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Helsinki, 26 October 2020

Addressees
Registrants of diuron listed in the last Appendix of this decision (registrant(s)[1])

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

Registered substance subject to this decision, hereafter 'the Substance'
Substance name: diuron
EC number: 206-354-4
CAS number: 330-54-1

DECISION ON SUBSTANCE EVALUATION

In accordance with Article 46(3) of Regulation (EC) No 1907/2006 (REACH), you must submit the following information on the Substance[2]:

Larval amphibian growth and development assay according to OECD test guideline 241 using African clawed frog (*Xenopus laevis)*, including measurements of plasma vitellogenin as further specified in Appendix 1.

Deadline to submit the requested information

Appendix 1: Section B.1 provides further details of how the deadlines were derived.

You must provide an update of the registration dossier(s) containing the requested information, including robust study summaries and, where relevant, an update of the chemical safety report by **3 May 2022**.

In addition to the robust study summary, you must submit the full study report by the same deadline. The full study report must be included as an attachment file to the relevant endpoint study record in IUCLID.

Appendices

The reasons of this decision and any further test specifications of the requirements are set out in Appendix 1. The procedural history is described in Appendix 2. Further information, observations and technical guidance as appropriate are provided in Appendix 3. Appendix 4 contains a list of registration numbers for the addressees of this decision. This

[1] The terms registrant(s), dossier(s) or registration(s) are used throughout the decision, irrespective of the number of registrants addressed by the decision.

[2] Testing on vertebrate animals can only be started or performed after the decision has been adopted according to Article 52 of REACH.

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appendix is confidential and not included in the public version of this decision.

Who performs the testing?

Based on Article 53 of the REACH Regulation, you are requested to inform ECHA who will carry out the study on behalf of all registrant(s) within 90 days. Instructions on how to do this are provided in Appendix 3.

Appeal

This decision can be appealed to the Board of Appeal of ECHA within three months of its notification. An appeal, together with the grounds thereof, has to be submitted to ECHA in writing. An appeal has a suspensive effect and is subject to a fee. Further details are described under: http://echa.europa.eu/regulations/appeals

Authorised[3] by Christel Schilliger-Musset, Director of Hazard Assessment

[3] As this is an electronic document, it is not physically signed. This communication has been approved according to ECHA's internal decision-approval process.

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Appendix 1: Reasons

Based on the evaluation of all relevant information submitted on the Substance and other relevant available information, ECHA concludes that further information is required to enable the evaluating Member State competent authority (MSCA) to complete the evaluation of whether the Substance constitutes a risk to the environment.

The evaluating MSCA will subsequently review the information submitted by you and evaluate if further information should be requested in another decision to clarify the concern, according to Article 46(3) of REACH.

A.1 The potential risk – environment

The identification of a potential risk is based on a combination of exposure and hazard information.

According to information in the registration dossier the Substance is used in industrial sites in the manufacture of rubber products and polymer preparations. In your comments you indicate that environmental exposure from chemical processes under REACH is considered limited and that there is no intended release of the Substance from rubber products. However, available information indicates wide dispersive use: release to the environment of the Substance 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 under release-promoting conditions or from construction or building materials due to weathering conditions. Furthermore, you did not provide any evidence that the release from these products is negligible or that no release from polymers would occur. The uses mentioned in your dossier can cause exposure and thus contribute to the potential risk. Therefore, significant exposure to the environment cannot be excluded.

It is noted that the Substance is also used as a biocide according to the biocidal products Regulation (EU) No 528/2012 and approved for use as a plant protection product in the EU according to Regulation (EC) No 1107/2009. You commented further that the potential risk to environment being overestimated because the use of the Substance in plant protection products (PPP) will be terminated. Furthermore, you pointed out that the environmental exposure from the biocidal (BP) use in material protection would be limited. However, these uses are not part of the potential risk of the present substance evaluation. Therefore, your comments concerning PPP and BP are not relevant for this substance evaluation decision, which justifies the request only based on uses identified under the REACH Regulation.

Based on information in the registration dossier and published literature as detailed below, there is a concern that the Substance may be an endocrine disruptor (ED) for the environment according to the World Health Organisation/International Programme on Chemical Safety working definition (WHO/IPCS, 2002).

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Based on this exposure and hazard information, there is a potential risk for the environment. As the available information is not sufficient to conclude on potential ED properties, further information is needed, as explained below.

A.2 The possible risk management measures – environment

If the obtained data from the Request is sufficient to confirm the suspected ED properties as defined in World Health Organisation/International Programme on Chemical Safety working definition (WHO/IPCS, 2002), the evaluating MSCA will assess the need for further regulatory risk management in the form of identification as a Substance of Very High Concern (SVHC) under Article 57 (f) of REACH and subsequent authorisation or restriction of the Substance.

A.3 Explanation of the testing strategy – environment

In the first decision on substance evaluation in 2016, it was decided to use a tiered approach for additional information requests, where environmental endocrine disrupting effects related to sex hormones would be studied first. Therefore, a fish sexual development test (FSDT, OECD TG 234) was requested. Further information requests, if needed, targeted at other hormonal modes of action (thyroid) in wildlife, would be assessed based upon the results of the first tier and all information then available.

The present information request constitutes the second tier in a testing strategy to clarify the concerns for endocrine disrupting properties targeted at a thyroid mode of action. The evaluating MSCA will review the information submitted by the registrant(s) as an outcome of the second tier of the testing strategy and all the information then available, and evaluate if further information should be requested to clarify the concern.

A.4 The concern(s) identified

Available *in vitro* and *in vivo* data indicates that the Substance might affect the endocrine system via interaction with the hypothalamic-pituitary-thyroid (HPT) and the hypothalamic-pituitary-gonadal (HPG) axis thus potentially causing endocrine disruption effects in wild-life species.

Experimental *in vivo* evidence suggests that the Substance can have adverse impacts on reproduction. The potential persistence of the Substance and the transformation products with hormonal receptor activity increase the probability of serious effects to the environment by enabling a long-lasting exposure of organisms to potentially endocrine disrupting compounds and, possibly, a simultaneous exposure to several such compounds. Moreover, possible biomagnification may increase the concern for ED effects.

