CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

International Chemical Identification:

diuron (ISO)

3-(3,4-dichlorophenyl)-1,1-dimethylurea

EC Number: 206-354-4

CAS Number: 330-54-1

Index Number: 006-015-00-9

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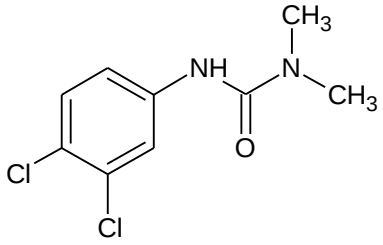
CONTENTS

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	*3-(3,4-Dichlorophenyl)-1,1-dimethylurea*
Other names (usual name, trade name, abbreviation)	diuron
ISO common name (if available and appropriate)	*diuron*
EC number (if available and appropriate)	206-354-4
EC name (if available and appropriate)	
CAS number (if available)	330-54-1
Other identity code (if available)	100
Molecular formula	$C_9H_{10}Cl_2N_2O$
Structural formula	
SMILES notation (if available)	
Molecular weight or molecular weight range	233.1 g/mol
Degree of purity (%) (if relevant for the entry in Annex VI)	970 g/kg (proposed by RMS)

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
see table 1			

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
-				

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
-					

Table 5: Test substances (non-confidential information) (this table is optional)

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Other information	The study(ies) in which the test substance is used
-				

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6: 1.1 Proposed harmonised classification and labelling according to the CLP criteria

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	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	Acute Tox. 4* Carc. 2 STOT RE 2* Aquatic Acute 1 Aquatic Chronic 1	H302 H351 H373** H400 H410	GHS09 GHS08 GHS07 Wng	H302 H351 H373** H410		M=10	
Dossier submitters proposal					**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	**Retain** GHS09 GHS08 GHS07 Wng	**Modify** H350 H373 (blood, bladder) **Retain** H410		**Modify** M = 100 M = 100	
Resulting Annex VI entry if agreed by RAC and COM					Carc. 1B STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H350 H373 (blood, bladder) H400 H410	GHS09 GHS08 GHS07 Wng	H350 H373 (blood, bladder) H410		M = 100 M = 100	

Table 7: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	*hazard class not assessed in this dossier*	No
Flammable gases (including chemically unstable gases)		
Oxidising gases		
Gases under pressure		
Flammable liquids		
Flammable solids		
Self-reactive substances		
Pyrophoric liquids		
Pyrophoric solids		
Self-heating substances		
Substances which in contact with water emit flammable gases		
Oxidising liquids		
Oxidising solids		
Organic peroxides		
Corrosive to metals		
Acute toxicity via oral route	***data conclusive but not sufficient for classification***	**Yes**
Acute toxicity via dermal route		
Acute toxicity via inhalation route		
Skin corrosion/irritation	*hazard class not assessed in this dossier*	No
Serious eye damage/eye irritation		
Respiratory sensitisation		
Skin sensitisation		
Germ cell mutagenicity	***data conclusive but not sufficient for classification***	**Yes**
Carcinogenicity	***harmonised classification proposed***	**Yes**
Reproductive toxicity	*hazard class not assessed in this dossier*	No
Specific target organ toxicity-single exposure		
Specific target organ toxicity-repeated exposure	***harmonised classification proposed***	**Yes**
Aspiration hazard	*hazard class not assessed in this dossier*	No
Hazardous to the aquatic environment	***harmonised classification proposed***	**Yes**
Hazardous to the ozone layer	*hazard class not assessed in this dossier*	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Diuron is an existing pesticide active substance approved in accordance Directive 91/414/EEC. The substance was initially discussed and classified in ECBI meeting 22/87 and was further discussed in subsequent ECBI meetings such as ECBI/27/97, ECBI/07/00, ECBI/44/04, ECBI/124/04 and ECBI/55/05**.**

Regarding health and environmental hazards, diuron (CAS-No. 330-54-1) has a legal classification (regulation (EC) No 1272/2008, translated from Annex I of Dir 67/548/EEC) which includes classification as Acute Toxicity Category 4* (H302), Carcinogenicity Category 2 (H351), Specific target organ toxicity-repeated exposure STOT RE Category 2* (H373**), Aquatic Acute Category 1 (H400) and Aquatic Chronic Category 1 (H410).

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Diuron is an active substance in the meaning of the Regulation EU No 1107/2009 and therefore subject to harmonised classification and labelling according to Article 36 of the CLP regulation (no further requirement for justification that action is needed at Community level).

