation. Endocrine disruption for the environment and human health were introduced in the CLP regulation only in 2023 (Commission delegated regulation (EU) 2023/707), i.e., in the final stages of this substance evaluation.

4. Regulatory follow-up actions at EU level

The current risk management measures in the registration dossier do not take into account the endocrine disrupting properties of the Substance for the environment. According to the information in the registration dossier the Substance is used in industrial sites in the manufacture of rubber products and polymer preparations. The evaluating MSCA considers that the available information indicates wide dispersive use: release of the Substance to the environment can occur from industrial use and, in addition, is likely to occur from outdoor use in long-life materials with high or low release rates e.g. from tyres or from construction and building materials due to weathering conditions.

During this substance evaluation, the combinations of persistent, mobile, and toxic (PMT) properties and very persistent and very mobile (vPvM) properties were introduced in the European Union legislation as hazard properties (Commission delegated regulation (EU) 2023/707). The further assessment of the PMT and PBT/vPvB properties of the Substance was identified in this substance evaluation as a necessary follow-up step to clarify whether the Substance has these hazard properties. The confirmation that the Substance has PMT, PBT, or vPvB properties could lead to improved risk management and mitigation of exposure which could be relevant to also address the initial concerns exposure of environment and ground and surface water pollution.

The evaluating MSCA notes that the endocrine disrupting property for the environment should be considered a hazard for which no safe threshold can be defined, unless otherwise justified, and this needs to be taken into account in the risk management. The potential PMT and/or PBT/vPvB properties are also properties which, if confirmed, would prevent the determination of a safe threshold for the Substance.

4.1 Harmonised Classification and Labelling

The harmonised classification of the Substance in Annex VI of Regulation (EC) 1272/2008 has been amended in Commission Delegated Regulation (EU) 2024/197 (Table 9).

Based on the available information relevant for the hazard classes in Commission delegated regulation (EU) 2023/707, the evaluating MSCA considers that a classification as endocrine disruptor for the environment in Category 1 is warranted for the Substance. A proposal for a harmonised classification and labelling for endocrine disruptor for the environment will be submitted.

The available information is not sufficient for identifying the Substance as an endocrine disruptor for human health. However, the evaluating MSCA considers that a classification of the Substance as an ED for human health would be unlikely to result in further risk management measures compared to the obligations coming from the classification as Carc. 1B in Commission Delegated Regulation (EU) 2024/197.

Regarding the hazard class "Persistent, Bioaccumulative and Toxic or Very Persistent, Very Bioaccumulative properties", the evaluating MSCA concludes that the Substance fulfils the toxicity (T) criterion and may fulfil the persistence (P/vP) and bioaccumulation (B/vB) criteria. Therefore, classification due to Persistent, Bioaccumulative and Toxic and/or Very Persistent, Very Bioaccumulative properties may be warranted.

Regarding the hazard class "Persistent, Mobile and Toxic or Very Persistent, Very Mobile properties" the evaluating MSCA concludes that the Substance fulfils the toxicity (T) criterion and may fulfil the persistence (P/vP) and mobility (M) criteria. Therefore, classification due to Persistent, Mobile and Toxic properties may be warranted.

Further assessment of the information relevant for P/vP, M, and B/vB criteria is needed before the evaluating MSCA can conclude on the need to propose an update to the current harmonised classification and labelling of the Substance due to the potential PMT and/or PBT/vPvB properties. This assessment will be done outside this substance evaluation. If the further assessment leads to the conclusion that CLP classification due to PMT, PBT, or

vPvB properties is warranted for the Substance, the evaluating MSCA will consider submitting a proposal for harmonised classification and labelling (CLH proposal). It would be decided at a later time whether the possible classification for PMT, PBT, or vPvB properties would be included in the same CLH proposal together with the endocrine disruption (environment) or in a separate CLH proposal with different time of submission.

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

The need for SVHC identification could be considered after the update of the harmonised classification of the Substance due to the endocrine disrupting properties for the environment and potentially also to PBT/vPvB and/or PMT properties. The need for SVHC identification based on the classification as Carc. 1B in Commission Delegated Regulation (EU) 2024/197 could also be considered.

4.3 Restriction

With the upcoming classification as Carc. 1B in the Annex VI of the Regulation EC No 1272/2008, the Substance will be restricted from consumer use according to Annex XVII, entry 28 of the Regulation EC No 1907/2006. This means that the Substance shall not be placed on the market for use by the general public as substance, or as constituent of other substances, or in mixtures when the concentration is equal to or greater than 0.1%. Diuron will also be covered by the Carcinogen and Mutagen Directive (CMD, Directive 2004/37/EC). The upcoming Carc. 1B classification is expected to also change the use and exposure at workplaces.

However, the classification as Carc. 1B might not be enough to restrict the emissions to the environment. The need for restriction (e.g. potential release to the environment) could be considered after the classification as endocrine disruptor for the environment in Category 1 (and potential PMT/PBT/vPvB properties).

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

Not applicable.

6. Tentative plan for follow-up actions

As indicated in Tables 2 & 3, 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 4 Follow-up actions

Follow-up action	Date for intention	Actor
Harmonised C&L	2026	Finland
Further assessment on PMT/vPvM properties	2026	Finland

Further assessment on PBT/vPvB properties	2026	Finland

Part B. Substance evaluation report

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 main scope of the evaluation was endocrine disrupting properties (human health and the environment). In addition, a review of information relevant to PBT/vPvB and PMT/vPvM properties has been done.

The present evaluation of Diuron is based on the

- updated registration dossier
- full study summaries provided by the Registrant
- open literature sources
- draft Renewal Assessment Report for Diuron under PPP Regulation
- information of an analogue substance Linuron
- ED-evaluation of Diuron under BPR Regulation

The ED properties of Diuron were discussed at the ECHA Endocrine Disruptor Expert Group meeting in 11-12 November 2014.

Before concluding the substance evaluation, a Decision to request further information was issued according to Article 46 on: 10 June 2016[3]

The Registrant updated the registration dossier with the requested new FSDT (OECD TG 234) test data on 28 June 2018.

A written procedure in the ECHA Endocrine Disruptor Expert Group was arranged on ED properties of Diuron in May 2019.

Before concluding the substance evaluation, a Decision to request further information was issued according to Article 46 on: 26 October 2020[4]

The Board of Appeal in its decision in Case No. A-002-2021[5] annulled in its entirety the Decision to request further information for Diuron.

The evaluating Member State concluded the evaluation without any further need to ask more information from the registrants under an Article 46 decision.

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 CHEM (europa.eu)

[3] https://echa.europa.eu/documents/10162/62a1d5bf-d174-7691-906e-4c1c51625336

[4] https://echa.europa.eu/documents/10162/8b0e9243-ae28-0a16-3bb8-27d448278910

[5] https://echa.europa.eu/documents/10162/f6a07364-4e25-10fb-23eb-726be390b66b

Synonyms: 3-(3,4-dichlorophenyl)-1,1-dimethylurea, Preventol A6

8.1. Type of Substance

Mono-constituent.

8.2. Other relevant information

Table 5 Other information relevant to the composition of the Substance

Type	Identity	Typical concentration	Concentration range	Remarks
Metabolite	3,4-dichloroaniline (3,4-DCA) EC 202-448-4			Relevant for the ED assessment
Metabolite	3-(3, 4-dichlorophenyl)urea (DCPU) CAS 35377-46-9			Relevant for the ED assessment
Metabolite	1-(3,4-dichlorophenyl)-3-methylurea (DCPMU) EC 622-767-6			Relevant for the ED assessment
Metabolite	3,4-dichlorophenyl-urea (DCPU) EC 622-620-6			Relevant for the ED assessment

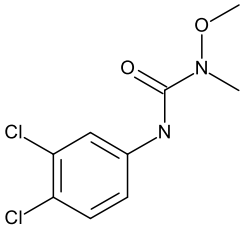
8.3. Analogue substance (read-across)

Diuron and Linuron belong to a group of phenyl urea herbicides and they are regarded as structural analogues sharing also similar transformation products (Badawi et al. 2009) (see identity information below and Table 13 for information on the transformation products). Linuron is regarded as a known antiandrogen (Category 1) in the ED priority list by EU COM (European Commission 2000).

Linuron, a structurally similar substance, is subject to harmonized classification as carcinogenic category 2 and as toxic to reproduction category 1B in the Annex VI of CLP Regulation (Regulation (EC) 1272/2008). The approval of Linuron for use in plant protection products under the PPP Regulation (Regulation (EC) No 1107/2009) was not renewed in 2017 because Linuron is classified as toxic for reproduction category 1B and negligible exposure could not be demonstrated. Furthermore, Linuron was considered to have endocrine disrupting properties in accordance with interim criteria for endocrine disrupters at that time.

Therefore, available relevant information on the ED effects of Linuron was also evaluated as supporting information together with the evaluation of ED effects of Diuron.

Table 6 Relevant analogue substance(s)

EC/List number	CAS RN	Public Substance name	Chemical structure
206-356-5	330-55-2	Linuron	

9. Physicochemical properties

Physicochemical properties of Diuron are presented below. In addition, some properties of Linuron are shown for comparison.

Table 7 Overview of physicochemical properties

Property	Diuron	Linuron
Molecular weight/weight range	233.095 g/mol	249.09 g/mol
Physical state at 20 °C and 101.3 kPa	solid at 20 °C and 101.3 kPa Form: powder Colour: whitely Odour: slight ammonia / amine odour solid at 20 °C and 101.3 kPa Form: powder Colour: white to beige Odour: slight	
Vapour pressure	7.6 x 10^{-9} hPa at 20 °C	5.1 x 10^{-3} Pa at 20 °C
Water solubility	29.0 mg/L (20 °C, pH 6.2 (unbuffered water)) 28.5 mg/L (20 °C, pH 4.02) 28.8 mg/L (20 °C, pH 7.01) 28.8 mg/L (20 °C, pH 9.01)	52.7 mg/l at pH5, 20 °C 63.8 mg/l at pH7, 20 °C 74.5 mg/l at pH9, 20 °C
Partition coefficient n-octanol/water (Log K_{ow})	2.84 (20 °C, pH 6.2 (unbuffered water)) 2.87 (20 °C, pH 4.03) 2.89 (20 °C, pH 7.01) 2.87 (20 °C, pH 9.00)	3.0
Partition coefficient organic carbon/water (Log K_{oc})		
Flammability	non-flammable solid, no pyrophoricity, no flammability in contact with water	
Explosive properties	not explosive	
Granulometry	D10: 0.458 µm D25: 2.023 µm D50: 5.739 µm D75: 10.12 µm D90: 14.84 µm (finest quality)	

Dissociation constant	no dissociation	
Melting/freezing point	156.0 °C at 1013 hPa	Melting point 93 - 95 °C
Boiling point	355 - 357 °C at 1013.3 hPa under decomposition	Not determined because the substance is a solid at room temperature.
Surface tension	72.1 mN/m at 20.0 °C (saturated aqueous solution) not surface active	
Viscosity	not applicable	
Auto flammability	401 °C	
Thermal stability	thermally stable	
Relative density	1.453 at 19.9 °C	

10. Manufacture and uses

10.1. Quantities

The aggregated tonnage (per year) of the Substance is 100 – 1,000 tonnes.

10.2. Overview of uses

The Substance is manufactured industrially in closed batch processes. Industrial uses of the Substance include use in rubber articles and in polymer preparations with subsequent article service life during use by workers.

The Substance is also used as a biocide e.g., in film preservatives (PT 7) and masonry preservatives (PT 10). The Substance was also approved for use as a plant as an active substance in plant protection product in the EU according to Regulation (EC) No 1107/2009 until September 2020. The application for renewal of approval of the Substance as an active substance under PPP Regulation was withdrawn by the applicant.

Table 8 Overview of uses

Main uses	**Key information**
Formulation	**Process category (PROC):** PROC 1: Use in closed process, no likelihood of exposure PROC 3: Use in closed batch process (synthesis or formulation) PROC 4: Use in batch and other process (synthesis) where opportunity for exposure arises PROC 5: Mixing or blending in batch processes for formulation of preparations and articles (multistage and/or significant contact) PROC 8b: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at dedicated facilities PROC 9: Transfer of substance or preparation into small containers (dedicated filling line, including weighing)

	PROC 14: Production of preparations or articles by tabletting, compression, extrusion, pelletisation **Environmental release category (ERC):** ERC 2: Formulation of preparations ERC 3: Formulation in materials
Industrial	**Use in rubber products:** **Process category (PROC):** PROC 1: Use in closed process, no likelihood of exposure PROC 3: Use in closed batch process (synthesis or formulation) PROC 5: Mixing or blending in batch processes for formulation of preparations and articles (multistage and/or significant contact) PROC 6: Calendering operations PROC 8b: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at dedicated facilities PROC 9: Transfer of substance or preparation into small containers (dedicated filling line, including weighing) PROC 13: Treatment of articles by dipping and pouring PROC 14: Production of preparations or articles by tabletting, compression, extrusion, pelletisation PROC 21: Low energy manipulation of substances bound in materials and/or articles **Chemical product category (PC**): PC 32: Polymer preparations and compounds **Environmental release category (ERC):** ERC 6d: Industrial use of process regulators for polymerisation processes in production of resins, rubbers, polymers ERC 10a: Wide dispersive outdoor use of long-life articles and materials with low release ERC 10b: Wide dispersive outdoor use of long-life articles and materials with high or intended release (including abrasive processing) ERC 11a: Wide dispersive indoor use of long-life articles and materials with low release **Sector of end use (SU):** SU 11: Manufacture of rubber products **Use in polymer preparations:** **Process category (PROC):** PROC 4: Use in batch and other process (synthesis) where opportunity for exposure arises PROC 7: Industrial spraying PROC 9: Transfer of substance or preparation into small containers (dedicated filling line, including weighing) PROC 10: Roller application or brushing PROC 13: Treatment of articles by dipping and pouring PROC 19: Hand-mixing with intimate contact and only PPE available **Chemical product category (PC):** PC 32: Polymer preparations and compounds

	Environmental release category (ERC): ERC 5: Industrial use resulting in inclusion into or onto a matrix ERC 6c: Industrial use of monomers for manufacture of thermoplastics
Article service life	**Article category (AC):** AC 10: Rubber articles **Process category (PROC):** PROC 14: Production of preparations or articles by tabletting, compression, extrusion, pelletisation PROC 21: Low energy manipulation of substances bound in materials and/or articles **Environmental release category (ERC):** ERC 6d: Industrial use of process regulators for polymerisation processes in production of resins, rubbers, polymers ERC 10a: Wide dispersive outdoor use of long-life articles and materials with low release ERC 10b: Wide dispersive outdoor use of long-life articles and materials with high or intended release (including abrasive processing) ERC 11a: Wide dispersive indoor use of long-life articles and materials with low release

11. Classification and labelling

Table 9 Classification of the Substance

Harmonised classification (Annex VI of CLP)	Self-classification in registrations	Self-classification in C&L notifications
Carc. 2, H351 Acute Tox. 4, H302 STOT RE 2, H373 Aquatic Acute 1, H400 Aquatic Chronic 1, H410, M=10 **Amended harmonised classification[4]** Carc. 1B, H350 **STOT RE 2, H373 (blood system)** Aquatic Acute 1, H400, M=100 Aquatic Chronic 1, H410, M=100	STOT RE 2 (inhalation, blood), H373	

The self-classification in the registration dossier classification follows the harmonised classification of Diuron in Annex VI of Regulation (EC) 1272/2008. There are no additional hazard classes notified among the aggregated self-classifications in the C&L Inventory.

The evaluating MSCA notes that the harmonised classification of has been amended in Commission Delegated Regulation (EU) 2024/197[6].

[6] https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:L_202400197

12. Environmental fate properties

12.1. Degradation

Information on degradation has been reviewed in 2014-2015 (i.e., during the initial SEv assessment). This assessment of degradation has been considered sufficient for the purpose to clarify the initial and additional concerns (see Section 1).

12.1.1. Abiotic degradation

Hydrolysis

Based on the OECD TG 111 hydrolysis test chemical hydrolysis can be excluded as a dissipation mechanism of Diuron in the environment. The degradation of Diuron was less than 10 % at pH 4, 7 and 9 and the corresponding half-life, at ambient temperature (25 °C) calculated using Arrhenius equation), estimated to be longer than 1 year.

Phototransformation/photolysis

Based on the Atkinson calculation method an atmospheric half-life of 2.9 to 3.1 h was obtained for Diuron. Therefore, Diuron will be rapidly photodegraded in air.

Phototransformation in water and soil was not included in this assessment.

12.1.2. Biodegradation in water

Estimated data

Estimated biodegradation data was not available in the registration dossier and has not been generated by the evaluating MSCA.

Screening tests

An OECD TG 301F ready biodegradability test is available in the registration dossier. This test reported 0% biodegradation after 28 days. Therefore, it is concluded that Diuron is not readily biodegradable.

Simulation tests (water and sediments)

A simulation test on the degradation and partitioning behaviour of Diuron in water-sediment systems is available in the registration dossier. The test followed SETAC-Europe Procedures. Diuron dissipated from water/sediment systems with a DT_{50} of 48-232 d at 20 ^{0}C. DCPU or/and m-CPDMU were reported as transformation products depending on the sample. Mineralization was 2-30% (based on percentage of applied radioactivity) after 120 d. Dissipation from water phase to sediment was rapid.

Table 10 Water/sediment simulation test.

Water/ sediment system	Tempe-rature	DT_{50} (dissipation)	Transformation products, not extracted fraction, and $^{14}CO_2$ (percentage of applied radioactivity in total water-sediment system)	Percentage of applied radioactivity recovered as $^{14}CO_2$	Method	Reference

River Erft	20 °C	48 d	DCPMU max. 4.4%, m-CPDMU not detected, non extracted max. 45.9%	max. 30.3%	U.S. EPA Pesticides Guidelines Subdivision N 162-1, corresponds to SETAC-Guidelines Part 1-1.1. No deviations. GLP, test period 120 d, test substance: [phenyl-UL-^{14}C]Diuron	Unpublished (2001)
Hönniger Weiher	20 °C	232 d	DCPMU max. 4.1%, m-CPDMU max. 15.2%, non extracted max. 17.5%	max. 2.0%	U.S. EPA Pesticides Guidelines Subdivision N 162-1, corresponds to SETAC-Guidelines Part 1-1.1. No deviations. GLP, test period 120 d, test substance: [phenyl-UL-^{14}C]Diuron	Unpublished (2001)

12.1.3. Biodegradation in soil

Two soil simulation studies were available in the registration dossier, following U.S. EPA Pesticides Guidelines Subdivision N 162-1 (Unpublished 1990a) and BBA IV, 4-1 (Unpublished 1996) guidelines. In addition, data obtained from the risk assessment conducted under the directive concerning the placing of plant protection products on the market (91/414/EEC) was used. These data were obtained from Draft assessment report (DAR) (RMS Denmark 2005) and from the Conclusion on the Peer review of Diuron (EFSA 2005).

In soils Diuron dissipated with a half-life of 20-372 d. Mineralization was at maximum 32% during 101 days. DCPU, DCPMU, and 3,4-DCA were the main transformation products.

Table 11 Soil degradation data for Diuron from the registration dossier and from the EU Risk assessment under the plant protection product regulation.

Soil	Temperature	Moisture	DT_{50} (dissipation)	Percentages of applied radioactivity detected as transformation products and not extracted fraction, $^{14}CO_2$	Percentage of applied radioactivity recovered as $^{14}CO_2$	Method	Reference
Silt loam	25 °C	75 % of the water content at 0.33 bar potential	372 d	DCPMU max. 22%, DCPU max. 0.7%, "polars" max. 3.1%, not extracted max. 14.9%,	max. 3%	US EPA 162-1, no deviations stated, GLP, test period 365 d.	Unpublished (1990a)[3, 4]
Slight humic sand	20 °C	not reported in dossier	118 d	DCPMU 19.1%, DCPU max. 1.6%, DCA not reported, not extracted max. 6%	max. 31.8%	BBA IV, 4-1 , no deviations stated, GLP, test period 101 d	Unpublished (1996a) [3, 4]
Sandy loam	20 °C	70 %WHC	51 d	DCPMU max. 33%, DCPU max. 4%, 3,4-DCA max. 3%,	not analysed	Danish Guidelines (1987), no deviations stated, GLP, test period 100 d	Unpublished (1993a)[4]
Sandy loam	10 °C	70 %WHC	143 d	DCPMU max. 23%, DCPU max. 2%, 3,4-DCA max. 2%,	not analysed	Danish Guidelines (1987), no deviations stated, GLP, test period 100 d	Unpublished (1993a)[4]
Sandy loam	20 °C	35 %WHC	27 d	DCPMU max. 33%, DCPU max. 11%, 3,4-DCA max. 2%,	max. 7%	Danish Guidelines (1987), no deviations stated, GLP, test period 100 d	Unpublished (1993a)[4]
Loamy sand	20 °C	70 %WHC	20 d	DCPMU max. 28%, DCPU max. 25%,	max. 5%	Danish Guidelines (1987), no	Unpublished (1993a)[4]

				3,4-DCA max. 2%,		deviations stated, GLP, test period 100 d	
Sand	20 °C	70 %WHC	119 d	DCPMU max. 27%, DCPU max. 1%, 3,4-DCA max. 2%,	max. 14%	Danish Guidelines (1987), no deviations stated, GLP, test period 100 d	Unpublished (1993a)[4]
Slight humic sand	not applicable	not applicable	112 d	not applicable	not applicable	Calculation using data from Unpublished (1996a)	Unpublished (1998)[4]

[3] Included in the registration dossier of Diuron [4] Included in the PPP risk assessment (RMS Denmark, 2005, EFSA 2005)

12.1.4. Non-standard published studies on biodegradation

Biodegradation of Diuron has been extensively studied in the scientific literature. Some studies on the microbial transformation and mineralization of Diuron, considered to be relevant for this assessment, were reviewed and are presented in Table 12.

Microorganisms capable of degradation and mineralization of Diuron have been isolated from environmental sites exposed to Diuron. Often improved degradation of Diuron is obtained with microbial consortia as compared to individual strains (Table 12). For example, some individual species are able to convert Diuron into 3,4-DCA but unable to metabolize 3,4-DCA further. Metabolism of Diuron has been reported also from a field site with no previous exposure to pesticides (Gooddy *et al*. 2002).

Table 12 Non-standard published biodegradation studies.

Study description	Studied microorganisms (or their source)	Biodegradation and growth results	Remarks	Reference
A field study to investigate the fate and transport of Diuron on an experimental plot established on a calcareous soil in southern England. Diuron was applied to the soil surface at a rate of 6.7 kg/ha along with a potassium bromide conservative tracer. Hand augured samples were taken at regular intervals over the next 50 days, with samples collected down to 54 cm. Porewaters	A calcareous soil field site with no previous pesticide applications for at least 30 years. The land is left under grass, being regularly cut during the summer months.	Diuron, DCPU, DCPMU, and 3,4-DCA were detected in soil (solid phase and porewater) samples. High concentrations of Diuron and metabolites were still present in the soil and soil solutions after 50 days.		Gooddy *et al*. 2002

Study description	Studied microorganisms (or their source)	Biodegradation and growth results	Remarks	Reference
were extracted from the soil cores.				
A field study was conducted to obtain an understanding of the fate of Diuron in a conventional sugarcane farming system in coastal Queensland, Australia. Surface runoff and the dissipation of Diuron and its metabolites were studied. The metabolites were 3-(3,4-dichlorophenyl)-1-methylurea (DCPMU), 3,4-dichlorophenylurea (DCPU) and 3,4-dichloroaniline (3,4-DCA).	A sugarcane farm situated on coastal sand classified as a Podosol or Podzol.1	Average concentrations of Diuron, DCPU and DCPMU in runoff were 93, 30 and 83–825 µg L^{-1} respectively. Their total loading in all runoff was >0.6% of applied Diuron. Diuron and DCPMU concentrations in stream sediments were between 3–22 and 4–31 µg kg^{-1} soil respectively. First-order *t*1/2 for dissipation of Diuron (based on dissipation functions calculated from soil concentration measurements) averaged 49 ± 0.9 days for the 0–15, 0–30 and 0–45 cm soil depths.	A spray application of Diuron was made. Diuron had not been applied to the trial area for at least 2 years prior to the spraying.	Stork *et al*. 2008
The main objective of this study was to investigate the use of biological treatment processes for Diuron elimination by contaminated water and to study the factors that affect its fate and enhance its possible bio-transformation. Activated sludge batch biodegra-dation experiments were conducted at Diuron concen-tration levels similar to those reported in the literature for runoff waters. The effect of aerobic and anoxic conditions, biomass acclimatization and presence or absence of supplemental substrate on Diuron biodegradation	Laboratory-scale sequencing batch reactors (SBRs) were used to simulate activated sludge process and to provide biomass for the biodegradation experiments. Two of them were operated in aerobic mode, while the others in anoxic mode. Activated sludge from a municipal wastewater treatment plant was used to seed the reactors, while municipal wastewater originating from the aforementioned plant was used as feed. To acclimatize biomass to Diuron, a known amount of this compound was daily added to the	Some of the conclusions by the authors were: Under aerobic conditions, almost 60% of Diuron was biodegraded, while its major metabolite was 3,4-DCA. The existence of anoxic conditions increased Diuron biodegradation to more than 95%, while the major metabolite was DCPU. Mass balance calculation showed that a significant fraction of Diuron is mineralized or biotransformed to other unknown metabolites. The role of sorption to biomass was not significant. The use of acclimatized		Stasinakis *et al*. 2009

Study description	Studied microorganisms (or their source)	Biodegradation and growth results	Remarks	Reference
potential was investigated. Biodegradation experiments were also performed under aerobic conditions for DCPMU, DCPU and 3,4-DCA.	influents, so as to achieve a concentration of 10 mg L^{-1}.	biomass under anoxic conditions enhanced Diuron biodegradation. Calculation of mass balances showed that at the end of the experiments Diuron was detected as DCPMU, DCPU and 3,4-DCA, while almost 30–49% had been mineralized or biotransformed to other unknown metabolites. Under aerobic conditions, Diuron metabolites were biodegraded faster than the parent compound. Half-lives equal to 19, 103 and 44 h were calculated for DCPMU, DCPU and 3,4-DCA, respectively.		
The objectives of this study were to establish anaerobic enrichment cultures with inoculum from pond sediment in the presence Diuron, determine the fate of Diuron in enrichment cultures under anaerobic conditions, and identify the major degradation product(s) formed.	Sediment samples were obtained from a pond treated with Diuron. Enrichment cultures were established with seven different media in the presence of Diuron (40 mg/liter).	According to the authors, all enrichment cultures completely degraded Diuron in 17-25 days. In all cultures showing Diuron degradation, the product identified as 3-(3-chlorophenyl)-1,1-dimethylurea appeared in approximately stoichiometric amounts. Reinjection of Diuron into each culture after 26 days resulted in rapid degradation of the parent herbicide with the appearance of proportionately more 3-(3-chlorophenyl)-1,1-dimethylurea. No other product was detected after 80 days in culture.		Attaway *et al*. 1982
The functioning of Diuron-degrading community was investigated. The study included experiments aiming	Soil was collected from upper horizon (0–20 cm) of a grass buffer strip (BS) located at the	Diuron-degrading *Arthrobacter* strains were isolated from soils and sediments. 3,4-DCA degrading *Achromobacter* strain		Devers-Lamrani *et al*. 2014

Study description	Studied microorganisms (or their source)	Biodegradation and growth results	Remarks	Reference
at isolating and characterizing members of Diuron -degrading microbial communities enriched from soil and sediment samples collected. Isolated members able to degrade Diuron or its aniline derivative 3,4-DCA were taxonomically characterized by sequencing 16S rRNA gene. Degrading genetic potential was monitored by studying genes involved in Diuron or 3,4-DCA transformation. The ability of these bacterial strains to degrade various phenylureas and anilines was studied.	interface between a vineyard and the Morcille river, a small firstorder stream (7 km long) subjected to a strong agricultural pressure (Beaujolais, France). Sediment samples (SED) were collected in the downstream section of the Morcille river. Enrichment cultures were done starting from either BS or SED samples. Mineral salt (MS) medium added with sodium citrate and Diuron as sole nitrogen source was used. After 3 enrichment cycles samples were plated onto solid Diuron or 3,4-DCA containing medium and pure colonies were selected for Diuron or 3,4-DCA degradation.	was isolated from sediments. *Arthrobacter* strain transformed Diuron to 3,4-DCA with PuhA enzyme. *Achromobacter* isolate transformed 3,4-DCA with DcaQ enzyme. Mixed culture of *Arthrobacter* and *Achromobacter* mineralized ^{14}C-Diuron to $^{14}CO_2$.		
Fungus-mediated bacterial transport, Diuron degradation pathways, and microbial interactions affecting fungal growth were studied using microbial cultures.	Fungal strains *Mortierella* sp. LEJ702 and *Mortierella* sp. LEJ703 Bacterial strains *Sphingomonas* sp. SRS2 and *Variovorax* sp. SRS16, *Arthrobacter. globiformis* D47	Mineralization of Diuron was observed with the studied bacterial and fungal strains and consortia. Some of the single strains and consortia degraded Diuron into the metabolites DCPMU, DCPU, 3,4-DCA, and 3,4-DCAA.	The bacterial single strains and consortia with purely bacterial strains degraded only minimal amounts of Diuron. The fastest mineralization of ^{14}C-labeled Diuron was seen in the consortium consisting of *Mortierella* LEJ702, *Variovorax* SRS16, and *A. globiformis* D47,	Ellegaard-Jensen *et al*. 2014

Study description	Studied microorganisms (or their source)	Biodegradation and growth results	Remarks	Reference
			as measured by evolved $^{14}CO_2$. The production of Diuron metabolites by this consortium was minimal.	
A two-member Linuron and Diuron-mineralizing consortium was constructed. The objective of this approach was to obtain Diuron-mineralizing bacterial cultures for use in bioremediation processes aimed at cleaning contaminated soils and water resources. The degradative capacity of the consortium was compared to that of each of the strains individually with regard to the ability to degrade and mineralize Diuron at ecologically relevant concentrations in both liquid media and soil.	*Arthrobacter. globiformis* D47 *Variovorax* sp. strain SRS16	Neither of the strains mineralized Diuron alone in a mineral medium, but combined, the two strains mineralized 31 to 62% of the added [*ring*-U-^{14}C]Diuron to $^{14}CO_2$. DCPMU, DCPU, and 3,4-DCA were reported as metabolites. Strain D47 was able to produce 3,4-DCA from Diuron, key enzymatic step is mentioned to be the hydrolysis of the amide bond of Diuron.	*Variovorax* sp. strain SRS16 was able to mineralize diuron in a pure culture when it was supplemented with appropriate growth substrates, making this strain the first known bacterium capable of mineralizing Diuron and representatives of both the *N*,*N*-dimethyl- and *N*-methoxy-*N*-methyl-substituted phenylurea herbicides. It was also concluded to be the first strain conclusively shown to mineralize the ring structure of Diuron. The two-member consortium showed a higher extent of Diuron mineralization than the strain SRS16 alone.	Sørensen *et al*. 2008
Biodegradation and metabolites of Diuron and two other phenylurea herbicides were studied on pure cultures of *Arthrobacter* sp. N2.	*Arthrobacter* sp. N2 was isolated from a soil contaminated with Diuron for many years (Widehem et al., 2002).	Diuron degradation by A*rthrobacter* sp. N2 resulted in the formation of 3,4-dichloroaniline. Diuron was quantitatively converted into 3,4-dichloroaniline,	The authors mention that, with the strain *Arthrobacter* sp. N2 the formation of the 3,4-dichloroaniline seems to come	Tixier *et al*. 2002

Study description	Studied microorganisms (or their source)	Biodegradation and growth results	Remarks	Reference
The toxicity of the identified degradation products was evaluated using the Microtox test and compared to that of parent molecule. In order to study the fate of the degradation product of Diuron in the environment, several fungal strains, known for their efficiency to degrade Diuron, were tested on 3,4-dichloroaniline.	Fungal strains were commercially available: *Aspergillus niger* van Tieghem ATCC 9142, *Beauveria bassiana* Balsamo ATCC 7159, *Cunninghamella elegans* var. *elegans* Lendner ATCC 9245 and *Mortierella isabellina* Oudem NRRL 1757.	which accumulated in the medium. The four fungal strains tested were able to degrade 3,4-dichloroaniline by more than 60% after five days (based on percentage depletion). An incubation assay of Arthrobacter sp. N2 with 3,4-dichloroaniline was also carried out. *Arthrobacter* sp. N2 was not able to degrade 3,4-dichloroaniline; this result is consistent with the accumulation of this metabolite during diuron degradation.	directly from the cleavage of the amide bond of Diuron. No-demethylation products were identified.	
The isolation and characterization of a bacterial strain, *Arthrobacter* sp. N2 is described. Its ability to aerobically transform Diuron into 3,4-dichloro-aniline in pure culture, either alone or in the presence of alternative carbon sources was studied.	The *Arthrobacter* sp. N2 strain was isolated from a soil treated over several years with Diuron.	*Arthrobacter* sp. metabolized Diuron and the final trans-formation product, 3,4-dichloroaniline, was produced in stoichiometric amounts in pure culture. 3,4-DCA was not further trans-formed. The bacterial activity was also evaluated in soil microcosms with a consequent disappearance of Diuron and concomitant appearance of 3,4-dichloroaniline, of which the concentration decreased thereafter.	The transformation of Diuron at different concentrations was more efficient in the presence of alternative sources of carbon and nitrogen.	Widehem *et al*. 2002

12.1.5. Transformation products and pathways

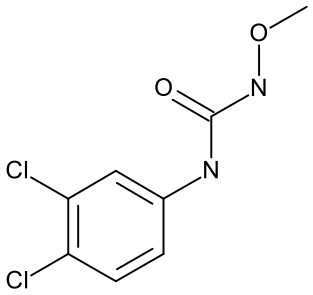
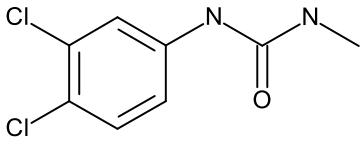
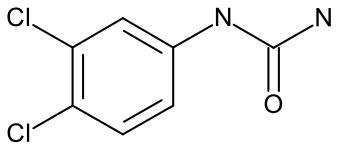
The transformation products of Diuron are presented in Table 13. The table also includes data on the transformation products of Linuron, a structural analogue of Diuron, which was used in the assessment. Proposed metabolic pathways for Diuron and 3,4-DCA are shown in Figure 1.