The concerns about the potential endocrine disrupting properties of the Substance in the

environment are based on the following observations:

- adverse *in vivo* effects on the reproduction and related pathways of aquatic and terrestrial invertebrates (*Daphnia, Eisenia*) and vertebrates (*Podarcis, Oreochromis*);
- *in vitro* evidence of hormonal activity of the Substance: antiandrogenic and weak (anti)estrogenic mode of action has been detected as well as thyroid peroxidase (TPO)-mediated and aryl hydrocarbon receptor (AhR)-mediated activity;
- the transformation products of the Substance (DCPU[4], DCPMU[5], 3,4-DCA[6], and 3,4-DCAA[7]) have been reported to bind to the androgen receptor *in vitro*;
- adverse *in vivo* antiandrogenic effect on fish (*Gasterosteus*), antiestrogenic effect on fish (*Pimephales*) and thyroid activity on amphibian (*Xenopus*) eleutheroembryo of the structurally similar substance, Linuron;
- due to the structural similarity with Linuron and the androgen receptor binding activity of both substances and their shared transformation products (DCPU, DCPMU and 3,4-DCA), the Substance may have the same kind of antiandrogenic effect on fish or amphibians as Linuron although the available *in vitro* studies indicate that there may be a difference in potency.

In addition, the Substance caused changes in thyroid hormone levels (T_3 and T_4) and thyroxin binding capacity in two sub-acute inhalation studies in rats. Linuron has shown similar effects on thyroid hormone levels in rats. It can be acknowledged that some endocrine mechanisms are evolutionarily conserved between aquatic vertebrates and mammals. However, extrapolation of study results between these taxonomic groups should be conducted carefully due to physiological differences and differences in exposure leading to differences in sensitivity.

Results from *in vitro* and *in vivo* studies suggest that the Substance may interact with the endocrine system through multiple endocrine modes of action (MoA). The negative outcome (i.e. no observed ED mediated adverse effects) of the FSDT thus does not rule out the concern for endocrine disruption. This is because the main MoA may be via thyroid hormone receptors or because effects may occur during life stages (e.g. reproductive phase of the life cycle) which are not covered by the FSDT. The Substance may also be more potent in other species or, with regard to systemic toxicity, the zebrafish might have been more sensitive to effects of the Substance than other species. In this situation, the existing data should be used to guide decision whether to conduct any further testing, e.g. modality for thyroid activity.

Information such as *in vitro* evidence of antiandrogenic, (anti)estrogenic and thyroidal activity, *in vivo* changes in biomarkers (gene expression, enzymatic levels and sex hormone levels) and *in vivo* antiandrogenic effects of the Substance in addition to thyroidal

[4] 3,4-dichlorophenylurea (DCPU)
[5] 3,4-dichlorophenyl-N-methylurea (DCPMU).
[6] 3,4-dichloroaniline (3,4-DCA)
[7] 3,4-dichloroacetanilide (3,4-DCAA)

effects of the structurally similar substance Linuron, indeed, suggests an ED MoA. Together with the thyroid findings in rodents exposed to the Substance, an ED concern for the environment cannot be ruled out.

Based on these observations, no conclusion can be drawn regarding the ED (androgenic or thyroid) activity of the Substance and, therefore, there is a concern about potential endocrine disrupting effects of the Substance leading to serious effects in the environment. According to the WHO/IPCS definition of endocrine disrupting chemicals (WHO 2002), a chemical is an ED if an adverse *in vivo* effect can be plausibly linked to MoA. Presently no clear link has been demonstrated between the observed ED mode of action and adverse effects e.g. on reproduction, but there is either no available information or evidence to overrule the concern of the Substance being an endocrine disruptor in the environment. In order to clarify the concern further and given that no studies are available for thyroid modality, more information on potential ED effects on wildlife (aquatic) is required.

In the following the underlying scientific evidence establishing the concern is described in more detail:

Concerns for Endocrine Disruption in Wildlife

In vitro data

(Anti)androgenicity and (anti)estrogenicity

Bauer et al. (1998) showed that the Substance has the 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 the Substance, also has an affinity to AR. Relative binding affinities (RBA) of the Substance (0.000024) and Linuron (0.0001) to bovine AR are much lower compared to an endogenous AR ligand DHT (RBA = 1.0). Linuron competed also 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). In a recombinant AR competitive binding assay, also Fang et al. (2003) showed that Linuron binds to AR. The RBA for Linuron was 0.0056 compared to synthetic androgen, R1181.

Vinggaard et al. (2008) tested the effect of the Substance (1, 3, 10 and 30 µM) on AR transactivation in a luciferase reporter assay in Chinese hamster ovary (CHO) cells expressing human AR. The Substance inhibited the AR transactivation by R1181. The concentration of the Substance showing 25% inhibition of 0.1 nM R1181-induced activity (IC_{25}) was within a range of 0.3 - 1 µM. Linuron also inhibited R1181-induced AR activation with the IC_{25} value between 1 - 3 µM.

Kojima et al. (2004) studied the effects of the Substance on human AR in a similar transactivation assay in CHO cells. The results of this study indicate also that the Substance has antiandrogenic potential. The Substance inhibited DHT-induced transcriptional activity of human AR. The RIC_{20} value for the Substance was 8.7 µM, i.e. this concentration caused 20% inhibition of androgenic activity by 0.1 nM DHT.

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Orton et al. (2009) have tested the Substance for endocrine disrupting potential *in vitro*. Recombinant yeast androgen screen (YAS) and yeast estrogen screen (YES) were used to detect agonistic or antagonistic effects on AR and ER (estrogen receptor). In this assay, the Substance (initial concentration range tested: 0.01 - 1000 µM) did not have any androgenic or estrogenic activity. The antagonistic effect was tested by coincubation of the Substance with AR agonist (2.5 nM testosterone) or ER agonist (0.25 nM 17β-estradiol). In YAS and YES assays, the Substance 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 the Substance 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 the Substance (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). Vinggaard et al. (1999) studied also the effects of the Substance on the activation of ER in yeast cells stably transfected to express human ERα and β-galactosidase as a reporter. The Substance or Linuron did not cause activation of ER. In another yeast-based assay, Noguerol et al. (2006) showed that the Substance 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_{50}) for the Substance was > 200 mg/L (> 850 µM).