In accordance with the alignment process with the renewal of the active substance under Regulation (EU) No. 1107/2007, it is necessary to prepare a targeted CLP report taking into account the few new data and the re-evaluation of the existing data. The minimum classification Acute Tox. 4* and STOT RE 2** will be addressed, in addition the classification Carc. 2 will be re-evaluated.

Considering the classification criteria diuron should not be classified for acute toxicity independently from the route of exposure. Due to effects on red blood cells and bladder in animal studies, classification with STOT RE 2 (H373) is confirmed. Taking the effect doses/concentrations for haematological effects into account, both the oral and the inhalative routes should be indicated as of concern. Classification of diuron as a carcinogen of the Category 1B (H350) is proposed, since malignant tumors of different types were observed in two independent studies in two species.

5 IDENTIFIED USES

Diuron is a systemic herbicide that controls monocotyledonous and dicotyledonous weeds as well as mosses. Diuron has phytotoxic action and is mainly absorbed by roots and translocated in the apoplast. Diuron is used as herbicide in agriculture, horticulture, non-cultivated lands and railway tracks.

6 DATA SOURCES

Renewal Assessment Report (RAR) – Diuron, 2018
Draft Assessment Report – Diuron – Volume 3, Annex B.2: Physical and Chemical Properties, 2017
Draft Assessment Report – Diuron – Volume 4 – Confidential Information, 2017
REACH registration dossier (full) – diuron (ISO); 3-(3,4-dichlorophenyl)-1,1-dimethylurea – 100-1000 tonnes per annum, last modified on 06-Feb-2019

7 PHYSICOCHEMICAL PROPERTIES

Table 8: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	colour: clear off-white (at room temperature) physical state: powder	Head (1995)	Visual assessment
Melting/freezing point	Melting point: 157.2 °C Melting point: 158.4 °C	Head (1995) Jean-Baptiste (2015)	EEC A.1; OECD 102 OECD 113; DSC method
Boiling point	No boiling before decomposition	Klusacek and Krasemann (1986)	OECD 113
Relative density	%	%	%
Vapour pressure	1.15 x 10^{-6} Pa at 25 °C	Barefoot and Cooke (1990)	OECD 104 Gas saturation method
Surface tension	72.1 mN/m (saturated aqueous solution) (20 °C)	Walter (2001)	EEC A.5
Water solubility	37.4 mg/L (25 °C) 35.6 mg/L (35 °C) There is no dissociation in water, therefore pH dependence on solubility is not necessary	Mayes (1995)	EEC A.6
Partition coefficient n-octanol/water	log Po/w = 2.87 (25 °C) log Po/w = 2.85 (pH 6.4, 19 ± 1 °C) Effect of pH was not investigated since there is no dissociation in water 1-(3,4-dichlorophenyl)-3-methylurea (20 ± 1 °C) log Po/w = 2.62 (pH 4) log Po/w = 2.59 (pH 7 and pH 10) 1-(3,4-dichlorophenyl)urea (20 ± 1 °C) log Po/w = 2.35 (pH 4) log Po/w = 2.32 (pH 7 and pH 10) 3,4-Dichoroaniline (20 ± 1 °C) log Po/w = 2.39 (pH 4) log Po/w = 2.37 (pH 7) log Po/w = 2.35 (pH 10) 1-(3-Chlorophenyl)-3-	Madsen and Yousuf (1995) Jean-Baptiste (2015) Nitzsche (2015) Nitzsche (2015) Nitzsche (2015) Nitzsche (2015)	EEC A.8 OECD 107 OECD 107 Shake flask method EEC A.8 OECD 117 EEC A.8 OECD 117 EEC A.8 OECD 117 EEC A.8

Property	Value	Reference	Comment (e.g. measured or estimated)
	methylurea (20 ± 1 °C) log Po/w = 1.81 (pH 4) log Po/w = 1.79 (pH 7 and pH 10) 3-Chlorophenylurea (20 ± 1 °C) log Po/w = 1.53 (pH 4) log Po/w = 1.51 (pH 7 and pH 10) 3-(3-Chlorophenyl)-1,1-dimethylurea (20 ± 1 °C) log Po/w = 1.88 (pH 4) log Po/w = 1.87 (pH 7 and pH 10)	Nitzsche (2015) Nitzsche (2015)	OECD 117 EEC A.8 OECD 117 EEC A.8 OECD 117
Flash point	not applicable as melting point > 40 °C	-	Comment (DAR)
Flammability	diuron is not highly flammable	Walter (2001)	EEC A.10
Explosive properties	diuron is not explosive	Franke (2001)	EEC A.14
Self-ignition temperature	No self-ignition up to 401 °C	Franke (2001)	EEC A.16
Oxidising properties	No oxidising properties	Franke (2004)	EEC A.17
Dissociation constant	No dissociation is observed for diuron in aqueous solution	Jean-Baptiste (2015)	OECD 112