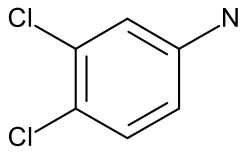
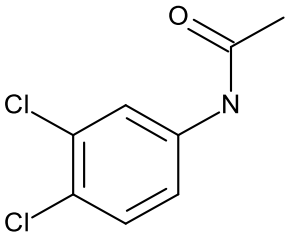
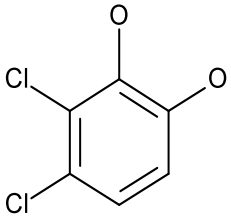
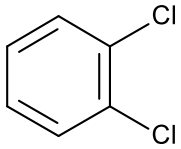
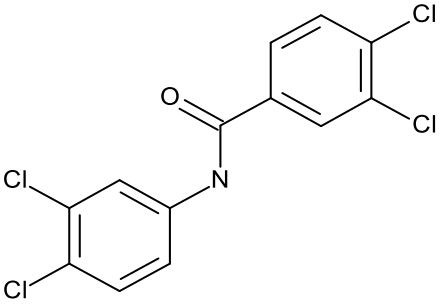
3,4-dichlorophenylurea (DCPU) and 1-(3,4-dichlorophenyl)-3-methylurea (DCPMU), were reported to be formed from Diuron in the simulation tests (Unpublished 1996a, Unpublished 2001, Unpublished 1993a in RMS Denmark 2005) and in published studies on microbial cultures (Attaway *et al*. 1982, Sørensen *et al*., 2008, Ellegaard-Jensen *et al*. 2014). DCPMU and DCPU have also been reported to be formed from Diuron in field studies (Gooddy *et al*. 2002, Stork *et al*. 2008).

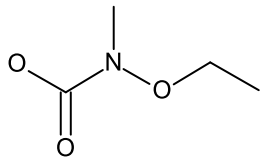
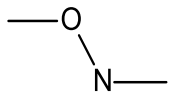
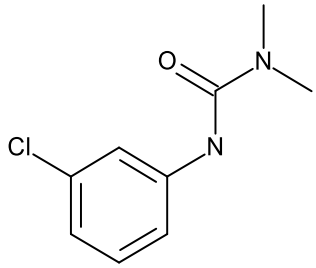
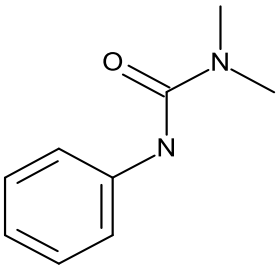
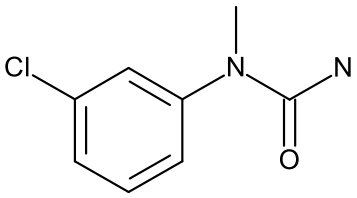
3,4-DCA has been reported to be formed from Diuron in a soil simulation test (Unpublished 1993a in RMS Denmark 2005), in microbial cultures (Widehem *et al*., 2002, Tixier *et al*. 2002, Sørensen *et al*. 2008, Devers-Lamrani *et al*. 2014, Ellegaard-Jensen et al. 2014) and in activated sludge reactors (Stasinakis *et al*. 2009). 3,4-DCA may be formed from Diuron directly (Tixier *et al*. 2002, Sørensen *et al*. 2008) or through DCPMU and DCPU (Ellegaard-Jensen *et al*. 2014). The formation of 3,4-DCAA from Diuron (Ellegaard-Jensen *et al*. 2014) and from 3,4-DCA (Tixier *et al*. 2002) in microbial cultures has been reported. DCPMU, DCPU, and DCA have been reported as metabolites of Diuron in rats (Da Rocha *et al*. 2013). In addition, 3,4-DCA has been reported to metabolize to 3,4-DCAA in fish (Allner 1997 as cited in European Chemicals Bureau 2006; Stahlschmidt-Allner *et al*. 1997).

The formation of 3-(3-chlorophenyl)-1,1-dimethyl urea (m-CPDMU) has been observed in anaerobic microbial cultures (Attaway *et al*. 1982) and in water/sediment systems (Unpublished 2001, U. S. EPA 2003).

Table 13 Transformation products proposed to occur in the microbial transformation of Diuron ((N'-(3,4-dichlorophenyl)-N,N-dimethyl urea) and Linuron (N'-(3,4-dichlorophenyl)-N, methoxy-N´-methyl urea).

Transformation product	**Diuron as proposed parent substance, references**	**Linuron as proposed parent substance, references**
3,4-dichlorophenyl-methoxyurea (desmethyl linuron) 	no information	RMS United Kingdom 1996, Badawi *et al*. 2009
N'-(3,4-dichlorophenyl)-N-methyl urea (DCPMU) (desmethoxy linuron) 	Attaway *et al*. 1982, Badawi *et al*. 2009, Unpublished 2001, Unpublished 1990a, Unpublished 1993a in RMS Denmark 2005, Ellegaard-Jensen *et al*. 2014, Gooddy *et al*. 2002, Giocomazzi and Cochet 2004, Unpublished 1996a, Stasinakis *et al*. 2009 Sørensen *et al*., 2008, Stork *et al*. 2008	RMS United Kingdom 1996, Badawi *et al*. 2004
N'-(3,4-dichlorophenyl) urea (DCPU) 	Attaway *et al*. 1982, Badawi *et al*. 2009, Unpublished 1990a , Unpublished 1993a as cited in RMS Denmark 2005, Ellegaard-Jensen *et al*. 2014, Giocomazzi and Cochet 2004, Notox B. V. 1996a, Sørensen *et al*., 2008	RMS United Kingdom, 1996, Badawi *et al*. 2009,
unknown transformation product (" a putative nonaromatic diol" (tentative structural formula is presented in the reference)	no information	Badawi *et al*. 2009

3,4-dichloroaniline (3,4-DCA) 	Devers-Lamrani *et al*. 2014, Unpublished 1993a in RMS Denmark 2005, Ellegaard-Jensen *et al*. 2014, Giocomazzi and Cochet 2004, Gooddy *et al*. 2002, Sørensen *et al*. 2008, Stasinakis *et al*. 2009, Tixier *et al*. 2002, Widehem *et al*., 2002	Badawi *et al*. 2009, Dejonghe *et al*. 2007
3,4-dichloroacetanilide 	Ellegaard-Jensen *et al*. 2014, Giocomazzi and Cochet 2004, Tixier *et al*. 2002 (3,4-DCA as parent substance),	Tixier *et al*. 2002 (3,4-DCA as parent substance), Proposed to be formed in transformation of 3,4-DCA (Giocomazzi and Cochet 2004)
dichlorocatechol 	Giocomazzi and Cochet 2004	Proposed to be formed in transformation of 3,4-DCA (Giocomazzi and Cochet 2004); therefore, a possible trans-formation product of Linuron
dichlorobenzene 	Giocomazzi and Cochet 2004	Proposed to be formed in transformation of 3,4-DCA (Giocomazzi and Cochet 2004); therefore, a possible trans-formation product of Linuron
3,4-dichloro-N-(3,4-dichlorophenyl)benzamide 	Giocomazzi and Cochet 2004	Proposed to be formed in transformation of 3,4-DCA (Giocomazzi and Cochet 2004); therefore, a possible trans-formation product of Linuron
Linear non-chlorinated compound	no information	Dejonghe *et al*. 2007

N-methyl-N-ethoxy-carbamic acid 	no information	Dejonghe *et al*. 2007
N,O-dimethyl hydroxylamine 	no information	Dejonghe *et al*. 2007
3-(3-chlorophenyl)-1,1-dimethyl urea m-CPDMU 	Attaway *et al*. 1982, Unpublished 2001, Giocomazzi and Cochet 2004	no information
1,1-dimethyl-3-phenylurea (PDMU) 	U.S. EPA 2003	no information
N-(3-chlorophenyl)-N'methylurea (mCPMU) 	U. S. EPA 2003	no information

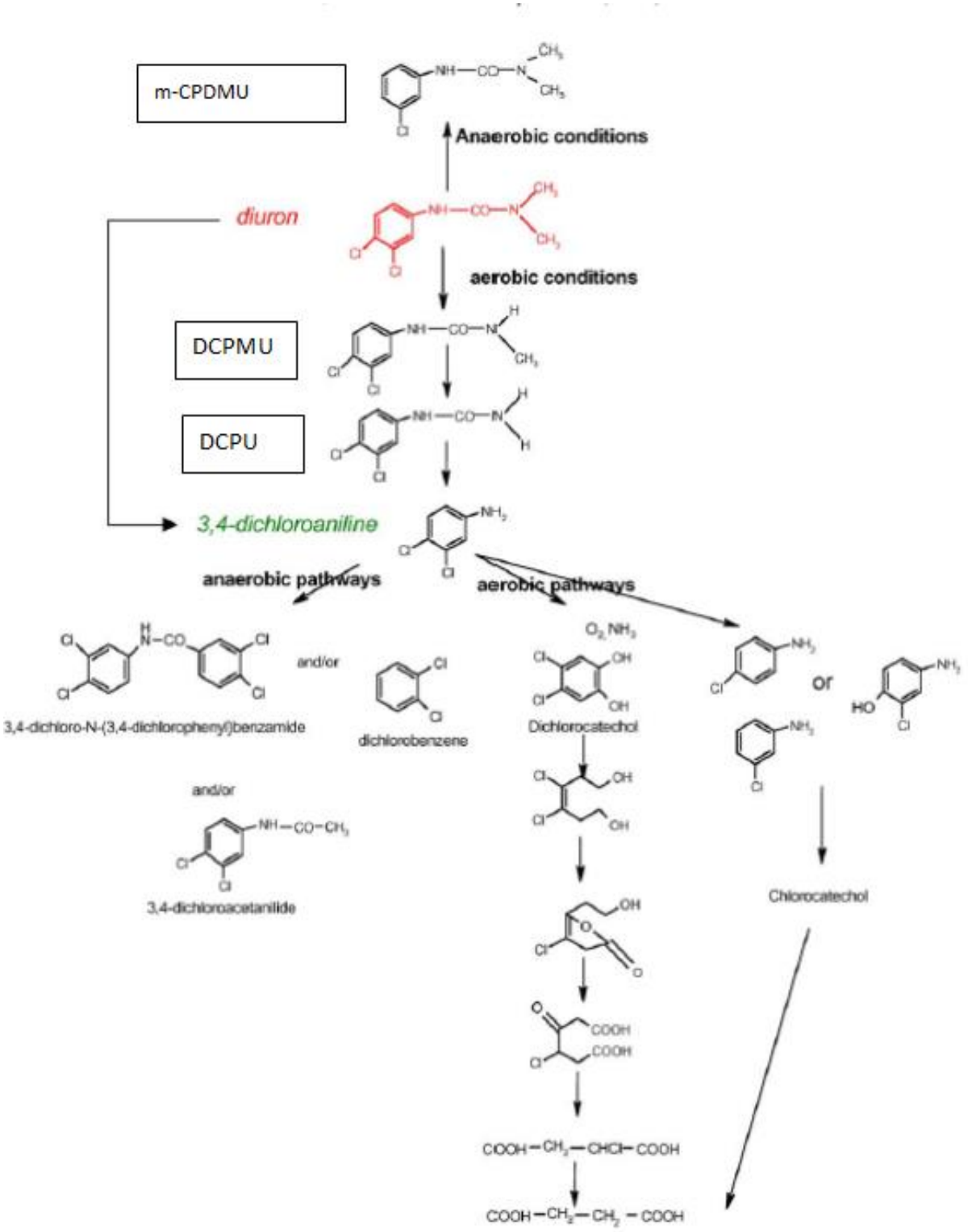


Figure 1 Metabolic pathways of bacterial degradation of Diuron and 3,4-dicholoroaniline. Reprinted from Chemosphere, 56, Giacomazzi, S., Cochet, N. Environmental impact of Diuron transformation: a review. Pages 1021–1032, Copyright (2004), with permission from Elsevier. (https://www.sciencedirect.com/journal/chemosphere). Substance names m-CPDMU, DCPMU, and DCPU have been added by evaluating MSCA.

12.1.6. Summary of degradation

- Chemical hydrolysis can be excluded as a dissipation mechanism of Diuron in the environment.
- Diuron is rapidly photodegraded in air (half-life of 2.9 to 3.1 h).
- Microorganisms capable of mineralizing Diuron have been isolated from environments exposed to Diuron. Microbial degradation pathways and transformation products have been identified. In laboratory simulation tests and in field studies microbial transformation has been observed also with environmental samples not previously exposed to Diuron.
- Diuron is not readily biodegradable (no biodegradation was observed in a ready biodegradability test).
- In water/sediment systems Diuron dissipated with a DT_{50} of 48-232 d at 20 ºC. DCPU or/and m-CPDMU were reported as transformation products depending on the sample. Mineralization was 2-30% of applied radioactivity after 120 d. Dissipation from water phase to sediment was rapid.
- In soils Diuron dissipated with a half-life of 20-372 d. Mineralization was at maximum 32% during 101 days. DCPU, DCPMU, and DCA were the main transformation products.

12.2. Environmental distribution

12.2.1. Adsorption/desorption

See soil adsorption/desorption studies in Section 12.3.1.

12.2.2. Volatilisation

Diuron is not volatile based on vapour pressure (7.6×10^{-9} hPa at 20 °C, Diuron registration dossier; 1.15×10^{-6} Pa at 25 °C (99.9%) (EFSA 2005). Diuron is not easily evaporated from water based on calculated Henry's Law Constant (2×10^{-6} Pa m^3/mole) (EFSA, 2005).

12.2.3. Distribution modelling

Mackay level I and level III distribution modelling

The distribution of Diuron in the environment was estimated using fugacity models by the evaluating MSCA. The level I Mackay model assumes a closed system where no degradation occurs. The evaluating MSCA performed a level I Mackay modelling using software "Level I Version 3.00" with standard settings (Table 14). According to a Mackay level I calculation Diuron will be distributed mainly in the water and soil compartments (Table 16).

The level III Mackay model assumes a standard environment which is in steady-state but not in equilibrium (input in and output from the model environment are occurring, as well as fluxes between the different environmental compartments). Level III model takes into account degradation processes. The modelling was done by assuming emissions to water compartment only as well as using the default emissions (equal emissions to water, soil, and air) (Table 15). The half-lives of 3 h in air, 232d in water and sediment (Unpublished 2001) and 372d in soil (Unpublished 1990a) were used as modeling parameters.

According to the results of Mackay level III environmental distribution modelling (Table 16), assuming default emissions of the model, i.e. equal emissions to air, soil, and water, 91.7% of Diuron will be distributed to the soil compartment, 8.2% to water, 0.1% to sediment and 0.01% to air. Assuming that all emissions are only to water, the majority of Diuron will be distributed to the water compartment (<0.001 of Diuron will be distributed to the soil compartment, 98.5% to water, 1.5% to sediment, and <0.001 to air).

Table 14 Parameters used for distribution modelling for the Level I Mackay model.

Media	air, soil, water, sediment
Calculation programme:	Level I Fugacity-Based Multimedia Environmental Equilibrium Partitioning Model. Version 3.00. 2004. www.trentu.ca/cemc
Environment	EQC Standard Environment
Input data:	
Amount of chemical	100000 kg
Molar mass	233.0945 g/mol
Data temperature	20 °C
Water solubility	29.0 mg/l (20 °C, pH 6.2 (unbuffered water))
Vapour pressure	7.6 x 10^{-9} hPa at 20 °C 0.00000076 Pa
Melting point	156
Log Kow	2.84 (20 °C, pH 6.2 (unbuffered water))

Table 15 Parameters used for distribution modelling for the Level III Mackay model.

Media	air, soil, water, sediment
Calculation programme:	Level III Fugacity-Based Multimedia Environmental Model. Version 2.80.1. Trent University. 2004. www.trentu.ca/cemc
Environment	EQC Standard Environment
Emission rates	Emission to: water 1000 kg/h soil 0 kg/h air 0 kg/h sediment 0 kg/h
Molar mass	233.0945 g/mol
Data temperature	20 °C
Water solubility	29.0 mg/l (20 °C, pH 6.2 (unbuffered water))
Vapour pressure	7.6 x 10^{-9} hPa at 20 °C 0.00000076 Pa
Log Kow	2.84 (20 °C, pH 6.2 (unbuffered water))

Melting point	156
Reaction half-life estimates	air 3 h water 232 d (5568 h) sediment 232 d (5568 h) soil 372 d (8928 h) suspended sediment: negligible aerosols: negligible aquatic biota: negligible

Table 16 Results of distribution modelling.

Model	**Emissions**	**Half-lifes (d): air/water/ sediment/ soil**	**Distribution in environmental compartments (%)**			
			Air	**Water**	**Soil**	**Sediment**
Mackay Level I	100000 kg	not applicable	<0.001	61.5	37.7	0.84
Mackay Level III	air 1000 kg/h; water 1000 kg/h; sediment 0 kg/h; soil 1000 kg/h	air 3 h; water 232 d; sediment 232 d; soil 372 d	0.01	8.2	91.7	0.1
Mackay Level III	Default value of the model (air 1000 kg/h; water 1000 kg/h; sediment 0 kg/h; soil 1000 kg/h)	air 3 h; water 232 d; sediment 232 d; soil 372 d	<0.001	98.5	<0.001	1.5

Fate of Diuron in wastewater treatment plant (STPWIN model)

To estimate the behaviour of Diuron in wastewater treatment plant, STPWIN model was used (model included in EpiSuite v 4.0). The half-life from water-sediment simulation test Unpublished (2001) was used as estimate of the half-life in STP. The modelling parameters are presented in Table 17.

Table 17 Parameters used for STPWIN modelling.

Calculation programme:	STPWIN model (included in EpiSuite v 4.0)
Water solubility	29 g/m^3
Vapour pressure	5.70047E-09 mmHg (0.00000076 Pa x 760 mmHg/101325 Pa)
Log Kow	2.84
STP half-life parameters	Bio P (primary clarifier): 232 d (5568 h) Bio A (aeration vessel): 232 d (5568 h) Bio S (settling tank): 232 d (5568 h)

The model results (Table 18) indicate that 0% of Diuron is emitted to air, 4.4% is adsorbed to sludge and 95.6% is released to water. Biodegradation (0.2%) is relatively low.

Table 18 Results of STPWIN.

Total removal (%)	Total biodegradation (%)	Total sludge adsorption (%)	Total to air (%)
4.6	0.2	4.4	0

12.2.4. Measured concentration in wastewater treatment plant effluents, surface water, and groundwater

Diuron has been detected in wastewater treatment plant (WWTP) effluents in an EU-wide monitoring survey (Loos *et al*. 2013). The study included samples from 90 WWTPs, mainly municipal WWTPs (some plants were dominated by industrial wastewaters). The detection frequency of Diuron was 77% and average concentration was 61.7 ng/l. Thus, WWTPs are not capable of preventing the emissions to the aquatic environment. Diuron has also been detected in surface waters (average concentration 41 ng/l (Loos *et al*. 2009) and in groundwater (Loos *et al.* 2010). The results indicate that a proportion of Diuron released to the environment (which can be from WWTPs as well as from other routes of exposure) is retained in, or transported into, surface water. The results also indicate that a proportion of released Diuron ends up in groundwater.

12.2.5. Summary and discussion of environmental distribution

Based on the Henry's law constants it can be concluded that Diuron is not easily evaporated from water. According to Level I Mackay model, Diuron will be distributed mostly to the water and soil compartments. However, Mackay level III model which takes into account degradation processes, indicates that 99% will be distributed in the water compartment when emissions are assumed to water compartment only. Assuming default emissions of the model, i.e., equal emissions to air, soil, and water, 92% of Diuron will be distributed to the soil compartment and 8% to water. The results indicate that in a closed system Diuron tends to distribute to soil and water but in a dynamic system where inflow of Diuron to the water compartment occurs Diuron is predominantly present in the water compartment. In wastewater treatment plant, ca. 5% of Diuron will be removed from water, mainly via distribution to the sludge whereas 95% will remain in the effluent, as estimated using the STPWIN model using degradation half-lives obtained from simulation tests. The reported presence of Diuron in effluents, surface waters and groundwater indicate that WWTPs are not capable of preventing the emissions to the aquatic environment and that proportions of Diuron released to the environment (which can be from WWTPs as well as from other routes of exposure) is retained in/transported into surface water and that a proportion of it ends up in groundwater. It should be noted that the Substance has also uses not belonging to the scope of the REACH regulation and the concentrations in the environment may be influenced by all uses of the Substance which cause release to the environment.

12.3. Mobility

Water solubility of 29 mg/L (20 °C, pH 6.2) was determined by flask method (OECD TG 105) for Diuron. No significant pH dependency of the water solubility was observed.

12.3.1. Soil adsorption/ desorption studies

In the available adsorption/desorption test (OECD TG 106) (Unpublished 2009) in the registration dossier the organic carbon-water partition coefficient (Koc) values ranged from 293 to 504 (log Koc 2.47-2.70) across five different investigated soils. The mean Koc value of 395 at 20 °C corresponds to log Koc value of 2.60.

Another adsorption/desorption test is available according to USEPA Pesticide Assessment Guideline, Subdiv. N, 163-1 in the registration dossier. Four different soils were studied (two sandy loams and two silty loams) with shaking time of 24 hours at 25 °C. Adsorption of Diuron on sandy loam soils were Koc 447.2 and 412.8 and on silty loam soils Koc 567.6 and 481.6. This corresponds to mean log Koc value of 2.63 for sandy loam soils and mean log Koc value of 2.72 for silty loam soils.

In the RMS Germany (2018) further adsorption/desorption tests are available for Diuron. Five different soils (sand, sandy clay loam, loam, silty clay, loam) were studied according to USEPA Pesticide Assessment Guideline, Subdiv. N, 163-1 (in line with OECD TG 106) and the Koc values were 578, 366, 1750, 410, 607, respectively. This corresponds to log Koc values from 2.56 to 3.24.

In another study according to OECD TG 106 five soils (two loamy sands, two silt loam and silty clay loam) were studied and the Koc values determined ranged from 543 to 959. This corresponds to log Koc values from 2.73 to 2.98.

One lysimeter study (Bergstroem *et al*. 1996) is available in the RMS Germany (2018). The mobility of Diuron was studied under non-steady state flow conditions in two sandy soils exposed to northern European climate conditions. The applied radioactivity in leachate at the end of the study were 0.6 % and 0.7 % in Långveka lysimeter and 0.2% and 0.6 % in Nåntuna lysimeter in low (2 kg/ha) and high (4 kg/ha) treatment rates, respectively. For lysimeters with high treatment rate Diuron was found in the leachate above 0.1 µg/L after first and second year. In the low treatment rate lysimeters, the concentrations of Diuron were below 0.1 µg/L. RMS Germany (2018) considers that the study shows there is leaching of Diuron under particular conditions.

The adsorption/desorption potential of degradation products of Diuron was not assessed by evaluating MSCA.

12.3.2. Estimated data on mobility, including read-across

KOCWIN v2.00 in EPISuite v4.10 predicts an estimated log Koc value of 2.3 based on experimental log Kow value of 2.68. Also in EPISuite, an experimental log Koc value of 2.4 is available for Diuron in the database (Meylan *et al*. 1992).

12.3.3. Summary of mobility

The evaluating MSCA agrees with the registration dossier that the results indicate that Diuron has a low potential to adsorb to soils considering the log Koc is mainly < 3, thus it could be considered as mobile.

12.4. Bioaccumulation

Information on bioaccumulation has been reviewed in 2014-2015 (i.e., during the initial SEv assessment). This assessment of bioaccumulation has been considered sufficient for the purpose to clarify the initial and additional concerns (see Section 1).

12.4.1. Aquatic bioaccumulation

Bioaccumulation potential was evaluated using Log K_{ow}, a *Mytilus edulis* bioaccumulation test (Unpublished 1993b) in the registration dossier and a non-guideline biomagnification study (Roche *et al*. 2009).

It is noted that in the risk assessment conducted under the directive concerning the placing of plant protection products on the market (91/414/EEC), no data was submitted nor required concerning bioconcentration as log PoW was <3 (EFSA 2005). It is noted, however, that information has been published indicating potential for bioaccumulation through biomagnification.

Log KoW

Log Kow for Diuron is 2.89, which is below the screening criterion for PBT assessment for bioaccumulation in aquatic organisms (4.5) and the cut-off value of the CLP regulation (>4), therefore suggesting a low potential for bioaccumulation in aquatic organisms.

Mytilus edulis bioaccumulation test

A *Mytilus edulis* bioaccumulation test (Unpublished 1993b) is available in the registration dossier. It is reported that the study was conducted according to OECD TG 305 modified for use with *Mytilus edulis.* The study was conducted according to the GLP. DMSO was used as a solvent and 0.096 ml/L DMSO was used as solvent control. Duration of exposure was 42 d and test temperature 15°C. In a range-finding/preliminary study a theoretical 96 h LC_{50} of 32 mg/L was adopted on which to base test concentrations. Test concentrations were 0.32 and 0.032 mg/L. Measured values did not deviate from nominal by more than 10%.

Glass aquaria were used as test vessels. Test vessels were aerated only during feeding periods. Flow rate of test solution was 500 L per 24 h, regulated by hydrostatic pressure. Number of organisms per vessel was 120. Test medium was seawater collected from clean source at Eastern Schelde estuary. A 16:8 h Light:dark regime was used.

The BCF after exposure to two concentrations of Diuron for 42 d was found to be 5.2 on average (range: 4.4 -6.6, based on wet weight tissue concentration of the substance). The values achieved in the low and high concentration are considered to be similar and it is assumed that the BCF in mussel tissue was concentration independent. Some mortality occurred but was deemed negligible (< 1%).

Calculated bioaccumulation data

BCF was estimated in the registration dossier using QSAR models based on correlations between BCF and log Kow. For example, the equation on page 91 of ECHA guidance R7.c (ECHA 2014) results in a BCF of 57.1:

$\log BCF = 0.85 \cdot \log Kow - 0.70$
$= 0.85 \cdot 2.89 - 0.7$
$= 1.7565$
$\Rightarrow BCF = 57.1$

Biomagnification in brackish water (Roche *et al*. 2009)

A biomagnification of Diuron as well as other organochlorines was shown in the Vaccarès Lagoon in France. Biomagnification was studied by sampling eels (four different growth stages) and a total of 17 other aquatic species. Eels were caught between 1996 and 2005 and the other species between 2001 and 2005. The studied organisms are described as follows: "*The studied organisms were zooplankton (copepods sp. + larvae), Sphaeroma sp, cokkles (Cerastoderma glaucum), mysids, gammarids (Gammarus salinus), pink shrimps (Crangon crangon), brown shrimps (Palaemonetes varians), sand smelts (Atherina boyeri), gobies (Pomatoschistus sp.), stickelbacks (Gastrosteus aculeatus), pipefish (Syngnatus acus), not to mention occasionally, common soles (Solea solea), breams (Abramis brama), pikeperch (Sander lucioperca), and juvenile mullets (Mugilidae sp). One plant of brackish and marine waters (Zostera noltii) (Helobiales), water and sedimentary organic matter (SOM) were also collected in spring 2005. The selected zooplankton, invertebrate and fish species differed in their trophic habits and ecological niches as estimated by the annual NNRC biodiversity report compiled by the French National Nature Conservancy Society (SNPN) (internal publication)*."

Lipid extraction was carried out prior to N isotopic analyses performed on the same sample. Individual data were recorded for fishes and pooled data for invertebrates. The

measurements were performed on the whole-body mass of organism with the exception of eel, sole, bream, pikeperch and common sunfish, for which only dorsal muscle was analyzed.

The trophic level was determined using $\delta^{15}N$ enrichment (Persic *et al*. 2004 as cited in Roche *et al*. 2009; Vollaire *et al*. 2007 as cited in Roche *et al*. 2009). The trophic level (TL) of consumers ranged from 2 in zooplankton to 4.39 in the yellow eels (Table 19).

The biomagnification factor between trophic guilds was calculated using the formula:

$$BMF = [(OC_{TGn}/OC_{TGn-1}/OC_{TGn-1})/(TL_{TGn}/TL_{TGn-1})]$$

Where OC TG_n and OC TG_{n-1} were the lipid-normalized concentration of organochlorines in trophic guilds.

Pesticides were extracted from muscle or whole organism lipids or from pools of individuals and purified by solid phase extraction (SPE) on florisil (MgO_3Si). The analyses of organochlorine compounds were achieved by gas chromatography using electron capture detection.

Table 19 Studied components of the Vaccarès food web (data source: Roche *et al*. 2009).

Guilds	**Species**
Producer	*Zostera* sp. Sedimentary organic matter
Consumer I	*Sphaeroma* sp. zooplankton cockles gammarids mysids
Consumer II-1	brown shrimp pink shrimp juvenile eel
Consumer II-2	pipefish mullet stickleback sand smelt goby sp. immature eel 1 common sole bream pikeperch immature eel 2
Consumer II-3	yellow eel

The results indicate that Diuron was concentrated along the food chain. A BMF value > 1 for Diuron was found in the four trophic sub-compartments (Table 20), showing according to the authors that uptake exceeds elimination. There was a statistically significant correlation between the $\delta^{15}N$ trophic level signature and Diuron concentration, although the correlation was weaker than for the other studied organochlorine pesticides (the lindane and endosulfane families, fipronil). The authors mention that BMF were <1 in consumers II-2 vs. consumers II-3 (top-consumers) for all pesticides biomagnified except Diuron (BMF=2).

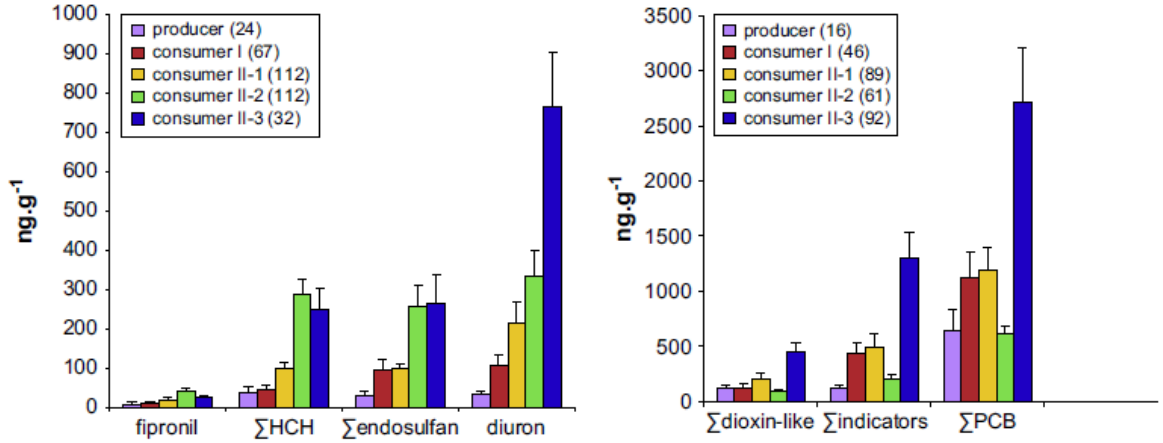


Figure 2 Global contamination profiles (on a dry weight basis) of the 5 trophic compartments of the Vaccarés Lagoon community sampled between 2001 and 2005. Reprinted from Environmental Pollution, 157, Roche, H., Vollaire, Y., Persic, A. Buet, A., Oliveira-Ribeiro, C., Coulet, E., Banas, D., Ramade, F. Organochlorines in the Vaccarés Lagoon trophic web (Biosphere Reserve of Camargue, France), Pages 2493-506, Copyright (2009), with permission from Elsevier. (https://www.sciencedirect.com/journal/environmental-pollution)

Table 20 Biomagnification factors of Diuron in biota from zooplankton to eels as a function of their trophic guilds. Number of contaminated samples was 302 (data source: Roche *et al*. 2009).

	producer vs. consumer I	**consumer I vs. consumer II-1**	**consumer II-1 vs consumer II-2**	**consumer II-2 vs consumer II-3**
Biomagnifi-cation factor	2.00	1.61	1.30	2.00

Diuron was the most abundant contaminant in the Vaccarés trophic web, notably in 2005. According to Roche *et al*. (2009) the high level of Diuron bioaccumulation in fish was in contradiction with the results of Tucker *et al*. (2003) who showed a rather low transfer to fish (channel catfish, *Ictalurus punctcatus)*, after experimental exposure consisting in nine consecutive weekly applications of Diuron at 0.01 mg/l.

According to Roche *et al*. (2009), the biomagnification of Diuron and other only slightly lipophilic molecules could be related to a retention at the lowest levels of the trophic web (sedimentary organic matter, phytoplankton). They further mention that, due to the retention, the bioavailability of such molecules for a direct bioconcentration (a passive transfer of contaminants from medium through teguments) should decrease and their potential trophic transfer should increase in the consumers.