Orton et al. (2009) have studied the effects of the Substance (6.25 and 62.5 µM) on ovulatory response and ovarian production of estradiol, testosterone and progesterone in frog (*Xenopus*) oocytes. The Substance (62.5 µM for 20 h) decreased testosterone levels and ovulation. Linuron had 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.

In a non-guideline *in vitro* assay (Jolly et al. 2009), with the structurally similar substance Linuron, DHT-induced spiggin production inhibition was studied in primed female stickleback kidney cell cultures after 48 h exposure to a range of concentrations of the test compound alone and together with 3 µg/l dihydrotestosterone (DHT). Linuron significantly inhibited DHT-induced spiggin production *in vitro* in a concentration-dependent manner at concentrations of 25 ng/l and higher, but showed no androgen agonistic activity.

All of the 15 ToxCast assays for estrogen receptor activity included in the U.S. EPA (2019) EDSP21 Dashboard were inactive (15 out of 18 assays used for the Estrogen Receptor Model). The ToxCast assay findings support the conclusions of the *in vitro* data that the Substance does not possess estrogen receptor-mediated endocrine activity.

The Substance induced a reduction in androgen receptor binding in an EDSP21 Dashboard assay measuring recombinantly expressed chimpanzee AR protein in the radioligand binding (NVS_NR_CAR assay).

These *in vitro* findings indicate that the Substance may have antiandrogenic activity, but the interaction with estrogen receptor is weaker or non-existent.

Thyroid-mediated activity

The three *in vitro* assays included in the U.S EPA EDSP21 Dashboard (2019b) studying promotion of thyroid receptor-mediated DNA transcription, agonism and antagonism of the thyroid receptor signalling pathway by thyroid receptor activation and inhibition were inactive.

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

The Substance induced a downregulation in rat thyroid tissue derived thyroid peroxidase (TPO) catalytic activity by 50% at 40 µM compared to DMSO in the ToxCast assay NCCT_TPO_AUR_dn (U.S. EPA 2019). Linuron caused a similar effect. The results suggest the potential of both substances to inhibit TPO.

Aryl hydrocarbon-mediated activity

The Substance has been shown to interact with aryl hydrocarbon receptor (AhR) *in vitro* (Noguerol et al. 2006, Zhao et al. 2006 and Takeuchi et al. 2008). In yeast AhR assay, Noguerol et al. (2006) showed that the Substance has significant interaction with human AhR. The EC_{50} for the Substance was 0.26 ± 0.10 mg/l (1.1 ± 0.4 µM), which 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 the Substance 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 the Substance. In these cells, the maximum Substance-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 the Substance-induced induction was relatively high (~ 8 µM) compared to that caused by TCDD (EC_{50} 0.12 nM) indicating much lower potency of the Substance.

In the other studied cell lines, the maximum of the Substance-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 the Substance (2 µM, exposure time 3.5 h) increases the expression of CYP1A1 mRNA, an endogenous AhR-responsive gene, in mouse hepatoma Hepa1c1c7 cells. They showed also by gel retardation analysis that the Substance is able to stimulate AhR transformation and DNA binding in guinea pig hepatic cytosol and in intact Hepa1c1ct cells.

The Substance-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, the Substance showed AhR agonistic activity. The relative the Substance-induced luciferase activity was about 80% of maximal activity induced by 0.1 nM TCDD. The potency of the Substance is clearly weaker than that of TCDD. The REC_{50} (Relative Effective Concentration) for the Substance 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).

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

Enzymes involved in the synthesis and metabolism of sex hormones

The Substance (10 nM - 100 µM) did not have any effect on aromatase activity (CYP19) in rainbow trout brain or ovarian microsomes (Hinfray et al. 2006). Similarly, the Substance (50 µM) or Linuron (50 µM) did not affect CYP19 aromatase activity, measured with ^3H2O assay using tritiated androstendione as a substrate, in human placental microsomes (Vinggaard et al. 2000).

The Substance had 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 (IC50 ≥ 24 µM) and only in the human prostate homogenates (Lo et al. 2007).

Thibaut and Porte (2004) studied the effect of the Substance on the activity of various enzymes involved in synthesis and metabolism of sex hormones in fish. Androstedione testicular metabolism was studied by incubating ^3H-androstenedione (0.1 µM) and the Substance (100 µM) with carp (*C. carpio*) 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. The Substance 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. The Substance (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 the Substance does not affect the activity of aromatase (CYP19) or 5α-reductase.
Four ToxCast assays indicate a downregulation of hormone synthesis involved in the steroidogenic pathway (U.S. EPA, 2019). The findings of the ToxCast assays support *in vitro* findings of potential antiandrogenicity of the substance.

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In vivo data

Aquatic animals, Daphnia

There were two guideline studies available for evaluation, based on the earlier test guideline OECD TG 202, Part II on *Daphnia magna* (Crustaceae) reproduction ([redacted] 1996, [redacted] 1989). 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.

It was concluded in the study of [redacted] (1996) that the Substance 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. No or little mortality was seen during the test, but the weight and length of the parental Daphnias were reduced. The acute toxicity (48 h-EC50) to Daphnia was 1.4 mg/l.

The test concentration range in the study of [redacted] (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 Daphnias between the control sample and the test samples during the 21 d semi-static test.

When assessing the two *Daphnia magna* studies, it is indicated that the Substance may interfere with Daphnia reproduction. 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.

Aquatic animals, fish

Katsiadaki et al. (2006) have studied the ED properties of the structural analogue Linuron with a method using three-spined stickleback (*Gasterosteus aculeatus*) as a sensitive *in vivo* test for detection of environmental antiandrogens. In the assay sexually mature female sticklebacks were simultaneously exposed to suspected anti-androgenic chemicals and a model androgen (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. The results showed that Linuron was antiandrogenic in the exposure concentrations of 15 and 150 µg/l in water. The inhibition of androgen-induced spiggin production in the highest Linuron concentration (150 µg/l) was statistically significant.

