EUROPEAN CHEMICALS AGENCY

Helsinki, 10 June 2016

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DECISION ON SUBSTANCE EVALUATION PURSUANT TO ARTICLE 46(1) OF REGULATION (EC) NO 1907/2006

For Diuron, CAS No 330-54-1 (EC No 206-354-4)

Addressees: Registrant(s)[1] of Diuron (Registrant(s))

This decision is addressed to the Registrant(s) of the above substance with active registrations pursuant to Article 6 of the REACH Regulation on the date on which the draft for the decision was first sent for comments. If Registrant(s) ceased manufacture upon receipt of the draft decision pursuant to Article 50(3) of the REACH Regulation, they did not become addressee(s) of the decision. A list of all the relevant registration numbers of the Registrant(s) that are addressees of the present decision is provided as an Annex to this decision.

Based on an evaluation by the Finnish Safety and Chemicals Agency as the Competent Authority of Finland (evaluating MSCA), the European Chemicals Agency (ECHA) has taken the following decision in accordance with the procedure set out in Articles 50 and 52 of Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH Regulation).

This decision is based on the registration dossier(s) on 10 July 2015, i.e. the day until which the evaluating MSCA granted an extension for submitting dossier updates which it would take into consideration.

This decision does not imply that the information provided by the Registrant(s) in the registration(s) is in compliance with the REACH requirements. The decision neither prevents ECHA from initiating compliance checks on the dossier(s) of the Registrant(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.

I. Procedure

Pursuant to Article 45(4) of the REACH Regulation the Competent Authority of Finland has initiated substance evaluation for Diuron, CAS No 330-54-1 (EC No 206-354-4) based on registration(s) submitted by the Registrant(s) and other relevant and available information and prepared the present decision in accordance with Article 46(1) of the REACH Regulation.

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 was included in the Community rolling action plan (CoRAP) for substance evaluation to be evaluated in 2014.

[1] The term Registrant(s) is used throughout the decision, irrespective of the number of registrants addressed by the decision.

The updated CoRAP was published on the ECHA website on 26 March 2014. The Competent Authority of Finland 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.

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.

The evaluating MSCA considered that, at this stage, further information is required to clarify the following concern: Environment/Potential endocrine disruptor. Therefore, it prepared a draft decision pursuant to Article 46(1) of the REACH Regulation to request further information. It submitted the draft decision to ECHA on 25 March 2015.

On 4 May 2015 ECHA sent the draft decision to the Registrant(s) and invited them pursuant to Article 50(1) of the REACH Regulation to provide comments within 30 days of the receipt of the draft decision.

Registrant(s) commenting phase

By 10 June 2015 ECHA received no comments from Registrant(s) of which it informed the evaluating MSCA without delay.

By 10 July 2015 Registrant(s) submitted update(s) of the registration dossier(s).

The evaluating MSCA considered the dossier update and did not amend the draft decision.

Commenting by other MSCAs and ECHA

In accordance with Article 52(1) of the REACH Regulation, on 21 of January 2016 the evaluating MSCA notified the Competent Authorities of the other Member States and ECHA of its draft decision and invited them pursuant to Articles 52(2) and 51(2) of the REACH Regulation to submit proposals to amend the draft decision within 30 days of the receipt of the notification.

Subsequently, MSCAs submitted proposals for amendment of the draft decision.

On 26 February 2016 ECHA notified the Registrant(s) of the proposals for amendment to the draft decision and invited them pursuant to Articles 52(2) and 5 1(5) of the REACH Regulation to provide comments on the proposals for amendment within 30 days of the receipt of the notification.

The evaluating MSCA reviewed the proposals for amendment and Registrants' comments and amended the draft decision.

Referral to Member State Committee

On 7 March 2016 ECHA referred the draft decision to the Member State Committee.

By 29 March 2016, the Registrant(s) provided comments on the proposals for amendment, in accordance to Article 51(5) and on the draft decision. The Member State Committee took the comments on the proposals for amendment of the Registrant(s) into account.

After discussion in the Member State Committee meeting on 25–29 April 2016, a unanimous agreement of the Member State Committee on the draft decision as modified at the meeting was reached on 26 April 2016.

ECHA took the decision pursuant to Article 52(2) and Article 51(6) of the REACH Regulation.

II. Information required

Pursuant to Article 46(1) of the REACH Regulation the Registrant(s) shall submit the following information using the indicated test method (in accordance with Article 13(3) and (4) of the REACH Regulation) and the registered substance subject to the present decision:

> Fish Sexual Development Test (FSDT, test method: OECD 234) with Japanese medaka *Oryzias latipes* or *Zebrafish Danio rerio* or Three-spined stickleback *Gasterosteus acuelatus*. The genetic sex determination and secondary sex characteristics shall also be included if the determination of the parameters is possible for the selected test species.