12.4.2. Bioaccumulation in air-breathing organisms

The possibility of a substance to accumulate in air-breathing organisms instead of aquatic organisms is indicated by the combination of a log Koa > 5 with a log Kow >2 (ECHA 2023). Diuron fulfils these criteria based on a log Kow 2.89 and predicted (KOAWIN v.1.10 in EPI Suite v4.10) log Koa values from 10.37 (using log Kow and Henry's law constant from experimental database of the software) to 10.58 (using a log Kow 2.89 and Henry's law constant from experimental database of the software).

12.4.3. Summary and discussion

The registrant(s) consider in the registration dossier that the Log Kow, the BCF values in a *Mytilus edulis* bioaccumulation test (OECD TG 305C) as well as the calculated BCF values, are indicating a low potential for bioaccumulation.

However, the evaluating MSCA notes that BMF values of 1.3 - 2 have been determined in a brackish water lagoon (Roche *et al*. 2009) indicating potential for bioaccumulation. According to the guidance R.7c (ECHA 2023) a reliable field BMF or TMF value significantly higher than 1 can be considered an indication of very high bioaccumulation. In addition, the screening criteria for bioaccumulation in air-breathing organisms are fulfilled.

13. Environmental hazard assessment

The environmental hazard assessment was focussed on the information relevant to the concern of endocrine disruption.

13.1. Aquatic compartment (including sediment)

Not assessed.

The environmental hazard assessment was focussed on the information relevant to the concern of endocrine disruption. More information is available in Section 15.

13.2. Terrestrial compartment

Not assessed.

13.3. Microbiological activity in sewage treatment systems

Not assessed.

13.4. PNEC derivation and other hazard conclusions

Not assessed.

13.5. Conclusions of the environmental hazard assessment and related classification and labelling

The Substance has a harmonised aquatic environmental classification as Aquatic Acute 1, H400 and Aquatic Chronic 1, H410 with M-factor of 100 according to Commission Delegated Regulation (EU) 2024/197. The evaluating MSCA considers that the environmental hazard information assessed in this substance evaluation (i.e., the information relevant to the concern of endocrine disruption) does not warrant changes to the current harmonised classification under the hazard class “Hazardous to the aquatic environment”. Regarding the conclusions for the other environmental hazard classes, please see Sections 15.3 and 16.5.

14. Human health hazard assessment

Information on human health has been assessed in more detail in 2014-2015 (i.e., during the initial SEv assessment). Before concluding the substance evaluation, a Decision to request further information was issued according to Article 46 to clarify additional concern for environmental endocrine disruption. After the information was provided in the registration dossier update in 2018, the ED assessment for human health was updated in 2018-2019. However, no new experimental information is available in the registration dossier after the initial assessment in 2014-2015 for human health. The Section 14 has

been updated in 2024 based on the RAC opinion[7] of new harmonised classification of the Substance.

14.1. Toxicokinetics

Table 21 Overview of toxicokinetic studies

Study	Remarks	Results	Reference
Absorption, distribution, metabolism and elimination of [^{14}C] Diuron in rats Comparable to EPA OPP 85-1 (Metabolism and Pharmacokinetics) GLP SD rat, male/female 5 male and 5 female rats Administration: oral, gavage Doses: 400 mg/kg bw/day once, 10 mg/kg bw once or multiple doses of 14 x 10 mg/kg bw/day. Urine faeces and organs were examined Exposure regime: single administration or once daily for 15 days	Reliability: 2 (reliable with restrictions) Test material: ^{14}C-radiolabelled Diuron, purity 98.3 %	Diuron was rapidly absorbed and widely distributed. Oral absorption was higher than 80 %. The vast majority was excreted as metabolites within the first 24 hours post dosing with 3,4-dichlorophenyl-urea (DCPU) being the main metabolite. Only small amount was excreted in unchanged form via faeces. Tissue residues were low. No evidence of accumulation was observed. A certain affinity to red blood cells was detected.	Unpublished (1996b) In RMS Germany (2018)
[Phenyl-UL-^{14}C]-Diuron. Investigation of the biokinetic behaviour in the rat. Comparable to EPA OPP 85-1 (Metabolism and Pharmacokinetics) GLP Wistar rat, male/female 5 male and 5 female rats. (for a preliminary study 2 male and female rats; bile fistulation study 5 males rats) Administration: oral or intravenous route Doses: 5 mg/kg once i.v. 5 mg/kg or 200 mg/kg p.o (once or 15 times)	Reliability: 2 (reliable with restrictions) Test material: ^{14}C-radiolabelled Diuron, purity 99.7 %	Diuron was almost completely absorbed (> 95 %). More that 97 % of the recovered radioactivity was excreted via urine (68-87 %) and faeces (13-32 %) within 72 hours. Metabolites were not measured. The highest residues were observed in the blood or haematopoietic organs, metabolism-excretion related organs and in females in the ovaries. No accumulation potential was observed.	Unpublished (1988) In the registration dossier

[7] RAC opinion (2021): https://echa.europa.eu/documents/10162/be583259-1abf-1995-118e-937efd0ce19f

Urine, faeces and organs were examined Exposure regime: single administration or once daily for 15 days			
The concentration of Diuron and its representative metabolites in the urine of male and female rats during a subacute inhalation study over eight weeks. non guideline study non-GLP Wistar rat, male/female (5 animals/sex/ dose) Administration: Inhalation Doses: Males: 4.1, 37.4 or 268.1 mg/m³ Females: 4.1, 37.4 or 268.1 mg/m³ Exposure regime: 4 or 8 weeks (6 h/ day,5 days/week) Urine was examined	Reliability: 2 (reliable with restrictions) Test material: Diuron, purity 98.4 %	The main metabolite was 3,4-dichlorophenyl-urea (DCPU) excreted in the urine, followed by 3, 4-dichloroaniline and N`-(3,4-dichlorophenyl)-N methylurea. No significant differences in the urinary excretion after 4 or 8 weeks. No accumulation potential was observed.	Unpublished (1986c) In the registration dossier

Following oral administration of radiolabelled Diuron to rats, it was almost completely absorbed, widely distributed, extensively metabolised and rapidly eliminated. No evidence of accumulation was obtained and no significant differences between males and females were observed.

In rats, N-(3,4-dichlorophenyl)-urea (DCPU) was the main metabolite in urine after exposure to Diuron via oral and inhalation route. Smaller amount of 1-(3,4-dichlorophenyl)-3-methylurea (DCPMU) and of 3,4-dichloroaniline (DCA) are also excreted in the urine. DCPU was also identified as the main metabolite in dogs (Hodge *et al*., 1967). In humans, Diuron is assumed to be metabolised by partial or complete demethylation and hydroxylation similar to the metabolisation in experimental animals. Similar metabolites have been found in human plasma and urine (Verheij *et al*. 1989, Van Boven *et al*. 1990, Da Rocha *et al*. 2013).

Abass *et al.* (2007) determined Diuron metabolites produced *in vitro* by human liver homogenates or species specific-mammalian liver microsomes as a function of incubation time and Diuron concentrations. Diuron was rapidly metabolized via N-methylation, with 3-(3,4-dichlorophenyl)-1-methylurea (DCPMU) being the major metabolite. The *in vitro* metabolism was similar between human liver homogenates or human, rat, mouse, dog, monkey, minipig and rabbit liver microsomes. Human-specific metabolites were not observed.

Mohammad *et al.* 2018 investigated the toxicokinetics of Diuron in *ex vivo* human placental perfusion and in *in vitro* human placental microsomes and human trophoblastic cancer cells (BeWo). Diuron crossed human placenta readily in placental perfusion indicating fetal exposure if pregnant women are exposed to Diuron during pregnancy. Diuron was metabolized into DCPMU in perfused placenta and in *in vitro* incubations using microsomes from placentas of tobacco smokers. In incubations with placental microsomes from non-smokers, and in BeWo cells, metabolism to DCPMU was detected but only with the highest used Diuron concentration. Diuron metabolism was inhibited upon addition of α-naphthoflavone, a CYP1A1 inhibitor, underscoring the role of CYP1A1 in the metabolism. In conclusion, it is evident that Diuron crosses human placenta and Diuron can be metabolized in the placenta to a toxic metabolite via CYP1A1.

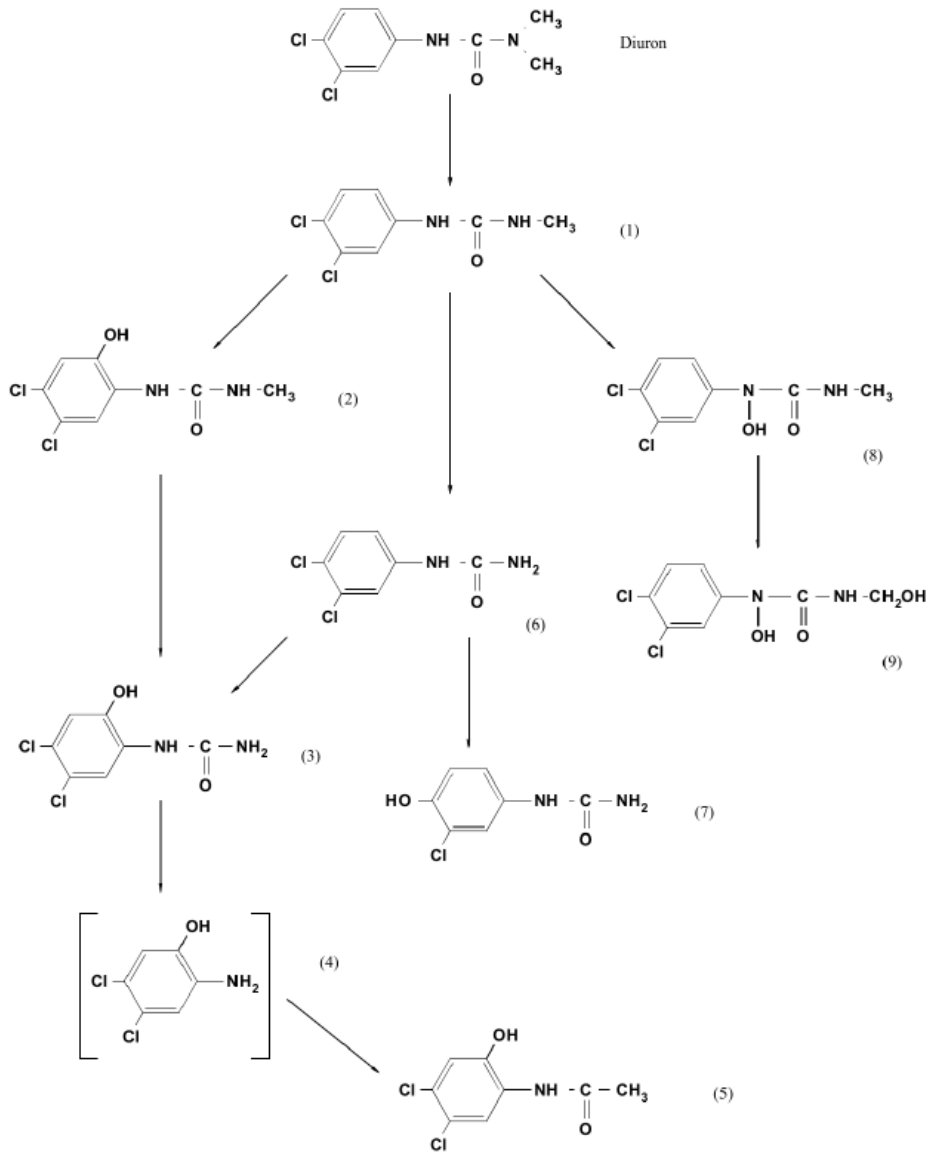


Figure 3 Proposed metabolic pathway for Diuron in the rat (RMS Germany 2018)

Table 22 ToxCast data on enzyme activity assays for Diuron

Assay	Hit Call	Analysis direction	Organism & cell origin	Scaled top	AC50	log AC50	Intended target family
TOX21_aromatase_inhibition	Inactive	Positive	Human, breast adherent MCF-7 cell line	0	1000	0	CYP
TOX21_VDR_BLA_agonist_ratio	Inactive	Positive	Human, kidney, HEK293T cell line	0	1000	0	CYP

TOX21_VDR_BLA_antagonist_ratio	Inactive	Positive	Human, kidney, HEK293T cell line	0	1000	0	CYP
NVS_ADME_rCYP3A1	Active	Positive	Rat, unspecified	2.70	5.36	0.730	CYP
NVS_ADME_rCYP2A1	Active	Positive	Rat, unspecified	4.60	0.278	-0.555	CYP
NVS_ADME_rCYP1A2	Active	Positive	Rat, unspecified	2.61	5.99	0.778	CYP
NVS_ADME_hCYP1A2	Active	Positive	Human, unspecified	3.91	1.48	0.171	CYP
CLD_CYP2B6_48hr	Active	Positive	Human primary hepatocytes	2.64	3.79	0.579	CYP
CLD_CYP1A2_48hr	Active	Positive	Human primary hepatocytes	6.25	10.5	1.02	CYP
CLD_CYP1A1_48hr	Active	Positive	Human primary hepatocytes	6.74	13.0	1.11	CYP
CLD_CYP2B6_24hr	Active	Positive	Human primary hepatocytes	3.38	1.90	0.278	CYP
CLD_CYP1A2_24hr	Active	Positive	Human primary hepatocytes	9.54	4.70	0.672	CYP
CLD_CYP1A1_24hr	Active	Positive	Human primary hepatocytes	10.1	8.01	0.903	CYP
CLD_CYP2B6_6hr	Active	Positive	Human primary hepatocytes	4.02	9.35	0.971	CYP
CLD_CYP1A2_6hr	Active	Positive	Human primary hepatocytes	4.09	2.76	0.441	CYP
CLD_CYP1A1_6hr	Active	Positive	Human primary hepatocytes	6.69	4.07	0.609	CYP
NCCT_TPO_AUR_dn	Active	Negative	Rat, thyroid gland	2.97	40.0	1.60	Oxidoreductase

From ToxCast Dashboard accessed on 5.4.2019 at https://actor.epa.gov/dashboard/

In addition to Table 22, the ToxCast assays in the database for CYPs 3A4, 2C19 and 2C9 using primary hepatocytes were inactive after 6 and 24 hours for induction and inhibition by Diuron, however the assay descriptions were lacking (U.S. EPA 2019a). Activation of rat CYPs, 3A1 and 2A1 and CYP1A2 and human CYP1A2 was measured by ToxCast assays using enzyme reporters. All of these assays were inactive with Diuron after an incubation time of 1 hour.

14.2. Acute toxicity and Corrosion/Irritation

Not assessed.

14.3. Sensitisation

Not assessed.

14.4. Repeated dose toxicity

Table 23 Overview of repeated dose toxicity studies via oral route

Study	Remarks	Results	Reference
Subchronic 90-day oral study in rats with recovery period of 90 days OECD Test Guideline 408 (Repeated Dose 90-Day Oral Toxicity in Rodents) EPA OPPTS 870.3100 (90-Day Oral Toxicity in Rodents) GLP Wistar rat, male/female (20 animals/sex/ dose) Administration: oral, feed 0, 100, 250, 2500 ppm (6.7, 17.0, 176 mg/kg bw/day/day in males; 8.7, 23.3, 214 mg/kg bw/day in females)	Reliability: 1 (reliable without restriction) Test material: Diuron, purity 99.5 %	LOAEL: 17.0 mg/kg bw/day in males and 8.7 mg/kg bw/day in females Decreased body weight, body weight gain and food consumption; haematologic effects, spleen and liver weight increased, hyperplasia of transitional epithelium in kidneys and urinary bladder (effects partly reversible) No effects on reproductive organs (weight and histopathology) Neurobehavioural test battery did not reveal indications of neurotoxicity	Unpublished (2004) In the registration dossier
Subchronic 90-day oral gavage toxicity study in rats OECD Test Guideline 408 (Repeated Dose 90-Day Oral Toxicity in Rodents), GLP Deviations (dosing only 5 days/week, no opthalmoscopy, no urinanalysis), reproductive organs were not examined. SD rat, male/female (12 animals/sex/dose) 0, 75, 250, or 500 mg/kg bw/day	Reliability: 2 (reliable with restrictions) Test material: Diuron, purity 98.5 %	Indications of toxic effects (haematological parameters, splenomaegaly, total bilirubin and blood urea nitrogen) were observed in all dose groups.	Unpublished (1996c) In RMS Germany (2018)

Study	Remarks	Results	Reference
Chronic oral toxicity study in dogs (12-month feeding study) Comparable to OECD Test Guideline 452 (Chronic Toxicity Studies) Non-guideline study GLP Beagle dog, male/female (6 animals/sex/dose) Administration: oral, feed 0, 50, 300, 1800 ppm (1.8, 11 and 64 mg/kg bw/day) Exposure regime: 12 months	Reliability: 1 (reliable without restriction) Test material: Diuron, purity 98.2-98.5 %	LOAEL: 11 mg/kg bw/day (Based on haemolytic anaemia, pigmentation of liver, kidneys and spleens and altered organ weights (liver, spleen). Relative testicle weights were significantly higher ($p<0,05$) at 1800 ppm. No corresponding histopathologic findings were observed. Increased testicle weights in the highest dose males were related to the reduced bw gain. A similar observation was made for relative ovary weights in top dose females. No corresponding histopathologic findings were observed.	Unpublished (1985a) In the registration dossier
6-month feeding study rats with examination of effects on blood Non-guideline study not GLP Wistar rat, male/female (10 animals/ sex/ dose) Administration: oral, feed 0, 4, 10, 25 ppm (nominal in diet 0.3, 0.7 and 1.6 mg/kg bw/day in males and 0.3, 0.8 and 1.8 mg/kg bw/day in females)) Exposure regime: up to 26 weeks Reproductive organs were not examined.	Reliability: 2 (reliable without restriction) Test material: Diuron, purity 98.8% Evaluating Member State considers the study is not comparable to a guideline study because clinical chemistry and urinalysis were not performed, since the study focussed on blood effects. The study had also limited determination of organ weights and histopathology. These can be considered significant deviations from the OECD guideline requirements for subchronic or chronic studies. Therefore a Klimisch score of 2 at most would apply.	LOAEL: 1.6 mg/kg bw/day in males and LOAEL: 1.8 mg/kg bw/day female in females effects to erythrocytes in males and pigment accumulation in spleens of both sexes.	Unpublished (1986a) In the registration dossier

The evaluating MSCA evaluated repeated dose toxicity endpoint only with respect to effects related to reproductive toxicity and ED properties.

There were no findings in oral repeated dose toxicity studies that would give an indication of potential ED properties of Diuron. In a one-year chronic dog study (Unpublished 1985a) statistically significant increase in relative weight of testicles was reported in top dose males but the effect was seen to be unrelated to the test substance because the corresponding bw gain was decreased and there were no histopathological findings. These

findings are discussed in the following chapter.

Increased testicle weights in a dog study (1985a, unpublished report)

In a chronic study with dogs comparable to OECD TG 452, groups of 6 beagle dogs/sex (20-27 weeks old, and weighed 6.2-9.9 kg at the beginning of the study) were given food containing Diuron at 0, 50, 300 or 1800 ppm dose daily for a period of 52 weeks, equivalent to 0, 1.8, 11 or 64 mg/kg bw/day. In the 1800 ppm group, feed consumption and body weights were decreased in both sexes towards the end of the study. At termination, gross organ weight and histopathology were examined. The mean testicle weights (absolute and relative) in the 300 ppm and 1800 ppm groups were higher than those of the control group. The difference was reported to be statistically significant for the relative testicle weight at 1800 ppm ($p<0.05$). Testicle to brain weight ratios were not reported. The variation of absolute testis weights in the 1800 group seems to be considerable large (individual absolute testis weights: 20.6, 24.7, 22.2, 14.9, 32.4, and 30.8 g). Two dogs had clearly heavier testicles than other four dogs. This seems to be due to differences in weights of dogs because two dogs were heavier at the beginning and the end of the study than other dogs. At study termination, lower body weight gains (males: 2.8 vs 3.7 kg; females: 2.6 vs 3.7 kg) and lower final body weights were observed at 1800 ppm. Histopathology did not reveal corresponding findings for the heavier testicles. These findings were reported as random findings unrelated to the test compound.

Table 24 Male dog body weight and relative testicle weight

Dose group (n=6/sex)	**0**	**50 ppm**	**300 ppm**	**1800 ppm**
Mean body weight gains difference week -1/week 52 [kg]	3.7	3.6	3.6	2.8
Mean body weight at end of study, week 52 [kg]	12.1	12.0	12.0	11.4
Absolute testicle weight [g ± std. dev.]	19.4 ± 2.77	19.1 ± 3.22	21.9 ± 4.48	**24.3 ± 6.55**
Relative testicle weight [g/kg ± std. dev].	1.620 ±0.142	1.595 ±0.340	1.820 ± 0.204	**2.110 ± 0.444***

*$p<0.05$

Effects on reproductive organs including testicles have been examined in several repeated-dose toxicity studies in rodents which were conducted according to OECD test guidelines or were comparable to them. No effects on testicles were reported in the subacute toxicity studies in rats via inhalation route (Unpublished 1986c, 1986d), 90-days repeated-dose toxicity study in rat via oral route (Unpublished 2004), or in the chronic/combined carcinogenicity studies in mice via oral route (Unpublished 1990b). In the chronic/combined carcinogenicity study in rats via oral route (Unpublished 1985b) the relative testicle weights were increased at highest dose (2500 ppm), but this effect was due to the significantly reduced body weights. No significant changes in absolute testicle weights were reported. In the two-generation reproductive toxicity study (Unpublished 1990c) relative testicle weights of P1/F1 males were increased in the highest dose group (1750 ppm), but this effect was due to the significantly reduced body weights at this dose level. No significant effects on absolute testicle weights or histopathology were reported. No effects on testicles were seen in two non-guideline studies from open literature (Fernandes *et al*. 2007, 2012) which examined effects on male reproductive system in rats.

Overall, the evaluating MSCA considers that the increased testicle weights in the dog study (Unpublished 1985a) have no toxicological significance due to the small number of animals/dose group, reduced body weight (due to the lower body weight gain) of high dose males, and because there were no corresponding histopathological findings. Furthermore, no clear effects on testicle were reported in other repeated dose toxicity studies in rodents via oral or inhalation route.

Table 25 Overview of repeated dose toxicity studies via dermal route in the registration dossier

Study	Remarks	Results	Reference
Repeated dose dermal toxicity study in rabbits US EPA F 82-2 Comparable to OECD Test Guideline 410 (Repeated Dose Dermal Toxicity: 21/28-Day Study) GLP New Zealand White rabbit, male/female (3 animals/ sex/ dose) 0, 50, 250 mg/kg bw/day (nominal concentration) Exposure: 6 hours (only 5 days per week) Exposure regime: 21 days	Reliability: 2 (reliable with restrictions (only 21 day exposure)) Test material: Diuron, purity 96.8 %	No LOAEL No significant toxic effects were observed. Reproductive organs not weighed, histopathologic examinations only for control and top dose animals which did not reveal any test substance related changes in reproductive organs.	Unpublished (1984)
Repeated dose dermal toxicity study in rats Comparable to OECD Test Guideline 411 (Subchronic dermal toxicity study: 90-day study) SD rat, male/female (12 animals/sex/group) 0, 250, 500 and 1000 mg/kg bw Exposure: 6 hours (only 5 days per week Exposure regime: 90-days	Reliability: 4 (not assignable) Test material: Diuron, purity 98.5 %	LOAEL: 250/250 mg/kg bw/day. Based on Decreased erythrocyte counts, haemoglobin and hematocrit, increased mean (red blood) cell volume (MCV) Haematological effects suggesting anemia were seen at all dose levels.	Unpublished (1996d)

There were no findings in dermal repeated dose toxicity studies that would give an indication of potential ED properties of Diuron.

Table 26 Overview of repeated dose toxicity studies via inhalation route in the registration dossier

Study	Remarks	Results	Reference
Repeated dose inhalation toxicity study in rats Comparable to OECD Test Guideline 412 (Repeated Dose Inhalation Toxicity: 28/14-Day) GLP Wistar rat, male/female (5 animals/ sex/ dose) Administration: inhalation, aerosol (nose/head only) 0, 20, 150, 1000 mg/m3 (nominal concentration) 4.1, 37.4, 268.1 mg/m3 (analytical concentration) Vehicle: 1:1 mixture of polyethylene glycol 400 and ethanol Exposure regime: 4 or 8 weeks (6 h/ day,5 days/week)	Reliability: 1 (reliable without restriction) Test material: Diuron, purity 98.4 %	LOAEC: 268.1 mg/m³ air in males. Based on adverse effects on haematologic parameters and dark, enlarged spleens LOAEC: 37.4 mg/m³ air in females. Based on adverse effects on haematologic parameters and dark, enlarged spleens Changes in haematological parameters and dark, enlarged spleens in the high dose group for both sexes after 4 weeks and 8 weeks exposure. Significantly increased levels of reticulocytes and Heinz bodies were also found in the middle dose group females, which had also dark and enlarged spleens. Decreased T_3 and T_4 values and increased thyroxin-binding capacity indicates that Diuron reduced the thyroid function. T_4 value was significantly decreased at top dose males (after 4 weeks: $p<0,01$, 8 weeks: $p<0,05$), T_3 value was significantly decreased at middle dose females ($p<0,01$) after 8 weeks, thyroxin-binding capacity was increased ($p<0,01$) at top dose females after after 4 weeks (8 weeks ($p<0,05$). No effects on the weights of thyroid or reproductive organs and no histopathologic findings.	Unpublished (1986c)
Repeated dose inhalation toxicity study in rats Comparable to OECD Test Guideline 412 (Repeated Dose Inhalation Toxicity: 28/14-Day) GLP Wistar rat, male/female (10 animals/ sex/ dose) Administration: inhalation, aerosol (nose/head only) 0, 20, 150, 1000 mg/m3	Reliability: 2 (reliable with restrictions) Test material: Diuron	LOAEC: 47.6 mg/m³ air in males and females Based on adverse effects on haematologic parameters. Enlarged and congested spleens in the middle and high dose level. Changes in haematological parameters in the middle and high dose females and in high dose males. Dark enlarged and congested spleens in middle and high dose males and females. Also increases in spleen weights were seen in both sexes in the mid and high dose levels. T_3 value was significantly decreased at top dose males	Unpublished (1986d)

Study	Remarks	Results	Reference
(nominal concentration) 0, 6.6, 47.6, 311 mg/m3 (analytical concentration) Vehicle: 1:1 mixture of polyethylene glycol 400 and ethanol Exposure regime: 21 day (6 h/ day, 5 days/ week)		($p<0,01$), T_4 value was significantly decreased at top dose males ($p<0,01$) and females ($p<0,05$), thyroxin-binding capacity was significantly ($p<0,01$) increased at middle and top dose males and females. The absolute weight of thyroid at 20 mg/m^3 females was significantly decreased ($p<0,01$) but relative thyroid weight was not changed. Decreased T_3 and T_4 values and increased thyroxin-binding capacity indicates that Diuron reduced the thyroid function at high dose males. No remarkable effects on the weights of thyroid or reproductive organs and no histopathologic findings.	

The available repeated dose toxicity studies do not give an indication of adverse effects on reproductive organs, which would indicate reproductive toxicity resulting from endocrine disrupting mode of action. In two subacute inhalation toxicity studies in rats (Unpublished 1986c and d) T_3 and T_4 hormone levels were reduced and thyroxin-binding capacity was increased in top dose animals. These findings indicate that the thyroid function activity was reduced in animals after Diuron treatment. However, there was no remarkable effects on the relative weights of thyroids and histopathology did not reveal any adverse effects on thyroids. Other findings related to potential endocrine disrupting properties of Diuron were not observed. Effects on thyroid hormone levels are discussed more detailed in the following chapter.

Effects on thyroid hormone levels

In a sub-acute inhalation study (Unpublished 1986c) comparable to OECD TG 412, Wistar rats were exposed to nominal doses of 0, 20, 150 and 1000 mg/m^3 (analytical conc. achieved: 0, 4.1, 37.4 and 268.1 mg/m³) of an aerosol of Diuron for 6 h per day, 5 days per week for 4 or 8 weeks. Clinical laboratory examinations were conducted after four or eight weeks on five rats per sex and dose. All rats survived the treatment period. Adverse effects on haematologic parameters and effects on spleen at higher dose levels were reported. In the 268.1 mg Diuron /m^3 group there were indications of decrease in activity of thyroid functioning - especially in males (Table 27).

In male rats decrease was statistically significant in the 268 mg/m^3 group for T_4 (after 4 and 8 weeks), but not for T_3 or for increase of thyroxin-binding capacity (TBK). In female rats there was statistically significant decrease of T_3 at the middle dose (150 mg/m^3) after 8 weeks. TBK was significantly increased at high dose females after 8 weeks but was lower after 4 weeks. There were no significant effects on weights of thyroid and histopathology did not reveal any adverse effect.

Table 27 Thyroid hormone levels and TBK. Values are given as 4 weeks values / 8 weeks values

Dose group [mg/m^3]	**0**	**4.1**	**37.4**	**268.1**
MALES				
Tri-iodothyronine(T_3) [nmol/l ± SD)	1.22± 0.21 / 0.91 ± 0.10	1.17 ± 0.21 / 0.92 ± 0.06	1.05 ± 0.14 /0.84 ± 0.12	0.97 ± 0.12 / 0.74 ± 0.09
Thyroxine (T_4) [nmol/l ± SD]	77 ± 6 / 52± 5	69 ± 10 / 46 ± 3	63 ± 8 / 44 ± 10	**47 ± 10**/ 36± 7***
Thyroxine binding capacity (TBK ± SD)	0.76 ± 0.03 / 0.78 ± 0.03	0.78 ± 0.03 / 0.79 ± 0.05	0.81 ± 0.03 / 0.78 ± 0.05	0.80 ± 0.03 / 0.83 ± 0.03
FEMALES				
Tri-iodothyronine(T_3) [nmol/l ± SD)	1.15 ±0.05 / 0.92 ± 0.15	1.28 ± 0.18 / 0.81 ± 0.07	1.23 ± 0.06 / **0.64 ± 0.07****	1.09 ± 0.13 / 0.72 ± 0.11
Thyroxine (T_4) [nmol/l ± SD]	50 ± 7 / 46 ± 3	54 ± 6 / 46 ± 4	54 ± 6 / 39 ± 8	42 ± 10 / 36 ± 8
Thyroxine binding capacity (TBK ± SD)	0.71 ± 0.02 / 0.80 ± 0.03	0.68 ± 0.07 / 0.84 ± 0.05	**0.63 ± 0.04**** / 0.86 ± 0.06	0.70 ± 0.06 / **0.85 ± 0.02***

* p<0.05, **p<0.01

Effects on thyroid function were observed in the presence of systemic toxicity. All male and female rats in the high dose treatment exhibited ungroomed fur in the last third of the study after each exposure and lasting till the next exposure. According to authors, all groups had low group mean weight gains not attributable to dosing but rather to treatment-induced stress due to the exposure in tubes. At the highest dose significant changes in many haematological parameters (Heinz bodies, reticulocytes, erythrocytes and haemoglobin) and dark enlarged spleens (increased absolute weight) were seen after 4 weeks and after 8 weeks exposure in males and females. Statistically significant increase in relative and absolute spleen weights were seen in males and females in the high dose group after week 4 and 8 (51 %/ 32% in males and 63 %/63 % in females at 268.1 mg/m3, respectively). In addition, significantly increased levels of reticulocytes and Heinz bodies were found also in the middle dose group females, which had also dark and enlarged spleens. Plasma protein and albumin levels were decreased in high dose males and females in the high dose group and in males in the middle dose group. An increased O-demethylation activity was pronounced in the high dose animals of both sexes especially after 8 weeks dosing indicating an enzyme induction in liver.

In a sub-acute inhalation study (Unpublished 1986d) comparable to OECD TG 412, Wistar rats were exposed (in tubes) to nominal doses of 0, 20, 150 and 1000 mg/m^3 (analytical concentrations were 0, 6.6, 47.6 and 311 mg/m³) of an aerosol of Diuron for 6 h per day, 5 days per week for 21 days. All rats survived the treatment period. Adverse effects on haematologic parameters and effects on spleen at higher dose levels were reported. Clinical laboratory examinations were conducted at the end of the study on ten rats per sex and dose. The thyroid function was affected (decreased) in high dose animals.

The statistically significant decrease in T_3 and T_4 values at 311 mg/m^3 in males, with roughly simultaneously increased TBK values at 47.6 and 311 mg/m^3, indicate decreased activity of thyroid functioning (Table 28). No clear dose-response was seen in decrease of T_3 and T_4. In females T_4 values were statistically significantly decreased at 311 mg/m^3 (no dose-response) and TBK values were increased at middle and high dose group. The absolute weight of thyroid at 20 mg/m^3 females was significantly decreased but relative

thyroid weight was not changed. Histopathology did not reveal any adverse effects on thyroid.

Table 28 Thyroid hormone levels and TBK

Dose group [mg/m3]	**Air**	**0**	**6.6**	**47.6**	**311**
MALES					
Tri-iodothyronine(T_3) [nmol/l ± SD)	1.01 ± 0.18	0.91 ± 0.13	0.92 ± 0.22	0.90 ± 0.20	**0.77 ± 0.10****
Thyroxine (T_4) [nmol/l ± SD]	63 ± 6	65 ± 7	62 ± 12	64 ± 8	**53 ± 7****
Thyroxine binding capacity (TBK) [TBI ± SD]	0.64 ± 0.07	0.68 ± 0.06	0.71 ± 0.08	**0.79 ± 0.10****	**0.84 ± 0.11****
FEMALES					
Tri-iodothyronine(T_3) [nmol/l ± SD)	0.91 ± 0.14	0.89 ± 0.17	1.04 ± 0.15	0.99 ± 0.09	0.91 ± 0.12
Thyroxine (T_4) [nmol/l ± SD]	54 ± 8	**70 ± 14****	59 ± 8	55 ± 9	**41 ± 11***
Thyroxine binding capacity (TBK) [TBI ± SD]	0.56 ± 0.05	0.61 ± 0.09	0.61 ± 0.08	**0.66 ±0.08****	**0.67 ± 0.06****

*p>0.05, **p<0.01

Effects on thyroid function were observed in the presence of systemic toxicity. All male and female rats in the high dose group exhibited piloerection after exposure. According to authors, all groups (including control) exhibited very low group mean weight gains or even weight losses which were attributed to treatment-induced stress due to the exposure in tubes but not to Diuron itself. Significantly reduced red blood cell parameters and increased reticulocytes and Heinz bodies were detected in the middle and high dose females and in high dose males. Plasma protein levels were decreased in the middle and high dose males and females. Dark enlarged and congested spleens were shown in middle and high dose males and females. Statistically significant increase in absolute and relative spleen weights were seen in males and females in the high dose group (absolute weight increased 33 % in males and 38 % in females at 311 mg/m^3, respectively). The increased O-demethylase activity pointed to an enzyme induction in the livers in high dose males and females.