Jolly et al. (2009) have also studied the (anti)androgenic impacts of Linuron using an *in*

vivo assay in the three-spined stickleback exposed to the test compound together with DHT (5 µg/l) for 21 days. Linuron induced also a significant decrease in DHT-induced spiggin production at a concentration of 100 µg/l and 250 µg/l when tested *in vivo*.

The androgenised female stickleback screen (OECD TG 230 modification, OECD GD 148, AFSS) 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 IC_{50} of 172 µg/L (Pottinger et al. 2013).

In a Fish Short Term Reproduction Assay (FSTRA, OCSPP 890.1350) 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 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 (*Pimephales promelas*) 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 (anti)androgenic effects of the Substance and its transformation products 3,4-dichloroaniline (3,4-DCA), 3,4-dichlorophenylurea (DCPU) and 3,4-dichlorophenyl-N-methylurea (DCPMU) on plasma hormone concentrations and spermatogenesis were evaluated. Sexually mature male nile tilapias (*Oreochromis niloticus*) were exposed to 200 ng/L of the Substance, or to one of its transformation products 3,4-DCA, DCPU, and DCPMU for 25 days. The Substance caused a decrease in testosterone (T) levels. The transformation products caused significant changes in plasma sex steroid T and 11-ketotestosterone (KT) levels, gonadosomatic index, diameters of seminiferous tubules and percentages of germ cells of testis.

In the *in vivo* test (Pereira et al. 2016), (anti)estrogenic effects on sexually mature female nile tilapia were exposed to 100 ng/L of the Substance, or to its transformation products 3,4-DCA, DCPU, and DCPMU for 25 days. The Substance caused variations in the quantitative percentage of germinative cells but no significant differences in 17β-estradiol (E_2) in plasma levels, gonadosomatic indices or final vitellogenic oocytes were observed. However, the transformation products were observed causing all the beforementioned

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effects. No significant changes in plasma concentrations of the oestrogen precursor and gonadal regulator 17α-hydroxyprogesterone(17α-OHP) were observed.

In order to study (anti)androgenic effects *in vivo* (Felício et al. 2016), juvenile male tilapias (*Oreochromis mossambicus*) were exposed for 7 days to the Substance, or to its transformation products 3,4-DCA, DCPU, and DCPMU at nominal concentrations of 40 and 200 ng/L. Expression of vitellogenin mRNA was induced and aromatase activity diminished at high concentrations of the Substance but no significant differences in 17β-HSD activity or expression of hepatic CYP3A were observed. However, the transformation products were observed causing all the before mentioned effects.

In the *in vivo* test (Boscolo et al. 2018), adult male nile tilapias were exposed for 10 days to the Substance or one of its transformation products 3,4-DCA and DCPMU at nominal concentrations of 100 ng/L. No significant differences in plasma concentration of T, KT or stress steroid (cortisol), in brain dopamine levels in or in behaviour were observed when exposed to the Substance. However, the transformation products 3,4-DCA and DCPMU caused the beforementioned (anti)androgenic effects except the changes of KT levels in plasma.

In response to the first substance evaluation decision and to clarify the (anti)androgenicity concern of the Substance, an *in vivo* study ([redacted] 2018) fish sexual development test (FSDT, OECD TG 234) with zebrafish (*Danio rerio*) was conducted. The mean measured concentrations of the Substance were 1.19, 3.26, 11.32, 32.51 and 105.44 µg/L. 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 macroscopically by inspection of the gonads and by histopathological verification. A LOEC of > 105.44 µg/L was determined based on the size and endocrine-related endpoints with the exception of statistically significant LOEC of 32.51 µg/L regarding the maturity index of the female fish. However, an effect without concentration-response relationship on post-hatch survival at 35 dpf and 63 dpf was observed between control and treatments at concentrations ≥ 3.26 µg/L. Based on the endpoint post-hatch survival, the NOEC of 1.19 µg/L was determined (see also section A.5).

Based on the *in vivo* studies described above, the Substance may cause antiandrogenic and estrogenic activity or interfere with fish reproduction.

Aquatic animals, amphibians

In the non-guideline *in vivo* test (Freitas et al. 2016), the impacts on metamorphosis and thyroid genetics were studied in American bullfrog (*Lithobates catesbeianus*) tadpoles exposed to 40 and 200 ng/L of the Substance or transformation product 3,4-DCA for 7 days at 28 °C and 34 °C. 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 the Substance at 28 °C and 34 °C, respectively.

thibz 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 the Substance 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) concentration were observed compared to controls. 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 claved frog (*Xenopus laevis*) as increasing concentrations of Linuron in the absence of T_3 and no effects in the presence of T_3 were observed in each of the laboratories which 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.

These *in vivo* findings indicate that the Substance may cause thyroid-mediated effects on amphibians.

Sediment dwelling organisms

Only weak impacts on the reproduction of freshwater snail (*Physella acuta*) (increase in the total number of egg sacs) were observed after exposure to the Substance at a concentration of 9.5 µg/L, found in actual fresh water environments (López-Doval et al. 2014). In a study with an ascidian (*Ciona intestinalis*) no effect on fertilization rate was discovered with the Substance exposure (2.33 mg/L), but the percentage of normal larvae was significantly decreased (Gallo and Tosti 2013).

Exposure experiments with cupped pacific oyster (*Crassostrea gigas*) led to a slight bioaccumulation of the Substance, with factor of 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). Akcha et al. (2012) observed increased percentage of abnormal D-larvae at concentrations of 0.05 µg/L upwards after 24 h exposure of the Substance. In the 24 h oyster embryo-larval bioassay (Behrens et al. 2016), a significant increase in the percentage of abnormal D-larvae was observed at a concentration of 0.01 µg/L of the Substance. The transformation products DCPMU and DCPU caused similar developmental abnormalities at concentrations of 0.01 µg/L and 0.05 µg/L, respectively.