8 EVALUATION OF PHYSICAL HAZARDS

Not relevant for this dossier

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 9: Summary table of toxicokinetic studies

Method	Results	Reference
Absorption, Distribution, and Elimination of [^{14}C]Diuron in Rats U. S. EPA Pesticide Assessment Guidelines, Subdivision F, 85-1 GLP	Absorption: almost completely absorbed (> 95 %) in Wistar rats following oral administration of single doses of 5 mg or 200 mg/kg bw or repeated daily doses of each 5 or 200 mg/kg bw Distribution: peak concentrations in blood reached between 1.7 and 6.8 hours post dosing, relatively highest residues were observed in the blood or to haematopoietic organs, in organs with metabolic or excretory function and, in females, in the ovaries. Excretion: rapidly eliminated; More than 97 % of the recovered radioactivity was excreted via urine (68 – 87 %) and faeces (13 – 32 %) within 72 hours; elimination with respiratory air was negligible (0.01 %) absorption, distribution and elimination depended to a low degree dependent on the sex of the animals, dose size, on the multiple-dose treatment and on the route of administration; major variations in kinetic parameters were due to changes of dose and frequency of treatment.	Anonymous 18, 1988
Absorption, Distribution, Metabolism, and Elimination of [^{14}C]Diuron in Rats U. S. EPA Pesticide Assessment Guidelines, Subdivision F, 85-1 GLP	Absorption: rapid, clearly higher than 80 % after oral exposure Distribution: low tissue residues, detection of a certain affinity to red blood cells; no evidence of accumulation Metabolism: 8 urine and 4 faeces metabolites were isolated and identified which indicated an extensive degradation of diuron (see Figure 1). Excretion: vast majority within the first 24 hours post dosing as metabolites with 3,4-dichloro-phenyl-urea as main metabolite via urine; very small amount of unchanged diuron via faeces. The impact of dose or repeated administration on the pattern of kinetics and metabolism was low. No consistent sex differences.	Anonymous 19, 1996
Metabolism, and Elimination of diuron in Rats during a Subacute Inhalation study over eight weeks No guideline, No GLP	N'-(3,4-dichlorophenyl) urea was confirmed as the major metabolite excreted in urine also following inhalation. extensive metabolism; no significant differences in urinary excretion after 4 weeks and 8 weeks inhalation indicating no accumulation of the active ingredient or its metabolites	Anonymous 13, 1986*
in-vitro Metabolism Study with Mammalian Hepatic Microsomes No guideline, No GLP	Rapid metabolism via N-demethylation, with 3,4-dichlorophenylurea (DCPU) as major metabolite Similar *in-vitro* metabolism of diuron between human liver homogenates or human, rat, mouse, dog, monkey, minipig and rabbit liver microsomes exposed to three different concentrations and incubation times of 20, 40 or 60 min. Human-specific metabolites were not observed.	Abass, 2007

* study is considered supplementary taking into account the limited scope of this investigation and that it is a non-Guideline and non-GLP study.

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

Following single oral administration of radiolabelled test substance to rats, diuron was virtually completely absorbed, widely distributed, extensively metabolised and rapidly eliminated. No evidence of accumulation was obtained and sex, dose or repeated dosing did not alter the toxicokinetic parameters to a significant extent.

In the study by Anonymous 18 (1988), ^{14}C-labelled diuron was almost completely absorbed (> 95 %) in Wistar rats following administration of single doses of 5 mg or 200 mg/kg bw or repeated daily doses of each 5 or 200 mg/kg bw with peak concentrations in blood reached between 1.7 and 6.8 hours post dosing. The radioactivity was rapidly eliminated from the body. More than 97 % of the recovered radioactivity was excreted via urine (68 – 87 %) and faeces (13 – 32 %) within 72 hours. Bile-cannulated male rats excreted around 38 % of the radioactivity via the bile but only 57 % via urine suggesting significant enterohepatic circulation of diuron in intact animals. Elimination in exhaled air was negligible. At 72 hours after dosing, only 0.5 – 2.5 % of the administered dose was still found in the body. The relatively highest residues were observed in the blood or in haematopoietic organs, in liver and kidneys and, in females, in the ovaries.

