Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

diuron (ISO); 3-(3,4-dichlorophenyl)-1,1-dimethylurea

EC Number: 206-354-4
CAS Number: 330-54-1

CLH-O-0000007019-74-01/F

Adopted

16 September 2021

16 September 2021

CLH-O-0000007019-74-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **diuron (ISO); 3-(3,4-dichlorophenyl)-1,1-dimethylurea**

EC Number: **206-354-4**

CAS Number: **330-54-1**

The proposal was submitted by **Germany** and received by RAC on **15 May 2020.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Germany has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **1 June 2020**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **31 July 2020**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Nathalie Printemps**

Co-Rapporteur, appointed by RAC: **Raili Moldov**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **16 September 2021** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	006-015-00-9	diuron (ISO); 3-(3,4-dichlorophenyl)-1,1-dimethylurea	206-354-4	330-54-1	Carc. 2 Acute Tox. 4* STOT RE 2* Aquatic Acute 1 Aquatic chronic 1	H351 H302 H373 ** H400 H410	GHS08 GHS07 GHS09 Wng	H302 H351 H373 ** H410		M = 10	
Dossier submitters proposal	006-015-00-9	diuron (ISO); 3-(3,4-dichlorophenyl)-1,1-dimethylurea	206-354-4	330-54-1	**Modify** Carc. 1B STOT RE 2 **Retain** Aquatic Acute 1 Aquatic Chronic 1 **Delete** Acute Tox. 4	**Modify** H350 H373 (blood, bladder) **Retain** H400 H410 **Delete** H302	**Retain** GHS08 GHS09 **Modify** Dgr **Delete** GHS07	**Modify** H350 H373 (blood, bladder) **Retain** H410 **Delete** H302		**Add** M = 100 **Modify** M = 100	
RAC opinion	006-015-00-9	diuron (ISO); 3-(3,4-dichlorophenyl)-1,1-dimethylurea	206-354-4	330-54-1	**Modify** Carc. 1B STOT RE 2 **Retain** Aquatic Acute 1 Aquatic Chronic 1 **Delete** Acute Tox. 4	**Modify** H350 H373 (blood system) **Retain** H400 H410 **Delete** H302	**Retain** GHS08 GHS09 **Modify** Dgr **Delete** GHS07	**Modify** H350 H373 (blood system) **Retain** H410 **Delete** H302		**Add** M = 100 **Modify** M = 100	
Resulting Annex VI entry if agreed by COM	006-015-00-9	diuron (ISO); 3-(3,4-dichlorophenyl)-1,1-dimethylurea	206-354-4	330-54-1	Carc. 1B STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H350 H373 (blood system) H400 H410	GHS08 GHS09 Dgr	H350 H373 (blood system) H410		M = 100 M = 100	

GROUNDS FOR ADOPTION OF THE OPINION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute toxicity: oral

There are four acute oral toxicity studies in rats. The studies were considered acceptable by the dossier submitter (DS). Six other studies were short summaries in the Renewal Assessment Report (RAR) but were not accepted due to missing information on the test method and results.

***Table**: Summary of acceptable acute oral gavage toxicity studies available in rats with diuron*

Species (no. /group)	LD_{50} (mg/kg bw)	Vehicle	Results (mortality)	Study	Remarks
Rats (5 F)	> 2000	Arachis oil	No information	2007	OECD TG 420, GLP Purity > 98%
Rats (5 M and 5 F)	> 2000	Propane-1,2-diol	No mortality	1993	OECD TG 401, GLP Purity: 98.5%
Rats (10 F)	4150	Cremophor EL	0 up to 1000 mg/kg 3/10 at 2500 mg/kg 5/10 at 5000 mg/kg 9/10 at 7100 mg/kg	1983	Similar to OECD TG 401, no information on GLP status, purity: 98.8%
Rats (10 M)	1017	Cotton seed oil	No information	1970	Non-guideline, non-GLP Purity: 95%

M: males, F: females

***Table:** Summary of acute oral toxicity studies available in rats with diuron: non-acceptable studies due to missing information*

Species (no./group)	LD_{50} (mg/kg bw)	Vehicle	Results (mortality)	Study	Remarks
Rats (5 M)	4138 mg/kg	Cremophor EL	No information	1984	Non-guideline, non-GLP No information on purity
Rats (5 M)	> 5000	Cremophor EL	No information	1981	Non-guideline, non-GLP No information on purity
Rats (10 M)	> 5000	Cremophor EL	No information	1981	Non-guideline, non-GLP No information on purity
Rats (10 M)	3000-5000	Cremophor EL	No information	1975	Non-guideline, non-GLP Purity: 99.4%
Rats	> 5000	Cremophor and water	1/10 at 2500 mg/kg 3/10 at 5000 mg/kg	1974	Non-guideline, non-GLP Purity: 98.5%
Rats	> 5000	Cremophor and water	2/15 at 2500 mg/kg 4/15 at 3500 mg/kg	1972	Non-guideline, non-GLP Purity: 97.3%

In the three studies conducted according or similar to OECD TG 401 or 420, LD_{50} values above 2000 mg/kg were obtained (Anonymous 5, 1983; Anonymous 17, 1993 and Anonymous, 2007). The fourth acceptable study is a non-guideline published study with limited reporting (Boyd and Krupa, 1970). The number of tested doses and animals that died was not reported. In this study, male rats were exposed to diuron by single oral gavage after 20-28 days feeding with three different diets. Diuron toxicity was higher in rats fed with low protein diet compared to rats fed with high protein-containing diet (LD_{50}=437 mg/kg *vs* LD_{50}=2390 mg/kg). The LD_{50} obtained in

rats fed with standard laboratory chow was 1017 mg/kg. The DS noted that the current harmonised classification has been based on the results of this study.

The DS gave higher weight to the three well-documented guideline studies. On this basis, the DS proposed to remove the existing classification Acute Tox. 4*, H302.

Acute toxicity: dermal

No classification was proposed by the DS for acute dermal toxicity as the LD_{50} values in the three relevant dermal toxicity studies in rats, using different vehicles and occlusive conditions, were above the thresholds for classification (> 2000 mg/kg). Two of the three studies were performed according to OECD TG 402 and were GLP-compliant.

Acute toxicity: inhalation

Three rat acute inhalation toxicity studies were available in the dossier and were considered acceptable by the DS. The studies were similar or performed according to OECD TG 403. The studies resulted in 4h LC_{50} values of > 0.223 mg/L or > 5.05 mg/L with diuron as an aerosol (Anonymous 5, 1983; Anonymous, 2007) and LC_{50} was > 7.1 mg/L for diuron administered as a dust (Anonymous 9, 1987). The substance was administered at the maximum achievable concentration in these three studies. On this basis, the DS proposed no classification.

***Table**: Summary of acceptable acute inhalation nose-only toxicity studies available in rats with diuron*

Species (no. /group)	LC_{50} (mg/L/4h)	Test substance, form and particle size	Results (mortality)	Study	Remarks
Rats (5M+5F)	> 5.05	Aerosol MMAD: 3.59µm, GSD:2.56	None	2007	OECD TG 403, GLP Purity > 98%
Rats (10M+10F)	> 7.1	Dust MMAD: 6-10µm	None	1987	Similar to OECD TG 403, GLP Purity: 99%
Rats (10M+10F)	>0.223	Aerosol Ethanol-lutrol mixture (1:1) Most of particles < 5µm	None	1983	Similar to OECD TG 403, no information on GLP status, purity: 98.8%

MMAD: Mean median aerodynamic diameter; GSD: geometric standard deviation

Additional studies identified by RAC

When examining the references used for acute toxicity classification, RAC identified two additional published studies (Gaines and Linder, 1986 and Hodge *et al*., 1967) that were not included in the RAR and CLH dossier. One of these studies provided results below 2000 mg/kg in rats (Gaines and Linder, 1986). In this study, male and female adult (minimum 90 days of age) Sherman rats were exposed to diuron by oral gavage. The vehicle used was peanut oil. Ten animals per group were used in the study. It is reported that 4 dosages were used for the calculation of the LD_{50} (detailed results not provided in the publication) and the dosing volume was 5 mL/kg. The LD_{50} was reported to be 1258 mg/kg in males (Confidence interval (CI) 95%: 999-1583) and 1182 mg/kg in females (CI95%: 995-1450). Purity of the test material was not specified.