Based on these *in vivo* studies, it seems that the Substance might have an impact on the reproduction of sediment dwelling organisms.

Terrestrial animals

There was one guideline study available on earthworm reproduction, based on the ISO

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11268-2 guideline, which is similar to the OECD TG 222 Earthworm reproduction test (*Eisenia fetida / Eisenia andrei*) ([redacted] 2001). The evaluation is based on the full study report.

The results of this study showed that the Substance had clear impacts on the reproduction (the mean number of juvenile earthworms) of *Eisenia fetida* with the NOEC value of 10.7 mg active ingredient (a.i.)/kg dry artificial soil and the LOEC value of 26.7 mg a.i./kg dry artificial soil. The results were statistically significant. The dose response was evident, and no mortality of the adults was seen nor impact on the growth of adults during the test indicating no general toxicity in the applied test concentrations. The exposure levels were 5.3, 10.7, 26.7, 133.3, 266.7 mg a.i./kg dry artificial soil and the test duration consisted of 28 d initial period and 28 d hatching period.

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% content of the Substance, 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 the Substance) and/or contaminated food (with 5.4 mg of the Substance). The amount and uptake of food was not given in the method description and neither was the uptake of contaminated drinking water, which is a shortcoming 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 cells. 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 $5.27 \pm 0.39 \times 10^{-3}$ (in the control group) to $2.3 \pm 0.18 \times 10^{-3}$ and $3.4 \pm 0.25 \times 10^{-3}$ in the the Substance 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 exposure groups in comparison with the control group. No estrogenic impact was observed as the 17β-estradiol plasma content was undetectable in all male

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lizards exposed to the Substance. It can be concluded that, despite of the shortcomings in the description and quantitative follow-up of the actual uptake, the results of the lizard study suggest that exposure to the Substance results in reproductive toxicity in male lizards.

Based on these *in vivo* studies, it seems that the Substance might have an impact on reproduction of terrestrial animals.

In vivo mammalian studies

Two *in vivo* inhalation studies in rats show comparable effects on thyroid hormones levels (T_3 and T_4) and thyroxin-binding capacity (TBC) suggesting that the Substance interacts with the hypothalamus-pituitary-thyroid (HPT) axis.

In a sub-acute inhalation study (Study report 1986a) comparable to guideline OECD TG 412, rats were exposed to doses of 0, 4.1, 37.4 and 268.1 mg/m³ of an aerosol of the Substance for 4 or 8 weeks. In males T_4 decreased statistically significantly at 268 mg/m³ after 4 and 8 weeks (Table 1). Although not statistically significant, a dose-dependent trend was also seen in decreased T_3 and increased TBC. In females there was a statistically significant decrease in T_3 at 37.4 mg/m³ after 8 weeks. TBC was significantly increased at 268.1 mg/m³ after 8 weeks.

Table 1. Thyroid hormone levels and TBC. Values are given as 4 weeks values/8 weeks values

Dose group [mg/m³]	**0**	**4.1**	**37.4**	**268.1**
Males				
T_3 [nmol/l)	1.22/0.91	1.17/0.92	1.05/0.84	0.97/0.74
T_4 [nmol/l]	77/52	69/46	63/44	47**/36*
TBC	0.76/0.78	0.78/0.79	0.81/0.78	0.80/0.83
Females				
T_3 [nmol/l)	1.15/0.92	1.28 /0.81	1.23/0.64**	1.09/0.72
T_4 [nmol/l]	50 /46	54/46	54/39	42/36
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 (Study report 1986b) comparable to guideline OECD TG 412, rats were exposed to doses of 0, 6.6, 47.6 and 311 mg/m³ of an aerosol of the Substance for 21 days. A statistically significant decrease in T_3 and T_4 values at

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311 mg/m^3 in males, with simultaneously increased TBC were observed at 47.6 and 311 mg/m^3 (Table 2). In females T_4 decreased statistically significantly at 311 mg/m3 and TBC was increased at 47.6 and 311 mg/m^3 .

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Table 2. Thyroid hormone levels and TBC

Dose group [mg/m3]	**Air**	**0**	**6.6**	**47.6**	**311**
Males					
T_3 [nmol/l)	1.01	0.91	0.92	0.90	0.77**
T_4 [nmol/l]	63	65	62	64	53**
TBC	0.64	0.68	0.71	0.79**	0.84**
Females					
T_3 [nmol/l)	0.91	0.89	1.04	0.99	0.91
T_4 [nmol/l]	54	70**	59	55	41*
TBC	0.56	0.61	0.61	0.66**	0.67**

*p<0.05, **p<0.01

In summary, exposure in two subacute inhalation studies in rats lead to effects on thyroid hormones levels in both genders indicating reduced thyroid function. There is a dose-dependent trend in the decreased thyroid hormone levels, at least in males. Thyroid-stimulating hormone (TSH) levels were not measured. No changes in thyroid gland weight or histopathology were observed. In both studies effects on thyroid function were seen in the presence of systemic toxicity (effects on hematology and spleen), but MTC (maximum tolerated concentration) was not exceeded (no toxicologically significant body weight decrease or severe clinical signs).

In vivo studies on Linuron have shown potential interaction of linuron with the HPT-axis in mammals characterized by changes in thyroid hormone levels in the absence of changes in thyroid weight or histopathology. Decreases in serum T_3 and/or T_4 levels were observed in 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 (U.S.EPA 2015).

In your comments on the draft decision, you did not consider the changes in T_3, T_4 and TBC observed in in vivo mammalian studies as a concern regarding an interaction with the HPT-axis. Consequently, the observed changes were considered being induced by systemic toxicity disturbing the normal homeostasis leading to a change in hormone levels. This was considered being supported by the fact that there were no reported indications of thyroid toxicity or disturbance of the HPT-axis in any other OECD level 4 or 5 studies. Therefore, exposure in two subacute inhalation studies in rats demonstrated effects on thyroid hormone levels in both genders indicating reduced thyroid function. Thyroid hormone levels were not measured in any other toxicity studies with the Substance, including OECD level 4 and 5 studies. 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

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T_4 without histopathological findings would still present a potential concern for neurodevelopment. It indeed seems that the effects on thyroid hormone levels were seen in the presence of systemic toxicity (effects on hematology and spleen), which may reduce the toxicological significance of these findings. However, similar effects have been shown with Linuron, a closely structurally related substance to the Substance. *In vivo* studies on Linuron have shown changes in thyroid hormone levels, namely decrease in the T_3 and T_4 levels, in the absence of changes in thyroid weight or histopathology.