In summary, the available two *in vivo* inhalation studies in rats show comparable effects on T_3, T_4 and TBC in both genders suggesting that Diuron interacts with the hypothalamus-pituitary-thyroid (HPT) axis by affecting thyroid hormone levels. There is a dose-dependent trend in the decreased thyroid hormone levels, at least in males. No historical control data from the test laboratory is available. Effects on thyroid hormone levels were observed in the presence of treatment-induced stress and marked systemic toxicity (effects on haematologic parameters and spleen).

Thyroid hormone levels were not measured in other available toxicity studies. The 90-day subchronic repeated dose toxicity study in rats and one-year chronic dog study via oral route (Unpublished 2004 and 1985a) did not reveal any adverse effects on weight of thyroid or histopathological changes. No histopathological changes were seen in pituitary. In the oral chronic toxicity/carcinogenicity studies in rats and mice (Unpublished 1985b, 1990b) no thyroid or pituitary effects were observed. In the chronic toxicity/carcinogenicity studies via oral route thyroid gland and pituitary was examined macroscopically and after

that microscopically, if abnormal or suspected of being neoplastic. Thyroid gland or pituitary were not weighed in these studies. In the two-generation reproductive toxicity study (Unpublished 1990c) thyroid glands were not weighed and examined histopathologically. No histopathological changes were seen in pituitary.

14.5. Mutagenicity

Not assessed.

14.6. Carcinogenicity

Table 29 Overview of combined chronic toxicity /carcinogenicity studies in the registration dossier

Study	Remarks	Results	Reference
Wistar rat: male/female (50 animals/ sex/ dose) Administration: oral (feed) 0, 25, 250 and 2500 ppm (nominal in diet) 0, 1.0, 10 and 111 mg/kg bw/day in males; 0, 1.7, 17 and 203 mg/kg bw/day in females (nominal concentration) Exposure: 12 months (interim sacrifice) 24 months (terminal sacrifice) EPA 83-1 OECD TG 453 (Combined Chronic Toxicity /Carcinogenicity Studies)	Reliability: 1 (reliable without restriction) Test material: Diuron	NOAEL (toxicity) not established LOAEL (toxicity) 1mg/kg bw/day in males, based on marginally increased heamosiderin deposits in spleen and 1.7 mg/kg bw in females based on anaemia and increased spleen weight Decreased body weights, bodyweight gain and food consumption at 111/203 mg/kg bw/day (males/females) NOAEL (carcinogenicity): 25 ppm (1.0/1.7 mg/kg bw/day in males/females) based on preneoplastic urinary bladder lesions from 17 mg/kg bw/day onwards in females and statistically significantly increased incidences of transitional epithelial carcinoma of urinary bladder in both sexes at 2500 ppm. The incidence of uterine adenocarcinoma was doubled at 203 mg/kg bw/day compared to controls. No treatment related findings regarding the mammary glands at any dose level. No remarkable histological findings or differences in weights of endocrine related organs	Unpublished (1985b)
NMRI mouse: male/female Chronic toxicity and carcinogenicity study Administration: oral (feed)	Reliability: 1 (reliable without restriction) Test material: Diuron	NOAEL (toxicity): 250 ppm (50.8/77.5 mg/kg bw/day in males/females) based on effects on red blood cells and increased liver weights and histopathological findings, reduced body weight and body weight gain	Unpublished (1990b)

Study	Remarks	Results	Reference
0, 25, 250 and 2500 ppm (nominal in diet) corresponding to 5.4, 50.8, 640.1 mg/kg bw/day in males and 7.5, 77.5, 867 mg/kg bw/day in females. Exposure: 24 months, interim sacrifice after 12 months (daily (continuous feeding)) OECD TG 453 (Combined Chronic Toxicity / Carcinogenicity Studies)		NOAEL (carcinogenicity): 250 ppm (77.5 mg/kg bw/day), based on statistically significantly increased incidence of adenocarcinomas in mammary gland and ovarian luteomas in females at 867 mg/kg bw/day	

The evaluating MSCA evaluated carcinogenicity endpoint only with respect to effects related to reproductive toxicity and ED properties. The published Renewal Assessment report for Diuron under PPP Regulation has been used as a basis of this evaluation (RMS Germany 2018).

Carcinogenic properties of Diuron have been recently assessed by the Risk Assessment Committee (RAC). The RAC opinion[8] concluded that there is sufficient evidence of carcinogenicity based on the clear increase in urinary tract malignant tumours in male and female rats. The presumed mechanism of action is cytotoxicity which leads to regenerative hyperplasia and subsequently to tumours. The threshold level is not known. There is also sufficient evidence of carcinogenicity in mice based on the statistically significant increase in mammary gland tumours. No increase in this tumour type was observed in rats. The increase in the incidence of malignant uterus tumours in rats and benign ovarian tumours in mice provide supportive evidence for classification. It could not be excluded that the tumours observed in uterus, mammary gland and ovary were endocrine-mediated, but no data are available to substantiate this hypothesis. RAC proposed a classification of Carc. Cat 1B for Diuron.

The registration dossier includes two chronic toxicity and carcinogenicity guideline studies with Diuron, one in a rat and one in a mouse (Unpublished 1985b, 1990b). In these studies, no effects on histology or on the weights of ED related organs were reported. Ovaries were not weighed in these studies. In the rat carcinogenicity study the incidence of transitional epithelial carcinoma of urinal bladder was statistically significantly increased in high dose (2500 ppm) males and females compared to controls (incidences 2%, 67% and 0%, 22%, in control males vs. high dose males and control females vs. high dose females, respectively). In addition to this tumor type for which mode of action is presumably not endocrine mediated, the incidence of uterine adenocarcinoma was doubled in high dose (2500 ppm) females compared to control group (incidences 10%, 10%, 10% and 20% in 0, 25, 250 and 2500 ppm). The incidence in high dose females was in the upper edge of the historical control incidence of the performing laboratory by far exceeding the mean incidence (range 2-20%, mean approximately 8 %). According to RMS Germany (2018) there was no information on statistical significance of this finding in the study report. It was also noted that sarcoma of the uterus endometrium was reported in two high dose females compared to zero incidences in other groups and squamous epithelial carcinoma of uterus occurred only in the two upper dose groups although only one animal per group was affected. According to RMS Germany (2018) the maximal tolerated dose was exceeded

[8] RAC opinion (2021) [04.01-ML-014.03] (europa.eu)

in high dose females in this study (21% decrease in body weight gain compared to controls) rendering toxicological significance of the uterine findings uncertain.

Due to uterine findings in rat, special morphometric measurements of uterus were conducted in the mice carcinogenicity study. An increased number of high dose mice had uterine horn lumen diameters greater than 2 mm but there were no differences in incidences of endometrial hyperplasia or in degree of its severity between the dose groups. No other differences in uterus morphometry or remarkable differences in uterine tumor incidences between dose groups were observed. However, statistically significantly increased incidences of mammary gland adenocarcinomas and benign luteomas of ovaries in high dose (2500 ppm) female mice compared to controls were reported in this study (incidences of adenocarcinoma 5.1%, 3.1%, 2.3% and 15.4% and luteomas 6.7%, 2.7%, 4.4% and 15.9% at 0, 25, 250 and 2500 ppm, respectively). According to RMS Germany (2018) these incidences are over the published historical control incidences in NMRI mice strain.

Tumors of uterus, mammary gland and ovaries may have endocrine-mediated mode of action (e.g. estrogenicity). No mechanistic information on uterine tumors have been provided in the registration dossier, RMS Germany (2018) or in published literature. Few published studies have addressed mammary gland adenocarcinoma. The studies by Grassi *et al*. (2011a,b) and De Moura *et al*. (2009) did not find evidence of a promoting potential of Diuron for mammary gland tumors in two-stage carcinogenesis model initiated with 7,12 -dimethylbenz(a)anthracene (DMBA) in female SD rats and Swiss mice. The evaluating MSCA notes that these studies were conducted with SD rat and a different mouse strain than used in the mouse carcinogenicity study of registration dossier (NMRI), and thus no firm conclusions on tumorigeneses in NMRI mice can be drawn. Regarding ovarian luteoma Grassi *et al*. (2011a) reported that dietary treatment of pregnant and nursing SD rats with 1250 ppm Diuron caused reductions in ovary weights and treatment with 750 ppm and 1250 ppm in the number of corpora lutea, of their female pups (see Section 14.7). However, the toxicological relevance of this finding is unclear.

In conclusion, in addition to the clear increase in the urinary tract tumours seen in rats, a slightly increased incidence of uterine adenocarcinomas in rat and statistically significantly increased incidences of mammary gland adenocarcinoma and ovarian luteomas in mice were reported in carcinogenicity studies with Diuron. The evaluating MSCA notes that the increased incidences of these tumours were observed in high dose animals only after two years Diuron administration. No remarkable differences were reported in uterine tumour incidences in mice or in mammary gland tumour incidences in rat and mechanistic studies failed to demonstrate promoting potential of Diuron for mammary gland tumours. No findings suggesting mode of action possibly related to these tumour types (e.g. estrogenicity) were reported in any of the available in vivo animal studies with Diuron.

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

14.7.1. Effects on fertility

Table 30 Overview of experimental studies on fertility in the registration dossier

Study	Remarks	Results	Reference
Two-generation reproductive toxicity study in rats According to EPA OPP 83-4 (Reproduction and Fertility Effects) Comparable to OECD TG 416 (Two-Generation Reproduction Toxicity Study) GLP Study was conducted before the OECD had updated the test guideline in 2001. Deviations: effects on spermatogenesis, on oestrus cycle and on the ovaries (corpora lutea, follicles) and the developmental of sexual maturation in the pups were not investigated. Organ weights of non-reproductive organs (brain, liver, kidneys, spleen, thyroid and adrenals) were not determined in the adults. As well as brain, spleen and thymus weights in the pups. Only testis weights are given. Water consumption was not measured. Crl:CD BR rat (30 animals/group) Administration: oral, feed 0, 10, 250 and 1750 ppm	Reliability: 1 (reliable without restriction) Test material: Diuron, purity 97.1 %	No impact on fertility or reproductive performance NOAEL (reproduction): 1750 ppm NOAEL (P): 14.8 mg/kg bw/day (male) based on reduced body weight, body weight gain, and food consumption in parental rats in the 1750 ppm dose groups.) NOAEL (P): 18.5 mg/kg bw/day (female) based on reduced body weight, body weight gain, and food consumption and on spleen enlargement and congestion NOAEL (F1/2): 18.9 mg/kg bw/day (male) based on reduced body weight in the F1/F2 pups in the 1750 ppm dose groups NOAEL (F1/2): 22.1 mg/kg bw/day (female) based on reduced body weight in the F1/F2 pups in the 1750 ppm dose groups	Unpublished (1990c)

14.7.2. Developmental toxicity

Table 31 Overview of experimental studies on developmental toxicity in the registration dossier

Study	Remarks	Results	Reference
Developmental toxicity study in rats Crl:SD BR rat (25 animals/group) Comparable to OECD TG 414 (Prenatal Developmental Toxicity Study) Study was conducted before the OECD had updated the test guideline in 2001. GLP Administration: oral, gavage 0, 16, 80 and 400 mg/kg bw/day (nominal concentration) Exposure regime: day 6 - 15 of gestation (once daily)	Reliability: 1 (reliable without restriction) Test material: Diuron, purity 99.0 %	LOAEL (maternal toxicity): 80 mg/kg bw/day based on decreased body weight and food consumption NOAEL (embryotoxicity): 80 mg/kg bw/day based on: test mat. LOAEL (embryotoxicity): 400 mg/kg bw/day based on reduced foetal body weight and delayed foetal ossification) Higher resorption rate was observed at 400 mg/kg bw/day.	Unpublished (1986a)
Developmental toxicity study in rabbits Comparable to OECD TG 414 (Prenatal Developmental Toxicity Study) Study was conducted before the OECD had updated the test guideline in 2001. New Zealand White rabbit (23-25 animals/group) Administration: oral, gavage 0, 2, 10, and 50 mg/kg bw/day Exposure regime: days 7 to 19 post-mating (once per day)	Reliability: 1 (reliable without restriction) Test material: Diuron, purity 99.0 %	LOAEL (maternal toxicity): 50 mg/kg bw/day based on decreased body weight gain and food consumption NOAEL (maternal toxicity): 10 mg/kg bw/day based on NOAEL (embryotoxicity): 50 mg/kg bw/day	Unpublished (1986b)

Table 32 Reproductive, fertility and offspring effects (RMS Germany (2018))

Concentration (ppm)	0 (Controls)	10	250	1750
P_1 Generation				
Mating index (%)	93.3 (28/30)	100.0 (30/30)	96.7 (29/30)	100.0 (30/30)
Fertility index (%)	78.6 (22/28)	83.3 (25/39)	79.3 (23/29)	96.7$^+$ (29/30)
Gestation length (days)	22.9	22.6	22.8	22.6
% of litters with at least one live pup	100	96	100	100
Lactation index	100	99.5	100	99.1
F_1 Generation				
Mating index (%)	96.7 (29/30)	86.7 (26/30)	93.3 (28/30)	93.3 (28/30)
Fertility index (%)	89.7 (26/29)	76.9 (20/26)	82.1 (23/28)	85.7 (24/28)
Gestation length (days)	22.6	22.7	22.1$^+$	22.5
% of litter with at least one live pup	100	100	100	100
Lactation index	97.6	99.4	100	100

$^+$Statistically different from control ($\alpha = 0.05$)

Pup weights (males and females) were decreased in both generations in the 1750 ppm dose group. Maternal weights were also decreased at this dietary level of Diuron. There were no compound-related gross observations seen in the offspring of either generation from either pups sacrificed by design or found dead.

Table 33 Mean pup weights (g), sexes not distinguished (RMS Germany (2018))

Concentration (ppm)	0 (Controls)	10	250	1750
F_1 Generation				
Day 0	7.0	7.0	7.0	6.6$^+$
Day 4 (post culling)	11.9	12.4	11.4	10.1$^+$
Day 21 (weaning)	58.9	61.3	57.9	48.4$^+$
F_2 Generation				
Day 0	6.7	7.0	6.9	6.5
Day 4 (post culling)	11.1	11.9	11.4	10.3
Day 21 (weaning)	62.7	64.7	61.1	51.1$^+$

$^+$Statistically different from control ($\alpha = 0.05$)

Under the conditions of this study, the NOAEL is 250 ppm (equals 14.8 to 22.1 mg/kg bw/day) for both adults and their offspring. The NOAEL for parental toxicity is based on reductions in body weight, body weight gain, food consumption and on spleen enlargement and congestion in adult females at the highest dose level. The NOAEL for offspring is based on lower birth weight and smaller pup weight gain in the 1750 ppm dose groups.

The study was conducted before the OECD had updated test guidelines 416 in 2001. No effects on spermatogenesis (semen quality: sperm numbers, morphology and motility), changes in oestrus cyclicity, effects on ovaries (corpora lutea, follicles), endpoints on

sexual maturation in the pups (age at vaginal opening and balano-preputial separation), changes in anogenital distance, or weight of other reproductive organs than testis were investigated (i.e. uterus, ovaries, epididymides, prostate, seminal vesicles) in P and F1 parental generations. Organ weights of non-reproductive organs (brain, liver, kidneys, spleen, thyroid and adrenals) were not determined as well as brain, spleen and thymus weights in the pups. Histopathology was conducted only for control and high dose P and F1 parental groups, however, thyroid and adrenals were not examined. Due to lack of many parameters, the study is acceptable only to assess fertility, reproductive performance and success. The study is not suitable to provide sufficient information to prove or exclude a potential for endocrine disruption.

Table 34 Overview of experimental studies on fertility provided in the published literature

Study	Remarks	Results	Reference
Reproductive toxicity in adult male rats non guideline study non-GLP study Wistar rat, male/female 18 rats/group Male 90 days old, female: 60 days old Administration: oral, gavage 0, 125 or 250 mg/kg bw/day Vehicle: corn oil (technical degree) Experiment 1 (9-10 rats/group, exposure time 30 days): body weight, reproductive organ weights, liver and kidney weights, measurement of Diuron concentrations in liver and kidney, plasma testosterone measurements, sperm counts and transit time. Experiment 2 (9-10 rats /group mated, dams killed on day 20 of gestation): sexual behavior, fertility and pregnancy outcome, histopathology and sperm morphology.	Test material: Diuron, (purity not given)	No consistent and dose-related effects on the body weight, sexual behavior, fertility and pregnancy outcome, reproductive organ weights, plasma testosterone concentrations, or sperm parameters were seen. No morphological alterations were reported in testes, epididymides or kidneys. Diuron residues were detected in all liver samples but only occasionally in the kidneys.	Fernandes *et al*. 2007
Developmental toxicity during prenatal life until peripubertal age in male rat Non-guideline study (assessment of reproductive/development	Test material: Diuron, Purity 99.0 %	Significantly reduced body weight gain (1 male/litter weighted) in male offspring at 750 ppm at PND 10, 21, and 42 ($p < 0.05$) compared to the control group. Reduction in body weight remained until PND 90.	Fernandes *et al*. 2012

al endpoints in the F1 generation followed the OECD TG 443 test guideline) non-GLP study SD rat, male/female 12 rats/group Administration: oral, feed Vehicle: Nuvilab CR-1 feed 0, 500, 750 ppm (nominal conc.) 8 wk-old female rats mated to 12-wk-old male rats. Exposure between GD 12 until the end of the lactation period PND 21. The male offspring: Exposure through diet until PND 42 (peripubertal age). At PND 42 and PND 90 adult male rats were killed.		Significant rise in relative testis weight at 750 ppm compared to control group and 500 ppm at PND 42 and 90. Probably due to reduced body weights. No significant change in absolute weights. No histopathological changes observed. No statistically significant changes in daily sperm production (testis), sperm morphology and motility (vas deferens) at PND 90. No significant difference in plasma testosterone levels among groups at PND 90	

Reproductive effects in male rats exposed to Diuron (Fernandes *et al*. 2007)

The Fernandes *et al*. (2007) study in adult Wistar rats was targeted to detect reproductive effects in adult male rats (groups of 18 young adults) after repeated dosing by gavage of Diuron at 0, 125 or 250 mg/kg bw/day for 30 days. During the study, the animals were monitored for mortality and clinical signs.

Nine or ten of the treated males were killed on the day of termination. The following parameters were investigated: body weight, reproductive organ weights (right testis, epididymis and vas deferens, ventral prostate and seminal vesicle), liver, and kidney weights; measurement of Diuron concentrations in liver and kidney; measurement of plasma testosterone and measurement of sperm parameters (daily sperm production per testis; sperm number and sperm transit time through epididymides).

The remaining males were mated with untreated adult females. Dams were killed on day 20 of presumed gestation and examined. The following parameters were examined: sexual behavior, fertility and pregnancy outcome after natural mating (*Corpora lutea*, implantations, resorptions and live fetuses were counted and weighed); testicular, epididymidal, kidney and liver histopathology and sperm morphology.

No significant differences in body weights occurred among the groups during the experimental period, but terminal body weight of animals from the high-dose group were reduced by approximately 5% from the control value. There were no significant differences between the treated and control groups in absolute or relative weights of the reproductive organs. However, when the two dose levels (125 and 250 mg/kg bw/day) were compared, the relative testis weight was lower at middle dose compared to high dose group and the absolute weights of the prostate and seminal vesicle were statistically significantly higher compared to high dose group.

Testosterone concentrations were increased at middle dose level (125 mg/kg bw/day), but this was not statistically significant increase (111.76±17.6, 146.63±19.5 and 115.0±24.6 ng/dL, mean ± SEM at 0, 125 and 250 mg/kg bw/day, respectively). Slight dose-related (not statistically significant) increase occurred in latencies to first ejaculation and first post-

ejaculatory intromission. In addition, latencies to first mount and first intromission were apparently higher at 125 mg/kg bw /day compared to controls and high dose group. Daily sperm production (testis and epididymides) was decreased at 125 mg/kg bw/day (30.10±0.73; 26.58±1.26; 27.98±0.74 x10^6/testis per day, mean±SEM at 0, 125 and 250 mg/kg bw/day, respectively), but this was not a statistically significant change and no clear dose-response was evident. In relation to fertility and reproductive performance, statistically significant reductions were observed in maternal weight, weight of uterus with fetuses and number of fetuses in the litters in untreated females mated with middle dose males (125 mg/kg bw/day). These effects were not confirmed in the high dose group.

Overall, there were no consistent and dose-related effects on the body weight, sexual behavior, fertility and pregnancy outcome, reproductive organ weights, or plasma testosterone concentrations. No significant effects on number of *corpora lutea* were observed. No effects on sperm counts in the testis and epididymides were observed. No significant alterations on sperm morphology (vas deferens) were seen. Morphology assessments of sperm (vas deferens) indicated the percentages of both abnormal and normal sperm were similar among all groups. No morphological alterations were reported in testes, epididymides or kidneys. Diuron residues were detected in all liver samples but only occasionally in the kidneys.

Summary of reproductive toxicity

Effects on fertility

The registration dossier contains a two-generation reproductive toxicity study (OECD TG 416, Unpublished 1990c) and two prenatal developmental toxicity studies (OECD TG 414, Unpublished 1986a & 1986b). The evaluating MSCA considered some additional studies from published literature relevant for the evaluation of effects on reproductive system.

The published renewal Assessment report for Diuron under the PPP Regulation has also been used as a source of this evaluation (RMS Germany (2018)).

The main findings from the studies can be summarised as follows:

Two-generation reproductive toxicity study (Unpublished 1990c)

In the two-generation reproductive toxicity study four groups of Crl:CD BR rat (30 males and 30 females per group) received Diuron at 0, 10, 250 or 1750 ppm in the diet for 73 days prior to mating.

No compound-related clinical signs or mortality were detected in the P/F1 generations. Final body weights, weight gains and overall food consumption of P/F1 males and females in the 1750 ppm groups were significantly lower than their respective controls. There were no significant changes compared to the control in body weight, food consumption, body weight gains and food efficiencies at dosage groups up to 250 ppm in P or F1 males.

There were no significant findings in histopathology of examined reproductive organs. The relative testis weights in P1 and F1 adult males in the 1750 ppm groups were increased, but this effect was due to the reduced body weights. No significant effects on absolute testicle weights or histopathology were reported. An increased incidence of enlarged spleens with microscopic evidence of congestions was observed in high dose P and F1 generation females. The enlarged spleens may be due to incomplete exsanguination at necropsy, however, enlarged spleen is a commonly noted sign following Diuron-treatment.

There were no compound related effects apparent in the mating index, fertility index, or gestation length in either the P or F1 parental generations. There were no compound-related effects on litter size or survival in either the F1 or F2 generation pups.

Developmental toxicity

The prenatal developmental toxicity studies in rat and rabbit (Unpublished 1986a, 1986b)

Rat

In the prenatal developmental toxicity study in Crl: COBS (SD) BR strain rat the dosing period (at 0, 16, 80, and 400 mg/kg bw/day) covers prenatal period (GD 6-15) of the development. On day 20 of presumed gestation, the female rats were sacrificed. The liver of each rat was weighed. The uterus was examined for pregnancy, number and placement of implantations, early and late resorptions and live and dead fetuses. Corpora lutea present in each ovary were counted. Each fetus was weighed and examined for external alteration. All fetuses were examined for skeletal alteration and one-half examined for visceral alteration.

Dosing 80 mg/kg/d and 400 mg/kg/d caused significantly reduced feed consumption and loss of body weight during the period of treatment. The NOAEL for maternal toxic effects is determined to be 16 mg/kg bw/day. Fetuses average body weights were significantly decreased at the high dosage group litters (400 mg/kg/d). In addition, significant skeletal alterations and delayed ossifications (vertebrae and sternum (central centres)) were observed for the high dosage group litters, as compared with control values. No visceral alterations were recorded. Higher resorption rate was observed at the highest dose level. No significant effects on number of corpora lutea were observed. The NOAEL of embryotoxic/ teratogenic effects is 80 mg/kg bw/day.

Rabbit

In the prenatal developmental toxicity study in white rabbits (unpublished report, 1986b) the dosing period (0, 2, 10, and 50 mg/kg bw/day) covers prenatal period (GD 7-19) of the development. A statistically significant decreasing average daily food consumption and body weight gain was observed for the top dose on days 13 to 20, resulting in significant weight loss to day 20. No significant effects on number of corpora lutea were observed. The NOAEL of maternal toxic effects is set 10 mg/kg bw/day. It appears questionable whether the highest dose level represents the MTD since maternal effects were weak. In the range-finding study, doses 250 and 350 mg/kg bw/day were too toxic. Diuron did not adversely affect the development of the offspring at the top dose. The NOAEL of embryotoxic / teratogenic effects is 50 mg/kg bw/day.

Under the conditions of these studies, the NOAEL for maternal animals was 10 mg/kg bw/day in rabbits and 16 mg/kg bw/day in rats, while the NOAEL for offspring was 80 mg/kg bw/day in rats. In rabbits, up to and including the highest dose tested, no effects were observed. The NOAEL used for chemical safety assessment by the registrant is $NOAEL_{oral}$: 80 mg/kg bw/day.

Effects of Diuron on male rat reproductive organs: a developmental and postnatal study (Fernandes *et al*. 2012)

The Fernandes *et al*. (2012) study in Sprague-Dawley (SD) rats was targeted to detect effects during androgen-dependent period of male sexual differentiation. Pregnant rats received a diet containing Diuron at 0, 500, 750 ppm (750 ppm ~ 67 mg/kg bw/day) from gestational day 12 (GD 12) until the end of lactation period (postnatal day 21). Males were further exposed to Diuron through diet until PND 42 (peripubertal age). The male exposure period covered prenatal period from the latter period of gestation (starting from GD 12) and postnatal development of offspring (until PND 42). At PND 42 and PND 90 (48 days after cessation of treatment) adult male rats were euthanised. According to authors, assessment of reproductive/developmental endpoints in the F1 generation followed the OECD 443 test guideline (Extended one generation reproductive toxicity study). However, only two dose levels were evaluated, and raw data is not available.

Assessment for effects on reproductive organs (weights of testis, epididymides, vas deferens, ventral prostate, and seminal vesicle; histopathology of testis and epididymides), sperm parameters (daily sperm production, sperm number, transit time in the

epididymides, sperm motility and morphology) and plasma testosterone at PND 90 was performed in one male per litter at both time points. In addition, organ weights of liver, kidney, and spleen were measured, and histopathology was performed at PND 42.

Up to 750 ppm there were no significant maternal effects. Dosing with 750 ppm caused significantly reduced body weight gain in male offspring (at PND 10, 21, and 42) and reduced body weight remained until sacrifice in PND 90.

At PND 90 no significant differences were seen in absolute or relative weights of ventral prostate, epididymides, vas deferens, full seminal vesicle, or empty seminal vesicle. There was a statistically significant increase in relative testis weights at 750 ppm (PND 90) probably due to the reduced body weights. No significant changes were detected in absolute testis weights. The morphology of testis and epididymides were not affected. There were no remarkable findings in liver or kidneys. A statistically significant increase in relative spleen weight was observed at 750 ppm. In addition, congestion of red pulp and reduction of white pulp were noted.

At PND 90 no effects on daily sperm production, motility or morphology were seen. Number of normal spermatozoids in vas deferens was slightly reduced in high dose offspring (number of normal spermatozoids: 177.58 ± 4.39, 176.36 ± 6.02 and 171.50 ± 4.77, mean ±SEM at 0, 500 and 750 ppm, respectively) but this was not a statistically significant change. At PND 90 no significant difference in plasma testosterone levels were detected at any dose level up to 750 ppm.

Assessment of female reproductive endpoints in Sprague-Dawley rats developmentally exposed to Diuron: potential ovary toxicity (Grassi *et al*. 2011a)

In the Grassi *et al*. study (2011a) it was evaluated whether early-life stage exposure alters onset or susceptibility to mammary carcinogenesis in female SD rats. Pregnant rats (12 per group) received in diet Diuron (purity is not given) at 500, 750 (~65 mg/kg bw/day) and 1250 ppm (~87 mg/kg bw/day), from gestational day 12 to the end of lactation (postnatal day 21). After weaning, female offspring continued receiving diet containing Diuron. AT PND 51, female offspring received a single dose of 50 mg/kg bw/day 7,12-dimethylbenz(a)antrachene (DMBA) for initiation of mammary carcinogenesis. The animals were sacrificed (PND 51, 75, and 226 to 233) for mammary gland morphology, reproductive organs and tumour analysis, respectively.

Investigation of reproductive organs (weight and histopathology of ovaries and uterus, ovarian follicles and corpora lutea on PND 75), oestrus cycle (PND 60 and 75), hormone concentrations (serum oestrogen and progesterone at PND 51, FSH and LH on PND 75) and sexual development (vaginal opening between PND 30-42 by daily) of female pups up to PND 75 were examined.

Serum oestrogen and progesterone measurements were carried out on 10 rats per group belonging at least 5 different litters. Oestrus cycle analysis was performed in 10-14 female offspring per group. Ovaries and the uterus were examined from mid, high and control female pups (1/litter, 9 per group). Ovarian follicles and corpora lutea were counted in six young females per group (from six different litters). FSH and LH were measured in 10 females per group, presumably in the same oestrus phase.

In pregnant dams, food consumption and body weight gain were reduced at the highest dose. Body weights of female pups were statistically significantly reduced at all dose levels compared to controls (at 500 ppm on PND 21, at 750 ppm on PND10 and 21 and at 1250 ppm on PND10, 21 and 51). Reproductive parameters including those for sexual maturation were not significantly altered i.e. oestrus cycle and vaginal opening. However, in female pups, significantly lower mean ovary weight at high dose (1250 ppm) and a statistically significantly reduced mean number of corpora lutea at intermediate (750 ppm) and high doses were observed on PND 75 (mean number of 32.67 ± 2.42, 18.80 ± 2.85* and 19.60 ±2.69* at control, 750 ppm and 1250 ppm, respectively). There were no differences in hormone concentrations among the treated and controls groups. No significant differences were seen among groups on mammary morphology or carcinogenesis.

Developmental exposure to Diuron causes spleen toxicity in male Sprague-Dawley rat pups (Domingues *et al*. 2012)

Domingues *et al*, (2012) investigated maternal toxicity in SD rats (12 pregnant dams/group) and effects on the lymphatic and haematopoietic system of male pups following dietary exposure at 500 (~49 mg/kg bw/day), 750 (65 mg/kg bw/day) and 1250 ppm (~87 mg/kg bw/day) during late gestation (GD 12-21) and lactation. Purity of the test material is not given. The statistically significant adverse effects on food consumption, body weight and body weight gain in the dams were seen at the highest dose level. In male pups (49-65 male pups/group), reduction of body weight, increased spleen weight, increased extramedullary haematopoiesis, congestion of red pulp, depletion of lymphoid follicles and a lower percentage of CD45RA-positive B cells was observed at the highest dose level. Effect to CD4+ B-lymphocytes is assumed to reflect spleen toxicity seen in the study.

14.7.3. Discussion on reproductive toxicity

The available reproductive toxicity studies provided in the registration dossier do not indicate clear effects that would give an indication of reproductive toxicity resulting from endocrine disrupting mode of action. Reduced pup weights seen (at 1750 ppm) in the two-generation reproductive toxicity and reduced fetus weights (at 400 mg/kg bw) and delayed ossification (vertebrae and sternum at 400 mg/kg bw) seen in the prenatal developmental toxicity study in rats indicate growth retardation, but clear conclusion on mechanistic background of these effects is not possible.

The available repeated dose toxicity or chronic toxicity/carcinogenicity studies in the registration dossier do not give an indication of effects on reproductive organs that would indicate reproductive toxicity resulting from endocrine disrupting mode of action. Increased relative testicle weights at the top dose males were reported in one chronic dog study via oral route (Unpublished 1985a), but the effect was considered unrelated to Diuron treatment due to reduced body weights of high dose animals and because there were no corresponding histopathologic findings.

The evaluating MSCA notes that the provided two-generation reproductive toxicity study (1990c) and prenatal developmental toxicity studies (Unpublished 1986a and 1986b) were conducted before the OECD had updated test guidelines 414 and 416 in 2001. The original OECD test guideline 416 (from 1986) and OECD test guideline 414 (from 1981) were revised to include additional endpoints for the evaluation of effects on male and female fertility and developmental toxicity. Several of these new endpoints are particularly sensitive also with respect to endocrine disruption. Thus, two-generation study conducted before the inclusion of sensitive endocrine endpoints by itself may not be considered adequate for demonstrating the probable absence of endocrine disrupting activity although it still provides valuable data mainly restricted to fertility and effects on reproductive organs (OECD, 2018a).