***Table**: References used for previous acute toxicity classification, not included in the CLH report*

Species	LD_{50} (mg/kg bw)	Vehicle	References
Rats	3400 (2900-4000)	Vehicle: peanut oil	Hodge *et al*., 1967
Rats	1258 (M) 1182(F)	Vehicle: peanut oil	Gaines and Linder, 1986

Comments received during consultation

One industry representative agreed with the DS proposal for acute toxicity (all routes). For acute oral toxicity, the industry representative noted that in the Boyd and Krupa (1970) study, a very high administration volume of oil-based formulation was given (20 mL/kg instead of 10 mL/kg recommended in the OECD TG). This deviation may have affected the study outcome as it may have resulted in passive reflux due to stomach overfilling.

Assessment and comparison with the classification criteria

Acute toxicity: oral

No data on potential human cases of poisoning were detailed in the dossier. The DS noted that the probable oral lethal dose in humans was reported to be in the range of 500 to 5000 mg/kg (US NIOSH's Registry of Toxic Effects of Chemical Substances and the US National Library of Medicine's Toxicology Data Bank). Nevertheless, the data were limited and the incidents with diuron included other substances, precluding their use for the determination of acute toxicity. In this context, RAC agrees with the DS that these human data cannot be used for classification. In addition, RAC agrees that the LD_{Lo} of 500 mg/kg reported in the same Data Bank, in mice exposed intraperitoneally to diuron is not suitable for acute oral toxicity classification.

Ten acute oral toxicity studies in rats were available in the CLH dossier. The LD_{50} was above 2000 mg/kg bw in all studies except in Boyd and Krupka (1970). In this study, an LD_{50} of 1017 mg/kg bw was obtained following a standard laboratory diet. The LD_{50} of 437 mg/kg bw obtained with animals fed with insufficient protein diet or the LD_{50} of 2390 mg/kg bw obtained with rats fed with protein test diet are not considered suitable for classification. In this study, the purity of the test material was lower than in other acceptable studies and the volume of administration may have been excessive. It may be noted that in this study, weanling rats were used (acquired as weanlings or 2 weeks after weaning) instead of 8- to 12-week old rats as recommended in the OECD TG. In addition, the DS noted poor reporting of the results. Moreover, an LD_{50} below 2000 mg/kg bw was obtained in the published study of Gains and Linden, 1986. Nevertheless, the purity of the test material was not available, and the results were only shortly reported.

Consequently, RAC agrees with the DS that based on the three most reliable studies, performed according to or similar to OECD TG for acute oral toxicity, **no classification for acute oral toxicity is warranted** for diuron.

Acute toxicity: dermal

Based on the two guideline, acute dermal toxicity studies and one published study performed on rats (1983, 1993, 2007), RAC concludes that **no classification for acute dermal toxicity is warranted** for diuron (LD_{50} > 2000 mg/kg bw).

Acute toxicity: inhalation

RAC notes that no death occurred in the three available acute inhalation toxicity studies. Although the negative result was observed in Anonymous 9 (1987) with diuron as a dust with a mass median aerodynamic diameter (MMAD) higher than 4 µm at the maximum attainable concentration, a lower MMAD was achieved in the most recent study from 2007 (3.59 µm), as recommended in the OECD TG. As no mortality was observed below 5 mg/L, **no classification is warranted for acute toxicity by inhalation**.

RAC evaluation of specific target organ toxicity-repeated exposure

Summary of the Dossier Submitter's proposal

The evaluation of the STOT RE endpoint was based on eleven repeated-dose toxicity studies. These comprised eight studies in rats: two oral non-guideline 4-week published studies, a 90-day guideline studies by oral and dermal route, an 8-week and a 21-day guideline study by inhalation and two 6-month mechanistic non-guideline feeding studies. In addition, a 3-week dermal toxicity study was available in rabbits, a 6-month mechanistic non-guideline feeding study was available in mice and a one-year guideline feeding study was available in dog. Two old 90-day oral toxicity studies in rats and one 2-year dog study were briefly summarised in the RAR but were not considered acceptable by the DS.

The blood system and the urinary tract were identified as the main target organs of diuron.

Blood system

In rats, mice and dogs, after repeated administration of diuron, haemolytic anaemia was observed. The following findings were noted by the DS in rats and dogs, relevant for classification:

- decreased erythrocyte count (RBC), haemoglobin (Hb), haematocrit (Ht),
- increase Heinz bodies and serum bilirubin,
- accumulation of pigments in liver, kidneys, and spleen, enlarged spleen,
- compensatory increase in reticulocytes and evidence of extramedullary haematopoiesis in the spleen,
- most of the effects were reversible.

Based on oral studies, haemolytic anaemia was observed below the dose levels relevant for classification asSTOT RE 1. The inhalation studies also support a classification of the substance as STOT RE. Nevertheless, due to the low magnitude of the observed effects, some of the findings may have been adaptative rather than adverse. Therefore, the DS proposed to keep the current existing classification of diuron STOT RE 2, H373 (but add blood and bladder as target organs).

Bladder

In the 90-day rat study (Anonymous 10, 2004), histopathological lesions (hyperplasia of the transitional epithelium) were noted in the bladder and kidneys in males and females, from 17 and 23.3 mg/kg bw/d onward, respectively. The effects were not observed in dogs. In the long-term study in rats, preneoplastic bladder lesions were observed in females from 17 mg/kg bw/d and at the top dose in males (111 mg/kg bw/d). The DS concluded that these findings fulfilled the criteria for classification of diuron as STOT RE 2, H373 (bladder).

Comments received during consultation

An industry representative agreed with the DS's proposal to classify the substance as STOT RE 2 for blood and bladder. A detailed analysis of the most reliable studies was also provided in an attachment to the comment. Four studies were considered as key studies for classification: 90-day oral rat study (2004), 8-week inhalation rat study (1986b), 90-day dermal study in rat (1996) and the 1-year dog study (1985).

Assessment and comparison with the classification criteria

Urinary tract

Transitional cell hyperplasia in the urinary tract was identified in the oral repeated-dose toxicity studies (including carcinogenicity) in rats and in the carcinogenicity study in mice. No effect on the urinary tract was reported in the dermal or inhalation studies in rats. Pigment deposit in the kidney of dog is considered related to the haemolytic anaemia. The effect observed in mice were observed at dose levels above the guidance value for classification as STOT RE 2.

Transitional cell hyperplasia seen in the urothelium are considered adaptive effects with no adverse consequences on cessation of exposure except possible development of neoplasia, which is addressed under the carcinogenicity hazard class as urinary tract tumours are observed. Therefore, as no other changes, indicative of urinary tract toxicity was noted, RAC considered that no classification for STOT RE is warranted for urinary tract system.

Haemolytic anaemia

Oral route

RAC agrees that there are 2 key studies for the classification of the substance by the oral route, the 90-day feeding study in rat and the 1-year study in dog. In addition to the studies taken into account by the DS for this endpoint, carcinogenicity studies performed in rats and mice were also considered relevant.

Signs of regenerative haemolytic anaemia was observed in rats, mice and dogs. The severity of the effects increased with dose levels. The rat was the most sensitive species. Effects at dose levels relevant for classification as STOT RE were only noted in rats and dogs. A summary of the relevant findings observed in the blood system of rats and dogs is provided in the in-depth analysis section below.

A decrease in Hb around 10 to 20% was found at doses relevant for classification STOT RE 2 in rats in the 90-day feeding study (Anonymous 10, 2004). Decreased Hb by more than 10% was also noted in the 1-year dog study and in the carcinogenicity study in rat but at dose levels above the guidance value relevant for classification. In the 90-day study, the decrease in Hb was associated with decreased haematocrit and red blood cells count (RBC). At the same time, an increase in reticulocytes and a marked increase in methaemoglobinaemia was noted (by 38% in males and females at 17/23 mg/kg bw/d). An increase in sulfhaemoglobin was also noted in both males and females, indicative of adversity as it may result in lower total oxygen-carrying capacity of the blood. The effects were reversible after 3-month cessation of exposure.

In this 90-day study, a significant increase in extramedullary haematopoiesis and congestion in the spleen was noted at all dose levels (≥ 8.7 mg/kg bw/d) in females. At the mid dose of 23 mg/kg bw/d, haematopoiesis in the bone marrow was noted in almost all females and 2/10 female had pigments in the spleen. Pigmentation in liver, spleen and kidney was also observed in the study in almost all animals at the top dose level only, above the guidance value for classification (176/214 mg/kg bw/d). The histopathological findings were reversible except that

haematopoiesis in bone marrow was still observed, without clear dose-response. Necrosis and degeneration were noted in a 4-week oral supplementary study but were not reported in longer-term studies.