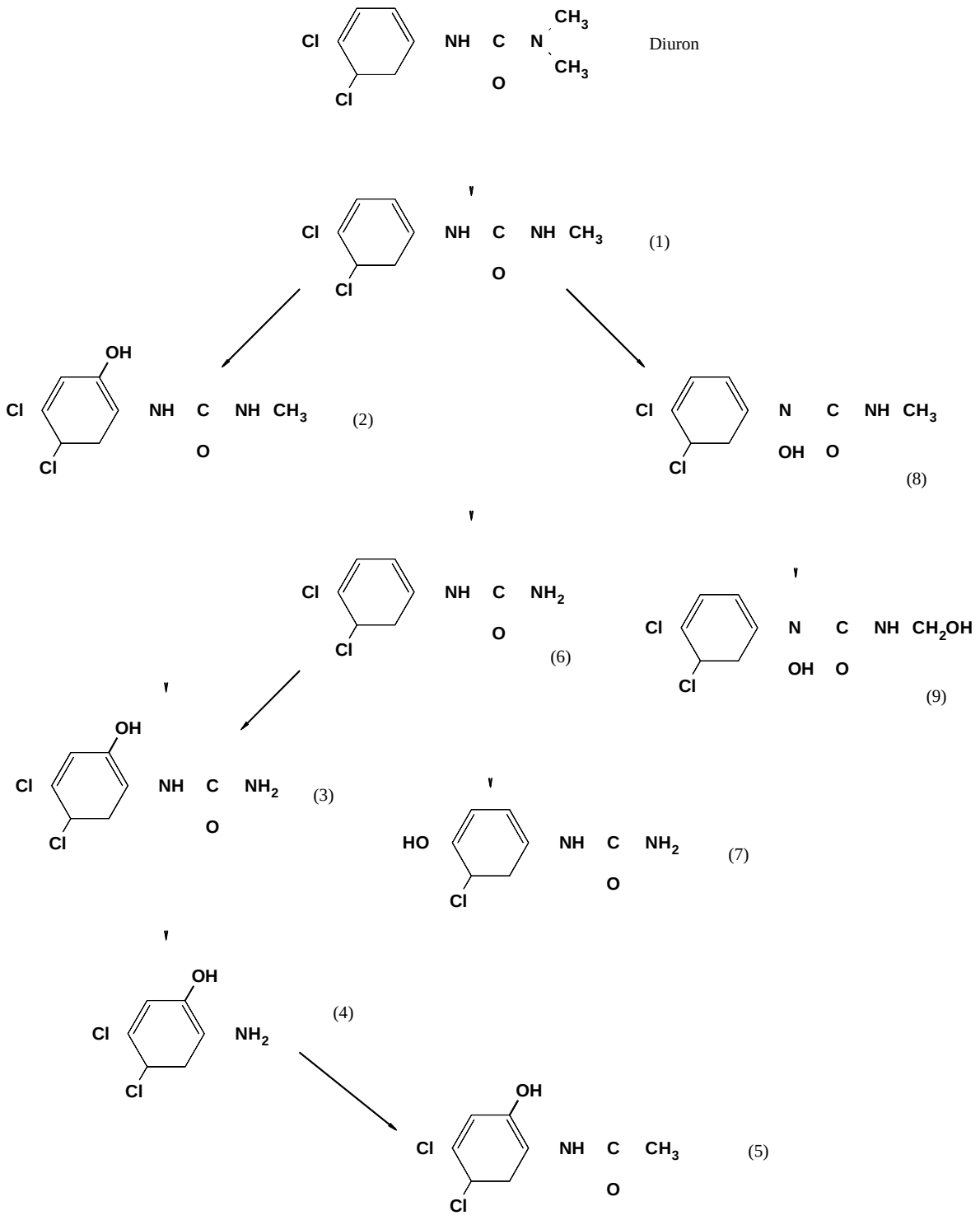
A second study (Anonymous 19, 1996) was performed in Sprague Dawley rats but the findings from the previous one were confirmed even though both the low and high doses were twice as high as in the older study. Diuron was rapidly and quantitatively absorbed and widely distributed. Again, oral absorption was clearly higher than 80 % and tissue residues were low. A certain affinity to red blood cells was confirmed which is well in line with the toxicological profile of diuron (see below). In this study, the metabolism of diuron was also investigated. The vast majority of diuron was excreted as metabolites, mainly N-dimethyl, phenyl ring-hydroxy and N-hydroxy derivatives and their glucuronide or sulfate conjugates with the 3,4-dichlorophenyl-urea (DCPU, IN-R915) being the main metabolite. Only a very small amount of diuron was excreted in unchanged form via faeces. However, a rather high percentage of metabolites in particular in urine could not be identified.