When compared to the updated test guidelines, the provided studies appear to have shortcomings with respect to evaluation of the concern for potential endocrine disrupting properties on the reproductive system. No effects on spermatogenesis (semen quality: sperm numbers, morphology and motility), changes in oestrus cyclicity, effects on ovaries (corpora lutea, follicles), endpoints on sexual maturation in the pups (age at vaginal opening and balano-preputial separation), changes in anogenital distance, or weight of other reproductive organs than testis were investigated (i.e. uterus, ovaries, epididymides, prostate, seminal vesicles, thyroid, adrenals). Histopathology was conducted only for control and high dose groups (thyroid and adrenals were not examined). Due to lack of many parameters, the evaluating MSCA considers that the study is acceptable only to assess fertility, reproductive performance and success. The study is not suitable to provide sufficient information to prove or exclude a potential for endocrine disruption.

In the prenatal developmental toxicity studies the dosing period (gestation day 6 -15 in rat and gestation day 7-19 in rabbits) does not cover the latter period of the gestation

which is a susceptible period for the developing reproductive system (e.g. sex organs). Consequently, effects will not be discovered if exposure is stopped earlier. In the updated OECD test guideline 414 the dosing period has been extended to cover at least the period from implantation (e.g., day 5 post mating) to scheduled caesarian section (day before birth, gestation day 21 in the rat). In studies where dosing is started before implantation, preimplantation loss may be assessed more reliably. In addition, the current test guideline includes more endpoints in respect of fetal development that may detect endocrine disruption (e.g. abnormalities of male and female genitalia).

The evaluating MSCA considers that there is uncertainty whether the reproductive toxicity studies provided in the registration dossier have the ability to detect possible endocrine disruption and all effects on reproduction (adverse effects on fertility and development) because the OECD test guidelines applied do not include all endpoints of the current OECD test guidelines. On the other hand, the evaluating MSCA considers some of the studies from open literature (i.e. Fernandes *et al*. 2007, 2012; Grassi *et al*. 2011a) relevant for the evaluation of effects on male and female reproductive system relevant for reducing these uncertainties and data gaps.

In the Fernandes *et al*. studies (2007, 2012) reproductive parameters in adult males and male offspring were not significantly altered i.e., no clear effects on reproductive organs (weight and morphology), sperm parameters (daily sperm production, sperm number, transit time in the epididymides, sperm motility and morphology) or plasma testosterone were detected. According to Fernandes *et al*. (2012), assessment of reproductive/developmental endpoints in the F1 generation followed the OECD 443 test guideline.

In the Grassi *et al*. study (2011a) reproductive parameters in female offspring were not significantly altered including those for sexual maturation (i.e oestrus cyclicity or vaginal opening) and hormone concentrations (serum oestrogen, progesterone, FSH or LH). However, in female pups, significantly lower mean ovary weight at high dose (1250 ppm) and significantly reduced mean numbers of corpora lutea at intermediate (750 ppm) and high doses were observed. This could indicate ovarian toxicity of Diuron. However, body weights of female pups were statistically significantly reduced at all dose levels (500 ppm, 750 ppm and 1250 ppm) in this study suggesting that Diuron exposure may have generally interfered with growth and development of the offspring. Therefore, the evaluating MSCA concludes that the toxicological relevance of ovarian findings in the study by Grassi *et al*. (2011a) is not clear. The evaluating MSCA further notes that in the two-generation reproductive toxicity study Diuron had no effect on fertility or reproductive performance up to highest dose (1750 ppm) and no remarkable findings were reported in the histopathology of ovaries in P1 and F1 dams.

No significant effect on number of corpora lutea was observed in two guideline prenatal developmental toxicity studies in adult rats (up to 400 mg/kg bw) and rabbits, or in the Fernandes *et al*. (2007) study (up to 250 mg/kg bw/day) in rats. In the two-generation reproductive toxicity study and in two prenatal developmental toxicity studies pup weights were decreased significantly at the highest dose levels. However, maternal weights were also significantly decreased at these dose levels.

Overall, on balance, the evaluating MSCA considers that available repeated dose toxicity, chronic toxicity/carcinogenicity studies and data from open literature are relevant for reducing the uncertainties related to the missing endpoints associated to endocrine disruption in the reproductive toxicity studies.

Final conclusion – Reproductive toxicity and endocrine disruption

Evaluating Member State concludes that the available data presently do not support classification for toxicity to reproduction and endocrine disruption of human health.

14.8. Hazard assessment of physicochemical properties

Not relevant for this evaluation.

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

Table 35 Critical DNELS/DMELS

Endpoint of concern	Type of effect	Critical study(ies)	Corrected dose descriptor(s) (e.g., NOAEL, NOAEC)	DNEL/ DMEL	Justification/ Remarks
Workers - Hazard via inhalation route	Systemic effects	Unpublished (1986c)	NOAEC: 2.00 mg/m^3 (based on AF of 12)	0.17 mg/m^3	The long-term inhalation DNEL is based on a reliable sub-acute study (eight weeks exposure) in rats comparable to OECD TG 412. In this study, the NOAEC was 4.1 mg/m³ for female rats based on no evident systemic toxicity.
Workers - Hazard via dermal route	Systemic effects	Unpublished (1984)	NOAEL: 250.20 mg/kg bw/day (based on AF of 43.3)	5.79 mg/kg bw/day	The long term dermal DNEL is based on a reliable 21-days dermal repeated dose toxicity study in rabbits equivalent or similar to OECD TG 410.

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

The conclusions relevant for the ED assessment of human health are summarised in Section 15.3. Diuron has a harmonised classification as Carc 1B, H350 and STOT RE 2, H373 (blood system) according to Commission Delegated Regulation (EU) 2024/197. The evaluating MSCA considers that the human health hazard information assessed in this substance evaluation (i.e., the information relevant to the concern of endocrine disruption) does not warrant changes to the current harmonised classification under the human health hazard classes.

15. Endocrine disrupting (ED) properties assessment

The evaluation was focused on endocrine disrupting properties of Diuron (both human health and environment). The relevant information of *in vivo* studies on human health (Section 14) are presented in the previous sections in detail and are also considered in this Section for the ED assessment. The relevant *in vivo* studies on environmental ED assessment are presented in this section.

In addition to the available information of ED effects of Diuron, relevant information on the ED effects of the structurally similar substance Linuron was also evaluated. The comparison of the properties of Diuron and Linuron and the conclusions on the structural similarity are described in detail in Section 8.

Linuron (3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea) (CAS 330-55-2) belongs to the same group of phenylurea herbicides as Diuron and they are regarded as structural analogues sharing also similar transformation products (Badawi *et al*. 2009). Linuron is regarded as a known antiandrogen (Category 1) in the ED priority list by EU COM (European Commission 2000).

Linuron, structurally similar substance, has a harmonized classification as carcinogenic category 2 and as toxic to reproduction category 1B in the Annex VI of CLP Regulation (Regulation (EC) 1272/2008). The approval of Linuron for use in plant protection products under the PPP Regulation (Regulation (EC) No 1107/2009) was not renewed in 2017 because Linuron is classified as toxic for reproduction category 1B and negligible exposure could not be demonstrated. Furthermore, Linuron was considered to have endocrine disrupting properties at that time in accordance with interim criteria for endocrine disrupters.

15.1. Endocrine disruption – Environment

In addition to the available information of ED effects of Diuron, relevant information on the ED effects of the structurally similar substance Linuron was also evaluated. *In vitro* activity information is also relevant for the ED human health assessment.

15.1.1. *In vitro* activity

(Anti)androgenicity and (anti)estrogenicity

Bauer *et al*. (1998) showed that Diuron has ability to bind and displace [^{3}H]dihydrotestosterone (^{3}H-DHT) from bovine androgen receptor (AR) in a radioreceptor assay with calf uterus cytosol. Linuron, a structurally similar compound to Diuron, has also affinity to AR. Relative binding affinities (RBA) of Diuron (0.000024) and Linuron (0.0001) to bovine AR are much lower compared to an endogenous AR ligand DHT (RBA = 1.0). Based on these results (experiments repeated only twice), Linuron has about 4 times higher affinity to AR than Diuron in this test system. In a recombinant AR competitive binding assay, Fang *et al*. (2003) showed also that Linuron binds to AR. The RBA for Linuron was 0.0056 compared to synthetic androgen, R118. Linuron competed with ^{3}H-testosterone for binding to rat AR in ventrate prostate cytosol. In this study, the IC_{50} for Linuron was 64 µM (Cook *et al*. 1993). This is higher than the IC_{50} values for DHT (1.4 nM) and flutamide (18 µM). The effect of Diuron (1, 3, 10 and 30 µM) on AR transactivation was tested in a luciferase reporter assay in CHO cells transfected to express human AR and luciferase reporter (Vinggaard *et al*. 2008). Diuron inhibited the AR transactivation by R1181. The concentration of Diuron showing 25% inhibition of 0.1 nM R1181-induced activity (IC_{25}) was within a range of 0.3 - 1 µM. The IC_{25} value for Linuron was between 1 - 3 µM. Thus, Diuron is more potent inhibitor of AR than Linuron in this *in vitro* transactivation assay (Vinggaard *et al*. 2008).

Kojima *et al*. (2004) studied the effects of Diuron on human AR in transactivation assay using Chinese hamster ovary (CHO) cells expressing these receptors and reporter gene constructs. They showed that Diuron has antiandrogenic potential. Diuron inhibited 5α-

dihydrotestosterone (DHT)-induced transcriptional activity of human AR. The RIC_{20} value for Diuron was 8.7 µM, i.e. this concentration caused 20% inhibition of androgenic activity by 0.1 nM DHT.

Orton *et al*. (2009) have tested Diuron for endocrine disrupting potential *in vitro*. A recombinant yeast androgen screen (YAS) and yeast estrogen screen (YES) were used to detect agonistic or antagonistic effects on AR and ER (estrogen receptors). In this assay, Diuron (initial concentration range tested: 0.01 - 1000 µM) and other tested pesticides did not have androgenic or estrogenic activity. The antagonistic effect was tested by coincubation of Diuron or other tested pesticides with AR agonist (2.5 nM testosterone) or ER agonist (0.25 nM 17β-estradiol). In YAS and YES assays, Diuron had both antiandrogenic and antiestrogenic activity. Linuron caused similar effects. In transactivation assay using CHO cells expressing human ERα and ERβ, Kojima *et al*. (2004) showed that Diuron neither transactivates these receptors nor inhibits estradiol-induced estrogenic activity.

In human MCF-7 breast adenocarcinoma cells (E3 clone), which can be used to study ER-dependent cell proliferation, neither Diuron (0.001, 0.1, 1 and 10 µM) nor Linuron had any effects on cell proliferation during exposure for up to 9 days (Vinggaard *et al*. 1999). The average increase in cell proliferation induced by 17β-estradiol (0.1 nM), was 3.6-fold and 7.7-fold at day 6 and day 9 compared to control cells, respectively. This indicates the functionality of the proliferation assay. Vinggaard *et al*. (1999) studied also the effects of Diuron on the activation of ER in yeast cells stably transfected to express human ERα and β-galactosidase as a reporter. In this yeast estrogen screen assay, Diuron or Linuron did not cause activation of ER. Both of these compounds were cytotoxic to yeast cells at concentrations between 30 - 63 µM (Vinggaard *et al*. 1999). In another yeast-based assay, Noguerol *et al*. (2006) showed that Diuron is able to interact with ER. However, this interaction appears to be very weak as measured by ER-mediated activation of β-galactosidase reporter. The Effective Concentration (EC) for Diuron was > 200 mg/L (> 850 µM).

In E-MORPH screening assay observing the inhibition of ER signaling in an MCF-7 breast cancer cell line, Diuron was determined as ER active (Klutzny *et al*. 2022). The EC_{50} value was 6.04 µM and the ER bioactivity compared to 7-α-Ethinylestradiol (1.00) was 0.59. Diuron also showed a weak estrogenic expression profile when the mRNA expression levels of the ERα target genes *BCL2L1*, *TFF1*, *PGR*, and *AREG* along with *ESR1* and *CDH1* were compared to expression profile of E2. In a 'pBIND-ERαvector assay', which allows the identification of substances that directly bind to the ERα ligand binding domain, Diuron also showed weak effects at 10 µM. Linuron was inactive in E-MORPH screening assay. All of the 15 ToxCast assays for estrogen receptor activity included in the U.S. EPA (2019b) EDSP21 Dashboard were inactive (15 out of 18 assays used for the Estrogen Receptor Model). The EDSP21 assay findings support the conclusions of the *in vitro* data that Diuron does not possess estrogen receptor mediated endocrine activity.

Diuron induced a reduction in AR receptor binding in an EDSP21 Dashboard assay measuring recombinantly expressed chimpanzee AR protein in the radioligand binding NVS_NR_CAR assay (U.S. EPA, 2019b). Exposure to Diuron for 72 hours resulted in a 5 % decrease in signal in AR receptor ligand binding compared to DMSO control. All assays included in the database measuring agonistic AR activity were inactive. Orton *et al*. (2009) have studied also the effects of Diuron (6.25 and 62.5 µM) on ovulatory response and ovarian production of estradiol, testosterone and progesterone in *Xenopus* oocytes. Diuron (62.5 µM for 20 h) decreased testosterone levels and ovulation. Linuron has similar decreasing effect on ovulation (statistically nonsignificant) and it increased progesterone levels but did not have any effects on testosterone levels. Neither of these compounds affected estradiol levels (Orton *et al*. 2009).

Jolly *et al*. (2009) have studied the (anti)androgenic impacts of four substances (one pharmaceutical, Flutamide, and three environmental contaminants, Fenitrothion, Vinclozolin and Linuron) using *in vitro* assays in the three-spined stickleback. In the *in vitro* assay spiggin production in primed female stickleback kidney cell cultures was studied as an endpoint after 48 h exposure to a range of concentrations of the test compounds alone

and together with 3 µg/L dihydrotestosterone (DHT). The exposure to the compounds alone showed no androgen agonistic activity for any of them. However, all the compounds significantly inhibited DHT-induced spiggin production in a concentration-dependent manner so that Linuron inhibited the production at concentrations of 25 ng/L and higher. In the *in vivo* test the female sticklebacks were exposed to the test compounds (6 concentrations of each) together with DHT (5 µg/L) for 21 days. Linuron caused a significant decrease in DHT-induced spiggin production at concentrations of 100 µg/L and 250 µg/L when tested *in vivo*.

These *in vitro* findings indicate that Diuron may have antiandrogenic activity, but the interaction with estrogen receptor is weaker.

Thyroid hormone mediated activity

Table 36 US EPA EDSP21 database assays on thyroid hormone receptor activity of Diuron

Assay	Hit Call	Analysis direction	Organism & cell origin	Intended target family
ATG_THRa1_TRANS_up	Inactive	Positive	human liver hepatoma cell line HepG2 (variant HG19)	Thyroid receptor
Tox21_TR_LUC_GH3_Agonist	Inactive	Positive	GH3 Rat pituitary tumour cells	Thyroid receptor
Tox21_TR_LUC_GH3_Antagonist	Inactive	Positive	GH3 Rat pituitary tumour cells	Thyroid receptor

From US EPA EDSP21 Dashboard, Diuron 330-54-1. Retrieved from https://actor.epa.gov/edsp21/ on 24.4.2019.

The three assays described in Table 36 studied promotion of thyroid receptor mediated DNA transcription, agonism and antagonism of the thyroid receptor signalling pathway by TRE activation and inhibition in response to treatment with Diuron (U.S. EPA, 2019b). All three assays were inactive with Diuron.

Diuron did not inhibit human iodothyronine deiodinase enzymes (i.e., deiodinase type 1, deiodinase type 2, and deiodinase type 3) at 200 µM in the three in vitro assays (Olker *et al*., 2019).

Diuron induced a downregulation in rat thyroid tissue derived thyroid peroxidase catalytic activity by 50% at 40 µM compared to DMSO in the ToxCast assay NCCT_TPO_AUR_dn (U.S. EPA, 2019b). The result of this screening assay (Table 22) suggests the potential of Diuron to inhibit TPO.

Linuron, a structurally similar compound to Diuron, induced also a downregulation in rat thyroid tissue derived thyroid peroxidase (TPO) catalytic activity in the ToxCast assay NCCT_TPO_AUR_dn.

Aryl hydrocarbon-mediated activity

Diuron has been shown to interact with aryl hydrocarbon receptor (AhR) *in vitro* (Noguerol *et al*. 2006, Zhao *et al*. 2006, Takeuchi *et al*. 2008 and Zhou *et al*. 2021). In yeast AhR assay, Noguerol *et al*. (2006) showed that Diuron has significant interaction with human

AhR. This is indicated by the calculated effective concentration. The EC_{50} for Diuron was 0.26 ± 0.10 mg/l (1.1 ± 0.4 µM). This value is close to that of a positive control (β-naphthoflavone), i.e., 0.14 ± 0.10 mg/l (0.6 ± 0.4 µM). Zhao *et al* (2006) showed that Diuron induced AhR-dependent reporter gene expression (luciferase) in recombinant rat (H4L1.1c4), mouse (H1L1.1c2), human (HG2L6.1c3) hepatoma cells, and in guinea pig intestinal adenocarcinoma (G16L1.1c8) cells. The clearest effect was observed in rat cells exposed for 4 h to Diuron. In these cells, the maximum Diuron-induced AhR-dependent reporter gene induction was greater than 90% of that induced by the well-known and most potent AhR ligand 2,3,7,8-tetrachlorodibenzo-p-dioxin (1 nM TCDD). However, the EC_{50} for Diuron-induced induction was relatively high (~ 8 µM) compared to that caused by TCDD (EC_{50} 0.12 nM) indicating much lower potency of Diuron. In the other studied cell lines, the maximum Diuron-induced AhR-mediated expression of luciferase was only 20-30% of that induced by 1 nM TCDD. In the same study, Zhao *et al*. (2006) showed that Diuron (2 µM, exposure time 3.5 h) increases the expression of CYP1A1 mRNA, an endogenous AhR-responsive gene, in mouse hepatoma Hepa1c1c7 cells. Abass *et al*. (2012) studied the ability of Diuron to activate the human pregnane X receptor (PXR) and mouse and human constitutive androstane receptor (CAR). In this assay CYP1A2, which in addition to CYP1A1 is regulated by AhR (Zhou, SF. *et al*., 2009), was strongly up-regulated by Diuron in HepaRG cells *in vitro*. Diuron-induced increase in CYP1A1 and CYP1A2 mRNA expression has been shown also *in vivo* in mice (Takeuchi *et al*. 2008). In this study, C57BL/6 mice were intraperitoneally exposed to Diuron (30, 100, and 300 mg/kg) for 14 h. The hepatic expression of CYP1A1 mRNA was increased by the highest Diuron dose and CYP1A2 mRNA by the two highest doses. Linuron had similar effects (Takeuchi *et al*. 2008).

Zhao *et al*. (2006) showed also by a gel retardation analysis that Diuron is able to stimulate AhR transformation and DNA binding in guinea pig hepatic cytosol and in intact Hepa1c1ct cells. Diuron-induced activation of AhR has also been shown in DR-EcoScreen cells, which are mouse hepatoma Hepa1c1c7 cells stably transfected with an AhR-mediated reporter gene (luciferase) construct (Takeuchi *et al*. 2008). In this study, Diuron showed AhR agonistic activity. The relative Diuron-induced luciferase activity was about 80% of maximal activity induced by 0.1 nM TCDD. The potency of Diuron is clearly weaker than that of TCDD. The REC_{50} (Relative Effective Concentration) for Diuron was 2.9 µM, i.e. the concentration showing 50% of the agonistic activity of 0.1 nM TCDD. Similar effects were caused by Linuron (Takeuchi *et al*. 2008).

Diuron did not exhibit AhR agonistic activity in the recombinant HepG2 cells, but a significant increase in fluorescence intensity was observed in transgenic (*cyp1a-12DRE:EGFP*) zebrafish at 4.29 µM (Zhu *et al*. 2021).

Diuron, DCPMU, DCPU, and 3,4-DCA was correctly docked in the ligand binding pocket of AhR. These compounds showed high affinity activity for AhR (Bao *et al*. 2022). The affinity value of Diuron, DCPMU, DCPU, and 3,4-DCA was -8.1, -7.8, -7.4, and -6.2 kcal/mol, respectively. The AhR agonist activity in vivo from high to low was DCPMU, DCPU, 3,4-DCA and Diuron.

The ability of Diuron to induce AhR-dependent effects in various *in vitro* assays suggest that it is potential AhR agonist. Environmental AhR agonists have been linked to ED-related effects (for a review, see Hotchkiss *et al*. 2008).

Table 37 Overview of Diuron-induced receptor effects related to ED mode of action.

***In vitro* assay**	**Receptor**	**Effect**	**Reference**
Radioreceptor assay with calf uterus cytosol	AR	Binding to AR	Bauer *et al* 1998
AR transactivation in Chinese hamster ovary (CHO) cells	AR	Antagonist	Kojima *et al*. 2004
AR transactivation in CHO cells	AR	Antagonist	Vinggaard *et al*. 2008

Yeast androgen screen	AR	Antagonist	Orton *et al*. 2009
Yeast estrogen screen	ER	No effect	Vinggaard *et al*. 1999
ER-dependent proliferation in human MCF-7 breast adenocarcinoma cells (E3 clone)	ER	No effect	Vinggaard *et al*. 1999
ER transactivation in CHO cells	ER	No effect	Kojima *et al*. 2004
Estrogen yeast assay	ER	Agonist (weak)	Noguerol *et al*. 2006
Yeast estrogen screen	ER	Antagonist	Orton *et al*. 2009
E-MORPH screening assay	ER	Agonist	Klutzny *et al*. 2022
ERα reporter gene assay	ER	Binding to ER (weak)	Klutzny *et al*. 2022
Yeast AhR assay	AhR	Agonist	Noguerol *et al*. 2006
AhR-mediated activity in recombinant cells (mouse, rat, guinea pig and human)	AhR	Agonist	Zhao *et al*. 2006
AhR-mediated activity in DR-EcoScreen cells	AhR	Agonist	Takeuchi *et al*. 2008
AhR mediated activity in Tg (*cyp1a-12DRE:EGFP*) transgenic zebrafish	AhR	Agonist	Zhu *et al*. 2021
Luciferase reporter assay (recombinant *HepG2-cyp1a1-12DRE-luciferase* cells)	AhR	No effect	Zhu *et al*. 2021

Enzymes involved in the synthesis and metabolism of sex hormones

Diuron (50 µM) or Linuron (50 µM) did not affect CYP19 aromatase activity, measured with ^3H_2O assay using tritiated androstendione as a substrate, in human placental microsomes (Vinggaard *et al*. 2000). In a similar aromatase activity assay, Diuron (concentration range 10 nM - 100 µM) did not have any effect on aromatase activity in rainbow trout brain and ovarian microsomes (Hinfray *et al*. 2006). According to study by Lo *et al*. (2007) Diuron has no effects on the activity of 5α-reductase, an enzyme needed in the synthesis of DHT, in human prostate homogenates and in human LNCaP prostate carcinoma cells. Linuron inhibited the activity of this enzyme but only at relatively high concentrations (IC_{50} ≥ 24 µM) and only in the prostate homogenates (Lo *et al*. 2007).

Thibaut and Porte (2004) studied the effect of Diuron on the activity of various enzymes involved in synthesis and metabolism of sex hormones. Androstenedione testicular metabolism was studied by incubating ^{3}H-androstenedione (0.1 µM) and Diuron (100 µM) with carp testicular microsomes. HPLC analysis revealed that androstenedione is metabolized in this test system to three metabolites: testosterone, 5α-androstane-3,17-dione and 5α-dihydrotestosterone. Diuron did not have any statistically significant effect on the formation of these metabolites suggesting that it does not affect the activity of 17β-hydroxysteroid dehydrogenase and 5α-reductase. Diuron (1 mM) did not have any statistically significant effect on the activities of testosterone UDP-glucuronosyltransferase (T-UGT) and estradiol UDP- glucuronosyltransferase (E_2-UGT) in microsomal fraction of carp liver.

Based on these few published *in vitro* studies, it seems that Diuron does not affect the activity of aromatase (CYP19) or 5α-reductase.

Upregulation and downregulation of the hormones involved in the steroidogenic pathway have been screened and included in the ToxCast database (U.S. EPA, 2019a). A human-derived, steroidogenically-competent adenocarcinoma cell line was used in all assays listed in Table 38. Four of the ToxCast assays indicate a downregulation of hormone synthesis. Namely, a decrease was observed in signals of testosterone, androstenedione, 11-deoxycortisol and 17α-hydroxyprogesterone as compared to DMSO control. The results may suggest a disturbance in steroidogenesis giving rise to decreased production of these hormones in the treated test system. Although not fit for purpose for stand-alone assessment or direct extrapolation to effects at organism-level, the assays provide a useful insight into any potential endocrine modes of action mediated via the steroidogenesis pathway. The findings of the ToxCast assays support *in vitro* findings of potential antiandrogenicity of Diuron.

Table 38 ToxCast data of steroidogenesis pathway activity assays with Diuron

Assay	Hit Call	Analysis direction	Organism & cell origin	Scaled top	AC50	log AC50	Intended target family
CEETOX_H295R_TESTO_dn	Active	Negative	Human, adrenal gland	1.77	29.8	1.47	Steroid hormone
CEETOX_H295R_ANDR_dn	Active	Negative	Human, adrenal gland	1.70	32.0	1.51	Steroid hormone
CEETOX_H295R_OHPROG_dn	Active	Negative	Human, adrenal gland	2.66	36.3	1.56	Steroid hormone
CEETOX_H295R_11DCORT_dn	Active	Negative	Human, adrenal gland	1.34	27.7	1.44	Steroid hormone
CEETOX_H295R_TESTO_up	Inactive	Positive	Human, adrenal gland	0	1000	0	Steroid hormone
CEETOX_H295R_PROG_up	Inactive	Positive	Human, adrenal gland	0	1000	0	Steroid hormone
CEETOX_H295R_PROG_dn	Inactive	Negative	Human, adrenal gland	0	1000	0	Steroid hormone
CEETOX_H295R_ESTRONE_up	Inactive	Positive	Human, adrenal gland	0	1000	0	Steroid hormone
CEETOX_H295R_STRONE_dn	Inactive	Negative	Human, adrenal gland	0	1000	0	Steroid hormone
CEETOX_H295R_ESTRADIOL_up	Inactive	Positive	Human, adrenal gland	0	1000	0	Steroid hormone
CEETOX_H295R_ESTRADIOL_dn	Inactive	Negative	Human, adrenal	0	1000	0	Steroid hormone

			gland				
CEETOX_H295R_DOC_up	Inactive	Positive	Human, adrenal gland	0.877	37.1	37.1	Steroid hormone
CEETOX_H295R_DOC_dn	Inactive	Negative	Human, adrenal gland	0	1000	0	Steroid hormone
CEETOX_H295R_CORTISOL_up	Inactive	Positive	Human, adrenal gland	0	1000	0	Steroid hormone
CEETOX_H295R_CORTISOL_dn	Inactive	Negative	Human, adrenal gland	0	1000	0	Steroid hormone
CEETOX_H295R_ANDR_up	Inactive	Positive	Human, adrenal gland	0	1000	0	Steroid hormone
CEETOX_H295R_OHPROG_up	Inactive	Positive	Human, adrenal gland	0	1000	0	Steroid hormone
CEETOX_H295R_OHPREG_up	Inactive	Positive	Human, adrenal gland	0	1000	0	Steroid hormone
CEETOX_H295R_OHPREG_dn	Inactive	Negative	Human, adrenal gland	0.761	10.3	1.01	Steroid hormone
CEETOX_H295R_11DCORT_up	Inactive	Positive	Human, adrenal gland	0	1000	0	Steroid hormone

From ToxCast Dashboard accessed on 5.4.2019 at https://actor.epa.gov/dashboard/
negative- a negative fitting direction measured by a loss of signal compared to DMSO baseline
Positive- a positive fitting direction measured by an increased signal compared to DMSO baseline
AC50- concentration in µM at 50% of maximum activity

In vitro activity of transformation products of Diuron

There is *in vitro* evidence that transformation products of Diuron may bind to the androgen receptor and replace testosterone. These transformation products are formed by metabolism of microorganisms or animals and include 1-(3,4-dichlorophenyl)-3-methylurea (DCPMU), 3,4-dichlorophenylurea (DCPU), 3,4-dichloroaniline (3,4-DCA), and 3,4-dichloroacetanilide (3,4-DCAA). DCPU, 3,4-DCA, and 3,4-DCAA were reported to bind to the bovine androgen receptor (Bauer *et al*. 1998). DCPMU and 3,4-DCA were reported to bind to the rat androgen receptor but for DCPU no binding was observed (Cook *et al*. 1993). In the human adrenal H295R cell line, 3,4-DCA showed a significant dose-dependent decrease of testosterone concentrations along with an increase in E2/T ratio (Bhuiyan *et al*. 2019). E2 levels were not changed after 3,4-DCA exposure. The *cyp*17 gene was significantly down-regulated and the *cyp*19A gene was significantly up-regulated by 3,4-DCA in the H295R cells. In H295R steroidogenesis assay (OECD TG 456) 3,4-DCA affected steroid hormone production similar to Linuron increasing pregnenolone, progesterone, the corticosteroids and the estrogens (estrone and estradiol) levels and decreasing androgen (dehydroepiandrosterone, androstenedione and testosterone) levels. (Ma *et al*. 2023). 3,4-DCA showed also AR antagonistic activity (IC50 12.3 µM) and higher efficacy for AR antagonism than Linuron (91 %) in AR EcoScreen assay (Ma *et al*. 2023).

The binding of Diuron and its transformation products/metabolites to the androgen receptor suggest a possible endocrine mode of action. See for further information on the transformation products in Section 12.1.5.

Estimated data

ToxCast bioactivity models for androgen and estrogen receptors are negative for Diuron. Linuron shows androgen antagonism in COMPARA model (antagonist score 1 and binding 1) and in ToxCast pathway model (Antagonist score 0.300). ToxCAST models are negative for transformation products DCPU and 3,4-DCA but positive for androgen antagonism for DCPMU in COMPARA (antagonist score 1).

Diuron shows positivity within prediction domain in Androgen Receptor Inhibition (Human *in vitro*), Androgen Receptor Binding, CoMPARA data (in vitro), Androgen Receptor Inhibition, CoMPARA data (in vitro), TPO inhibition, Sodium/iodide symporter (NIS), Peroxisome Proliferator-Activated Receptor gamma (PPAR-γ) Inhibition, Arylhydrocarbon Receptor (AhR) Activation and Constitutive Androstane Receptor (CAR) Activation in Danish QSAR Database.

Linuron shows positivity within prediction domain in Androgen Receptor Inhibition (Human in vitro), Androgen Receptor Binding, CoMPARA data (in vitro), Androgen Receptor Inhibition, CoMPARA data (in vitro), TPO inhibition, Sodium/iodide symporter (NIS), Thyroid Receptor β Binding (Human in vitro), Peroxisome Proliferator-Activated Receptor gamma (PPAR-γ) Inhibition, Arylhydrocarbon Receptor (AhR) Activation and Constitutive Androstane Receptor (CAR) Activation in Danish QSAR Database.

3,4-DCA shows positivity within prediction domain in TPO inhibition and Constitutive Androstane Receptor (CAR) Activation and outside prediction domain in Androgen Receptor Inhibition (Human in vitro), Androgen Receptor Inhibition, CoMPARA data (in vitro) and Arylhydrocarbon Receptor (AhR) Activation in Danish QSAR Database.

3,4-DCAA shows positivity within prediction domain in Androgen Receptor Inhibition (Human in vitro), Androgen Receptor Binding, CoMPARA data (in vitro), Androgen Receptor Inhibition, CoMPARA data (in vitro), TPO inhibition, Sodium/iodide symporter (NIS), Arylhydrocarbon Receptor (AhR) Activation and Constitutive Androstane Receptor (CAR) Activation and outside prediction domain in Peroxisome Proliferator-Activated Receptor gamma (PPAR-γ) Inhibition in Danish QSAR Database.

DCPMU shows positivity within prediction domain in TPO inhibition, Sodium/iodide symporter (NIS), Arylhydrocarbon Receptor (AhR) Activation and Constitutive Androstane Receptor (CAR) Activation and outside prediction domain in Androgen Receptor Inhibition (Human in vitro), Androgen Receptor Binding, CoMPARA data (in vitro), Androgen Receptor Inhibition, CoMPARA data (in vitro)and in Peroxisome Proliferator-Activated Receptor gamma (PPAR-γ) Inhibition in Danish QSAR Database.

DCPU shows positivity within prediction domain in Androgen Receptor Inhibition (Human in vitro), domain in TPO inhibition, Peroxisome Proliferator-Activated Receptor gamma (PPAR-γ) Inhibition, Arylhydrocarbon Receptor (AhR) Activation and Constitutive Androstane Receptor (CAR) Activation and outside prediction domain in Androgen Receptor Binding, CoMPARA data (in vitro), Androgen Receptor Inhibition, CoMPARA data (in vitro)and Sodium/iodide symporter (NIS) in Danish QSAR Database.

15.1.2. In vivo studies on fish

Information on ED related in vivo effects of Diuron, as well as on Linuron (antiandrogenicity) are summarised in the table and text below.

Table 39 In vivo effects on fish.