In dogs, after 1-year exposure to diuron, pigment deposits were increased in spleen and kidney at 11 mg/kg bw/d (Anonymous 8, 1985), a dose relevant for classification as STOT RE 2.

According to the CLP regulation, the main criteria for haemolytic anaemia classification is "*any consistent and significant adverse changes in haematology*". Classification is warranted if haemolytic anaemia induces one or more of the serious effects listed in Annex I.

- *Morbidity or death resulting from repeated or long-term exposure*. Mortality or severe clinical signs resulting from anaemia were not reported in the studies.
- *Any consistent and significant adverse effect in clinical biochemistry, haematology or urinalysis parameters.* Reduction of -Hb- ≥ 20% was not observed in the studies. In the 90-day study, functional Hb was reduced by more than 20 % (by 22%) considering a combination of Hb reduction and Met Hb increase only at the top dose level, above the guidance value for classification (> 100 mg/kg bw/d).
- *Evidence of appreciable cell death (including cell degeneration and reduced cell number) in vital organs incapable of regeneration*. Significant increase in haemosiderosis was noted in the spleen of females at a dose relevant for classification STOT RE 2 in the 90-day study (23 mg/kg bw/d) in combination with significant haematological findings (Hb reduction ⩾ 10%). It may be noted that degeneration and necrosis was seen in the liver in the 28-day study in rat. In addition, after long-term 2-year exposure spleen fibrosis was noted in a few rats at a dose relevant for classification STOT RE 2, indicative of non-reversible changes. In a weight of evidence (WoE) analysis, the CLP criteria are considered fulfilled.

Changes observed at dose levels relevant for STOT RE 1 were not severe enough for classification (e.g. Hb decreased by less than 10%, marginal changes at necropsy). Therefore, RAC agrees with the DS that a classification as STOT RE 2, H373 for blood system by oral route is warranted.

<u>Dermal route</u>

Diuron was administered by dermal exposure in two studies, in a 28-day study in rabbits and in a 90-day study in rats. No effects suggestive of anaemia were noted in the rabbit study. In the 90-day dermal rat study, a decrease in Hb (14-16%), associated with a significant decrease in RBC (13-16% in males and 22-24% in females) and increased MCV (> 10% in female only) was noted at a dose relevant for classification. Nevertheless, no other indicator of haemolytic anaemia was noted in the study. Therefore, the observed effects after dermal administration are of doubtful toxicological importance and may be rather considered adaptative.

<u>Inhalation route</u>

There are two studies that were conducted by inhalation in rats. One is a 3-week study, and one is a combined 4- and 8-week exposure study (5d/week). In the combined 4- and 8-week studies, a significant decrease in RBC parameters and increased reticulocytes and Heinz bodies was noted. The decrease in Hb was above 10% at the top dose, relevant for classification as STOT RE 2 in females only. At necropsy, dark and enlarged spleen was observed.
In the 3-week inhalation study, decreased RBC, and increase MCV, reticulocytes and Heinz bodies were observed. Dark, swollen and congested spleen was observed from the mid dose onward. Haemosiderin in spleen was also reported to be present but incidence and significance was not detailed in the study.

Overall, based on the 3- or 8-week studies, haematological parameters changes, indicative of haemolytic anaemia were noted. The effects observed by inhalation are supporting the classification for STOT RE.
Haemolytic anaemia cannot be conclusively excluded by other routes than oral route as effects indicative of haemolytic anaemia were observed by all routes of exposure (oral, inhalation, dermal).
RAC agrees to **classify diuron as STOT RE 2 for blood system**, not specifying the route of exposure.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The DS concluded that based on the available *in vitro* data, diuron did not cause gene mutation in bacteria or mammalian cells. An *in vitro* unscheduled DNA synthesis assay (UDS) was negative in hepatocytes. In contrast to these negative results, contradictory results were obtained in the *in vitro* chromosome aberration assays.

In vivo, one micronucleus assay was positive, using intraperitoneal route of exposure. Nevertheless, a second assay, using the same route of administration up to higher dose levels failed to reproduce the positive assay. In addition, the substance was negative in an oral micronucleus assay.

The DS pointed out that the positive results obtained in an *in vivo* UDS assay in bladder cells could be in line with the bladder tumours observed in the long-term study in rats. Nevertheless, the DS considered that the data rather suggested stimulation of mitosis than true genotoxicity.

The DS stressed that the available studies were not performed according to the latest version of OECD TG but were still considered acceptable or supplementary. Available positive published data on non-mammalian organisms were briefly reported in the RAR but were not considered acceptable for classification of the substance as there are no existing current guidance documents to assess how to consider the studies.

Overall, based on a WoE analysis, the DS concluded that diuron does not fulfil the CLP criteria for germ cell mutagenicity.

Comments received during consultation

An industry representative agreed with the DS' proposal and provided an in-depth analysis of the studies available *in vitro* and *in vivo*.

Assessment and comparison with the classification criteria

In vitro data

Gene mutation in bacteria

The DS reported seven Ames assays. The studies were performed according to or were similar to OECD TG 471. Based on all the available data, all recommended tested strain were tested. Dose levels up to precipitation of the test item were tested. The studies used either preincubation methods or plate incorporation. Positive results were only noted in two recent experiments dated

2008 in TA100 at ⩾2500µg/plate with metabolic activation in presence of toxicity and precipitation of the test item. Nevertheless, the increase was not reproducible in the studies. Two additional studies specifically investigating the mutagenic potential of the substance in TA100, failed to reproduce the positive results as no biological increase was noted and toxicity was confirmed at concentration ⩾ 2500 µg/plate with and without metabolic activation.

Therefore, RAC concludes that diuron is not mutagenic in bacteria in presence or absence of metabolic activation.

Mammalian cells results

Regarding *in vitro* gene mutation in mammalian cells, negative results were observed in the two available HPRT gene mutation studies in Chinese hamster ovary cells or human lymphocytes (Anonymous, 1998 and Anonymous, 1985). The studies were performed according to or were similar to OECD TG 476.

An *in vitro* UDS is available in rat hepatocytes. A significant dose-related increase in net grain count was noted at 0.33mM, 1 and 20 mM. At these dose levels, severe cytotoxicity was reported. Precipitation was noted at ⩾ 1mM. Following analysis, it was concluded that the increase was due to a decrease in cytoplasmic grain counts and not due to the nuclear grain count. The authors concluded that the positive results were caused by cytotoxicity rather than by DNA repair synthesis. RAC agrees with this conclusion.

There are three chromosomal aberration studies in mammalian cells reported in the dossier. The studies were performed according to or were similar to OECD TG 473. In Anonymous, 1989, a statistically significant increase in chromosomal aberration was noted in human lymphocytes at ⩾ 250 µg/mL without metabolic activation and at 750µg/mL with metabolic activation (excluding gaps). It is reported that based on mitotic index, the effects were observed at cytotoxic concentration. The extent of the decrease in mitotic index was not specified. It is therefore unclear whether the decrease in mitotic index was above the threshold of 50%. In Anonymous (1999), no increase in chromosomal aberration was noted in Chinese hamster ovary cells up to 360 µg/mL in presence or absence of chromosomal aberration. Mitotic index was 55% in presence of S9 and 60% in absence of S9 at the top dose. In the third summarised study, published by Federico *et al*. (2011), diuron induced an increase in the percentage of aberrant cells in Chinese hamster ovary and epithelial liver cells at ≥ 0.45 µg/mL. According to the author of the study, mitotic index was not reduced by more than 65% of the control values. As mentioned by the industry representative during public consultation of the dossier, the doses selected for testing were not explained. Moreover, the reliability of the study is questionable as the mitotic index was below 10% in all groups (including negative and positive controls).

Overall, RAC agrees with the DS that the substance did not induced gene mutation in bacteria or mammalian cells *in vitro* but that equivocal results were observed for clastogenic potential of diuron *in vitro*.