Deadline for submitting the required information

Pursuant to Article 46(2) of the REACH Regulation, the Registrant(s) shall submit to ECHA by **18 December 2017** an update of the registration(s) containing the information required by this decision[2], including a robust study summary and a full study report, and, where relevant, an update of the Chemical Safety Report.

The need for further information requests will be evaluated by the evaluating MSCA based on all available relevant information when the required information have been provided in the dossier update.

III. Statement of reasons

Fish Sexual Development Test (FSDT, OECD TG 234)

The present evaluation shows that Diuron can have an endocrine disrupting mode of action (MoA) and that it can cause adverse effects on animals, which are possibly endocrine mediated. Therefore, information on endocrine disrupting effects in the environment is required in order to enable the evaluating MSCA to assess the properties of the substance and to decide whether the suspected concern (Environment/Potential endocrine disruptor) may be realised or not. Without the requested information it will not be possible to verify further whether there remains an uncontrolled risk with Diuron that should be subject to further risk management measures.

[2] The deadline set by the decision already takes into account the time that registrants may require to agree on who is to perform any required tests and the time that ECHA would require to designate a registrant to carry out the test(s) in the absence of the aforementioned agreement by the registrants (Article 53(1) of the REACH Regulation).

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). Therefore relevant data on ED related properties of Linuron have been evaluated together with Diuron as supporting information.

Concerns for Endocrine Disruption in Wildlife

In vitro data

(Anti)androgenicity and (anti)estrogenicity

Bauer et al. (1998) showed that Diuron 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 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). 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 Diuron (1, 3, 10 and 30 µM) on AR transactivation in a luciferase reporter assay in Chinese hamster ovary (CHO) cells expressing human AR. 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. Linuron also inhibited R1181-induced AR activation with the IC_{25} value between 1 - 3 µM.

Kojima et al. (2004) studied the effects of Diuron on human AR in a similar transactivation assay in CHO cells. The results of this study indicate also that Diuron has antiandrogenic potential. Diuron inhibited 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*. 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, Diuron (initial concentration range tested: 0.01 - 1000 µM) did not have any androgenic or estrogenic activity. The antagonistic effect was tested by coincubation of Diuron 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). 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. Diuron or Linuron did not cause activation of ER.

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_{50}) for diuron was > 200 mg/L (> 850 µM).

Orton et al. (2009) have studied the effects of Diuron (6.25 and 62.5 µM) on ovulatory response and ovarian production of estradiol, testosterone and progesterone in frog (*Xenopus*) oocytes. Diuron (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.

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

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 and Takeuchi et al. 2008). In yeast AhR assay, Noguerol et al. (2006) showed that Diuron has significant interaction with human AhR. The EC_{50} for Diuron 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 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. They showed also by 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).

The ability of Diuron 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

Diuron (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, Diuron (50 µM) or Linuron (50 µM) did not affect CYP19 aromatase activity, measured with $^{3}H2O$ assay using tritiated androstendione as a substrate, in human placental microsomes (Vinggaard et al. 2000).

Diuron 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 Diuron on the activity of various enzymes involved in synthesis and metabolism of sex hormones in fish. Androstedione testicular metabolism was studied by incubating ^{3}H-androstenedione (0.1 µM) and Diuron (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. 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.

In vivo data

Aquatic animals, Daphnias

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(s). 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 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 Daphnias were impacted (reduced). The acute toxicity (48 h-EC50) to Daphnia was 1.4 mg/l, which was higher than the lowest effective concentration on reproduction. The results were statistically significant.

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 the semi-static test.

However, while looking at the raw data of reproduction in the study, it can be seen that 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 the [redacted] (1989) test (1 mg/l) was approximately the same as the LOEC concentration in the [redacted] (1996) test (0.97 mg/l). Therefore it can be concluded that the results of the [redacted] (1989) test may well be in concordance with the [redacted] (1996) 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.

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 both *in vitro* assay (spiggin production in primed female three-spined 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) and *in vivo* assay in the three-spined stickleback exposed to the test compound together with DHT (5 µg/l) for 21 days. 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. 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*.

Sediment dwelling organisms

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 fresh water 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 was significantly decreased (Gallo and Tosti 2013).

Exposure experiments with cupped oyster (*Crassostrea gigas*) led to a slight bioaccumulation of Diuron, 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).