An overview on assumed metabolic pathways of diuron in the rat is depicted in Figure 1.

The metabolism of diuron was also studied in an inhalation study by Anonymous 13 (1986) in which Wistar rats were exposed to actual concentration of 268.1, 37.4 or 4.1 mg/m^3 for 8 weeks (6 hours per day, 5 days a week). Rather extensive metabolism was seen with DCPU being the main metabolite, followed by 3,4-dichloraniline and N'-(3,4-dichlorophenyl)-N-methyl urea. Unchanged diuron was found in the two highest dose groups only. No significant differences were found in the urinary excretion after 4 weeks and 8 weeks inhalation indicating that neither the active ingredient nor its metabolites were accumulated.

Published data on poisoning incidents confirmed abundance of DCPU and at least some other of these rat metabolites also in man following oral intake of diuron (Geldmacher et al. 1970; Verheij et al. 1989, Van Boven et al. 1990). DCPU was also identified as the main metabolite in dogs (Hodge et al. 1967).

The new data requirement of a comparative *in vitro* study on metabolism in cells from different species including man was addressed in a publication by Abass et al. (2007). In this study, diuron was rapidly metabolised via N-demethylation, with 3,4-dichlorophenylurea (DCPU) being the major metabolite in all species. The *in vitro* metabolism of diuron was assessed in human liver homogenates or human, rat, mouse, dog, monkey, minipig and rabbit liver microsomes exposed to three different concentrations and incubation times of 20, 40 or 60 min. Reaction rates for N-dealkylation of diuron were similar (within one order of magnitude) in liver microsomes from rat, mouse, dog, monkey, minipig and rabbit. Human-specific metabolites were not observed. Like a previous publication by Suzuki and Casida (1981) comparing *in vitro* metabolism of diuron by mouse liver microsomes to the results of *in vivo* data obtained in rats, this new study suggests that there are no significant interspecies differences in the metabolic fate of diuron in mammals.



(1) Urea, N-(3,4.dichlorophenyl)-N'-methyl-
(2) Urea, 1-(4,5-dichloro-2-hydroxyphenyl)-3-methyl-
(3) Urea, (4,5-dichloro-2-hydoxyphenyl)-
(4) Phenol, 2-amino-4,5-dichloro-
(5) Acetamide, N-(4,5-dichloro-2-hydroxyphenyl)-
(6) Urea, (3,4-dichlorophenyl)-
(7) Urea, (4-chloro-3-hydroxyphenyl)-
(8) Urea, N-(3,4-dichlorophenyl)-N-hydroxy-N'-methyl-
(9) Urea, N-(3,4-dichlorophenyl)-N-hydroxy-N'-hydroxymethyl-

Figure 1: Proposed metabolic pathway for diuron in the rat

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

There is a current classification and labelling of diuron as "Acute Tox. 4 - Harmful if swallowed" (H302) due to the "translation" of a former classification as "Harmful" (Xn, R22) to the Annex VI of Regulation (EC) No. 1272/2008 (CLP Regulation) (see Section 10.1.3 for more details). As relevant data in experimental animals are available for classification purposes, the acute oral toxicity of diuron is addressed in the present CLH dossier.

10.1 Acute toxicity - oral route

Diuron proved to be of low acute oral toxicity. A large number of acute toxicity studies was reported in the RAR but many of them are considered not acceptable for classification. The lack of acceptability is primarily due to missing information on diuron purity, batch number, rat strain, body weights, or specifications with respect to administration of the oral dose. Also, symptoms of toxicity, time of death or results of pathological examinations were either not or only very poorly reported. The two studies from the RAR considered suitable for acute oral toxicity evaluation are compiled in Table 10.