In vivo data

Assay	Test system	Test condition	Results	Effective dose level	Reference
Germ cells					
Dominant lethal (similar to OECD TG 478)	Male mice	Oral: gavage 2500 mg/kg bw	-	/	Anonymous, 1986
Dominant lethal (similar to OECD TG 478)	Male mice	Intraperitoneal (single dose) 170 or 340 mg/kg	+	170 mg/kg bw	Argawal *et al*., 1997
Chromosomal aberration (OECD TG 483)	Male mice	Oral: gavage (single dose) 500-5000 mg/kg bw spermatogonia	-	/	Anonymous 16, 1988
Somatic cells					
Unscheduled DNA synthesis in bladder urothelial cells (similar to OECD TG 486)	Rat (M+F)	Oral: dietary (7-day exposure)	Equivocal	104 mg/kg bw	Klein, 1986
Chromosomal aberration (similar to OECD TG 475)	Rat (M+F)	Oral: gavage (single dose) Bone marrow cells, 500-5000 mg/kg bw Sampling at 6, 24 or 48h	-	/	Anonymous 1, 1985/1997
Chromosomal aberration (OECD TG 475)	Chinese Hamster (M + F)		-	/	Anonymous, 1987a
Sister chromatid exchange	Chinese Hamster	Oral gavage (single dose) 500-5000 mg/kg bw, sampling at 24h	-	/	Anonymous 16, 1987b
Micronucleus in bone marrow cells (Similar to OECD TG 474)	Mouse (M+F)	Oral: gavage (single dose) Sampling 30, 48h and 72h	-	/	Anonymous, 1983
	Mouse (M+F)	i.p. (single dose) 85-340 mg/kg bw Sampling: 30, 48, 72h	+	178 mg/kg bw at 30 and 48h only (dose-related), 340 mg/kg bw induced toxicity	Agrawal *et al*., 1996
	Mouse (M+F)	i.p. (single dose), 700 mg/kg bw Sampling: 16, 24, 48h	-	/	Anonymous 7, 1998

-: negative, +: positive

Two dominant lethal tests in male mouse were reported. In Anonymous, 1985, male mice received 0 or 2500 mg/kg bw diuron. Males were mated for 12 periods with untreated females. The substance did not show an effect on fertility or implantation. However, no positive control was included in the study. Therefore, the results are not considered reliable. In the study published by Agrawal *et al*., 1997, an increase in dominant lethality was observed after single intraperitoneal administration of the substance at ≥ 170 mg/kg bw. The assay is not considered

reliable by the DS as no positive control was used and as the purity of the test material was not reported.

A negative chromosomal aberration study was performed on male mice germ cells according to OECD TG 483. The main limitation of the study was the low number of metaphases scored compared to the number recommended in current test guideline.

An *in vivo* UDS test is available in bladder urothelial cells (Klein, 1986). The test was considered as supplementary due to missing information on purity of the test item and the absence of GLP. A significant increase in grain count per cell was noted at ≥ 250 mg/kg. A dose-related increase in S phase cell proportion was also observed. As the increase was only observed in cells with 3 silver grains (and not 4 or 5), the delay in DNA repair may not have been due to a direct genotoxic effect. RAC agrees with this conclusion. The DS noted that this result might indicate a mitogenic potential effect that would be in line with the results of the carcinogenicity study.

Two chromosomal aberration studies were performed on bone marrow cells and were similar or conducted according to OECD TG 475. The main limitation of these studies was the low number of metaphase score compared to the current OECD TG. Although an increase in chromosomal aberration was noted at 5000 mg/kg bw in Anonymous 1, 1985/1997, the increase was inside the historical control data (HCD) and in presence of cytotoxicity. Therefore, the two studies are considered negative.

The reported sister chromatid exchange performed in Chinese hamster at 500 to 5000 mg/kg diuron was negative *in vivo*. Toxicity was noted in this study at all dose levels.

Three bone marrow micronucleus assays were available in mice. The studies were similar to or were performed according to OECD TG 474. In the study dated 1983, considered as supplementary by the DS, mice were treated orally at 2500 mg/kg bw diuron. No general toxicity and no increase in micronuclei in polychromatic erythrocytes were noted. In the study published by Agrawal *et al*., 1996, considered as supplementary due to insufficient reporting, a single intraperitoneal dose of 85, 170 or 340 mg/kg bw was given to mice. The top dose was considered the maximum tolerated dose by the authors. A dose-related statistically significant increase in micronucleated polychromatid erythrocytes was noted after 30 and 48h at ≥ 170 mg/kg bw. The effect was not observed at 72h. The DS noted that the negative and the positive controls were very low, possibly due to a late reading of the test. Nevertheless, RAC notes that no further data were provided to substantiate this statement (1.6 MNPCE per 1000 in the control of the published study vs 1.7 and 1.8 per 1000 in the two study reports). In the third study, dated 1998, a single intradermal administration of 700 mg/kg bw was given to mice. In this study, dose levels were chosen based on a pilot test. The study failed to reproduce the positive results obtained in Agrawal *et al*., 1996 although higher dose levels were used.

Comparison with classification criteria

There is no human data available. Therefore, Category 1A is not warranted.

One positive result was obtained from *in vivo* heritable germ cell assay (Dominant lethal) test after intraperitoneal administration of diuron. Nevertheless, although the assay may indicate and intrinsic potential of the substance, negative results were obtained in an oral dominant lethal assay up to 2500 mg/kg bw diuron. In addition, a chromosomal aberration assay was negative in spermatogonia with diuron. Therefore, no classification in Category 1B is considered warranted.

In somatic cells, a positive micronucleus assay was reported after intraperitoneal administration of diuron. Nevertheless, the results were not reproduced in another intraperitoneal micronucleus study using higher dose levels or in an oral gavage micronucleus study. Regarding the equivocal

in vivo UDS assay, the increase in net grain count may not have been due to a direct genotoxic effect. On this basis, no classification in Category 2 is warranted.

Overall, RAC agrees with the DS that **diuron does not fulfil the CLP criteria for germ cell mutagenicity classification**.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The carcinogenicity potential of diuron was investigated in two-year carcinogenicity studies in rats and mice. In addition, several mechanistic studies were available to investigate the potential tumour mode of action (MoA).

The following neoplastic findings were observed in the rats and mice carcinogenicity studies:

- Increased incidence of malignant transitional cell epithelium carcinoma in the urinary bladder in both sexes and in the renal pelvis in males,
- Increased incidence of malignant uterus adenocarcinoma in female rats,
- Increased incidence of malignant mammary gland adenocarcinoma in female mice,
- Increased incidence of benign ovary luteoma in female mice.

According to the DS, the current classification of diuron as Carc. 2 is based on the bladder tumours in rats. The DS noted that the mice tumours were not taken into consideration during the previous assessment of carcinogenicity although the mice carcinogenicity study was available.

The DS pointed out that there are mechanistic data available on bladder tumours in rats, suggesting that urinary solids do not contribute to the development of the neoplastic findings. The assumption that cytotoxicity was due to physical irritation is not anymore supported. On balance the DS stated that the additional available mechanistic data showed that diuron and/or its metabolites is cytotoxic to the urothelium leading to regenerative hyperplasia and subsequently urinary tract tumours. In addition, tumour promotion in the bladder has been demonstrated in a two-stage carcinogenesis model in mice.
There are a few mechanistic data available on mammary gland carcinogenesis. Diuron was not a promoting agent for mammary tumours as negative results were obtained in two-stage carcinogenesis models in mice and rats (Grassi *et al*., 2011a and De Moura *et al*., 2009). There is no mechanistic information on other tumour types. Hormonal disturbance has not been investigated but the DS commented that it could not be excluded that the tumours observed in uterus, mammary gland and ovary were endocrine-mediated.

The DS stressed that although the increases in tumour incidences were seen at the top dose levels, the doses were well below 1000 mg/kg bw/d. In addition, the DS highlighted that there is no evidence that the tumours would not be relevant to human.

Based on tumours observed in two-species, multi-site response with progression to malignancy, the DS proposed to classify diuron as Carc. 1B, H350.

Comments received during consultation

An industry representative disagreed with the DS' proposal to change the current classification Carc. 2 to Carc. 1B and provided an in-depth analysis of the carcinogenicity studies and of the HCD.