Study	Remarks	Results	Reference
21 d *in vivo* test with three-spined stickleback (*Gasterosteus aculeatus*) (AFSS)	Non-guideline study Reliability: 2 (reliable with restrictions) Test material: Linuron	Linuron was antiandrogenic in the exposure concentrations of 15 µg/L and 150 µg/L in water.	Katsiadaki *et al*. 2006
21 d *in vivo* test with three-spined stickleback (*Gasterosteus aculeatus*) (AFSS) - independent and combined effects with four potential antiandrogenic substances, including Linuron	Non-guideline study Reliability: 2 (reliable with restrictions) Test material: Linuron (and vinclozolin, fenithrotion, flutamide)	Linuron was antiandrogenic and the least potent of the four substances with IC_{50} of 172 µg/L. The androgen receptor antagonists acted in in an additive fashion in fish.	Pottinger *et al*. 2013
21 d *in vivo* test with three-spined stickleback (*Gasterosteus aculeatus*) (AFSS)	Reliability: 2 (reliable with restrictions) Experimental result Test material: Linuron (and vinclozolin, fenithrotion, flutamide	Linuron caused a significant decrease in DHT-induced spiggin production at concentrations of 100 µg/L and 250 µg/L.	Jolly *et al*. 2009
21 d *in vivo* test with fathead minnow (*Pimephales promelas*) (FSTRA)	Reliability: 2 (reliable with restrictions) Test material: Linuron	The plasma vitellogenin concentrations were significantly reduced in females exposed to linuron at 1 µg/L and 100 µg/L.	Marlatt *et al*. 2013
21 d *in vivo* test with fathead minnow (*Pimephales promelas*) (FSTRA)	GLP study in accordance with the TG Reliability: 1 (reliable without restrictions) Test material: Linuron	Effects including changes in gonadal staging, decreased yolk synthesis and increased oocyte atresia of predominantly pre-vitellogenic follicles and no egg production were observed at the highest test concentration (9.1 mg/L) in the presence of systemic toxicity. In the absence of systemic toxicity, fecundity and fertility were significally reduced at the mid concentration (0.92 mg/L).	U.S. EPA 2015

Study	Remarks	Results	Reference
25 d *in vivo* test with male nile tilapia (*Oreochromis niloticus*)	Non-guideline study Reliability: 2 (reliable with restrictions) Test material: Diuron (and 3,4-DCA, DCPU, DCPMU)	Diuron caused a decrease in testosterone levels and minor effects on gonadal histology at concentration of 200 ng/L. Diuron transformation products 3,4-DCA, DCPU and DCPMU decreased levels of plasma testosterone and 11-ketotestosterone, gonadosomatic index, diameters of seminiferous tubules and percentages of germ cells of testis.	Pereira *et al*. 2015
25 d *in vivo* test with female nile tilapia (*Oreochromis niloticus*)	Non-guideline study Reliability: 2 (reliable with restrictions) Test material: Diuron (and 3,4-DCA, DCPU, DCPMU)	Concentration of 100 ng/L diuron and its transformation products caused variations in the quantitative percentage of germinative cells. Diuron transformation products caused increases of 20 % of E_2 in plasma levels, 30 % of gonadosomatic indices and 30 % of final vitellogenic oocytes.	Pereira *et al*. 2016
7 d *in vivo* and *in vitro* test with male tilapia (*Oreochromis mossambicus*)	Non-guideline study Reliability: 2 (reliable with restrictions) Test material: Diuron (and 3,4-DCA, DCPU, DCPMU)	Diuron transformation products induced expression of hepatic CYP3A and, as well as Diuron, expression of vitellogenin mRNA. Aromatase activity was diminished in treatments with Diuron and DCPMU and DCPU. 17βHSD activity was reduced with DCPMU and 3,4-DCA. Depending on the endpoint, the observations were made in concentrations of 40 and 200 ng/L.	Felício *et al*. 2016
10 d *in vivo* test with male nile tilapia (*Oreochromis niloticus*)	Non-guideline study Reliability: 2 (reliable with restrictions) Test material: Diuron (and 3,4-DCA, DCPMU)	Diuron transformation products 3,4-DCA and DCPMU significantly reduced plasma testosterone concentrations, dopamine levels in the brain and aggressive behaviour observed as well as increased plasma cortisol concentrations at concentrations of 100 ng/L.	Boscolo *et al*. 2018

Study	Remarks	Results	Reference
OECD TG 234 Fish Sexual Development Test with zebrafish (*Danio rerio*) (FSDT)	GLP study in accordance with the TG Reliability: 2 (reliable with restrictions) Test material: Diuron 98.7%	For post-hatch survival, NOEC of 1.00 µg/L was determined. No effects were determined for the growth or the endocrine-related endpoints. Furthermore, no indicative effects on gonad maturation or lesions were found in histopathological evaluations. No redistribution of fish or reduction of replicates was performed according to the guideline. Thus, the study has low weight in ED assessment.	Unpublished (2018) In the registration dossier
180 d *in vivo* test with marine medaka (*Oryzias melastigma*) (F0) following 270 d indirect exposure of F1 generation.	Non-guideline study Reliability: 2 Experimental results shown in different articles. Test material: Diuron (>98%) (and DCPMU, DCPU, 3,4-DCA)	Diuron caused decreased gonadosomatic index (GSI) (5 ng/L), histological changes of testes in F0 (spermatogonia, spermatocytes), decreased mobility of sperm (500 ng/L) and reduced fecundity in F1 (sexual behaviour, copulation time and success of spawning, fertilization, and hatching). Increases in gonadotropin releasing hormone (GnRH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), 17β-estradiol and ratio of E2/T was observed across treatments in F1 males. Down-regulation of *vtg1*, *vtg2*, *chgh* and *chgl* transsciption and decreased GnRH, LH and E2 levels were observed in F1 females. Parental exposure (F0) of Diuron decreased GSI and gonad development in F1 females at 500 and 5000 ng/L. In silico and in vivo models identified Diuron, DCPMU and DCPU as aryl hydrocarbon receptor agonist (AhR). DCPMU and DCPU induced the gene expression of AhR signalling and metabolizing enzymes (such as cyp1a1) in the livers.	Zhou *et al*. 2022 Bao et a. 2022

Study	Remarks	Results	Reference
90 d (until sexual maturity) in vivo test with Japanese medaka (Oryzias latipes)	Non-guideline study Reliability: 2 Test material: Diuron (>98%) Nominal concentrations were analytically verified during the first 10 days of experiment.	Diuron decreased survival (63%) at 41 ng/L. Increased percentage of phenotypic females (88-89%) and genetic males with female phenotype was observed at both concentrations of 41 and 205 ng/L. Significant up-regulation of *cyp19a* mRNA expression.	Li *et al*. 2021
96h OECD TG 236 Fish embryo toxicity test with zebrafish (*Danio rerio*) (FET)	Reliability: 2 Test material: Diuron (>98%) No analytical monitoring (semi-static daily renewal)	Acute toxicity: 96h LC50 value of 6.31 mg/L Diuron also induced abnormalities during the development of embryos, and effects such as lack of pigmentation, deformations of head or tail, column malformations, yolk sac or pericardial edema and underdevelopment were observed. Behavioural changes also observed (decreased spontaneous coiling movement of embryo and reduction of thigmotaxis in larvae).	Velki *et al*. 2017
21d in vivo test with Javanese medaka (*Oryzias javanicus*)	Non-guideline study Reliability: 2 No analytical monitoring (semi-static renewal after every 3 days) Test material: Diuron	Diuron caused histopathological alterations in gonads (ovary and testis).	Kamarudin *et al*. 2020
21d in vivo test with Javanese medaka (*Oryzias javanicus*)	Non-guideline study Reliability: 2 Test material: 3,4-DCA (98%)	3,4-DCA reduced eggs spawned, female gonadosomatic index, gonadal development stage and oocyte atresia at 250 µg/L. No effects on hepatosomatic index were observed.	Ibrahim *et al*. 2021

Study	Remarks	Results	Reference
15d in vivo test with inland silverside (*Menidia beryllina*)	Non-guideline study Reliability: 2 Test material: (Diuron ≥ 98%)	Diuron increased body homogenate T3 and T4 (0.1 µg/L), decreased TRα and GHR receptor gene expression (1 µg/L) and increased Dio 1 gene expression (1µg/L) at control conditions of 10 °C and 10 ‰ salinity. Diuron reduced growth at ≥0.1 µg/L.	Moreira *et al*. 2018
60d in vivo test with red seabream (*Pagrus major*) and black rockfish *(Sebastes schlegelii*)	Non-guideline study Reliability: 2 Test material: (Diuron ≥ 98%)	Significant decrease in 17β–estradiol and 11–ketotestosterone levels and gonadosomatic index were observed on day 60 in fish exposed to 10 µg/L Diuron.	Nam *et al*. 2023
21d in vivo test in line with OECD TG 229 with zebrafish (*Danio rerio*)	Reliability: 2 Test material: 3,4-DCA (≥98%)	3,4-DCA decreased reproduction (spawning events and cumulative embryo count) at 0.38 mg/L and decreased sex hormone levels (T, E_2 and E_2/T ratio) was observed for both males and females. Diuron caused also down-regulation of *cyp*19b, ERα (male), *fsh*6, *fshr* (female), *star*, *cyp*11α and *cyp*19α and up-regulation of *gnrh1* (female), *gnrh2* (female), *gnrh3* (male), *gnrh4* (male), *ptgs2* (male) and 36*hsd* (female) gene expression.	Bhuiyan *et al*. 2021
14d in vivo test according to OECD TG 204 with zebrafish (*Danio rerio*).	Reliability: 2 Test material: 3,4-DCA (≥98%)	3,4-DCA decreased testosterone (0.38 mg/L) and 17β–estradiol (1.9 mg/L) concentrations and increased E2/T ratio (1.9 mg/L) in male zebrafish. 3,4-DCA also caused down-regulation of *star* and *cyp17* gene expression.	Bhuiyan *et al*. 2019
28d in vivo test with African sharptooth catfish (Clarias gariepinus)	Non-guideline study Reliability: 4 (only abstact assessed) Test material: Diuron (99%)	Diuron caused concentration dependent decrease in T4, T3 and 17β-estradiol levels.	Joseph *et al*. 2024

Katsiadaki *et al*. (2006) have studied the ED properties of Linuron with a sensitive *in vivo* test using three-spined stickleback (*Gasterosteus aculeatus*) for detection of environmental antiandrogens. In the so called AFSS assay (androgenised female stickleback screen) sexually mature female sticklebacks were simultaneously exposed to the suspected antiandrogenic chemicals and a model androgen (500 ng/L of 17α-methyltestosterone) during a limited part of their life-cycle (21 days). The endpoint that indicates the (anti)androgenic activity is the level of spiggin (glue protein normally produced in male kidneys) in the female stickleback kidneys.

Spiggin is an adhesive glycoprotein produced in the kidney of male sticklebacks during the breeding season and is subsequently secreted into the urinary bladder from where it is employed for nest building. Androgen inducible spiggin is also present in the kidneys of females but normally at very low levels and this feature has been exploited to provide a bioassay for anti-androgenic activity. The use of females, with normally low spiggin levels, provides a relatively constant baseline from which consistent androgen-induced spiggin levels can be achieved.

The results showed that Linuron was antiandrogenic in the exposure concentrations of 15 and 150 µg/L in water (Katsiadaki *et al*. 2006). Due to the structural similarity with Linuron and the androgen receptor binding activity, Diuron may have the same kind of antiandrogenic effect on fish as Linuron. However, there may be a difference in potency.

The same AFSS assay (OECD 230 modification, OECD GD 148) was used to investigate the combined effects of four antiandrogenic chemicals (vinclozolin, fenithrotion, flutamide, Linuron). The results showed that the androgen receptor antagonists acted in concert in an additive fashion in fish. The tested chemicals inhibited spiggin induction in a concentration-dependent manner, confirming that the AFSS effectively detects AR antagonists. Linuron was the least potent of the four substances with IC50 of 172 µg/L (Pottinger *et al*. 2013).

Jolly *et al*. (2009) have studied the (anti)androgenic impacts of four substances (one pharmaceutical Flutamide and three environmental contaminants, Fenitrothion, Vinclozolin and Linuron) using both *in vivo* and *in vitro* assays in the three-spined stickleback. In the *in vivo* test the female sticklebacks were exposed to the test compounds (6 concentrations of each) together with DHT (5 µg/L) for 21 days. Linuron caused a significant decrease in DHT-induced spiggin production at concentrations of 100 µg/L and 250 µg/L when tested *in vivo*.

In a Fish Short-Term Reproduction Assay (FSTRA) by Marlatt *et al*. (2013), the mode of action of Linuron was examined in male and female fathead minnows (*Pimephales promelas*) using the biomarker vitellogenin (VTG), nuptial tubercle formation, gonadal-somatic index (GSI), egg hatching and larvae survival at nominal concentrations of 1, 10 and 100 µg/L. The plasma vitellogenin concentrations were significantly reduced in females exposed to linuron at 1 µg/L and 100 µg/L. No other significant effects were observed in the study.

In a FSTRA (OCSPP 890.1350) by U.S. EPA (2015), adult fathead minnows were exposed to Linuron at mean measured concentrations of 0.099, 0.92 and 9.1 mg/L. Effects including changes in gonadal staging, decreased yolk synthesis and increased oocyte atresia of predominantly pre-vitellogenic follicles and no egg production were observed at the highest test concentration (9.1 mg/L) in the presence of systemic toxicity (decreased survival and clinical signs of toxicity). In the absence of systemic toxicity, fecundity and fertility were significantly reduced at the mid concentration (0.92 mg/L) as well. There were no other significant effects observed in the study.

In the *in vivo* test (Pereira *et al*. 2015), the effects of diuron and its transformation products on plasma hormone concentrations and spermatogenesis were evaluated. Sexually mature male nile tilapias (*Oreochromis niloticus*) were exposed to 200 ng/L of Diuron as well as to its transformation products 3,4-dichloroaniline (3,4-DCA), 3,4-dichlorophenylurea (CDPU)

and 3,4-dichlorophenyl-N-methylurea (DCPMU) for 25 days. Exposure concentrations were confirmed by chemical HPLC. Diuron caused a decrease in testosterone (T) levels. However, the transformation products 3,4-DCA, DCPU and DCPMU reduced levels of plasma sex steroid T and 1—ketotestosterone (KT) levels about 11 % as well as decreased gonadosomatic index, diameters of seminiferous tubules and percentages of germ cells of testis.

In the *in vivo* test (Pereira *et al*. 2016), sexually mature female nile tilapia were exposed to 100 ng/L of Diuron, 3,4-DCA, CDPU or DCPMU for 25 days. Exposure concentrations were confirmed by chemical HPLC. Diuron transformation products caused increases of 20 % of 17β-estradiol (E_2) in plasma levels, 30 % of gonadosomatic indices and 30 % of final vitellogenic oocytes. Diuron and its transformation products caused variations in the quantitative percentage of germinative cells. No significant differences in plasma concentrations of the oestrogen precursor and gonadal regulator 17α-hydroxyprogesterone(17α-OHP) were observed.

In the *in vivo* test (Felício *et al*. 2016), juvenile male tilapias (*Oreochromis mossambicus*) were exposed for 7 days to Diuron and the transformation products 3,4-DCA, DCPU and DCPMU at nominal concentrations of 40 and 200 ng/L. Expression of hepatic CYP3A was induced in high concentration of the Diuron transformation products. Expression of vitellogenin mRNA was induced high concentrations of Diuron, in both concentrations of DCPMU and DCPU as well as in low concentration of 3,4-DCA. Aromatase activity was diminished in high concentration of Diuron treatment, in both concentration of DCPMU and in the low concentration of DCPU. 17β-HSD activity was reduced in the low concentrations of DCPMU and 3,4-DCA.

In the *in vivo* test (Boscolo *et al*. 2018), adult male nile tilapias were exposed for 10 days to Diuron and the transformation products 3,4-DCA and DCPMU at nominal concentrations of 100 ng/L. Exposure concentrations were confirmed by chemical HPLC. Compared to Diuron and the control group, the 3,4-DCA and DCPMU significantly reduced plasma testosterone concentrations, dopamine levels in the brain and aggressive behaviour observed as well as increased the concentrations of the stress steroid (cortisol) in plasma. No difference of KT in plasma levels was observed between groups.

In the *in vivo* test (Zhou *et al*. 2022, Bao *et al*. 2022), marine medaka (*Oryzias melastigma*) were continuously exposed to 0 (0,005% DMSO), 5, 50, 500, and 5000 ng/L Diuron from embryo (0 dpf) to adult (180 dpf). Also between 150^{th} and 170^{th} day of exposure, some adult male fish was mated with non-treated female and the hatching time of F1 embryo was recorded until 20 dpf. Concentrations of Diuron and the metabolites DCPMU, DCPU and 3,4-DCA in seawater and in muscle were analysed by LC-MS system. Gonadosomatic index in male marine medaka was significantly decreased after exposure to Diuron at each concentration after 180 days. Also body mass index was significantly increased while liver somatic index was significantly decreased in the 50 and 500 ng/L groups. Brain somatic index and heart somatic index was significantly increased at 5000 ng/L.

Diuron significantly inhibited germ cells maturation, which caused significant increases in the percentage of spermatogonia (500 and 5000 ng/L) and decreases in the percentage of spermatocytes (50 and 5000 ng/L). Significant changes in the motility of sperm were also observed. Curvilinear velocity was significantly decreased after exposure to 500 ng/L and beating cross frequency was consistently decreased. In the male muscle, 5000 ng/L Diuron exposure led to a significant increase in the level of GnRH. The levels of FSH significantly increased in the 50, 500, and 5000 ng/L groups. The levels of LH, 17β-estradiol and the ratio of E_2/T exhibited a significant increase in the 500 ng/L group. No significant change in the levels of testosterone was shown. In the male brains, the relative mRNA expression of *sgnrh*, *mgnrh*, and *lhβ* was significantly up-regulated in the 50 and 500 ng/L groups. Exposure to 5 ng/L Diuron resulted in a significant down-regulated in the relative mRNA expression of *fshβ*. The relative mRNA expression of androgen receptor α (*araα*) showed a significant up-regulated in the 50, 500, and 5000 ng/L groups. The relative mRNA expression of arβ was significantly up-regulated in the 50 and 500 ng/L groups. The relative mRNA expression of estrogen receptor γ (*erγ*) showed a significant down-regulated after exposure to each concentration Diuron. In the male testes, the relative mRNA expression

of follicle stimulating hormone receptor (*fshr*) was significantly down-regulated in the 5, 50, and 500 ng/L groups compared with the control group. Exposure to 500 ng/L Diuron resulted in a significant increase in the relative mRNA expression of *cyp19a*. The relative mRNA expression of *arβ* was significantly down-regulated in the 5000 ng/L group. Significant down-regulated was observed in the relative mRNA expression of erα at the 5, 500, and 5000 ng/L groups. In the male livers, exposure to 50, 500, and 5000 ng/L Diuron led to a significant down-regulated in the relative mRNA expression of vitellogenin 1 (*vtg1*), *vtg2*, and *erα*.

The residue of DCPMU, DCPU and 3,4-DCA was significantly increased after exposure to 5000 ng/L of Diuron indicating that Diuron was metabolised more into DCPMU and DCPU with increasing of exposure concentration.

The relative mRNA expression of *ahr2* in male livers was significantly up-regulated after exposure to 5000 ng/L Diuron. Significant up-regulated was observed in the relative mRNA expression of *arnt, cyp1a1, cyp1b1*, and glucuronosyltransferase 1 (*ugt1*) at the 500 and 5000 ng/L groups. Exposure to 50, 500, and 5000 ng/L Diuron resulted in a significant up-regulated in the relative mRNA expression of *ugt2*.

The alteration of sexual behaviours is an important indicator for reproductive effects and fecundity. The percentage of following time was significantly decreased after exposure to 5000 ng/L Diuron. Exposure to 500 and 5000 ng/L Diuron resulted in a significant decreased in the times of copulation. Spawning success exhibited a slightly decreasing trend after Diuron exposure. Fertilization success and hatching rate in F1 embryos showed a significant decrease after exposure to 500 and 5000 ng/L Diuron. The hatching time of F1 embryos was significantly increased in the 50 and 500 ng/L groups. The relative mRNA expression of hatching enzyme (high choriolytic enzyme, *hce*) in F1 embryo was significantly down-regulated in the highest concentration group. Furthermore, exposure to 500 and 5000 ng/L Diuron resulted in a significant increase in mortality rate of F1 embryos and malformation rate of F1 larvae. Significant decrease was observed in the body length of F1 larvae and swim-up success rate at the 50 and 5000 ng/L groups. Compared to the control group, Diuron caused several developmental malformation and growth retardation of F1 embryos and larvae, including spinal curvature, yolk sac edema, and pericardial edema. Diuron exposure of F0 exerted negative effects on gonad development in F1 females while testis development in F1 males was unaffected. The gonadosomatic index of F1 females decreased at 500 and 5000 ng/L and increase in postvitellogenic oocytes and decrease in mature oocytes were also observed (500 ng/L). Significant down-regulation of GnRH and LH levels (500 and 5000 ng/L) and concentration dependent decrease in levels of 17β-estradiol (significant at 5000 ng/L) were observed in F1 females. The transcription of *vtg1, chgh* and *chgl* was significantly downregulated (500 ng/L and 5000 ng/L), and the transcription of *vtg2* was significantly downregulated (5000 ng/L) in a concentration dependent manner. The transcriptional levels of the DNA methyltransferase gene *dnmt1* were significantly upregulated in F1 female brains, ovaries and livers suggesting potential multigenerational effects of Diuron.

In the *in vivo* test (Li *et al*. 2021), Japanese medaka (*Oryzias latipes*) were exposed (semi-static with daily renewal) to nominal concentration of 41 ng/L and 205 ng/L of Diuron from embryo until sexual maturity (circa 3 months). The concentration of Diuron was analytically measured during the first 10 days of experiment (larvae phase). Analytically verified concentrations were 81% and 82% of nominal, respectively. Diuron decreased the survival of fish (68% survival) at the highest concentration of 205 ng/L. Diuron increased the percentage of phenotypic females (89% and 88%, respectively) and increased genetic males with female phenotypic changes at both concentrations. Morphological changes were observed with decreased anal fin rays with papillary processes (41 ng/L), increased decree of urogenital protuberance (205 ng/L) and increased gender score for feminization (205 ng/L). All morphological changes were similar to positive control 17β-estradiol. In medaka also up-regulation of mRNA expression of *cyp*19a was observed for both exposure concentrations.

In the *in vivo* test (Velki *et al*. 2017), zebrafish (*Danio rerio*) larvae were exposed (semi-static with daily renewals) to nominal concentrations of 1 and 10 mg/L of Diuron according

to OECD TG 236 (FET). Acute toxicity 96h LC50 value of 6.31 mg/L was observed for Diuron. Diuron also induced abnormalities during the development of embryos, and effects such as lack of pigmentation, deformations of head or tail, column malformations, yolk sac or pericardial edema and underdevelopment were observed. Furthermore, for the investigation of behaviour effects, larvae were exposed to nominal concentrations of 1, 2 and 3.8 mg/L of Diuron according to similar protocol. Diuron caused reduction of spontaneous coiling movement of embryos (3.8 mg/L) and reduction of thigmotaxis (swimming behaviour) in larvae (1 mg/L).

In the *in vivo* test (Kamarudin *et al*. 2020), Javanese medaka (*Oryzias javanicus)* were exposed (semi-static) for 21 days to nominal concentrations of 1, 50, 100, 500 and 1000 µg/L of Diuron. Diuron caused decreased ovarian gonadal staging at 50 µg/L and increase in oocyte atresia with concentration-response. Diuron also caused decreased gonadal staging in testes at 50, 100 µg/L (gonadal median stage 3), 500 and 1000 µg/L (gonadal median stage 1). Diuron caused testicular damage such as incomplete seminiferous lobes containing germs cells and disintegration of the Sertoli cells.

In the *in vivo* test (Ibrahim *et al*. 2021), Javanese medaka (*Oryzias javanicus)* were exposed (semi-static) for 21 days to nominal concentrations (2-day renewal) of 10, 25, 50, 125, and 250 µg/L of 3,4-DCA. 3,4-DCA reduced eggs spawned, female gonadosomatic index, gonadal development stage and oocyte atresia at 250 µg/L. No effects on hepatosomatic index or male gonads were observed. Degeneration of ovarian cells and high proportion of perinucleolar oocytes was observed at 250 µg/L.

In the *in vivo* test (Moreira *et al*. 2018), inland silverside (*Menidia beryllina*) were exposed (semi-static, renewal every 2 days) for 15 days to nominal concentrations of 0.1 and 1 µg/L of Diuron. Diuron exposure reduced fish growth at both concentrations and increased body homogenate T3 and T4 (0.1 µg/L), decreased *TRα* and GHR receptor gene expression (1 µg/L) and increased *Dio1* gene expression (1µg/L) at control environmental conditions of 10 °C and 10 ‰ salinity. The concentrations of Diuron were analytically measured by HPLC. Increased expression of deiodinases was observed in *M. beryllina* exposed to Diuron combined with higher salinity (20‰) at 10 °C (*Dio3*) and 20 °C (*Dio1*), indicating the influence of salinity on deiodination pathway activation.

In the *in vivo* test (Nam *et al*. 2023), red seabream (*Pagrus major*) and black rockfish (*Sebastes schlegelii*) were exposed to concentrations 0.1, 1 and 10 µg/L of Diuron for 60 days. Significant decreases in 17β–estradiol and 11–ketotestosterone levels and gonadosomatic index were observed on day 60 in fish exposed to 10 µg/L Diuron. VTG concentrations decreased at 10µg/L for black rockfish but not for red seabream. Parameters of immunity, such as alternative complement activity, lysozyme activity, and total immunoglobulin levels, were significantly lowered by 60-day exposure to 10 µg/L in both fish. Significant decreases in the hepatic enzyme activities of alanine transaminase and aspartate transaminase were observed with an induction of cortisol on day 60 in fish exposed to 10 µg/L Diuron. Intracellular malondialdehyde and glutathione levels were significantly increased by 10 µg/L Diuron at day 60 with an increase in the enzymatic activities of catalase and superoxide dismutase.

In the *in vivo* test according to OECD TG 204 (Bhuiyan *et al*. 2019), zebrafish (*Danio rerio*) were exposed to nominal concentrations of 0.024, 0.12, 0.6, or 3.0 mg/L of 3,4-DCA for 14 days. The concentrations of 3,4-DCA were analytically measured with UHPLC. 3,4-DCA decreased testosterone (0.38 mg/L) and 17β–estradiol (1.9 mg/L) concentrations and increased E2/T ratio (1.9 mg/L) in male zebrafish. 3,4-DCA also caused down-regulation of star and cyp17 gene expression in male zebrafish gonads. In the *in vivo* test according to OECD TG 229 (Bhuiyan *et al*. 2021), zebrafish (*Danio rerio*) were exposed to nominal concentrations of 0.024, 0.12 and 0.6 mg/L of 3,4-DCA for 21 days. The concentrations of 3,4-DCA were analytically measured with UHPLC. 3,4-DCA decreased reproduction (spawning events and cumulative embryo count) and decrease in sex hormone levels for both sex (E_2, T and E_2/T ratio) at 0.38 mg/L. A significant up-regulation of gonadotropin releasing hormone 2 (*gnrh2*) gene was observed in female brain at 0.38 mg/L and *gnrh3* gene was up-regulated in the male fish. Following exposure to 3,4-DCA, two pituitary genes

(*gnrhr2* and *gnrhr4*) were up-regulated in the male fish, but *gnrhr1* was up-regulated only in the female at 0.38 mg/L. A significant down-regulation of follicular stimulating hormone b (*fshb*) and the *cyp19b* was also observed. A significant down-regulation of estrogen receptor a (*era*) was observed in the female. The expression of *era, er2b,* and *ar* genes were not affected in the male fish following 3,4-DCA exposure. After exposure to 3,4-DCA, *fshr* gene was down-regulated only in the ovary while no changes were observed for *lhr* gene in both male or female gonads. In addition, significant downregulations of cytochrome P450 family 11 subfamily A (*cyp11a*) and 17b-hydroxysteroid dehydrogenase (*17bhsd*) were observed by 3,4-DCA. Oocyte maturation related gene *ptgs2* was down-regulated in the testis by 3,4-DCA.

In a non-guideline study (Joseph 2024) African sharptooth catfish (*Clarias gariepinus*) was exposed to Diuron concentrations of 0.09, 0.18, 0.26 and 0.35 mg/L for 28 days (only abstract assessed). Concentration and exposure duration dependent decrease was observed for T_4, T_3 and 17β-estradiol levels.

Fish sexual development test with zebrafish Danio rerio (OECD TG 234)

The fish sexual development test OECD TG 234 (Unpublished 2018) is available in the registration dossier. The test was performed under GLP. Zebrafish (*Danio rerio*) was used as test organism.

The nominal concentrations of the test item were 1.0, 3.16, 10.0, 31.6 and 100 µg/L in the test and the mean measured concentrations were 1.19, 3.26, 11.32, 32.51 and 105.44 µg/L (of active ingredient), respectively. The measured concentrations were used in calculation of results. The initial stock solution of Preventol A 6 was prepared in acetone.

Controls and all test concentrations were run in four replicates in glass aquaria, each with total volume of 28 l and with 25 l of test solution, in a flow through system. 120 eggs (4 x 30) were used for each test concentration. Test conditions were 12/12 hours light/dark (light intensity 1000 lux). Water temperature was set to 27 ± 2 °C and oxygen concentrations was maintained not lower than 60%.

The active substance (Diuron) was analysed using LC-MS/MS. For determination of the vitellogenin levels, an enzyme-linked immunosorbent assay (ELISA) raised to zebrafish (*Danio rerio*) VTG (homologous ELISA kit, Biosense, Bergen, Norway) was used.

The evaluating MSCA considers that the validity criteria are met. However, according to the study report no redistribution of fish or reduction of replicates was performed after the observed treatment-related effect on survival due to excessive larval mortality during dpf 10-21. According to the FSDT guideline (para 27): "No later than 28 days post fertilisation the number of fish per replicate should be redistributed, so that each replicate contains as equal a number of fish as possible. If exposure related mortality occurs, the number of replicates should be reduced appropriately so that fish density between treatment levels is kept as equal as possible".

Results

Mortality

Hatching success of fertilized eggs was 96.7 – 100 % and was completed at 6 to 8 dpf in all replicates. Increased larval mortality was observed between 11 and 21 dpf across the test levels, mainly during the phase of transition from yolk sac feeding to external feeding. Post-hatch survival rate met the validity criteria in the control (83.1 % at 21 dpf and 82.3 % at 35 dpf). However, there was a statistically significant difference in post-hatch survival in treatments ≥ 3.26 µg a.i./L compared to control. The survival ranged from 39.6 % (32.51 µg/L) to 63.3 % (11.32 µg/L) at 21 dpf and 35 dpf, without any concentration-response relationship. No significant further increase in mortality was seen at termination (63 dpf) but the mortality occurred mainly at early life stage. Therefore, the NOEC for the endpoint post-hatch survival was determined to be ≥ 1.19 µg a.i./L at 35 and 63 dpf.

There was no concentration-response relationship in growth of fish exposed to the test item compared to controls. On average fish grew somewhat larger and gained more weight in treatments than in controls. No impaired growth in terms of length and weight of males and females were observed. Moreover, no other indication of generic toxicity was seen except for the effect on post-hatch survival.

Sex ratio

The sex ratio in the replicates was determined macroscopically by inspection of the gonads and, furthermore, histopathologically in order to determine the sex of very immature and to verify the sex of the other individuals. The examined fish were assigned to three different categories: female, male or transitional phase. The latter described a juvenile, immature individual that was most probably a male, but still in the natural transitional phase from juvenile ovary to testis.

The sex ratio (in % females) was determined to be between 46.3 % and 62.1 % females. This ratio matched the requirements of a sex ratio between 30 – 70 % females. Compared to the control, no test item-related effect or significant change of the percentage of fish in transitional phase were observed. As no statistically significant differences in the sex ratio was observed, the NOEC for the endpoint sex ratio was determined to be ≥ 105.44 µg a.i./L.

Maturation and maturity index

Maturation stage of ovaries and testis was determined histopathologically. The maturation stages range from 0 (very immature) to 4 (fully mature). Out of 54 female fish in the control the maturations stage of ovaries was 0 in 21 fish and 4 in 13 fish, the rest of them being at stage 1 to 3. The control males totalled 41, out of which 3 fish were at stage 0 and no fish reached maturation stage 3 or 4. Most of them were at stages 1 and 2.

The average maturity index of females ranged between 2.7 (controls) and 4.3 (32.51 µg a.i./L) and showed a concentration-related increase, which was statistically significant at the two highest concentrations. Histopathological lesions in the ovaries were not frequent or severe. The high percentage of females with egg debris at a concentration of 32.51 µg a.i./L was due to the low number of females, all showing egg debris, in one replicate. Based on that information, the NOEC for maturity index of females was determined to be ≥ 11.32 µg a.i./L.

Testis maturity indices ranged between 2.3 (3.26 µg a.i./L) and 2.8 (11.32; 32.51, and 105.44 µg a.i./L) and showed no clear nor statistically significant concentration-related changes. Single immature oocytes were found in the testis tissue (testis-ova) of some individuals but no concentration-related change in frequency or severity of this effect could be identified. Pathological alterations of testicular morphology were not observed. Thus, the NOEC for the maturity index of male was determined to be ≥ 105.44 µg a.i./L.

Vitellogenin content in blood plasma

For determination of the vitellogenin levels, an enzyme-linked immunosorbent assay (ELISA) raised to zebrafish was used. Due to the early and different developmental stages of fish, VTG values were low for single individuals (irrespective of the sex) and showed high variability in general. Values that were below the LOQ at the lowest dilution (1:50) were considered as zero for statistical analysis.

No statistically significant differences between controls and treatments were determined for the VTG content of female fish. For controls, mean values of 301.0 ng VTG/µg protein were measured, while treatments displayed VTG concentrations between 568.1 ng VTG/µg protein (1.19 µg/L) and 991.2 ng VTG/µg protein (32.51 µg/L). For males, mean measured VTG values ranged between 0.06 ng VTG/µg protein (105.44 µg/L) and 1.03 ng VTG/µg protein (3.26 µg/L, with no statistically significant differences between controls and treatments. Based on the data, the NOEC for the VTG content in blood plasma was determined to be ≥ 105.44 µg a.i./L.