Overall, ECHA considers, despite the relatively minor changes in T_3, T_4 and TBC which in isolation are of limited toxicological significance, that the weight of evidence (including also the data available in amphibians) gives cause for concern which may be related to HPT-axis interaction.

Transfomation products of the Substance with potential ED relevance

There is *in vitro* evidence that transformation products of the Substance may bind to the androgen receptor and replace testosterone. These transformation products are formed by metabolism of microorganisms or animals and include DCPMU, DCPU, 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). The binding of the Substance and its metabolites to the androgen receptor suggest a possible endocrine mode of action.

DCPU and DCPMU were reported to be formed from the Substance in the simulation tests ([redacted] 1996, [redacted] 2001, [redacted] 1993 as cited in RMS Denmark 2005) and in published studies on microbial cultures (Sørensen et al., 2008, Ellegaard-Jensen et al. 2014). DCPMU and DCPU have also been reported to be formed from the Substance in field studies (Gooddy et al. 2002, Stork et al. 2008).

3,4-DCA has been reported to be formed from the Substance in a soil simulation test ([redacted] 1993 as cited 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 the Substance 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 the Substance (Ellegaard-Jensen et al. 2014) and from 3,4-DCA (Tixier et al. 2002) in microbial cultures has been reported. DCPMU, DCPU, and 3,4-DCA have been reported as metabolites of the Substance 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).

It is noted that DCPMU and DCPU have been identified as transformation products also for Linuron in simulation tests (RMS United Kingdom 1996) and in a soil fungus study (Badawi

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et al. 2009). Badawi et al. (2009) also reported 3,4-DCA as a transformation product of Linuron. In addition, 3,4-DCAA has been reported to be formed from 3,4-DCA in microbial cultures (Tixier et al. 2002, Giacomazzi and Cochet 2004) and therefore 3,4-DCAA can be expected to be formed from Linuron through 3,4-DCA.

The available information did not enable the evaluating MSCA to conclude on ED potential of the Substance to wildlife species.

A.5 Why new information is needed

According to the OECD (GD 150) Conceptual Framework for Testing and Assessment of Endocrine Disrupting Chemicals (OECD 2018a), in a situation when FSDT (OECD TG 234) gives a negative result, a careful consideration of any existing data is needed. In this specific case, although the conducted FSDT test gave negative result, there is not enough information to conclude that the Substance is not an endocrine disruptor due to other indications of antiandrogenic and (anti)estrogenic MoA. In addition, a concern related to thyroid modality is raised.

Information both on endocrine mode of action and endocrine mediated adverse effects are required to conclude on the endocrine disrupting properties. In order to confirm whether the assumed thyroid mode of action exists and results in adverse effects, an aquatic test including population relevant adverse endpoints is required. Data on amphibians could be used for assessing the T modality (thyroid hormone), as well as EA ((anti)estrogenic, (anti)androgenic) modalities, in the form of Larval Amphibian Growth and Development Assay (LAGDA, OECD TG 241). Therefore, the LAGDA, including measurements of plasma vitellogenin, is needed to clarify the concern.

A.6 Considerations on the test method

Taking into account the available information, a test conducted on the Substance according to OECD test guideline 241 (the Larval Amphibian Growth and Development Assay, LAGDA) is needed. The test guideline assesses endocrine disrupting effects of the Substance by identifying disturbance on development, metamorphosis and growth from fertilization to early juvenile period of African claved frog (*Xenopus laevis*). In addition to effects mediated via estrogenic and androgenic modalities, the guideline identifies thyroid modality mediated interference with the thyroid pathway or function of HPT axis.

In your comments to the draft decision, you considered the test guideline of the LAGDA is insufficiently validated and testing laboratories not having enough experience with the test which might, accordingly, lead to an invalid study and there is a high risk of false-positive results. You also acknowledged that adequate ED testing on the Substance is needed but it should be performed using established and reliable test guidelines at laboratories that have demonstrated proficiency conducting them. ECHA does not agree with your comments as the LAGDA is a validated test method and, therefore, should be considered

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fit to provide the information needed. Taking into account the fact that the Amphibian Metamorphosis Assay (AMA, OECD TG 231) has been the only test available on amphibians for a long time, ECHA agrees that the laboratories might have more experience conducting the AMA compared to the LAGDA or the Xenopus eleutheroembryonic Thyroid Signaling Assay (XETA, OECD TG 248). However, the lack of experience gained on the test method shouldn't be used as a reason to request an alternative test.

Five test concentrations must be used in the requested study in order to obtain a robust concentration response and to increase the probability to derive a precise NOEC/LOEC or ECx to be used for further risk management considerations. Results from the existing studies on the Substance can be used to determine the highest test concentration in order to avoid overly toxic concentrations. In addition to the existing data, conducting a range-finding study could be considered beneficial in order to exclude acutely toxic concentrations. However, the highest test concentration must be sufficiently high to give a clear systemic (non endocrine-specific) response in order to ensure that a sufficiently wide range of exposure levels are tested and to obtain the NOEC/LOEC value.

The OECD test guideline 241 includes an optional endpoint of plasma VTG measurement. There are indications of the Substance having antiandrogenic and (anti)estrogenic MoA and, therefore, plasma VTG must be measured in the study. The measurement of plasma VTG is required as it may be useful for understanding study results in the context of antiandrogenic and (anti)estrogenic MoA. The measurements must be carried out according to the guideline. Furthermore, the inclusion of plasma VTG measurements in the requested LAGDA does not include additional use of laboratory animals. You must submit the full study report for the Request. Considering the complexity of the case as described above, a complete rationale of test design and interpretation of results and access to all information available (implemented method, raw data collected, interpretations and calculations, consideration of uncertainties, argumentation, etc.) are needed. This will allow the evaluating MSCA to fully assess all the provided information, including the statistical analysis, and to efficiently clarify the concern for ED. The full study report must be included as an attachment file to the relevant endpoint study record in the IUCLID file.