The industry representative commented that except the urinary tract tumours in rats, the tumours observed in other organs were not relevant for classification. The main arguments were:

- Inconsistent picture between rats and mice tumour profiles.
- Rat uterine adenocarcinoma were inside appropriate HCD. In addition, the increase was of borderline statistical significance and observed in presence of excessive toxicity.
- The increase in rat urinary bladder tumours was a high dose effect related to cytotoxicity, observed only at excessive dose levels above the maximum tolerable dose in rats. Indeed, they commented that, based on mechanistic data, high dose levels were required to produce irritant metabolites at urinary concentration that would be cytotoxic. These high dose levels are not relevant when compared to occupational environmental levels.
- In mice, the mammary gland tumours may have been age-related. Industry highlighted the very long study duration of the diuron study. In the HCD, all the studies were 20 or 21 months whereas the diuron mice study was 24 months. In addition, industry questioned the reliability of the study as due to the long study duration, the survival in mice was below 50% at the end of the study. The Industry representative also pointed out that there were no pre-neoplastic findings in the mammary gland and that mammary gland was not the target organ in repeated-dose toxicity studies.
- No evidence of a treatment-related effect in ovary in the absence of effects on combined sex cord stromal tumours.
- Lack of mechanistic evidence (no genotoxic potential, no pre-neoplastic findings) for female tumours.
- No epidemiological evidence in human of a carcinogenic potential of the substance.

Assessment and comparison with the classification criteria

Two carcinogenicity assays were included in the CLH report, one in Wistar rats (Anonymous 14, 1985) and one in NMRI mice (Anonymous, 1990).

Rats

In Wistar rats, 50 animals/sex/group were exposed to diuron during 2 years at 0, 1.0, 10, 111 mg/kg bw/d in males and 0, 1.7, 17 and 203 mg/kg bw/d in females. The study was similar to OECD TG 453. Some limitations were noted compared to the OECD TG as some parameters were not included (e.g. clinical chemistry). In addition, mammary glands were not examined in the initial study report. As tumours were seen in mice, histopathological examination on residual mammary tissue attached to the salivary glands from the interim groups and all main groups were conducted in an additional analysis (Information retrieved in APVMA, 2011).

No treatment-related effect on survival was noted in the study. Clinical signs were reported at the top dose in males (reddish discoloured or bloody urine). A significant decrease in body weight gain (-18% in males and -21% in females) and food efficiency was noted in both sexes at the top dose. Effects on blood system (haemolytic anaemia and compensatory haematopoiesis) was observed at mid dose and high dose in males and at all dose levels in females.

<u>Transitional cell tumours in rats</u>

A strong statistically significant increase in the incidence of urinary bladder carcinoma (transitional cell) was observed at the top dose in both sexes. No HCD were provided but this type of tumours is rare in rats.
In addition, one high dose male had transitional cell papilloma and two high dose males had transitional cell carcinoma in the renal pelvis. The increase in transitional cell carcinoma in the renal pelvis resulted in a significant trend according to the DS calculation. Pre-neoplastic findings (transitional cell hyperplasia) were noted in both male and female rats. Indeed, an increase in

severity of transitional cell hyperplasia was observed at interim and terminal kill at the top dose in males and at the mid and top dose in females, indicative of dose-response. RAC notes that the absence of dose-response for urinary tract tumours may have been due to the large dose-spacing.

***Table**: Summary of transitional cell neoplastic incidences in urinary bladder in the rats (Anonymous 14, 1985)*

	Males				Females			
Dose (mg/kg bw/d)	**0**	**1.0**	**10**	**111**	**0**	**1.7**	**17**	**203**
Number of animals	50	50	50	50	48	50	50	50
Carcinoma	1	0	1	33*	0	0	0	11*
Papilloma	0	0	0	3	1	0	2	2

*p≤0.01 (Cochrane Armitage linear trend test, two-sided)

The increase in transitional cell carcinoma observed in males and females in the urinary bladder and in males in the renal pelvis are considered treatment-related and relevant for classification. Although the increase in tumours was noted in presence of general toxicity (e.g. body weight gain changes), the effect is not considered a secondary consequence unrelated to the intrinsic properties of the substance. Indeed, the urinary tract system is a target organ of the substance. Pre-neoplastic lesions were noted at the top dose but also at the mid dose in females and in the mice carcinogenicity study in the presence of only mild general toxicity. In addition, RAC notes that transitional cell hyperplasia was already noted after 90-day exposure in rats in both males and females at ≥17 mg/kg bw/d (Anonymous 10, 2004).

Uterus tumours in rats

An increase in the number of malignant neoplasia was observed in the uterus at the top dose. The most notable increase was the increase in uterus adenocarcinoma. Nevertheless, the increase was not statistically significant (pair wise comparison). The DS calculated a borderline Cochrane Armitage linear trend (p≤0.036, one-sided and p ≤0.07, two-sided). No statistically significant increase in non-neoplastic findings in the uterus was observed.

***Table**: Summary of uterine tumour incidence (%) in rats (Anonymous, 1985)*

Dose (mg/kg bw/d)	**0**	**1.7**	**17**	**203**	**Historical control**
Number of animals	48	50	50	50	
Uterus adenocarcinoma, malignant	5 (10%)	5 (10%)	5 (10%)	10* (20%)	Range: 0-20% Mean: 8%
Endometrial sarcoma, malignant	0	0	0	2 (4%)	
Squamous cell carcinoma, malignant	0	0	1 (2%)	1 (2%)	
Polyps, benign	7 (15%)	7 (14%)	6 (12%)	3 (6%)	
Combined uterine polyps and adenocarcinoma	12	12	11	13	

*p<0.05 (Cochrane Armitage linear trend test, one-sided)

An Industry representative provided HCD from 20 studies performed in the same laboratory and the same strain of rats, in a relevant period of time (1979-1984). The increase in uterine tumours observed in the diuron study was at the upper end of the historical control range but was far above the mean. In addition, the current control of the study was inside the historical control range. Previous studies performed in the laboratories from the same testing facility, same strain and breeder between 1975 and 1980 were reported to be between 0 and 16.3% (mean 7.8%)

and 0 to 20% (mean 5.7%) in studies performed between 1975 and 1994. These ranges are considered of lower relevance. Industry representative also provided published HCD from the same strain for uterus and vagina tumours: range 1.1 to 25%. Nevertheless, as the studies were performed in different laboratories and different periods, this published HCD range is not considered relevant.

Although no effects on combined benign and malignant tumours were observed, the decrease in the incidence of benign lesions in the uterus (polyps) suggests a trend towards increasing malignancy.

Although no HCD are available, squamous cell carcinoma rarely occurs spontaneously. Nevertheless, as only one incidence at the mid and high dose levels was observed, the effect is of uncertain toxicological relevance. Similarly, the increase in malignant endometrial sarcoma is of uncertain biological relevance due to the low incidence observed at the top dose.

Overall, the increase in uterine adenocarcinoma may be treatment related but was of borderline significance and at the upper end of the HCD. In addition, the increase in uterine tumours was only noted at the top dose associated with general toxicity (decreased body weight gain and food efficiency). Therefore, RAC considers that the increase in uterus adenocarcinoma in the rat carcinogenicity study does not provide strong indication of carcinogenicity.

Mice

In the carcinogenicity study available in mice, male and female NMRI mice were exposed for 2 years to diuron at 0, 5.4/7.5, 50.8/77.5, 640/867 mg/kg bw/d in males/females, respectively. The study was similar to OECD TG 453. No treatment-related effect on survival was noted in the study. The decrease in survival noted in all dose groups was below 50% only at the end of the study. Therefore, RAC agrees with the DS that the study can be considered acceptable. The decrease in body weight gain in female mice was 12% at the top dose. No treatment-related clinical signs were noted in the study. Effects on blood systems were noted in both males and females at the top dose. Liver changes (weight, hypertrophy, single cell necrosis) was also observed in liver at the top dose. RAC considers that the high dose level in the study was not associated with excessive toxicity.

No increase in tumours were observed in male mice.

<u>Mammary gland tumours in NMRI mouse</u>

In female mice, a statistically significant increase in mammary gland adenocarcinoma was observed.
An Industry representative provided HCD from the same laboratory and the same strain of mice derived from 10 studies conducted during a relevant time period (1981 to 1984). Indeed, the study was apparently performed in 1981-1983 and reported later in 1990. The increase observed in the diuron study was slightly outside the testing facility's HCD. Although the maximum observed incidence was 5/39 in one study, this maximum may have been an outlier considering the mean of the historical control database of 3.2%. The HCD are limited as the studies were of shorter duration (20 or 21 months compared to 24 months in the diuron study) and as this type of tumour may appear with advanced age. Nevertheless, the concurrent control in the diuron study were still inside the historical control database. In addition, as a high variability was observed in the historical control database, the concurrent control of the study is the most reliable control. No information on the time at which the tumours occurred was available in the CLH dossier. RAC notes that due to the large dose-spacing, a dose-response could not be detected.