Terrestrial animals

There was one guideline study available on earthworm reproduction, based on the ISO 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 Diuron 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% 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 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 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 exposure groups in comparison with the control group.

No estrogenic impact was observed as the 17β-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 of the lizard study suggest that Diuron exposure results in direct male reproductive toxicity.

Environmental fate of Diuron

The potential bioaccumulation and persistence of Diuron and persistence of its transformation products with potential ED relevance increase the concern for possible adverse effects in the environment. Therefore, these properties were evaluated in addition to ED properties.

Transformation products of Diuron with potential ED relevance

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). The binding of Diuron and its metabolites to the androgen receptor suggest a possible endocrine mode of action.

DCPU and DCPMU were reported to be formed from Diuron in the simulation tests (. 1996, 2001, 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 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 (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 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 3,4-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).

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 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, Giocomazzi and Cochet 2004) and therefore 3,4-DCAA can be expected to be formed from Linuron through 3,4-DCA.

Persistence of Diuron and its transformation products

The persistence of Diuron and its transformation products can increase the probability for serious effects to the environment by resulting in longer exposure times of animals as well as higher environmental concentrations, compared to non-persistent substances. It is noted, however, that persistence in the context of the PBT assessment is out of the scope of the present assessment.

In the degradation simulation tests (1990, 1996, 2001, 1993 as cited in RMS Denmark 2005) mineralization of Diuron was at highest 30-32% of applied radioactivity after 102-120 days (a soil simulation test by (1996) and a water-sediment simulation test by (2001)). DCPMU, DCPU, and 3-(4-chlorophenyl)-1,1-dimethylurea (m-CPDMU) were the main transformation products. The Registrant(s) consider that Diuron meets the criteria for persistent (P) and very persistent (vP) substances. For the transformation products DCPMU, DCPU, 3,4-DCA and 3,4-DCAA no persistence assessments are available. However, 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 suggests potential persistence. It is also noted that DCPMU and DCPU are structurally relatively similar to Diuron and 3,4-DCA and therefore may be similar in persistence. Due to the observed low-rate transformation of Diuron and its transformation products in the environment organisms in the environment can be simultaneously exposed to Diuron and its transformation products.

Bioaccumulation potential of Diuron

The potential of a substance to bioaccumulate can increase the probability of serious effects to the environment because bioaccumulation may result in longer exposure times of animals as well as higher concentrations in the environment and in organisms, compared to non-bioaccumulative substances. It is noted, however, that bioaccumulation in the context of the PBT assessment is out of the scope of the present evaluation.

Although measured (1993) and calculated (CSR) bioconcentration factors (BCF) for Diuron are well below the B criterion, biomagnification factors (BMF) of 1.3 - 2 have been determined in a brackish water lagoon (Roche et al. 2009) indicating potential for bioaccumulation, which may promote potential ED concern. Biomagnification is not included in the bioaccumulation criteria in the PBT assessment under the REACH Regulation. However, in the Guidance on Information Requirements and Chemical Safety Assessment Chapter R.11: PBT/vPvB assessment (R.11.4.1.2 p52, Version 2.0 November 2014) it is stated that a relevant BMF or TMF value higher than 1 can also be considered as an indication of very high bioaccumulation and that for aquatic organisms, this value indicates an enhanced accumulation due to additional uptake of a substance from food next to direct accumulation from water. Therefore, the observed biomagnification potential (BMF > 1) can increase the concern for environmental endocrine disruption.

Conclusion regarding available information and concern for endocrine disruption to wildlife species

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

Experimental *in vivo* evidence suggests that Diuron can have adverse impacts on reproduction. The potential persistence of Diuron 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, biomagnification may increase the concern for ED effects.

The concerns about the potential endocrine disrupting activity of Diuron in the environment are based the following key observations:

- adverse *in vivo* impacts on the reproduction and related pathways of aquatic and terrestrial invertebrates (*Daphnia, Eisenia*) and vertebrates (*Podarcis*);
- *in vitro* evidence of hormonal activity of Diuron: antiandrogenic and weakly (anti)estrogenic mode of action has been detected as well as aryl hydrocarbon receptor (AhR) -mediated activity;
- the transformation products of Diuron DCPU, DCPMU, 3,4-DCA, and 3,4-DCAA have been reported to bind to the androgen receptor *in vitro*;
- adverse *in vivo* antiandrogenic impact on fish (*Gasterosteus*) 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) 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.

In addition, endocrine disrupting properties of Diuron in mammalians could not be concluded with the information available to the evaluating MSCA, because for instance not all the endocrine sensitive endpoints have been studied in the reproductive toxicity studies.