Table 40 Summary of NOEC/LOEC determination during the course of the study.

Parameter	**NOEC/LOEC Nominal concentration Preventol A 6 [µg/L]**	**NOEC/LOEC Mean measured concentration a.i. [µg/L]**
Post-hatch survival at 35 dpf	1.00 / 3.16	1.19 / 3.26
Length at 35 dpf	≥ 100.00 / > 100.00	≥ 105.44 / > 105.44
Post-hatch survival at 63 dpf	1.00 / 3.16	1.19 / 3.26
Length (decrease) at 63 dpf (all fish)	≥ 100.00 / > 100.00	≥ 105.44 / > 105.44
Weight (decrease) at 63 dpf (all fish)	≥ 100.00 / > 100.00	≥ 105.44 / > 105.44
Length (decrease) at 63 dpf (males)	≥ 100.00 / > 100.00	≥ 105.44 / > 105.44
Length (decrease) at 63 dpf (females)	≥ 100.00 / > 100.00	≥ 105.44 / > 105.44
Weight (decrease) at 63 dpf (males)	≥ 100.00 / > 100.00	≥ 105.44 / > 105.44
Weight (decrease) at 63 dpf (females)	≥ 100.00 / > 100.00	≥ 105.44 / > 105.44
Pseudo-spec. growth rate	≥ 100.00 / > 100.00	≥ 105.44 / > 105.44
Sex ratio	≥ 100.00 / > 100.00	≥ 105.44 / > 105.44*
VTG content [ng/µg protein]; females	≥ 100.00 / > 100.00	≥ 105.44 / > 105.44*
VTG content [ng/µg protein]; males	≥ 100.00 / > 100.00	≥ 105.44 / > 105.44**
Maturity index (females)	10.00 / 31.60	11.32 / 32.51
Maturity index (males)	≥ 100.00 / > 100.00	≥ 105.44 / > 105.44
Histopathology (females)	≥ 100.00 / > 100.00	≥ 105.44 / > 105.44

If not indicated else, statistical evaluations were performed with the Jonckheere-Terpstra Test
* Dunnett's t-test, α=0.05, two-sided
** Bonferroni-Holm median test, α=0.05, two-sided

Generic toxicity

It is noted that a statistically significant post-hatch mortality was seen in the treatments but without concentration-response relationship. No other indications of generic toxicity were observed. Fish exposed to the test item were larger and gained weight compared to controls. According to evaluating MSCA this was related to the lack of redistribution of fish after the observed high early mortality and low stocking density of fish at the highest concentrations at the end of the study.

Sex ratio

Statistical analysis of the fish sex ratio showed that the chemical treatment with Diuron did not have a statistically significant effect on sexual differentiation. Therefore, the slight feminization observed in the low exposure groups that did not occur in the high exposure groups was considered as natural variation of fish sex ratio instead of endocrine-related effects of the test item.

Maturity index

The chemical exposure seemed to have a slightly stimulating, statistically significant, effect on female gonad maturation. The effect was most likely related to the overall increased size of the fish (Spence *et al*. 2008) at higher concentrations, most likely due to the lower number of individuals promoting faster growth and development (Parachy *et al*. 2009). Thus, the effects were considered as secondary effect.

Histopathological lesions in females (extended areas of egg debris and atresia combined with inflammation) were not very pronounced, not concentration-related and rare. In males, no pathological lesions could be diagnosed but all groups except for the exposure with 32.51 µg a.i./L had a low percentage of male fish who were categorized as testis-ova. However, the rate was not significantly higher in these groups compared to the control group and, therefore, considered as not related to endocrine disruption potential of the test item.

The variation in maturation stage is rather high and large share of fish remained immature by 63 dpf. However, high variation of developmental stages was acknowledged also in the validation report (OECD 2011).

Vitellogenin

It was concluded in the validation report of the FSDT test (OECD 2011) that VTG results alone should be interpreted carefully due to the relatively high variability of this parameter in fish of different size and at different developmental stage. VTG should be seen in connection with the sex ratio because the skewing of the sex ratio where genetic sex is changed phenotypically can affect VTG. For example, it is significantly different between genetic males and phenotypically sex reversed females.

Although not statistically significant, there was a slight increase in VTG concentrations of female fish and fewer individuals having VTG value below the LOQ in treatments compared to control. High control variability in the FSDT vitellogenin should not affect the results because induction in VTG is normally massive in response to chemicals and because it should be always connected to the sex ratio (OECD 2011).

Regarding the suspected endocrine mode of action for Diuron (it is a potential androgen receptor antagonist), VTG not a suitable endpoint for androgen antagonism, but sex ratio i.e. increase in females and intersex are relevant endpoints (OECD 2011).

Conclusion

Zebrafish *(Danio rerio)* was selected as test organism in the fish sexual development test (OECD 234). The nominal concentrations of the test item (98.7 % pure Diuron) used were 1.0, 3.16, 10.0, 31.6 and 100 µg/L in the test and the mean measured concentrations

were 1.19, 3.26, 11.32, 32.51 and 105.44 µg/L (of active ingredient), respectively. The test was performed under GLP.

Endpoints determined included hatching success and rates and mortalities during the early life stage and juvenile growth. Size (length and weight) and vitellogenin content in blood plasma were measured and sex ratio determined by macroscopically by inspection of the gonads and histopathological verification.

Evaluation of all endpoints revealed an effect without concentration-response relationship on post-hatch survival at 35 dpf and 63 dpf. Based on the endpoint post-hatch survival, the following NOEC of 1.19 µg a.i./L was determined. No effects were determined for the growth in terms of length and weight.

Furthermore, no effects were determined for the endocrine-related test endpoints sex ratio and VTG content in female and male blood plasma. Also, histopathological evaluations showed no indicative effects on gonad maturation or lesions. Thus, based on endocrine-related endpoints, the following NOEC of ≥ 105.44 µg a.i./L was determined.

The results of the performed test are considered negative regarding endocrine-related interference to sexual development and differentiation. However, no redistribution of fish or reduction of replicates was performed although that is required by OECD TG 234. The evaluating MSCA considers that this resulted in highly biased test results as effects on individuals in replicates with a lower number of individuals have higher statistical weight. The observed increase in length, weight and maturation of fish due to low stocking density (caused by the lack of redistribution) may have masked the potential ED effects. Thus, the FSDT study is considered to have a low weight in the ED assessment. Nevertheless, the negative outcome of the FSDT study should not rule out the ED mediated adverse effects observed in other studies and mainly in the key study Li *et al*. 2021.

15.1.3. *In vivo* studies on amphibians and reptiles

There is one non-guideline study related to *in vivo* effects of Diuron and one validation report on *in vivo* effects of Linuron on amphibians available. The studies are presented below.

Table 41 *in vivo* effects on amphibians and reptiles.

Study	Remarks	Results	Reference
7 d *in vivo* test with american bullfrog (*Lithobates catesbeianus*)	Non-guideline study Reliability: 3 (not reliable) Test material: Diuron (and 3,4-dichloroaniline)	Tadpoles exposed to 3,4-DCA at 34 °C presented decreases in T_3 levels. Also changes in thyroid-hormone induced proteins as well as in gene expressions were observed.	Freitas *et al*. 2016
96 h *in vivo* test with African claved frog (*Xenopus laevis*)	Reliability: 2 (reliable with restrictions) Test material: Linuron	Linuron was detected acting on the thyroid system from the lowest test concentration of 2.5 mg/L upwards.	OECD 2018b

Study	Remarks	Results	Reference
Impacts of Diuron on the reproductive system of male lizard (*Podarcis sicula)*	Non-guideline study Reliability: 2 (reliable with restrictions) Test material: Diuron 50% (Toterbane 50F)	E.g. - Intertubular tissue increased considerably in volume, and contained numerous lymphocytes, neutrophils and some monocytes; - Epididymis appeared regressed with abundant connective tissue; - The mean gonadosomatic index (GSD) was reduced; - A clear reduction in seminiferous tubule diameter - A significant decrease in all germ cells; - Decrease of testosterone values.	Cardone *et al*. 2008
Test 1: Embryo Teratogenesis Assay—Xenopus (FETAX) (ASTM 1991) Test 2: Chronic toxicity test with Pacific treefrogs (*Pseudacris regilla*), bullfrogs (*Rana catesbeiana*), red-legged frog (*Rana aurora*) and African clawed frog (*Xenopus laevis*)	Non-guideline study Reliability: 2 (reliable with restrictions) Test material: Diuron 99.8%	4d LC50 (mortality): >29.1 mg/L (*X. laevis* & *P. regilla*) 10d EC50 (deformity): 22.2 mg/L (*P. regilla)* Lowest LC50 (14d) value of 8.1 mg/L was observed for *X. laevis.* Diuron caused effects on length, wet weight and deformities to *P. regilla* and *R. catesbeiana.* Diuron also caused delay of appearance of forelimbs and hindlimbs following 14 days exposure in *R. aurora*.	Schuytema & Nebeker 1998

In the non-guideline *in vivo* test (Freitas *et al*. 2016), the impacts on metamorphosis and thyroid genetics of American bullfrog (*Lithobates catesbeianus*) tadpoles exposed to 40 and 200 ng/L of Diuron and transformation product 3,4-DCA for 7 days at 28 °C and 34 °C were studied. Significant increases in thyroid hormone-induced bZip protein (*thibz*) transcripts were observed as well as on the relative abundance of Krüppel-like factor 9 (*klf9*) expression at 200 ng/L of Diuron at 28°C and 34°C, respectively. t*hibz* was significantly increased at 200 ng/l of 3,4-DCA at 28°C. Iodothyronine deiodinase type II (*dio2*) was significantly increased only at 200 ng/l Diuron at 34°C and at both concentrations of 3,4-DCA at 28°C and at 40 ng/l at 34°C.

No effects on developmental stages, metamorphosis, mortality, weight or alterations of plasma 3,3′,5-triiodothyronine (T_3) concentrations were observed when compared to control. No histopathological examinations were conducted.

In Xenopus Eleutheroembryonic Thyroid Assay (XETA, OECD TG 248) draft validation report (OECD, 2018b), Linuron was identified being a thyroid receptor agonist in African clawed frog (*Xenopus laevis*) as increasing concentrations of Linuron in the absence of T_3 and no effects in the presence of T_3 are observed in each laboratory conducted ring test with Linuron. Linuron was detected acting on the thyroid system from the lowest test concentration of 2.5 mg/L upwards. The observations were statistically significant in two of the three laboratories.

In a study with a lizard species (*Podarcis sicula*) the test animals (sexually mature males) were captured from nature from an uncontaminated area during gonadal full activity (Cardone *et al*. 2008). The animals were first adapted to test conditions and then three separate exposure groups were exposed to commercial product Toterbane 50 F, with 50% Diuron content, via soil, drinking water, food and a combination of these for 3 weeks. There was a control test and one level of exposure concentration for each treatment.

Morphology of testis and epididymis showed negative effects following the treatment with contaminated soil (sprayed with 3.75 L/ha of Toterbane, reflecting average recommended dose in agricultural use) and contaminated drinking water (with 1.08 µg/mL of Diuron) and/or contaminated food (with 5.4 mg of Diuron). The amount and uptake of food was not given in the method description and neither was the uptake of contaminated drinking water, which are shortcomings of the study description.

The seminiferous tubules of lizard were markedly reduced in cross-sectional area, probably due to collapse of the seminiferous epithelium or different degrees of degenerative changes. There was also a greatly reduced lumen - or no lumen whatsoever - in the tubules. Histological changes in each lizard were uniform throughout each testis, and in the most severely damaged tubules only Sertoli cells and some spermatogonia were present, while complete loss of all the stages of the germ cell. Additionally, the intertubular tissue increased considerably in volume, and contained numerous lymphocytes, neutrophils and some monocytes. The epididymis appeared regressed with abundant connective tissue and the epithelial cells were low, without secretory granules.

Quantitative changes were also discovered. The mean gonadosomatic index (GSD) was reduced from 5.27±0.39x10-3 (in the control group) to 2.3±0.18x10-3 and 3.4±0.25x10-3 in the Diuron exposed groups. A clear reduction in seminiferous tubule diameter also occurred and a significant decrease in all germ cells was observed. Apoptotic (TUNEL-positive) cells were not detected either in the seminiferous epithelium or the interstitial space in the exposed groups. The decrease of testosterone values varied from 34% to 52% in different groups in comparison with the control group. No estrogenic impact was observed as the 17b-estradiol plasma content was undetectable in all Diuron-exposed male lizards. It can be concluded that, despite of the shortcomings in the description and quantitative follow-up of the actual uptake, the results with lizard suggest that Diuron exposure results in direct male reproductive toxicity.

In an *in vivo* test (Schuytema & Nebeker 1998), survival and growth of Pacific treefrog (*Pseudacris re*gilla), bullfrog (*Rana catesbeiana*), red-legged frog (*Rana aurora*), and African clawed frog (*Xenopus laevis*) embryos and tadpoles were determined in static-renewal tests (acute embryo toxicity tests according to FETAX test protocol and chronic toxicity tests). *P. regilla* and *X. laevis* embryos had reduced growth and developed increased deformities in Diuron concentrations over 20 mg/L. Hindlimb bud and forelimb development were retarded in *R. aurora* following 14 days exposure to Diuron concentrations of >7.6 mg/L. Mean 14d LC_{50} for *P. regilla* and *X. laevis* tadpoles were 15.2 and 11.3 mg/L Diuron, respectively. The 21d LC_{50} for *R.catesbeiana* tadpoles was 12.7 mg/L Diuron. The 14d LC_{50} for *R. aurora* tadpoles was 22.2 mg/L. The lowest NOECs calculated in chronic tests were: *P. regilla*, 14.5 mg/L (14 days); *R. catesbeiana*, 7.6 mg/L (21 days); *R. aurora*, 7.6 mg/L (14 days); and *X. laevis* >29.1 mg/L (14 days).

15.1.4. *In vivo* studies on invertebrates

Aquatic invertebrates

There were two guideline studies available for evaluation, based on the earlier test guideline OECD TG 202, Part II on *Daphnia magna* (Crustaceae) reproduction in the registration dossier. Full study reports were submitted to the evaluating MSCA by the registrant. Neither of these studies included recording of the production of male neonates, and therefore the adverse effects on reproduction cannot be linked to ED mode of action. The studies are presented below.

Table 42 Effects on aquatic invertebrates

Study	Remarks	Results	Reference
OECD TG 202, Part II *Daphnia magna* reproduction	Key study Reliability: 1 (reliable without restrictions) Test material: Diuron	LOEC 0.97 mg/l NOEC 0.56 mg/l	Unpublished (1996e) In the registration dossier
OECD TG 202, Part II Daphnia magna reproduction	Supporting study Reliability: 1 (reliable without restrictions) Test material: Diuron	LOEC > 1 mg/l NOEC > 1 mg/l	Unpublished (1989) In the registration dossier

It was concluded in Unpublished (1996e) that Diuron impacts the reproduction of Daphnia by reducing the number of offspring per parent and by delaying the time of breeding. The NOEC was 0.56 mg/l and LOEC 0.97 mg/l. The test concentrations ranged from 0.032 to 1.8 mg/l, and they remained rather stable in the test solutions during the test. No or little mortality was seen during the test, but the weight and length of the parental Daphnids were impacted (reduced). The acute toxicity (48 h-EC_{50}) to Daphnia was 1.4 mg/l, which was clearly higher than the lowest effective concentration on reproduction. The results were statistically significant.

The test concentration range in the study of Unpublished (1989) was from 0.0003 to 1.0 mg/l. Both the NOEC and LOEC were concluded to be >1 mg/l. There was no significant difference in the mortality of Daphnids between the control sample and the test samples during the 21 d the semi-static test.

However, while looking at the raw data of reproduction in the study, the number of offspring was 68.1% of the number in the control test in the highest 1.0 mg/l test concentration. The authors conclude that the result was not statistically significant. There was also a shortcoming in the test setup as the parental animals (10/concentration) were put in the same vessel for the first 9 days of the test, instead of using separate beakers for each animal as guided in the protocol. This may also limit the power of the statistical analysis of the results.

When comparing the two *Daphnia magna* studies, it should be noted that the highest test concentration in Unpublished (1989) test (1 mg/l) was approximately the same as the LOEC concentration in Unpublished (1996e) test (0.97 mg/l). Therefore, it can be concluded that the results of Unpublished (1989) test may well be in concordance with Unpublished (1996e) test and both tests indicate that Diuron may interfere with Daphnia reproduction although the applied lower concentration range in the latter test shows only weak impact.

ED mode of action for the observed disturbance in reproduction of Daphnia cannot be concluded from the test results, as no endpoints providing data on hormonal impacts were included in the tests conducted with the earlier version of the test protocol.

Sediment organisms

Some studies on the impacts of Diuron on the reproduction of sediment dwelling organisms have been published.

Table 43 Effects on sediment organisms.

Study	Remarks	Results	Reference
Impacts on reproduction of freshwater snail (*Physella acuta*)	Non-guideline study Reliability: 2 (reliable with restrictions) Test material: Diuron	Weak impacts (increase in the total number of egg sacs) followed by Diuron exposure were observed in 9.5 µg/L	López-Doval *et al*. 2014
Effects on reproduction of ascidian (*Ciona intestinalis*) of antifouling compounds	Non-guideline study Reliability: 2 (reliable with restrictions) Test material: Diuron	No effect on fertilization rate with Diuron exposure (2.33 mg/L), but the percentage of normal larvae significantly decreased	Gallo *et al*. 2013
Exposure experiments with cupped oyster (*Crassostrea gigas*)	Non-guideline study Reliability: 2 (reliable with restrictions) Test material: Diuron	Some physiological effects were observed in terms of reproduction (partial spawning) and histopathology (atrophy of the digestive tubule epithelium)	Buisson *et al*. 2008
24 h *in vivo* test with oyster	Non-guideline study Reliability: 3 (not reliable) Test material: Diuron	Diuron decreased the percentage of normal D-larvae from the lowest tested concentration of 0.05 µg/L. Genotoxicity was observed from concentration of 0.25 µg/L upwards but no effects on sperm viability was detected.	Akcha *et al*. 2012
6 h and 24 h *in vivo* tests with pacific oyster (*Crassostrea gigas*)	Non-guideline study Reliability: 2 (reliable with restrictions) Test material: Diuron (and 3,4-DCA, CDPU, DCPMU, ascorbic acid)	Diuron and the transformation products DCPMU and DCPU caused abnormalities in D-larvae from 0.05 µg/L. Also genotoxicity was observed from 0.05 µg/L of Diuron and all the transformation products. Combined exposure of Diuron and the transformation products DCPMU and DCPU with ascorbic acid were observed decreasing the genotoxicity and developmental abnormalities.	Behrens *et al*. 2016

Study	Remarks	Results	Reference
30 min and 48h *in vivo* test with sea urchin (*Paracentrotus lividus*)	Non-guideline study Reliability: 2 (reliable with restrictions) Test material: Diuron (97-99.8%)	Diuron caused reduction of the fertilization ability of exposed sperms (NOEC 0.5 mg/L) and concentration dependent developmental anomalies in the fertilized eggs after 48 exposure.	Manzo *et al*. 2005

Only weak impacts on the reproduction of freshwater snail (*Physella acuta*) (increase in the total number of egg sacs) followed by Diuron exposure were observed in 9.5 µg/L concentration, found in actual freshwater environments (López-Doval *et al*. 2014). In a study with an ascidian (*Ciona intestinalis*) no effect on fertilization rate was discovered with Diuron exposure (2.33 mg/L), but the percentage of normal larvae significantly decreased (Gallo *et al*. 2013). Exposure experiments with cupped oyster (*Crassostrea gigas*) led to slight accumulation of two substituted ureas (Diuron and Isoproturon), with factor around 7, and some physiological effects were observed in terms of reproduction (partial spawning) and histopathology (atrophy of the digestive tubule epithelium) (Buisson *et al*. 2008).

In the *in vivo* tests (Akcha *et al*. 2012), embryotoxic and genotoxic effects on oyster were studied. After 24 h of exposure to nominal concentrations of 0.05, 0.10, 0.25 and 0.50 µg/L of Diuron, embryotoxicity was studied using the embryo-larval bioassay after in accordance with the standardized AFNOR procedure (AFNOR XP-T-90-382) and increased percentage of abnormal D-larvae was detected at concentrations of 0.05 µg/L upwards. Effects of genotoxicity were studied by combining information of sperm viability by comparing intracellular ATP titration together with measurements of sperms ability to reduce resazurin and by using alkaline comet assay. After 1 h exposure, no effects on viability were observed. However, in two out of the three alkaline comet assays genotoxic effects were seen on spermatozoa at concentrations of 0.25 and 0.50 µg/L upwards. Also, sperm membrane integrity and mitochondrial function were studied using flow cytometry analysis but no effects were observed.

In the *in vivo* tests (Behrens *et al*. 2016), the implications of both biotransformation and reactive oxygen species (ROS) production, Diuron and its main transformation products (DCPMU, DCPU and 3,4-DCA) at nominal concentrations of 0.002, 0.01, 0.05 and 0.5 µg/L with and without ascorbic acid were studied of in pacific oyster (*Crassostrea gigas*) larvae by the embryo-larval bioassay on D-larvae and the comet assay on trochophore larvae. The production of ROS on trochophore larvae exposed to Diuron and ascorbic acid was also measured. In the 24 h embryo-larval bioassay, a significant increase in the percentage of abnormal D-larvae was observed from concentration of 0.01 µg/L of Diuron and transformation product DCPMU and 0.05 µg/L of transformation product DCPU. The transformation product 3,4-DCA, however, did not show any sign of embryotoxicity. Concerning genotoxicity in comet assay (6h), damage was detected from the concentration of 0.05 µg/L of Diuron and its transformation products. When performed in presence of known oxidant scavenger, ascorbic acid, a decrease in genotoxicity and developmental abnormalities caused by Diuron and its transformation products DCPMU and DCPU was observed.

In the *in vivo* tests (Manzo *et al*. 2005), sea urchin sperm was exposed to Diuron at the concentrations of 0.25, 0.5, 1, 2, 2.5, 5, and 7.5 mg/L. Fertilization rate shows a significant progressive decrease due to a reduction of the fertilization ability of exposed sperms (NOEC 0.5 mg/L). Treated sperm that retained fertilization ability produced offspring that develop abnormally 48 hours post fertilization.

15.1.5. ED related adversity

Experimental *in vivo* evidence suggests that Diuron can have adverse effects on sexual development (sex ratio), reproduction and sexual behaviour. Key *in vivo* evidence for endocrine activity and adversity (EAS modalities) is shown in Table 44**Error! Reference source not found.**. In summary, Diuron caused exposure related feminisation of genetic male fish (*Oryzias latipes*) and decrease in male secondary sex characteristic (Li *et al*. 2021), decrease in fecundity, hatching success and sexual behaviour of *Oryzias melastigma* (Zhou *et al*. 2022), decrease in specific male histopathology (testis & sperm) across different species and taxonomic groups (Cardone *et al*. 2008, Kamarudin *et al*. 2020 and Zhou *et al*. 2022) and decrease in specific female gonadal histopathology (Kamarudin *et al*. 2020). Decrease in male testosterone levels in multiple species and taxonomic groups (Cardone *et al*. 2008, Nam *et al*. 2023 and Pereira *et al*. 2015) support potential for male reproductive impairment.

Experimental *in vivo* evidence of Diuron metabolites (3,4-DCA, DCPU, DCPMU) also suggest that the metabolites can cause ED related adverse and mechanistic effects (Pereira *et al*. 2015, Boscolo *et al*. 2018, Bhuiyan *et al*. 2019, Bhuiyan *et al*. 2021 & Ibrahim *et al*. 2021). Metabolite 3,4-DCA decreased reproduction of *Danio rerio* and sex hormone levels for both female and male fish in OECD TG 229 (Bhuiyan et a. 2019). Observed decrease in male sex hormone levels, male specific histopathology, and spermatogenesis in other studies support antiandrogenic mode of action (MoA) for metabolites. DCPU and DCPMU also show higher AhR agonistic activity compared to Diuron *in vivo* (Zhou *et al* 2022).

The results of the OECD TG 234 test are considered negative regarding endocrine related interference to sexual development and differentiation. However, no redistribution of fish or reduction of replicates was performed as required in OECD TG 234. The evaluating MSCA considers that this resulted highly biased test results as effects on individuals in replicates with a lower number of individuals have higher statistical weight. The observed increase in length, weight and maturation of fish due to low stocking density (caused by the lack of redistribution) may have masked the potential ED effects. Thus, the OECD TG 234 study is considered to have a low weight in the ED assessment. Nevertheless, the negative outcome of the FSDT study should not rule out the ED mediated adverse effects observed in other studies and mainly in the key study Li *et al*. 2021.

Table 44 Assembling and integration of key in vivo evidence for endocrine activity and adversity (EAS modalities) related to environmental ED properties.

	Type of evidence	Lines of evidence	Species	Exposure period	Effect concentration µg/L	Observed effects	Effect observed only in presence of excessive general systemic toxicity (Y/N)	Assessment of evidence	Assessment of integrated evidence
Integrated evidence for endocrine activity	In vivo mechanistic	VTG in males	*Oryzias melastigma*	180	*0.05*	Dose depended decrease of mRNA expression of vtg1 and vtg2 in liver	N	Supporting evidence	Overall supportive but not conclusive for endocrine activity
			Oreochromus mossambica	7	0.2	Increased expression of vitellogenin mRNA at	N		

						highest concentration			
			Danio rerio	63	105.44	No effect			
		VTG in females	*Danio rerio*	63	105.44	No effect		No evidence	
		VTG in fish before sex differentiation	*Sebastes schlegelii*	60	10	Decrease at the highest concentration	N	Supporting evidence	
			Pagrus major	60	10	No effect			
		Estradiol level in males	*Oryzias melastigma*	180	0.5	Increase at one concentration	N	Supporting evidence	
			Podarcis sicula	21	1.08	No effect			
		Estradiol level in females	*Oreochromis niloticus*	25	0.1	No effect		No evidence	
		Estradiol level in fish before sex differentiation	*Pagrus major*	60	10	Decrease at the highest concentration	N	Supporting evidence	
			Sebastes schlegelii	60	10	Decrease at the highest concentration	N		
		Testosterone level in males	*Oryzias melastigma*	180	5	No effect		Supportive: Decrease in testosterone levels observed in multiple studies and species.	
			Oreochromis niloticus	25	0.2	Decrease	N		
			Oreochromis niloticus	25	0.1	No effect			
			Podarcis sicula	21	1.08 (drinking water)	Decrease	N		
		Testosterone level in fish before sex differentiation	*Pagrus major*	60	10	Decrease at the highest concentration (11-ketotestosterone)	N		

			Sebastes schlegelii	60	10	Decrease at the highest concentration (11-ketotestosterone)	N		
		mRNA expression of cyp19a in testis	*Oryzias melastigma*	180	0.5	Increase at one concentration	N	Supporting evidence	
			Oryzias latipes	10	0.033	Increase at mid concentration	N		
Integrated evidence for adversity	EATS mediate parameters	Specific male histopathology	*Oryzias melastigma*	180	0.005	Decrease in sperm motility	N	Supportive: Decrease in specific male histopathology across different species and taxonomic groups	Overall positive evidence for adversity
			Oryzias melastigma	180	0.05	Decrease in testis histopathology (various parameters and effect levels)	N		
			Oryzias javanicus	21	50	Consistent Decrease in gonadal staging	N		
			Oreochromis niloticus	25	0.2	No effect on diameter of seminiferous tubule			
			Podarcis sicula	21	1.08 (drinking water)	Decrease in testis histopathology	N		
		Specific female histopathology	*Oryzias javanicus*	21	50	Consistent Decrease in gonadal staging	N	Supportive: Decrease in female histopathology	
		Sex ratio	*Oryzias latipes*	90	0.041	Consistent increase of genetic males with female phenotype (%). Similar effect as positive control (17β-estradiol 0.2 µg/L)	N	Sufficient: exposure related feminisation of genetic male fish comparable to positive control (17β-estradiol)	
			Danio rerio	63	105.44	No effect			

		Secondary sex charasteristics	*Oryzias latipes*	90	0.041	Consistent decrease of anal fin rays with papillary processes.	N	Sufficient: Decrease in male secondary sex charasteristics	
	Sensitive to, but not diagnostic of, EATS	Fecundity	*Oryzias melastigma*	180	0.5	Decrease in fertilisation success (F1)	N	Supportive: Concentration related decrease in fecundity in one study	
		Hatching success	*Oryzias melastigma*	180	0.5	Decrease in hatching rate (F1)	N	Supportive: Concentration related decrease in hatching success in one study	
		Sexual behaviour	*Oryzias melastigma*	180	0.5	Decrease in time of copulation and following time (5 µg/L)	N	Supportive: Decrease in male sexual behaviour	

15.1.6. ED activity

The available *in vitro* and *in vivo* data support that Diuron have antiandrogenic and aryl hydrocarbon receptor (AhR) -mediated activity (Table 37). The interaction with estrogen receptor is weaker. Metabolites DCPU, DCPMU, 3,4-DCA, and 3,4-DCAA have been reported to bind to the androgen receptor and 3,4-DCA can affect steroidogenesis *in vitro*. The information available for Diuron and its metabolites provide supporting evidence for ED activity and that Diuron can act through multiple ED modes of actions.

Due to the structural similarity with Linuron and the androgen receptor binding activity of both Diuron and Linuron and their shared transformation products (DCPU, DCPMU, and 3,4-DCA), the information available supports that Diuron may have the same kind of antiandrogenic effect on fish as Linuron although the available *in vitro* studies indicate that there may be a difference in potency.

15.1.7. Mode of action analysis

Results from *in vitro* and *in vivo* studies suggest that Diuron may interact with the endocrine system through multiple endocrine modes of actions (MoA). The available *in vitro* and *in vivo* data support that Diuron have antiandrogenic and aryl hydrocarbon receptor (AhR) -mediated activity. This is supported by Danish QSAR data and ToxCAST model predictions for metabolite DCPMU. Due to the potential cross-talk between multiple ED related pathways and the potential impact of ED active metabolites of Diuron, it is likely that no single molecular initiating event (MIE) is responsible for the observed effects *in vivo*. Zhou *et al*. 2020 showed that the residues of DCPMU, DCPU and 3,4-DCU in fish muscle increased together with increasing exposure concentration of Diuron. Thus, in many studies it might not be possible to demonstrate whether the observed adverse effects are induced by Diuron or the metabolites (or combination).

ED mediated adversity has been demonstrated in feminisation of genetic male fish (*Oryzias latipes*), decrease in male secondary sex characteristic, decrease in specific male histopathology (testis & sperm) across different species and taxonomic groups, and decrease in specific female gonadal histopathology. Especially, the skewed sex-ratio supports EAS related MoA. Even though the skewed sex ratio was determined based on secondary sex characteristics in Li *et al*. 2021, thus being not as strong an indicator as if based on gonad histopathology, it can be considered as strong evidence for population relevant ED mediated adversity. 17β-Estradiol was used as positive control in the Li *et al*. 2021 study and showed similar response to Diuron (difference in potency). According to ECHA & EFSA 2018 a change in sex ratio of fish accompanied by gonad histopathological findings is seen as both adverse and highly likely to be EAS mediated. In such case, conclusion on biologically plausible link can be reached without detailed MoA analysis based on existing knowledge and the lack of non-ED MoA. In the case of Diuron gonad histopathological findings were not studied together with the sex ratio in Li *et al*. 2021 but were demonstrated in other studies (Table 44).

For AhR mediated pathway there is one non endorsed AOP 310 (Embryonic Activation of the AhR leading to reproductive failure, via epigenetic down-regulation of GnRHR) available in AOP wiki for fish reproduction failure (Table 45**Error! Reference source not found.**). AOP is rather specific and includes many key events leading to adverse outcome (decreased reproduction). Based on the available information the AOP 310 is plausible but there is not enough information available on all key events. Limited information is available for mechanisms related to gonadotrophin releasing hormone receptor and linking it to the observed decrease in fecundity and spawning in fish.

Zhao *et al*. 2022 showed that exposure to Diuron for 180 days could have an effect on hypothalamus-pituitary-gonadal-liver axis manifested by sexual behaviour disorder, inhibited testis maturation, decreased sperm quality, abnormal growth and development of F1 embryo and larvae. Authors of Zhou *et al*. 2022 suggested that mechanism of developmental toxicity in F1 embryo and larvae may be related to AhR signalling pathway and suggested potential cross-talk between AhR and ER signalling pathways. AhR pathway is known to cross-talk with estrogen, progestin, androgen, glucocorticoid and thyroid hormone receptor pathways (Petersen *et al*. 2006). AhR ligands both positively and negatively modulate ER and AR signaling pathways (Beischlag & Perdew 2005, Matthews *et al*. 2005), liganded AhR acts as an E3 ubiquitin ligase, and degrades ER and AR *in vitro* and *in vivo* (Ohtake *et al*. 2007). Well known AhR agonist, TCDD, has been observed to cause post-hatch mortality, stunted growth, hemorrhage, craniofacial malformations and yolk sac edema in early fish life stages (King-Heiden *et al*. 2012). Similar effects have also been observed for Diuron (OECD TG 234, Velki *et al*. 2017 & Zhou *et al*. 2022).

Adverse effects on male and female gonads have been observed for Diuron and its metabolites across multiple studies and taxonomic groups (fish and reptiles). There is also information on decreasing parameters relevant for reproduction success, which is shown together with changes in reproductive hormones. However, due to potential cross-talk with multiple endocrine related pathways and modes of actions, detailed MoA for decreasing reproduction success is not discussed further in this evaluation.