***Table**: Summary of mammary gland tumours in mice (Anonymous 3, 1990)*

Dose (mg/kg bw/d)	0	7.50	77.5	867	Historical control
No. of mice [1]	50/39	47/32	49/44	50/39	
Adenocarcinoma, malignant	2 (5.1%)	1 (3.1%)	1 (2.3%)	6* (15%)	0-12.8% mean: 3.3%
Carcinoma, anaplastic, malignant	0	1 (2%)	0	0	

*p≤0.05 (trend test); [1] number of mice for gross examination/ for microscopic examination.

Overall, the statistically significant increase in mammary gland tumours in mice is considered treatment-related and should be considered relevant for classification.

Ovarian luteoma in NMRI mouse

A statistically significant increase in ovarian luteoma was observed at the top dose in mice. No preneoplastic lesions in sex cord/stromal cells were reported in the study.

The industry provided HCD from the same laboratory and the same strain of mice derived from 11 studies conducted during a relevant time period (1981 to 1984). The increase in ovary luteoma in mice exposed to diuron at the top dose level was above the provided HCD range. Nevertheless, as commented above, the HCD are limited as the studies were of shorter duration than the diuron study and as this type of tumour may appear with advance age, depending on the susceptibility of the strain. The highest incidence observed in the HCD were 3 in two studies (3/44, 3/45 mice).

***Table**: Summary of ovarian gland tumours in mice (terminal kill)*

Dose (mg/kg bw/d)	0	7.50	77.5	867	Historical control
No. of mice[1]	50/45	47/37	49/46	50/44	
Luteoma, benign					0-6.7%
- unilateral	3/6%	0	2/4.4%	7*/16%	Mean: 1.8%
- bilateral	0	1/2.7%	0	0	
Combined sex cord stromal tumours[2]	11 24%	7 19%	15 33%	14 32%	

*p≤0.05 (Peto and/or Cochran Armitage trend test). Not statistically significant according to Fisher 's exact test. [1] number of mice for gross examination/ for microscopic examination. [2] Including granulosa cell tumours, luteomas, thecomas, Sertoli cell tumours of the ovary, Leydig cell tumours, androblastoma, arrhenoblastoma and lipid cell tumours (APVMA, 2011)

Ovarian luteoma (cells of sex cord origin) are rare in contrast to ovarian granular cell tumours. Nevertheless, as it may be difficult to differentiate the different type of ovarian tumours, combined sex cord stromal tumours may be taken into account to conclude on the carcinogenic potential of the substance in the ovary.

Overall, RAC considered that the increase in luteoma might be treatment-related. Nevertheless, ovarian luteoma did not progress to malignancy and no increase in combined sex cord stromal tumours were noted. Therefore, the increase in benign ovary luteoma in mice does not provide strong a indication of carcinogenicity.

Mode of action and human relevance

Seven mechanistic studies were summarised by the DS on urinary tract tumours in rats and mammary gland tumours in mice.

Urinary tract tumour

In a 26-week feeding male rat study (Anonymous 15, 1987), metaplasia in some animals and an increase in the incidence and severity of urothelial hyperplasia was observed in animals dosed at 2500 ppm diuron (equivalent to ~ 200 mg/kg bw/d).

In Da Rocha *et al*. (2010), precipitates and magnesium ammonium phosphate crystals similar to controls were present in the urine of male Wistar rats treated with diuron (2500 ppm, equivalent to ~ 135 mg/kg bw/d) for 15, 25 or 30 weeks. Increased incidence and severity of hyperplasia in the urinary bladder was also noted in all treated group. An extra group of animals was treated with 135 mg/kg bw/d diuron and NH4Cl for 25 weeks. The additional treatment did not affect the incidence or severity of urothelial lesions induced by diuron, suggesting that urinary solids do not contribute to cytotoxicity or to the development of pre-neoplastic urothelial lesions caused by diuron.

In a 20-week dietary rat study, Cardoso *et al*. (2013) found hyperplasia and a higher proliferation index of bladder and kidney urothelium from 500 ppm (equivalent to ~ 40.5 mg/kg bw/d) onwards with a NOAEL at 125 ppm (equivalent to ~ 10.1 mg/kg bw/d). Further examination of biological samples from this 20-week feeding experiment by microarray analysis (Ihlaseh *et al*., 2011), show a difference in gene expression at 500 ppm compare to lower dose levels.

Da Rocha *et al*. (2012) exposed male Wistar rats at 2500 ppm diuron (equivalent to ~ 295 mg/kg bw/d) for different time periods from one day up to 8 weeks. As early as on day 1, there was already urothelial cell swelling, whereas by day 28, extensive necrosis, exfoliation and piling up of cells suggestive of hyperplasia had become apparent. In the cellular and molecular pathways analysis, the most significant diseases and biological function pathways altered in high-dose animals included cancer, amino acid metabolism, small molecule biochemistry, and cell death.

In the study of Da Rocha *et al*. (2013) five male rats were exposed 8 weeks to 0 or 2500 ppm diuron and the relative cytotoxicity of urine metabolites of diuron were investigated *in vitro*. The metabolite with the highest concentration in the urine of male Wistar rats treated with the carcinogenic dose of diuron (~295 mg/kg bw/d) was N-(3,4-dichlorophenyl)urea (DCPU). The authors suspected that DCPU, as the main urinary metabolites of diuron, might be responsible for the urothelial lesions.

In a 2-year stage carcinogenicity model in Swiss mice, diuron was found to be a promoting agent to the urinary bladder (De Moura *et al*., 2009)

For the occurrence of bladder carcinoma in the long-term study, a non-genotoxic MoA with urothelial necrosis induced by direct cytotoxicity, regenerative cell proliferation and sustained urothelial hyperplasia is a plausible MoA that would ultimately lead to bladder tumours. This proposed MoA would be in line with the increase in DNA synthesis and S-phase cells observed in an UDS assay on rat bladder cells. RAC notes that there is no indication that this MoA would not be relevant to human. Nevertheless, a practical threshold is expected for these tumours, decreasing the concern.

Uterine adenocarcinoma

Two studies were retrieved in the literature. Grassi *et al*. (2011a) and De Moura *et al*., 2009 did not find evidence of a promoting potential of diuron for mammary tumours in a two-stage carcinogenesis model in female Sprague Dawley rats after 25-week exposure or in Swiss mice after 13-week exposure to diuron up to 2500 ppm.

No data on potential endocrine properties of the substance were available in the dossier. An endocrine MoA may be hypothesised for the observed tumours in mice, but no data are available to substantiate this hypothesis.

Comparison with classification criteria

As there are no reliable epidemiological studies in humans reported in the dossier, classification in Category 1A is not appropriate.

Animal studies provided sufficient evidence of carcinogenicity in the urinary tract system in both males and females and in the uterus in female mice. According to the CLP Regulation (Annex I: 3.6.2.2.4), additional considerations like human relevance and background incidences as part of a WoE approach have to be taken into account for a classification for carcinogenicity.

Tumour type Considering background incidence and HCD	Urinary bladder transitional cell carcinoma in male and female rats, transitional cell carcinoma in the renal pelvis of male rats. Strong increase, statistically significant. Low background incidence in rats.	Sufficient evidence
	Uterus adenocarcinoma in rats. Borderline statistical significance. High variability in background incidence. At the upper end of the HCD range.	Insufficient evidence
	Benign ovary luteoma in mice. Statistically significant. Above available HCD (limited relevance). No increase in combined sex cord tumours.	Insufficient evidence
	Mammary gland adenocarcinoma. Statistically significant. Above HCD (limited relevance).	Sufficient evidence
Multi-site response	No, one site in mice and one site in rats.	/
Progression of lesion to malignancy	Yes	/
Whether responses are in single sex or both	Both sexes in rats reported tumours.	/
Whether responses are in a single species or several	Tumour formation occurred in rats and mice. No consistent pattern between the species.	/
Structural similarity to (a) substance(s) for which there is good evidence of carcinogenicity	Diuron is structurally related to monuron and linuron classified as Carc. 2 in the CLP Regulation. Structural similarity analysis was not performed in the CLH dossier.	/
Route of exposure	Oral route is a relevant route of exposure.	/
Comparison of ADME between test animals and humans	No species-specific differences identified in the available toxicokinetics studies.	/

The possibility of a confounding effect of excessive toxicity at test doses	The tumours are not considered secondary to unspecific excessive toxicity	/
Mode of action and its relevance for humans	Non-genotoxic MoA. Urinary tract tumours may be related to cytotoxicity and threshold-based. No MoA demonstrated for other tumour types.	Human relevance is plausible.