A.7 Alternative approaches and proportionality of the request

The request for the LAGDA is suitable and necessary to obtain information that will allow to clarify whether there is a potential risk for the environment. More explicitly, between different available alternatives it is the least onerous way to obtain information. In your comments you did not consider the LAGDA justified taking into account the available information. Instead, you prefer to perform the XETA, one of the possible alternatives for further evaluation of ED properties to your knowledge. ECHA notes that the XETA, a screening assay representing the level 3 of the OECD ED conceptual framework (OECD 2018a), is not suitable for investigating other modalities than thyroid. The LAGDA, as placed in the level 4 of the OECD ED CF, can be used directly to address the population relevant adverse endpoint for estrogen, androgen, thyroid (steroidogenic) (EAT(S)) modalities.

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You commented that the LAGDA requires from 480 to 640 animals and, in order to avoid unnecessary testing on animals, an alternative test with less or no animals should be conducted. As examples, you mentioned the XETA and the fish short-term reproduction assay (FSTRA, OECD TG 229). XETA is not applicable as stated above and, furthermore, it covers less thyroidal mode(s) of action compared to the AMA or the LAGDA. Regarding the FSTRA, ECHA acknowledges that the method (240 fish) uses less animals (50%) compared to the LAGDA method. However, the FSTRA is not a suitable test because it does not cover the T modality.

When comparing the AMA to the LAGDA, the minimum amount of tadpoles needed in the AMA (320 larvae for three test concentrations plus the control) is indeed lower compared to the LAGDA (480 larvae for four test concentrations plus control). However, as more (i.e. four or five) test concentrations are recommended for the AMA to reduce the possibility of false negative results and to derive a robust dose-response relationship to calculate NOEC/LOEC to be used for further risk management processes, the only significant difference between the AMA and the LAGDA regarding the number of test animals is the number of replicates in the control: four for the AMA and eight for the LAGDA assuming an equal number of test concentrations. Furthermore, in the LAGDA the higher number of replicates in the control addresses the variability better and, therefore, helps to ensure appropriate statistical power of the test. Moreover, if the AMA results are positive or inconclusive, a follow-up test may be required in the form of the LAGDA.

In the comments you provided, you noted that no evidence of (anti-)androgenic or estrogenic activity was observed in the FSDT. Regarding the alternative testing strategy discussed in your comments on the draft decision, in addition to the AMA and the XETA you also mentioned testing on fish with thyroid endpoints introduced being an alternative and pointed out that as published by Knacker et al. (2010), the most sensitive endpoint with relevance for fish populations for estrogenic and anti-androgenic mode of action is the reproduction in terms of fecundity or fertility.

As a comment to your reference to the most sensitive endpoints regarding population level effects, also information on sex ratio is considered relevant (Knacker et al. 2010) in addition to reproduction for estrogenic mode of action. With regard to studies on fish, it is shown that toxicity on fish and amphibians could correlate and that fish would be more sensitive compared to amphibians (Weltje et al. 2013). ECHA is also aware of the work done in order to introduce the thyroid endpoints in fish toxicity tests. However, ECHA notes that the T-mediated (thyroid hormone) endpoints for the fish are not yet validated and as there are validated methods available, testing using unvalidated methods is not recommended. Furthermore, ECHA considers that the LAGDA can provide additional information on the EA-mediated effects ((anti)estrogenic, (anti)androgenic) compared to the tests already available and, therefore, a negative LAGDA (i.e. no ED mediated effects observed) could be used to conclude the absence of EA-mediated effects as well. ECHA also notes that a valid FSDT has already been conducted and, in addition, several non-guideline *in vivo* studies on fish are available. Looking at the available information on endocrine disrupting properties caused by estrogen, androgen and steroidogenic modalities, no further testing on fish is considered justified at this stage of the assessment,

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even taking into account that the test concentrations might not have been optimal[8] or the exposure not long enough to detect ED related effects and the reproduction phase not being covered in the FSDT. In this regard, neither the medaka extended one generation reproduction test (MEOGRT, OECD TG 240) nor the zebrafish extended one generation reproduction test (ZEOGRT, draft OECD TG) is considered an appropriate test method despite the possible estrogenic or antiandrogenic effects of the Substance and although the reproductive phase could be the most sensitive part of the development regarding this MoA.

As theoretical alternatives, *in vitro* studies could be contemplated to clarify the potential interference with the HPT axis further. However, no standardized guidelines for *in vitro* tests assessing interactions with hypothalamic or pituitary regulation of thyroid hormone production are available.

In summary, there are no adequate studies available on thyroid modality of the Substance. Therefore, for clarifying the potential thyroid-mediated endocrine disruptive properties in addition to further clarification of EA modalities mediated effects, the LAGDA is considered to be the most suitable test in aquatic wildlife.

According to the EFSA/ECHA guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009 (ECHA & EFSA 2018), in order to have the thyroid activity sufficiently investigated, the results from all the T-mediated parameters from both human and mammalian studies and other non-target organisms (like fish and amphibians) should be examined and, therefore, requesting the LAGDA is considered adequate and justified.

ECHA notes that there is no experimental study available at this stage to generate the necessary information whilst avoiding testing in vertebrate animals. More explicitly, there is no equally suitable alternative way available of obtaining this information.

B.1 Consideration of the time needed to perform the requested studies

The deadline for provision of the requested data takes into account the time that you may need to agree on which of the registrant(s) will perform the required tests (3 months is allocated for this) and include the time required for developing an analytical method, conduct of the study according to OECD test guideline 241, preparation of the study report and reporting in IUCLID.

For the Request, ECHA considers that 18 months is a sufficient time for conduct and reporting of the study. In your comments you requested flexibility on the 18 months deadline for the LAGDA testing. However, you did not provide any concrete evidence to substantiate this request.