In addition to the RAR, two other studies considered by the REACH registrant as supportive evidence for acute oral toxicity were identified in the REACH registration dossier (see Table 11).

Table 10: Summary table of animal studies on acute oral toxicity (from the RAR)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
Oral (gavage) Similar to OECD 401 No mention of GLP Deviation: 10 female rats tested per dose; limited data reporting of effects observed at each dose	Rat, Wistar Bor: WISW (SPF-Cpb), 10 F	Diuron (98.8 %), in Cremophor EL and distilled water (10 mL/kg bw)	25, 50, 1000, 2500, 5000, 7100 mg/kg bw single dose followed by 14-d post-treatment observation period	4150 mg/kg bw	Anonymous 5 1983
Oral (gavage) OECD 401 GLP No deviations that would affect the study outcome	Rat, Sprague Dawley, 5 M & 5 F	Diuron (98.5 %) in propan-1,2-diol (1.0-.1.1 mL/animal)	2000 mg/kg (limit test) single dose followed by 14-d post-treatment observation period	> 2000 mg/kg bw	Anonymous 17 1993

Table 11: Summary table of animal studies on acute oral toxicity (from the REACH registration dossier; ECHA, 2019)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
Oral (gavage) Non-guideline acute oral toxicity study No mention of GLP Limited reporting of study outcomes (e.g. number of doses tested or animals with effects)	Rat, Wistar, M 10 per group for test substance; 15 for control	Diuron (labelled as Technical Diuron, 95% purity) in cottonseed oil 20 mL/kg bw	Not specifically reported (described in study as "given in a series of doses estimated to produce mortality rates of from just above 0% to just below 100%) Single dose followed by post-treatment observation period of up to 5 days	1017 mg/kg bw (rats fed standard laboratory chow) 437 mg/kg bw (rats fed protein-deficient diet) 2390 mg/kg bw (rats fed protein test diet-normal)	Boyd & Krupa, 1970
Acute oral toxicity – fixed dose method OECD 420 GLP No deviations mentioned	Rat, Sprague-Dawley CD (Crl: CD (SD) IGS BR) 5 F	Diuron (reported as Diuron Technical, >98% purity) in arachis oil	2000 mg/kg Single dose followed by 14-d post-treatment observation period	>2000 mg/kg bw	Anonymous 2007 (retrieved from ECHA, 2019)

There are no relevant human data on acute oral toxicity of diuron available for evaluation. Limited poisoning incidents with diuron also included other substances, thereby precluding the determination of acute oral toxicity in humans that is specific to diuron exposure.

10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

Clinical signs of toxicity in an acute oral study (conducted similarly to OECD Guideline 401 with some deviations) in female Wistar rats by Anonymous 5 (1983) included breathing difficulties, staggering, spastic gait, and in some cases lying in side position or on stomach. These signs were reported to first appear after minutes in the lethal range and approximately 2 hours post-dosing at lower doses starting at 50 mg/kg bw. The signs lasted up to 8 days except for behavioural disorders, which lasted up to 12 days. However, it is not specified in the study report which signs (and how severe or any recovery) were observed at each dose. Mortality was seen from a dose of 2500 mg/kg bw onwards.

In the oral limit test by Anonymous 17 (1993) that was performed in accordance with OECD Guideline 401 and under GLP, no mortalities occurred. Apparent toxicity was confined to transient lethargy for two hours following dosing. No pathological changes were discovered in any organ in any of the exposed animals.

In the scientific publication by Boyd and Krupa (1970), male Wistar rats (acquired as weanlings or 2 weeks after weaning) were fed for 20-28 days before exposure to diuron on one of the three diets

with different protein content: a protein-deficient diet containing 3.5% protein as casein, a protein test diet-normal containing 26% casein, and a standard laboratory chow. Afterwards, a single administration of diuron (suspended in cottonseed oil; 20 mL/kg bw) was given orally to the rats via gavage. The tested doses were not specifically reported in the publication, but it was mentioned that a series of doses estimated to generate just above 0% to near 100% mortality was administered. It was shown in this study that rats fed on diet with lower protein content were the most susceptible to diuron toxicity (LD_{50} of 437 mg/kg bw) compared to rats fed on higher protein-containing diet or standard laboratory chow (LD_{50} of 2390 mg/kg bw or 1017 mg/kg bw, respectively).