Overall, there is sufficient evidence of carcinogenicity in animals based on the strong increase in urinary tract malignant tumours in male and female rats. Although the MoA may be threshold-based, the exact level is not known.

There is sufficient evidence of carcinogenicity in mice based on the statistically significant increase in mammary gland tumours. Although no increase in this tumour type was observed in rats, a difference in sensibility between the species cannot be excluded.

The increase in the incidence of malignant uterus tumours in rats and benign ovarian tumours in mice also provide supportive evidence for classification.

On the basis of tumours observed in two sexes in rats and in two species, RAC agrees with the DS to **classify diuron as Carc. 1B, H350**.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Current entry in Annex VI, CLP Regulation: Aquatic Acute 1, H400, Aquatic Chronic 1, H410, M-factor=10.

The physico-chemical characteristics indicate that diuron has moderate water solubility (37.4 mg/L at 25 °C) and a low vapour pressure of 1.15E-06 hPa at 25 °C indicating that it is not considered volatile. A low potential for adsorption onto sediment/soil and other particulate organic matter is also indicated, log Pow = 2.87 and log Pow = 2.85 (pH 6.4, 19 ± 1 °C).

The DS proposed to retain classification as Aquatic Acute 1 and Aquatic Chronic 1 and to add M-factors of 10 for both.

Degradation

Abiotic degradation

A summary of the relevant information on rapid degradability is provided in Table 33 of the CLH report.

Diuron is hydrolytically stable at pH 4, 5, 7 and 9 as less than 5% of the substance were degraded after 30 days at 25 °C. During hydrolysis, no degradation products in concentrations > 2 % were found (Williams, 1995; Hawkins, 1988).

A DT_{50} of 491 d has been shown using a kinetics model for diuron regarding aerobic mineralisation in surface waters within 60 days. Degradation products in water accounted for < 1 % in surface water and suspended sediment experiment (Swales, 2016).

Biodegradation

Ready biodegradation

No studies are presented on ready biodegradability so the active substance diuron is considered as not 'readily biodegradable' by default.

The DS concluded that based on the available information, diuron does not fulfil the criteria to be considered as rapidly degradable in the aquatic environment.

Bioaccumulation

A summary of the available information on bioaccumulation is provided in Table 38 of the CLH report and relevant information on the metabolites in Table 8 of the CLH report.

No experimental study characterising the bioconcentration potential in fish is available. The DS concluded that since the log P_{ow} value of diuron (log P_{ow} = 2.87) and its major metabolites DCPMU (log P_{ow} = 2.59), mCPDMU (log P_{ow} = 1.79) and DCPU (log P_{ow} = 2.23) (Nitzsche, 2015) are below the trigger of 4, there is no potential of bioaccumulation of the substance according to CLP criteria.

The DS considers diuron as having no potential for bioaccumulation based on available data for the substance and its metabolites.

Aquatic toxicity

Aquatic acute toxicity

A summary of the relevant information on aquatic acute toxicity is presented in Table 39 of the CLH report.

For acute aquatic toxicity, studies were presented for all trophic levels, fish, invertebrates and algae and other aquatic plants. Two valid acute fish toxicity studies were available. The OECD TG 203 study on *Oncorhynchus mykiss* shows an LC_{50} of 12.6 mg/L (nom) after 96h exposure under the test conditions. The other 96 h FIFRA guideline study presents an LC_{50} of 14.2 mg/L (nom) in a similar range for *Pimephale promelas*.

For invertebrates, two reliable acute studies were provided, one with *Daphnia magna* and the other with the saltwater mysid *Americamysis bahia*. The water flea immobilisation test assesses the acute toxicity of diuron under static conditions in freshwater according to the OECD TG 202. Under the test conditions, the LC_{50} for *Daphnia magna* was determined to be > 5.0 mg/L (nom) after 48h exposure.

Mysids (*Americamysis bahia*) were used to evaluate the acute toxicity of diuron in static conditions according to a FIFRA Guideline. Under the test conditions, a 96h LC_{50} value of 1.1 mg/L (nom) was determined.

Four reliable studies were presented for autotrophs. The toxicity of diuron to the cyanobacterium *Synechoccus leopoliensis* was determined according to the OECD TG 201, presented as the key study. The 72h E_yC_{50} and E_rC_{50} values were determined to be 0.0028 mg/L (nom) and 0.0078 mg/L (nom), respectively. For the other species (*Navicula pelliculosa*, *Anabaena flos-aquae* and *Lemna gibba*) the obtained EC_{50} values were in a similar range and varied from 0.011 to 0.026 mg/L (nom).

According to these valid studies, fish were found to be the least sensitive species followed by invertebrates in sensitivity. The lowest E_rC_{50} is obtained with *Synechoccus leopoliensis* (0.0028 mg/L) and the DS proposed to classify diuron as Aquatic Acute 1 (H400) with an M-factor of 100, based on the $L(E)C_{50}$ between 0.001 and ≤ 0.01 mg/L.

Aquatic chronic toxicity

The valid data for chronic aquatic toxicity is presented in Table 42 of the CLH report.

Four long-term studies with fish were presented in the CLH report. The prolonged toxicity study according to OECD TG 204 on rainbow trout (*Oncorhynchus mykiss*) was conducted and a 28-day NOEC of 0.41 mg/L (mm) was derived. As the analytical verification showed deviations of more than 20 % to nominal, the results were related to measured concentrations.

The study re-evaluated to consider the requirements of OECD TG 210 with *Cyprinodon variegatus* (study F4, 1992) showed a NOEC of 1.70 mg/L (mm) based on the most sensitive parameter (post-hatch survival) after 32 days of exposure. Reliable estimation of EC_{10} values for hatchability, post-hatch survival, fresh weight and fish length was not possible due to a lack in the dose response. The recommendations of EPA Guideline concerning the temperature of the test medium were not met.

The FIFRA Guideline study with fathead minnows exposed to diuron presented a NOEC of 0.0033 mg/L (mm). The number of abnormal fry at time of transfer and survival through 60 days were the main affected parameters.

The fish sexual development test following OECD TG 234 presented a NOEC for post hatch survival of *Danio rerio* of 0.00119 mg/L (mm).

For invertebrates, the DS reported two tests one performed with water fleas and the other with harlequin fly. The *Daphnia magna* reproduction test (semi-static, 21 d) of diuron re-evaluated according to OECD TG 211 indicated that the most sensitive endpoint was growth (dry weight) with a NOEC of 0.096 mg/L (mm). Reliable estimation of EC_{10} values was not possible due to a lack in the dose response (Heimbach, 1996).

The effects of diuron on *Chironomus riparius* were determined in a static 28-day chronic toxicity study according to OECD TG 219. The 28-day NOEC for the most sensitive endpoints (development rate) was determined to be 1.55 mg/L (mm) in overlying water, pore water and sediment. The EC_{10} value was 4.04 mg/L, corresponding to > 3.95 mg/L initially measured concentrations of overlying water, pore water and sediment (Gonsior, 2016).

In a static growth inhibition test with the cyanobacteria *Synechococcus leopoliensis* the effects of diuron were assessed over a test period of 72h. The NOEC values were estimated to be 0.000632 mg/L (nom) for growth rate and yield, E_rC_{10} of 0.0037 mg/L (nom) and E_yC_{10} 0.00133 mg/L (Wenzel, 2015).

In a static growth inhibition test with the diatom *Navicula pelliculosa* the effects of diuron were assessed over a test period of 72 h. The nominal E_rC_{10} value 0.0071 mg/L (nom) and E_yC_{10} was 0.0033 mg/L (nom). The overall NOEC was determined to be at 0.00428 mg/L (nom). (Falk, 2016)

Three OECD TG 239 studies have been given on three different submerged rooted macrophytes (*Ceratophyllum demersum*, *Chara globularis* and *Elodea canadensis*) *Ceratophyllum demersum* being the most sensitive of the three. Dry weight was the most sensitive growth rate and yield parameter. The NOEC for both parameters was 0.000463 mg/L (mm) and the E_rC_{10} for growth rate and dry weight was 0.000267 mg/L (mm).

Chara globularis and *Elodea canadensis* showed lower toxicity with growth rate NOEC values of 0.00326 mg/L (mm) and 0.00586 mg/L (mm) for total shoot length and fresh weight,

respectively. The E_rC_{10} values for growth rate and shoot length were 0.00311 mg/L (mm) and 0.000278 mg/L (mm), respectively (Wenzel, 2016).