Table 45 AOP 310 Embryonic Activation of the AHR leading to Reproductive failure, via epigenetic down-regulation of GnRHR

	Brief description	**Supporting evidence**
MIE	Activation, AhR	Positive results for Diuron from ToxCast. AhR agonist activity detected for Diuron, DCPMU, and DCPU *in vivo* in *tg*(*cyp1a-12DRE:egfp*) larvae (Zhou *et al*. 2022).
KE	Dimerization,AhR/ARNT	No evidence
KE	Increase, DNMT expression	Significant upregulation of *dnmt1* in brain, ovary and liver in F1 females (marine medaka) caused by Diuron exposure in the parent generation (F0) (Bao *et al*. 2022).

KE	Increase, hypermethylation of the promoter region of GnRHR	No evidence
KE	Decrease, expression of GnRHR	No change observed in F0 male (marine medaka) (Zhou *et al*. 2022). Decrease in F1 female from parental (F0) exposure (Bao *et al*. 2022).
KE	Reduction, 17beta-estradiol synthesis by ovarian granulose cells	No evidence
KE	Reduction, 17beta-estradiol concentrations	Significantly (dose dependent) lowered E2 concentration in F1 females (marine medaka) caused by F0 Diuron exposure (Bao *et al*. 2022). Decrease in 17β–estradiol levels after Diuron exposure (Nam *et al*. 2023 & Joseph *et al*. 2024) and 3,4-DCA exposure (Bhuiyan *et al*. 2021)
KE	Reduction, vitellogenin synthesis in liver	Significantly lowered *vtg1* and *vtg2* expression in F1 females (marine medaka) caused by F0 Diuron exposure (Bao *et al*. 2022). - *vtg1* and *vtg2* expression in liver down-regulated in males (marine medaka) following Diuron exposed (Zhou *et al*. 2022).
KE	Reduction, plasma vitellogenin concentrations	Decrease of VTG concentrations in some fish species (Nam *et al*. 2023)
KE	Reduction, Vitellogenin accumulation into oocytes and oocyte growth/development	Significant increase in previtellogenic oocytes and decrease in vitellogenic and mature oocytes in F1 females (marine medaka) caused by F0 Diuron exposure (Bao *et al*. 2022). Decreased median gonadal stage in female Javanese medaka (*O. javanicus*) exposed to Diuron (Kamarudin *et al*. 2020). Reduction in gonadal stage in females exposed to 3,4-DCA (Ibrahim *et al*. 2021).
AO	Reduction, cumulative fecundity and spawning	Decreased hatching rate and fertilization rate, increased malformations and mortality rate in F1 generation (marine medaka) caused by Diuron exposure of F0 males (Zhou *et al* 2022). Reduction of spawned eggs after exposure to 3,4-DCA (Ibrahim *et al*. 2021 & Bhuiyan *et al*. 2021).

In addition, Diuron caused impacts on thyroid hormone levels (T_3 and T_4) and thyroxin binding capacity in two inhalation studies in rats. Linuron has shown similar impacts on thyroid hormone levels in rat. Linuron is also thyroid active substance in amphibian (*Xenopus laevis*) eleutheroembryo in XETA (OECD TG 248). Both Diuron and Linuron suggest TPO inhibition in ToxCast assay. Diuron caused deformities and effects on length and wet weight to the amphibians *Pseudacris regilla* and *Rana catesbeiana*. Diuron also caused delay of appearance of forelimbs and hindlimbs in *Rana aurora*. However, based on the available information evaluating MSCA considers that no further conclusion is possible regarding environmental ED concern for thyroid modality.

15.2. Endocrine disruption – Human health

Sex hormones

The available *in vitro* data indicate that Diuron may have antiandrogenic and aryl hydrocarbon receptor (AhR) -mediated activity. The interaction with estrogen receptor is weak. All of the 15 ToxCast *in vitro* assays for estrogen receptor activity included in the U.S. EPA (2019b) EDSP21 Dashboard were inactive. Transformation products of Diuron DCPU, DCPMU, 3,4-DCA, and 3,4-DCAA have been reported to bind to the androgen receptor *in vitro*.

The available reproductive toxicity studies provided in the registration dossier do not indicate effects that would indicate reproductive toxicity resulting from endocrine disrupting mode of action. Reduced pup weights seen in the two-generation reproductive toxicity study (Unpublished 1990c) and reduced fetus weights and delayed ossification (vertebrae and sternum) seen in the prenatal developmental toxicity study in rats (Unpublished 1986a) indicate growth retardation, but clear conclusion on mechanistic background of these effects is not possible. No effects were seen in reproductive organs in the available repeated dose toxicity that would indicate effects resulting from endocrine disrupting mode of action. Increased relative testicle weights at the top dose males were reported in one chronic dog study via oral route (Unpublished 1985a), but the effect was considered unrelated to Diuron treatment due to reduced body weights of high dose animals and because there were no corresponding histopathologic findings.

In carcinogenicity studies increased incidences of tumour types that may have endocrine-mediated mode of action were reported (uterine adenocarcinoma in rat, mammary gland adenocarcinoma and ovarian luteoma in mice). However, these tumour findings were not consistent between species and mechanistic studies do not support tumorigenesis in mammary gland. No findings suggesting mode of action possibly related to these tumour types (e.g., estrogenicity) were reported in any of the available other in vivo studies with Diuron. The RAC opinion[9] concluded that there is sufficient evidence of carcinogenicity based on the clear increase in urinary tract malignant tumours in male and female rats. The presumed mechanism of action is cytotoxicity which leads to regenerative hyperplasia and subsequently to tumours. The threshold level is not known. There is also sufficient evidence of carcinogenicity in mice based on the statistically significant increase in mammary gland tumours. No increase in this tumour type was observed in rats. The increase in the incidence of malignant uterus tumours in rats and benign ovarian tumours in mice provide supportive evidence for classification. It could not be excluded that the tumours observed in uterus, mammary gland and ovary were endocrine-mediated, but no data are available to substantiate this hypothesis. The evaluating MSCA note that the provided two-generation reproductive toxicity study (Unpublished 1990c) and prenatal developmental toxicity studies (Unpublished 1986a and 1986b) were conducted before the OECD had updated test guidelines 414 and 416 in 2001. The original OECD test guideline 416 (from 1986) and OECD test guideline 414 (from 1981) were revised to include additional endpoints for the evaluation of effects on male and female fertility and developmental toxicity. Several of these new endpoints are particularly sensitive also with respect to endocrine disruption. Thus, two-generation study conducted before the inclusion of sensitive endocrine endpoints by itself may not be considered adequate for demonstrating the probable absence of endocrine disrupting activity although it still provides valuable data mainly restricted to fertility and effects on reproductive organs (OECD, 2018).

The evaluating MSCA considers that there is uncertainty whether the reproductive toxicity studies provided in the registration dossier have the ability to detect possible endocrine disruption and all effects on reproduction (adverse effects on fertility and development) because the OECD test guidelines applied do not include all endpoints of the current OECD test guidelines. On the other hand, the evaluating MSCA considers some of the studies

[9] RAC opinion (2021) [04.01-ML-014.03] (europa.eu)

from open literature (i.e. Fernandes *et al*. 2007, 2012; Grassi *et al*. 2011) relevant for the evaluation of effects on male and female reproductive system relevant for reducing these uncertainties.

In the Fernandes *et al*. studies (2007, 2012) reproductive parameters in adult males and male offspring were not significantly altered i.e. no effects on reproductive organs (weight and morphology), sperm parameters (daily sperm production, sperm number, transit time in the epididymis, sperm motility and morphology) or plasma testosterone were detected. According to authors of the Fernandes *et al*. study (2012), the assessment of reproductive/developmental endpoints in the F1 generation followed the OECD 443 test guideline.

In the Grassi *et al*. study (2011a) reproductive parameters in female offspring were not significantly altered including those for sexual maturation (i.e oestrus cyclicity or vaginal opening) and hormone concentrations (serum oestrogen, progesterone, FSH or LH). However, in female pups, significantly lower mean ovary weight at high dose (1250 ppm) and significantly reduced mean numbers of corpora lutea at intermediate (750 ppm) and high doses were observed. This could indicate ovarian toxicity of Diuron. However, body weights of female pups were statistically significantly reduced at all dose levels (500 ppm, 750 ppm and 1250 ppm) in this study suggesting that Diuron exposure may have generally interfered with growth and development of the offspring. Therefore, the evaluating MSCA concludes that the toxicological relevance of ovarian findings in the study by Grassi *et al*. (2011a) is not clear. The evaluating MSCA notes that in the two-generation study Diuron had no effect on fertility or reproductive performance up to highest dose (1750 ppm) and no remarkable findings were reported in the histopathology of ovaries in P1 and F1 dams.

No significant effect on number of corpora lutea was observed in two guideline prenatal developmental toxicity studies in adult rats (up to 400 mg/kg bw) and rabbits, or in the Fernandes *et al*. (2007) study (up to 250 mg/kg bw/day) in rats. In the two-generation reproductive toxicity study and in two prenatal developmental toxicity studies pup weights were decreased significantly at the highest dose levels. However, maternal weights were also significantly decreased at these dose levels.

Overall, on balance, the evaluating MSCA considers that available repeated dose toxicity, chronic toxicity/carcinogenicity studies and data from open literature are relevant for reducing the uncertainties related to the missing endpoints associated to endocrine disruption in the reproductive toxicity studies.

Thyroid hormones

The three *in vitro* assays included in the EDSP21 Dashboard studying promotion of thyroid receptor mediated DNA transcription, agonism and antagonism of the thyroid receptor signalling pathway by TRE activation and inhibition were inactive. Diuron did not inhibit human iodothyronine deiodinase enzymes (i.e., deiodinase type 1, deiodinase type 2, and deiodinase type 3) at 200 µM in the three in vitro assays (Olker *et al*., 2019).

Diuron induced a downregulation in rat thyroid tissue derived thyroid peroxidase catalytic activity by 50% at 40 µM compared to DMSO in the ToxCast assay NCCT_TPO_AUR_dn. The result of this screening assay suggests the potential of Diuron to inhibit TPO.

Diuron exposure in two subacute inhalation studies in rats lead to effects on thyroid hormones levels (T_3 and T_4) and thyroxin-binding capacity (TBC) indicating reduced thyroid function.

In a sub-acute inhalation study (Unpublished 1986c) comparable to guideline OECD TG 412 (Klimisch 1), Wistar rats were exposed to doses of 0, 4.1, 37.4 and 268.1 mg/m³ of an aerosol of Diuron for 6 h per day, 5 days per week for 4 or 8 weeks. Clinical laboratory examinations were conducted after four or eight weeks on five rats per sex and dose. In males T_4 decreased statistically significantly at 268 mg/m^3 after 4 and 8 weeks. No statistically significant changes were observed in T_3 or TBC. In females there was statistically significant decrease in T_3 at the middle dose level (37.4 mg/m^3) after 8 weeks. TBC was significantly increased at high dose females after 8 weeks. Thyroid-stimulating

hormone (TSH) levels were not measured. No changes were observed in thyroid gland weight or histopathology. Effects on thyroid functioning were observed in the presence of systemic toxicity (hematology and spleen).

Table 46 Thyroid hormone levels and TBC. Values are given as 4 weeks values / 8 weeks values

Dose group [mg/m^3]	**0**	**4.1**	**37.4**	**268.1**
MALES				
Tri-iodothyronine(T3) [nmol/l)	1.22/0.91	1.17/0.92	1.05/0.84	0.97/0.74
Thyroxine (T4) [nmol/l]	77/52	69/46	63/44	47**/36*
Thyroxine binding capacity (TBC)	0.76/0.78	0.78/0.79	0.81/0.78	0.80/0.83
FEMALES				
Tri-iodothyronine(T3) [nmol/l)	1.15/0.92	1.28 /0.81	1.23/0.64**	1.09/0.72
Thyroxine (T4) [nmol/l]	50 /46	54/46	54/39	42/36
Thyroxine binding capacity (TBC)	0.71/0.80	0.68 /0.84	0.63**/0.86	0.70/0.85 *

* p<0.05, **p<0.01

In a second sub-acute inhalation study (Unpublished 1986d) comparable to guideline OECD 412 (Klimisch 2), Wistar rats were exposed (in tubes) to nominal doses of 0, 6.6, 47.6 and 311 mg/m³ of an aerosol of Diuron for 6 h per day, 5 days per week for 21 days. Clinical laboratory examinations were conducted at the end of the study on ten rats per sex and dose. The statistically significant reduction in T_3 and T_4 values at 311 mg/m^3 in males, with roughly simultaneously increased TBC values were seen at 47.6 and 311 mg/m^3. In females T_4 values were statistically significantly decreased at 311 mg/m^3 and TBC values were increased at middle and high dose group. TSH levels were not measured. The absolute weight of thyroid at 20 mg/m^3 females was significantly decreased but relative thyroid weight was not changed. No changes were observed in thyroid gland histopathology. Effects on thyroid functioning were observed in the presence of systemic toxicity (hematology and spleen).

Table 47 Thyroid hormone levels and TBC

Dose group [mg/m3]	**Air**	**0**	**6.6**	**47.6**	**311**
MALES					
Tri-iodothyronine(T3) [nmol/l)	1.01	0.91	0.92	0.90	0.77**
Thyroxine (T4) [nmol/l]	63	65	62	64	53**
Thyroxine binding capacity (TBC) [TBI]	0.64	0.68	0.71	0.79**	0.84**
FEMALES					
Tri-iodothyronine(T3) [nmol/l)	0.91	0.89	1.04	0.99	0.91
Thyroxine (T4) [nmol/l]	54	70**	59	55	41*

Thyroxine binding capacity (TBC) [TBI]	0.56	0.61	0.61	0.66**	0.67**

*p<0.05, **p<0.01

In summary, the available two *in vivo* inhalation studies in rats show comparable effects on T_3, T_4 and TBC in both genders suggesting that Diuron interact with the hypothalamus-pituitary-thyroid (HPT) axis by affecting on thyroid hormone levels. There is a dose-dependent trend in the decreased thyroid hormone levels, at least in males.

Thyroid hormone levels were not measured in other available toxicity studies. The 90-day subchronic repeated dose toxicity study in rats and one-year chronic dog study via oral route (Unpublished 2004 and 1985a) did not reveal any changes in thyroid gland weight or histopathology. No histopathological changes were seen in pituitary. In the oral chronic toxicity/carcinogenicity studies in rats and mice (Unpublished 1985b, 1990b) no effects on thyroid or pituitary were observed. In the chronic toxicity/carcinogenicity studies thyroid gland and pituitary was examined macroscopically and after that microscopically, if abnormal or suspected of being neoplastic. Thyroid gland or pituitary were not weighed in these studies. In the two-generation reproductive toxicity study (Unpublished 1990c) thyroid glands were not weighed and examined histopathologically. No histopathological changes were seen in pituitary.

According to the EFSA/ECHA guidance (Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009, Appendix A, page 103), a decrease in T_4 hormone levels should act as a trigger for further studies. Substances that alter the circulating levels of T_3 and/or T_4 without histopathological findings would still present a potential concern for neurodevelopment.

Overall, based on the available information, the evaluating MSCA considers that the data from subacute inhalation studies represent a borderline case for requesting further studies.

Structurally similar substance – Linuron

Linuron, structurally similar substance, has a harmonized classification as carcinogenic category 2 and as toxic to reproduction category 1B in the Annex VI of CLP Regulation (Regulation (EC) 1272/2008). The approval of Linuron for use in plant protection products under the PPP Regulation (Regulation (EC) No 1107/2009) was not renewed in 2017 because Linuron is classified as toxic for reproduction category 1B and negligible exposure could not be demonstrated. Furthermore, Linuron was considered to have endocrine disrupting properties in accordance with interim criteria for endocrine disrupters. Linuron has shown to impair fertility and overall reproductive performance and to produce developmental toxicity in several studies (EFSA 2016, Renewal Assessment Report on Linuron, February 2016). Linuron has shown to be antiandrogenic and has adverse effects on different endocrine organs. In the published literature, male pups have shown increased retention of areolae/ nipples, induction of epididymal malformations, testicular lesions and slight decreases in anogenital distance. Mechanistic studies indicated that Linuron is an anti-androgenic substance.

The endocrine disrupting potential of Linuron has also been evaluated as part of the U.S. EPA's Endocrine Disruptor Screening Program (EDSP). The Endocrine Disruptor Screening Program`s (EDSP) Tier 1 assay battery is designed to provide the data to evaluate the potential of chemicals to interact with the estrogen (E), androgen (A) or thyroid (T) signaling pathways. In addition to the available Tier 1 assay data, other scientifically relevant information, including general toxicity data and open literature studies of sufficient quality were considered in this weight of evidence assessment.

According to the EDSP Weight of Evidence Conclusions on the Tier 1 Screening Assays for Linuron (U.S. EPA 2015), Linuron appears to act as an anti-androgen both in vitro and in vivo (see website: https://www.epa.gov/endocrine-disruption/endocrine-disruptor-screening-program-tier-1-screening-determinations-and). Linuron *in vitro* exhibited competitive binding to the AR and activated AR transcriptional activity in multiple species

in vitro (human, fathead minnow, and rainbow trout). Linuron also inhibited *ex vivo* testosterone production in fetal rat testes. The findings of multiple Hershberger assays, male pubertal assays, and reproductive developmental toxicity studies in rats, as well as assays measuring the inhibition of androgen-induced spiggin production by female stickleback fish kidney cells, support that Linuron exhibits anti-androgenic effects *in vivo*. In mammalian studies, Linuron caused changes in testes and epididymal weights and histopathology in the testes and epididymides.

There was evidence of potential interaction of Linuron on thyroid signaling in mammals characterized by changes in thyroid hormone levels in the absence of changes in thyroid weight or histopathology in several studies. Although no thyroid histopathology effects were observed in any of the studies, decreases in serum T_3 and/or T_4 levels were observed across multiple studies including the EDSP Tier 1 female pubertal assay, a 12-week female rat thyroid toxicity study, and two 15-day adult male rat screening assays.

In the Tier 1 female pubertal assay, dose-dependent decreases in T_4 and pituitary weight were observed, but no corresponding changes were seen in thyroid weight or histopathology. The thyroid effects observed occurred only in the presence of overt toxicity (signs of systemic toxicity). In the validation of the male pubertal assay, serum T_4 levels were decreased at the high dose (100 mg/kg/day), TSH was decreased at the low dose (50 mg/kg/day), and pituitary weights were decreased at both dose levels; however, the high dose animals in this study exhibited signs of overt toxicity. Dose-dependent decreases in both T_3 and T_4 levels were observed in the 15-day adult rat screening study, with no changes in TSH levels or thyroid histopathology or weights; the effects in the high dose occurred in the presence of overt toxicity. Dose-dependent decreases in T_3 and T_4 levels were also observed in a thyroid toxicity study at doses of 16.3 and 65.5 mg/kg/day. No overt toxicity was noted in this study. None of the above studies showed changes in thyroid weight or histopathology, and changes in pituitary weight were only noted in the female pubertal assay and the validation trial of the male pubertal assay. As a consequence of thyroid findings, special thyroid assay in pregnant animals, postnatal, and adult animals was recommended for Tier 2 testing to generate specific data on the thyroid to protect the developing nervous system from thyroid hormone disrupting chemicals.

In summary, *in vivo* studies and *in vitro* studies on Linuron, shows that Linuron has antiandrogenic properties. Although no anti-androgenic effects have been seen in mammals *in vivo,* the available *in vitro* data indicate that Diuron may have antiandrogenic activity.

In vivo studies on Linuron, such as Diuron, has shown potential interaction of Linuron with the HPT axis in mammals characterized by changes in thyroid hormone levels. The mechanistic background of potential interaction of Linuron with the HPT axis is not well known. Both Diuron and Linuron induced a downregulation in rat thyroid tissue derived thyroid peroxidase (TPO) catalytic activity in the *in vitro* ToxCast assay NCCT_TPO_AUR_dn (U.S. EPA 2019b).

Conclusion on endocrine disrupting effects in mammalians

Based on the available information the ED concern for human health can be considered as inconclusive as the data is not sufficient for ED conclusion. The RAC opinion[10] concluded that there is sufficient evidence of carcinogenicity based on the clear increase in urinary tract malignant tumours in male and female rats. However, it could not be excluded that the tumours observed in uterus, mammary gland and ovary were endocrine-mediated, but no data are available to substantiate this hypothesis.

[10] RAC opinion (2021) [04.01-ML-014.03] (europa.eu)

15.3. Conclusions of the endocrine disrupting properties assessment and related classification and labelling

15.3.1. ED environment

According to the hazard class for endocrine disruption for the environment in CLP regulation a substance can be classified in Category 1 if it meets all the following criteria:

a) endocrine activity;
b) an adverse effect in an intact organism or its offspring or future generations;
c) a biologically plausible link between the endocrine activity and the adverse effect.

The evaluating MSCA considers that the observed skewed sex-ratio demonstrates strong evidence for population relevant ED mediated adversity. EA(T)S mediated parameters provide overall positive evidence for adversity. The available *in vitro* and *in vivo* data also support that Diuron have antiandrogenic and aryl hydrocarbon receptor (AhR) -mediated activity. According to ECHA & EFSA 2018 a change in sex ratio of fish accompanied by gonad histopathological findings is seen as both adverse and highly likely to be EAS mediated. In such case, conclusion on biologically plausible link can be reached without detailed MoA analysis based on existing knowledge and the lack of non-ED MoA. The evaluating MSCA considers that this applies to the present case with Diuron. There is also no evidence demonstrating that the observed adverse effects are solely non-specific consequences of other toxic effects.

Based on the available information, the evaluating MSCA considers that Diuron can be classified as endocrine disruptor for the environment in Category 1.

15.3.2. ED human health

During the initial SEv assessment in 2014-2015 the evaluating MSCA deemed it at that stage appropriate to wait for i) the RAC opinion on the proposed harmonised classification of Diuron in relation to carcinogenicity under the CLP Regulation (Regulation (EC) 1272/2008) and ii) the outcome of the forthcoming ED-evaluation of Diuron under the Biocidal Products Regulation (Regulation (EU) 528/2012) in 2020, before considering further information requests on human health. This was considered necessary in order to align the assessments under different regulatory frameworks in the EU.

The RAC opinion[11] concluded that there is sufficient evidence of carcinogenicity in animals based on the strong increase in urinary tract malignant tumours in male and female rats. According to RAC there is also sufficient evidence of carcinogenicity in mice based on the statistically significant increase in mammary gland tumours. The increase in the incidence of malignant uterus tumours in rats and benign ovarian tumours in mice also provide supportive evidence for classification. However, RAC stated that the tumours observed in uterus, mammary gland and ovary may be endocrine-mediated, but there are no data to substantiate this hypothesis.

The concern for endocrine disruption (human health) can be considered inconclusive as the information available is neither sufficient for identifying the Substance as an endocrine disruptor for human health nor for excluding the concern. However, the evaluating MSCA considers that due to the ongoing ED assessment under Biocidal Products Regulation (Regulation (EU) 528/2012) and the fact that the ED concern is confirmed for the environment, it is currently not justified to request further information under REACH Regulation. The evaluating MSCA considers that a classification of the Substance as an ED for human health would be unlikely to result in further risk management measures

[11] RAC opinion (2021) [04.01-ML-014.03] (europa.eu)

compared to the obligations coming from the classification as Carc. 1B in Commission Delegated Regulation (EU) 2024/197.

16. PBT/vPvB and PMT/vPvM assessment

A definitive assessment of PBT/vPvB or PMT/vPvM properties was not in the scope of this substance evaluation. However, information on these properties have been partly reviewed to obtain a view of the environmental fate of the Substance. Therefore, the available information relevant for PBT/vPvB or PMT/vPvM properties of the Substance (presented in the previous sections) is summarised in the sections below.

16.1. Persistence

The Substance is not readily biodegradable. Simulation tests for freshwater water-sediment systems and soils are available (summarized in Table 48). In freshwater water-sediment systems dissipation half-lives were 48-232 days and in soils 20-352 days.

The registrants concluded that the Substance is not readily biodegradable and that therefore in the sense of PBT assessment the Substance is persistent "P".

The evaluating MSCA considers that as the Substance is not readily biodegradable and as dissipation half-lives in freshwater sediments and soils higher than 180 days have been reported, it may fulfil the persistence (P) and very persistence (vP) criteria, also considering that most of the studies have been conducted at 20 °C and that the reference temperature for providing results is 12 °C (ECHA 2023). However, full assessment regarding the reliability and relevance of the studies and the derivation of the half-lives has not been conducted by the evaluating MSCA.

In the simulation tests, transformation/degradation products and non-extracted residues were identified but their PBT properties have not been analysed in the present assessment. DCPMU, DCPU, m-CPDMU and 3,4-DCA were the main transformation/degradation products. For the transformation/degradation products no previous persistence assessments are available. Considering the degradation data for 3,4-DCA (European Chemicals Bureau 2006) 3,4-DCA would potentially fulfil the P and vP criteria as it is not readily biodegradable and DT50 for soil is >470 days. In addition, the fact that DCPMU and/or DCPU were formed and remained at levels of 0.5-22% of applied radioactivity in the simulation tests on the Substance, suggests potential persistence of these transformation/degradation products. It is also noted that DCPMU and DCPU are structurally relatively similar to the Substance and 3,4-DCA and therefore may be similar in persistence.

Based on the available information, the evaluating MSCA considers that the Substance and some of its transformation/degradation products may fulfil the persistence (P/vP) criteria of the REACH Regulation (EC) No 1907/2006 and the Commission delegated regulation (EU) 2023/707.

Table 48 Simulation test results relevant for P/vP assessment for Diuron.

Compartment	Details of samples	Tempera-ture	Dissipation half-life (DT_{50})	Mineralization
Water/sediment system (freshwater)	two different water/sediment systems	20 °C	48-232 d	max. 2-30% during 120 d
Soil	one soil	25 °C	352 d	max. 14.9% during 365 d

Soil	one soil	20 °C	118 d	max. 31.8% during 101 d
Soils (three samples, one with two different treatments)	three soils, one of which was studied at two different moistures	20 °C	20-119 d	not analysed
Soil	one soil	20 °C	112 d	not applicable (recalculation study)
Soil	one soil	10 °C	143 d	not analysed

P/vP criteria for soil and for freshwater sediment: $T_{1/2}$ >120 (P); $T_{1/2}$ >180 (vP)

16.2. Bioaccumulation

Log Kow for Diuron is <3 and does not suggest a potential for bioaccumulation in aquatic organisms. The BCF values in a *Mytilus edulis* bioaccumulation test (OECD 305C) and calculated BCF values (CSR) are below the B/vB criteria. However, BMF values of 1.3 - 2 have been determined in a brackish water lagoon (Roche *et al*. 2009). According to the guidance R.7c (ECHA 2023) a reliable field BMF or TMF value significantly higher than 1 can be considered an indication of very high bioaccumulation. In addition, the screening criteria for bioaccumulation in air-breathing organisms are fulfilled. Based on these observations the evaluating MSCA considers that the Substance may fulfil the bioaccumulation (B/vB) criteria of the REACH Regulation (EC) No 1907/2006 and the Commission delegated regulation (EU) 2023/707.

16.3. Mobility

Log Koc values according to OECD TG 107 for Diuron are mainly <3 and suggest a potential for mobility. The available information suggest that Diuron meets the mobility (M) criterion (log Koc ≤3) for PMT according to the Commission delegated regulation (EU) 2023/707).

16.4. Toxicity

Diuron meets the toxicity criteria (T) of the REACH Regulation (EC) No 1907/2006 and the Commission delegated regulation (EU) 2023/707 based on the following:

- The Substance is classified for Specific target organ toxicity – repeated exposure, category 2 (STOT RE 2) (according to Regulation EC No 1272/2008).
- The harmonised classification of the Substance in Annex VI of Regulation (EC) 1272/2008 has recently been amended for carcinogenicity in category 1B (Carc. 1B).
- NOEC value (survival) below 0.01 mg/l has been reported for zebrafish (*Danio rerio)* in FSDT study.

The evaluating MSCA considers that the Substance fulfils the toxicity (T) criterion of the Commission delegated regulation (EU) 2023/707) also on the following basis, in addition to those listed above:

- Endocrine disrupting properties for the environment, fulfilling the criteria for classification as endocrine disruptor for the environment in Category 1.

In addition, NOEC values below 0.01 mg/L have been reported for the alga *Navicula pelliculosa,* the cyanobacterium *Synechococcus leopoliensis* and three different

macrophytes (*Ceratophyllum demersum, Chara globularis and Elodea canadensis*)[12]. The toxicity (T) criterion can be met also based on the NOECs from these studies.

16.5. Conclusions of the PBT/vPvB/PMT/vPvM assessment and related classification and labelling

Regarding the hazard class "Persistent, Bioaccumulative and Toxic or Very Persistent, Very Bioaccumulative properties", the evaluating MSCA concludes that the Substance fulfils the toxicity (T) criterion and may fulfil the persistence (P/vP) and bioaccumulation (B/vB) criteria. Therefore, classification due to Persistent, Bioaccumulative and Toxic and/or Very Persistent, Very Bioaccumulative properties may be warranted.

Regarding the hazard class "Persistent, Mobile and Toxic or Very Persistent, Very Mobile properties" (Commission delegated regulation (EU) 2023/707) the evaluating MSCA concludes that the Substance fulfils the toxicity (T) criterion and may fulfil the persistence (P/vP) and mobility (M) criteria. Therefore, classification due to Persistent, Mobile and Toxic properties may be warranted.

The evaluating MSCA considers that further assessment of M/vM, B/vB and P/vP criteria is needed before it is possible to conclude on the need to propose an update of the current harmonised classification and labelling of Diuron due to the potential PMT and/or PBT/vPvB properties. This assessment will be done outside this substance evaluation.

17. Exposure assessment

Quantitative exposure assessment was out of the scope of this substance evaluation. According to the information in the registration dossier the Substance is used in industrial sites in the manufacture of rubber products and polymer preparations. The evaluating MSCA considers that available information indicates wide dispersive use: release of the Substance to the environment can occur from industrial use and, in addition, is likely to occur from outdoor use in long-life materials with high or low release rates e.g. from tyres or from construction or building materials due to weathering conditions.

The exposure of environment is of concern due to the identified hazards of the Substance (including e.g. endocrine disruption), uses, and the information available on the persistence, bioaccumulation, mobility, and environmental presence of the Substance. Due to the same reasons, as well as the predicted environmental distribution (Section 12.2.3) and the reported presence of the Substance in wastewater treatment plant effluents, surface water, and groundwater (Section 12.2.4) as well as in biota (Section 12.4.1), the initial concern for ground and surface water pollution is considered relevant. It should be noted that the Substance has also uses not belonging to the scope of the REACH regulation and the concentrations in the environment may be influenced by all uses of the Substance which cause release to the environment. The reported concentrations in effluents and in the environment inform on the presence and distribution of the Substance in the environment but not explicitly on exposure from REACH uses.

The evaluating MSCA notes that the endocrine disrupting property for the environment should be considered as a hazard for which no safe threshold can be defined, unless otherwise justified, and this needs to be taken into account in the risk management. The potential PMT and/or PBT/vPvB properties are also properties which, if confirmed, would prevent the determination of a safe threshold for the Substance.

[12] RAC opinion (2021): [04.01-ML-014.03] (europa.eu)

17.1. Human health

Not assessed.

17.2. Environment

Not assessed.

17.3. Combined exposure assessment

Not assessed.

18. Risk characterisation

Not assessed.

19. References

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20. Abbreviations

AFSS	Androgenised female stickleback screen
AhR	Aryl hydrocarbon receptor

AR	Androgen receptor
BCF	Bioconcentration factor
BPR	Biocidal products regulation (EU) 528/2012
BMF	Biomagnification factor
CAS RN	CAS registry number
CCH	Compliance check
CLP	Classification, labelling and packaging
CoRAP	Community rolling action plan
CYP	Cytochrome P450
DMEL	Derived minimal effect level
DNEL	Derived no-effect level
DNMT	DNA methyltransferase
EAS	Estrogen, Androgen, Steroidogenesis
EC	European community
ECHA	European chemicals agency
ED	Endocrine disruption
ER	Estrogen receptor
EU	European union
FSDT	Fish Sexual Development Test
FSH	Follicle-stimulating hormone
FSTRA	Fish Short-Term Reproduction Assay
GnRHR	Gonadotropin-releasing hormone receptor
GLP	Good laboratory practice
GSI	Gonado-somatic index
LH	Luteinizing hormone
LOAEL	The lowest observed adverse effect level
LOAEC	The lowest observed adverse effect concentration
LOEC	The lowest observed effect concentration
MoA	Mode of Action
MSCA	Member state competent authority
NOAEC	No observed adverse effect concentration
NOAEL	No observed adverse effect level
NOEC	No observed effect concentration
NONs	Notification of new substances
OECD	Organisation for economic co-operation and development
PBT	Persistent, bioaccumulative and toxic
PMT	Persistent, mobile, and toxic
PNEC	Predicted no-effect concentration
POP	Persistent organic pollutants
PPP	Plant protection products regulation EC 1107/2009
QSAR	Quantitative structure-activity relationship
RAR	Risk assessment report
REACH	Regulation No 1907/2006 concerning registration, evaluation, authorisation, and restriction of chemicals
RMS	Rapporteur Member State
STOT RE	Specific target organ toxicity – repeated exposure
STOT SE	Specific target organ toxicity – single exposure
SVHC	Substances of very high concern
TG	Test guideline
TPE	Testing proposal examination
TMF	Trophic magnification factor
UNEP	United nations environment program
UVCB	Unknown or variable composition, complex reaction products or of biological materials.
WHC	Water holding capacity
vPvB	Very persistent and very bioaccumulative
vPvM	Very persistent and very mobile

VTG	Vitellogenin