[8] No range-finding study was performed but the test concentrations were based on other fish toxicity studies (on fathead minnow and rainbow trout) available at that time.

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ECHA considers therefore that the following deadlines are sufficient for conduct and reporting of the requested study:

Test requested	**Deadline**
Larval Amphibian Growth and Development Assay (LAGDA, OECD TG 241)	18 months

The final deadline is based on the duration of the requested test, Larval Amphibian Growth and Development Assay (LAGDA, OECD TG 241), for which 18 months is allocated.

B.2 Explanation of the Grouping of substances and read-across approach applied for the substance evaluation

Analysis of the grouping and read-across approach

Linuron (3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea) (CAS 330-55-2) belongs to the same group of phenylurea herbicides as the Substance and they are regarded as structural analogues sharing also similar transformation products (Badawi et al. 2009).

Conclusion on the read-across approach

Based on the structural analogy and similar transformation products, relevant data on ED related properties of Linuron have been evaluated together with the Substance as supporting information.

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Appendix 2: Procedural history

12-month evaluation

On the basis of an opinion of the ECHA Member State Committee and due to initial grounds for concern relating to Human health/Potential endocrine disruptor; Exposure/Wide dispersive use, ground and surface water pollutant, Diuron CAS No 330-54-1 (EC No 206-354-4) was included in the Community rolling action plan (CoRAP) for substance evaluation to be evaluated in 2014. The updated CoRAP was published on the ECHA website on 26 March 2014. The competent authority of Finland (hereafter called the evaluating MSCA) was appointed to carry out the evaluation.

In the course of the evaluation, the evaluating MSCA identified additional concerns regarding endocrine disruption in the environment, which is closely linked to the initial grounds for concern about potential endocrine disrupting effects on human health. Based on the results of the present evaluation of available information no final conclusion could be drawn on the initial and additional concern about endocrine disrupting effects. In consequence the evaluating MSCA decided to use a tiered approach for additional information requests, where environmental endocrine disrupting effects related to sex hormones will be studied first. Further information requests, if needed, targeted at other hormonal mode of actions (thyroid) in wildlife will be assessed based upon the results of the first tier and all information then available.

It was decided that further information requests, if needed, related to human health (endocrine disruption and reproductive toxicity) concern, will be assessed based upon the results of the first tier and all information then available.

In accordance with Article 46(1) of the REACH Regulation, a substance evaluation decision was issued on 10 June 2016 requesting further information. You submitted all the requested information on 28 June 2018. The evaluating MSCA carried out the evaluation of the information in your updated registration(s) and other relevant and available information.

Based on the results of the present evaluation of available information no final conclusion could be drawn on the initial and additional concern about endocrine disrupting effects. In consequence the evaluating MSCA decided to request testing targeted at environmental endocrine disrupting effects related to sex hormones and thyroid mode of action.

The evaluating MSCA deemed it at this stage appropriate to wait i) the RAC opinion on the proposed harmonised classification of the Substance as Carc. 1B under the CLP Regulation (EC No. 1272/2008) and ii) the outcome of the forthcoming ED-evaluation of the Substance under the Biocidal Products Regulation (EU No 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. The need for further information requests under the REACH Regulation, related to environment or human health concern will be assessed based upon the results of the environmental assessment and all information then available.

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The evaluating MSCA considered that further information was required to clarify the following concern: Environment/Potential endocrine disruptor. Therefore, it prepared a draft decision under Article 46(3) of the REACH Regulation to request further information. It subsequently submitted the draft decision to ECHA on 28 June 2019.

Decision-making

ECHA notified you of the draft decision and invited you to provide comments.

For the purpose of this decision-making, dossier updates made after the date the draft of this decision was notified to you (Article 50(1) of REACH) will not be taken into account.

You were exceptionally given extra time to update the dossier after the draft decision was notified to you, but no updates were received by the deadline given.

(i) Registrant(s)' commenting phase

ECHA received your comments and forwarded them to the evaluating MSCA without delay. The evaluating MSCA took your comments into account and they are reflected in the reasons (Appendix 1). The request(s) and the deadline were not amended.

(ii) Proposals for amendment by other MSCAs and ECHA and referral to the Member State Committee

The evaluating MSCA notified the draft decision to the competent authorities of the other Member States and ECHA for proposal(s) for amendment.
As no amendments were proposed, ECHA took the decision according to Articles 52(2) and 51(3) of REACH.

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Appendix 3: Further information, observations and technical guidance

1. This decision does not imply that the information provided by you in the registration(s) is in compliance with the REACH requirements. The decision neither prevents ECHA from initiating compliance checks on your dossier(s) at a later stage, nor does it prevent a subsequent decision under the current substance evaluation or a new substance evaluation process once the present substance evaluation has been completed.

2. Failure to comply with the request(s) in this decision, or to otherwise fulfil the information request (s) with a valid and documented adaptation, will result in a notification to the enforcement authorities of your Member State.

3. In relation to the required experimental study, the sample of the substance to be used ('test material') has to have a composition that is within the specifications of the substance composition that are given by all registrant(s). It is the responsibility of all the registrant(s) to agree on the tested material to be subjected to the test(s) subject to this decision and to document the necessary information on the composition of the test material. The substance identity information of the Substance and of the sample tested must enable the evaluating MSCA and ECHA to confirm the relevance of the testing for the substance subject to substance evaluation.

4. In relation to the experimental study the legal text foresees the sharing of information and costs between registrant(s) (Article 53 of the REACH Regulation). You are therefore required to make every effort to reach an agreement regarding each experimental study for every endpoint as to who will carry out the study on behalf of the other registrant(s) and to inform ECHA accordingly within 90 days from the date of this decision under Article 53(1) of the REACH Regulation. This information should be submitted to ECHA using the following form stating the decision number above at:

   https://comments.echa.europa.eu/comments_cms/SEDraftDecisionComments.aspx?CaseNumber=DEC-SEV-206-354-4-1-1

5. Further advice can be found at http://echa.europa.eu/regulations/reach/registration/data-sharing. If ECHA is not informed of such agreement within 90 days, it will designate one of the registrants to perform the study on behalf of all of them.