Based on these observations there is a concern about potential endocrine disrupting effects of Diuron, which can lead to serious effects in the environment. The potential to persist and to bioaccumulate in organisms further increases the probability of 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 endocrine mode of action (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 Diuron being an endocrine disruptor in the environment. In order to clarify the concern further information on potential ED effects on wildlife (aquatic) is required.

Required testing of fish

In the OECD Conceptual Framework 150 (CF 150) (OECD 2012) guidance is given to select appropriate test methods for ED identification. Level 4 *in vivo* assays provide data on adverse effects on endocrine-relevant endpoints and may provide information about the ED potency of a compound. The OECD TG 234 Fish Sexual Development Test (FSDT) (level 4) is considered the most appropriate test to provide sufficient information of the potential ED impacts of Diuron on wildlife. The test allows evaluation of estrogen-mediated activity (agonistic and antagonistic) and androgen-mediated activity (agonistic and antagonistic) as well as interference with steroidogenesis (e.g. aromatase inhibitors) through a combination of endpoints such as biased phenotypic sex ratio, induction of intersex fish, increase of sexually undifferentiated fish, vitellogenin induction/depression in males and/or females and specific gonad histopathologic findings. Hence, the test enables ED identification and can establish a link between the observed endocrine mode of action and adverse effects on wildlife caused by Diuron.

The alternative test methods with fish and ED related endpoints would be level 3 (CF 150) screening methods (OECD 229 Fish short-term reproduction assay, OECD 230 21-day fish assay and OECD Guidance Document 148 Androgenised female stickleback screen (AFSS), which offer more limited information for ED identification than OECD 234. With OECD 229 it is generally not possible to link the ED MoA observed with adverse effects (changes in fecundity can be adverse but it is not an endocrine specific endpoint). OECD 230 does not address apical signs of adverse effects that could be attributed to a single EATS (estrogenic, androgenic, thyroid, steroidogenesis) modality (fecundity and histopathology are not included). OECD GD 148 (AFSS) addresses only androgen-mediated activity, which would exclude the estrogen-mediated activity seen in some of the *in vitro* tests. The advantage of the FSDT test (OECD 234) is that impacts on the sex ratio are both indicative of mode of action and reflect population relevant changes.

The choice of the OECD TG 234 test is appropriate also considering that the surface water compartment is regarded as a relevant compartment for testing based on distribution modeling as well as reported concentrations of the substance in wastewaters and surface waters. According to the results of Mackay level III environmental distribution modelling, 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). The half-lives of 3 h in air (1991), 232 d in water and sediment (2001) and 372 d in soil (1990) were used as modeling parameters.

Diuron has also been detected in waste water 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. Diuron has also been detected in surface waters (average concentration 41 ng/l) (Loos et al. 2009). The common presence of Diuron in effluents and in surface waters indicates that a proportion of Diuron released to WWTP's finds it way to the aquatic ecosystem and may persist in surface water.

Conclusion

Therefore, pursuant to Article 46(1) of the REACH Regulation, the Registrant(s) are required to carry out the following study using the registered substance subject to this decision:

Fish Sexual Development Test OECD TG 234 with Japanese medaka *Oryzias latipes* or *Zebrafish Danio rerio* or Three-spined stickleback *Gasterosteus acuelatus*. The genetic sex determination and secondary sex characteristics shall also be included if the determination of the parameters is possible for the selected test species.

IV. Adequate identification of the composition of the tested material

In relation to the required experimental stud(y/ies), the sample of the substance to be used shall 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 composition of the test material. The substance identity information of the registered 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. Finally, the test(s) must be shared by the Registrant(s).

V. Avoidance of unnecessary testing by data- and cost-sharing

In relation to the experimental stud(y/ies) the legal text foresees the sharing of information and costs between Registrant(s) (Article 53 of the REACH Regulation). Registrant(s) are therefore required to make every effort to reach an agreement regarding each experimental study for every endpoint as to who is to 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

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 stud(y/ies) on behalf of all of them.

VI. Information on right to appeal

An appeal may be brought against this decision to the Board of Appeal of ECHA under Articles 52(2) and 51(8) of the REACH Regulation. Such an appeal shall be lodged within three months of receiving notification of this decision. Further information on the appeal procedure can be found on the ECHA's internet page at http://www.echa.europa.eu/regulations/appeals. The notice of appeal will be deemed to be filed only when the appeal fee has been paid.

Authorised[3] by Leena Ylä-Mononen, Director of Evaluation

Annex: List of registration numbers for the addressees of this decision. This annex is confidential and not included in the public version of this decision.

[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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