Since chronic aquatic toxicity information were available for all three trophic levels the DS considered, based on the E_rC_{10} of 0.000267 mg/L for higher aquatic plants (*Ceratophyllum demersum*) and supported by the NOEC for growth for algae *Synechoccus leopoliensis* of 0.000632 mg/L and that the substance is not rapidly degradable, that diuron fulfils the criteria for classification as Aquatic Chronic 1 with an M-factor of 100, based on the NOEC between 0.0001 and 0.001 mg/L.

Comments received during consultation

Three MSCAs and an industry representative supported the proposed environmental classification Aquatic Acute 1, H400 (M=100) and Aquatic Chronic 1, H410 (M=100) based on the available data for the most sensitive species (Algae and aquatic plant species: *Synechoccus leopoliensis*; 72h-E_rC_{50}=0.00788 mg/L (nom) and *Ceratophyllum demersum* 14d- E_rC_{10}=0.000267 mg/L (mm)), respectively.

One MSCA commented on the NOE_rC of 0.000463 mg/L (lowest concentration tested) indicating that 12.1% inhibition of the growth rate was seen at this concentration, although not considered statistically significant. Furthermore, the MSCA added that the 14d-EC_{10} (= 0.000267 mg/L - geom. mean) was extrapolated and may contain remarkable uncertainties.

One MSCA commented that the study with *Elodea canadiensis* could additionally be considered a key study in a WoE approach for the chronic classification and that more information is required to confirm the Chronic M-factor of 100. Additionally, the MSCA stated that the 95% confidence intervals support a well-defined E_rC_{10} endpoint of 0.0037 mg/L that should be considered the most appropriate long-term endpoint in preference to the NOE_rC for *Synechoccus leopoliensis*, which would result in a Chronic M-factor of 10.

The DS agreed with the comments and added that the coefficient of variation, combined with the fact that the EC_{10} is extrapolated outside of the tested concentration range, casts some doubt on the robustness of the reported E_rC_{10} of 0.000267 mg a.s./L. However, the NOE_rC based on the same endpoint is 0.000463 mg a.s./L. This is also in the concentration range of 0.0001 to 0.001 mg/L, which would likewise lead to the classification of Aquatic Chronic 1 with a chronic M-factor of 100. The DS considered *Ceratophyllum demersum* the most sensitive of the tested species, hence the assessment should be based on this species and adds that NOE_rC and E_rC_{10} are in a similar concentration range and the growth rate was already reduced by 12 % at the NOE_rC. Therefore, the DS does not see it justified to classify diuron with a chronic M-factor lower than 100.

One MSCA commented that in the study on *Synechococcus leopoliensis* the lowest test concentration (0,1 µg/l) is outside ±20% of the nominal concentrations so the concentrations should be reported as geometric mean concentrations. At the same time the MSCA added as only the lowest test concentration is outside the ±20% range, and all higher concentrations are within the range of ±20%, the E_rC_{50} could be based on nominal concentrations because the deviations in measured concentrations at a concentration of 0.1 µg/l do not affect the outcome of the E_rC_{50}. The DS explained further that the recovery of diuron at the lowest tested concentration (0.1 µg/L) varied between 131 and 212 % of nominal, while the measured concentrations in all other treatments were very close to the nominal concentrations (89.5 – 107 %). As relevant effects were only seen in concentrations higher than 0.632 µg a.s./L and the deviations in the 0.1 µg/L treatment will have negligible effect on the outcome of the EC_x-calculations, the DS found it acceptable to base the calculations on nominal concentrations. The same MSCA also brought a ready biodegradability study available in the REACH registration dossier to the DS's attention.

The commenting MSCA was of the view that the study indicated the diuron is not readily biodegradable, according to OECD TG 301 F.

Assessment and comparison with the classification criteria

Degradation

The CLH report did not include information on diuron behaviour in the water sediment systems. However, the accompanying RAR document indicated calculated valid DT_{50} values for the supernatant water phase of 4 days (9 days recalculated to 12 °C) and 232 days (493 days recalculated to 12 °C) for the total system, indicating a low potential for adsorption. One major metabolite, mCPDMU, was observed in the test system Hoenninger Pond and amounts to 6.7 % in the water phase (1 x >5 %) and 8.5 % in the sediment after 55 days (2 x >5 % in consecutive samples). Mineralisation of 2.05 % was reached, while 17.5 % non- extractable residues were formed after 120 d (Sneikus, 2001).

RAC agrees with the DS that diuron can be considered hydrolytically stable in the environment.

RAC takes note of the ready biodegradability screening study in water introduced during the consultation showing no biodegradation (0 % after 28 d) under test conditions but points out no full study report is available for this study.

According to the criteria on rapid degradability defined in the Section 4.1.2.9 of the CLP Regulation and on the basis of the valid and available data presented by the DS, RAC agrees to consider diuron as not rapidly degradable for classification purposes.

Bioaccumulation

RAC notes that no experimental BCF data is available but considers the available log Pow information sufficient to come to conclusion on the bioaccumulation potential of the substance. According to the Section 4.1.2.8 of the CLP Regulation partitioning data is normally considered when determining the bioaccumulation of a substance and any other relevant data is considered supportive. Therefore, RAC agrees with the DS and concludes that diuron has a low potential for bioaccumulation based on the available information on the partition coefficient as it is well below the cut-off value of 4.

Aquatic toxicity

RAC notes that reliable data are available for all three trophic levels for both acute and chronic hazards, with algae being the most sensitive group.

RAC agrees with the DS that the most sensitive species under acute testing is *Synechoccus leopoliensis* (Wenzel, 2015) and that the acute endpoint value (E_rC_{50} 0.0078 mg/L) is reliable for acute aquatic classification use. RAC also agrees on the use of the E_rC_{10} from the *Synechococcus leopoliensis* study based on nominal concentrations that is justified as measured concentrations remained within 20% of nominal. The single variation at the lowest dose gives little effect to the calculations of the effect concentrations. The NOEC was estimated to be 0.000632 mg/L. RAC also notes that the E_rC_{10} of 0.0037 mg/L, although obtained with a high CI, is not the lowest valid effect concentration value obtained for this species and should not be preferred when applying chronic classification procedure.

RAC agrees with the DS that *Ceratophyllum demersum* can be considered the most sensitive species under chronic testing (14d E_rC_{10} = 0.000267 mg/L) (Wenzel, 2016; LDG-001/4-12/B). However, RAC points out that the *Elodea canadiensis* E_rC_{10} of 0.000278 mg/L (Wenzel, 2016) is in the same order of magnitude and should be considered as supportive when classifying diuron. RAC also notes the E_rC_{10} for *E. canadiensis* (Wenzel, 2016; LDG-001/4-12/C) is extrapolated

outside of the tested concentration range and that the coefficient of variation is higher for the EC_{20} but does not support the use of EC_{20} values for classification purposes.

In conclusion, RAC agrees with the DS that algae and plants are the most sensitive trophic level with *Synechoccus leopoliensis* providing an E_rC_{50} value of 0.00788 mg/L, which results in a classification of Aquatic Acute 1 for diuron. According to Table 4.1.3 in the CLP Regulation, an M-factor of 100 is warranted for $L(E)C_{50}$s ranged from 0.001 to 0.01 mg/L.

RAC agrees with the DS that the lowest chronic endpoint of 14d E_rC_{10} for *Ceratophyllum demersum* of 0.000267 mg/L results in a classification of Aquatic Chronic 1 for diuron as a non-rapidly degradable substance. According to Table 4.1.3 in the CLP Regulation an M-factor of 100 for not rapidly degradable substances is warranted between the range of 0.0001 and 0.001 mg/L.

In conclusion, RAC agrees with the DS that **Diuron warrants classification as:**

Aquatic Acute 1 (H400), M=100

Aquatic Chronic 1 (H410), M=100

Additional references

APVMA, 2011. Australian Government, Australian Pesticides and Veterinary Medicines Authority. Diuron human health assessment.

Gaines and Linder, 1986. Acute toxicity of pesticide in Adult and weanling rats. Fundamental and applied toxicology 7, 299-308.

H.C. Hodge, W.L. Downs, B.S. Panner, D.W. Smith, E.A. Maynard, J.W. Clayton, R.C. Rhodes. Oral toxicity and metabolism of diuron (N-(3,4-dichlorophenyl)-N',N'-dimethylurea) in rats and dogs. Food and Cosmetics Toxicology, Volume 5, 1967, 513-531

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).