A GLP-compliant acute oral toxicity (fixed dose method) study performed in accordance with OECD 420 in female Sprague-Dawley rats as reported in the REACH registration dossier revealed a LD_{50} of >2000 mg/kg bw with no indications of macroscopic abnormalities. No further results were provided in the dossier.

10.1.2 Comparison with the CLP criteria

Table 12 presents the results of the valid toxicological studies in comparison with the CLP criteria for acute oral toxicity. The oral LD_{50} values exceeded the highest dose of 2000 mg/kg bw for classifying acute toxicity hazard categories.

Table 12: Results of acute oral toxicity in comparison with CLP criteria

Result of the toxicological studies	CLP criteria
LD_{50} = 4150 mg/kg (oral, gavage) in rat LD_{50} > 2000 mg/kg (oral, gavage) in rat (limit test) Reported LD_{50} values here determined from studies performed similar to or in accordance with OECD guideline studies for acute oral toxicity (e.g. OECD 401 or 420)	Cat. 4 (H302): 300 < LD_{50} ≤ 2000 mg/kg (oral) Cat. 3 (H301): 50 < LD_{50} ≤ 300 mg/kg (oral) Cat. 2 (H300): 5 < LD_{50} ≤ 50 mg/kg (oral) Cat. 1 (H300): LD_{50} ≤ 5 mg/kg (oral)

10.1.3 Conclusion on classification and labelling for acute oral toxicity

There is a current classification and labelling of diuron as “Acute Tox. 4 - Harmful if swallowed” (H302) due to the “translation” of a former classification as “Harmful” (Xn, R22) to the Annex VI of Regulation (EC) No. 1272/2008 (CLP Regulation). The existing classification may have been based on the oral LD_{50} of 1017 mg/kg bw in rats as reported by Boyd and Krupa (1970) as well as data from chemical registries and databases, e.g. the US NIOSH’s Registry of Toxic Effects of Chemical Substances and the US National Library of Medicine’s Toxicology Data Bank, which reported LD_{Lo} of 500 mg/kg in mice exposed intraperitoneal to diuron (which is not a human-relevant route of exposure) and a range of 500 mg-5 g/kg as probable oral lethal dose in humans (no further information available for assessment). These latter values in mice and humans were not considered suitable for classification purposes.

In this assessment, 4 acute oral toxicity studies in rats, including the Boyd and Krupa (1970) study, were evaluated for classification purposes. Although the Boyd and Krupa (1970) study reported the lowest rat oral LD_{50} of 1017 mg/kg bw, higher weight was given to the studies that were performed

similarly to or in accordance with OECD guideline for acute oral toxicity (e.g. OECD 401 or 420). The main reason for this is that the limited reporting of results from the Boyd and Krupa (1970) study, e.g. number of tested doses and of animals that died or with observed effects, precluded setting this study as key evidence for classification purposes. Also, deviations from this study from OECD guidelines on acute oral toxicity test (e.g. administered dose volume of 20 mL/kg bw in comparison to the recommended 10 mL/kg bw) could have an influence on the study outcome.

On the other hand, the other 3 acute oral toxicity studies (Anonymous 5, 1983; Anonymous 17, 1993; Anonymous, 2007 from the REACH dossier) all reported an oral rat LD_{50} of >2000 mg/kg bw. In particular, the study of Anonymous 17 (1993) is well-documented with clear reporting of the methodologies and outcomes, and therefore, it is considered justified to use the LD_{50} of >2000 mg/kg bw as the basis for acute oral toxicity classification.

Taking a weight-of-evidence approach, diuron should not be classified for acute oral toxicity.

10.2 Acute toxicity - dermal route

Diuron proved to be of low acute dermal toxicity. The studies are compiled in Table 13 (identified in the RAR) and Table 14 (additionally identified in the REACH registration dossier).

Table 13: Summary table of animal studies on acute dermal toxicity (from the RAR)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Value LD_{50}	Reference
Dermal Non-guideline study (method according to Noakes and Sanderson, 1969) No mention of GLP	Rat, Wistar Bor: WISW (SPF-Cpb), 5 M & 5 F	Diuron (98.8 %), in physiological saline solution	2500, 5000 mg/kg bw, single dose, occlusive, 24 h	> 5000 mg/kg bw	Anonymous 5 1983
Dermal OECD 402 GLP No deviations that would affect the study outcome	Rat, Sprague Dawley, 5 M & 5 F	Diuron (98.5 %) in propan-1,2-diol	2000 mg/kg (limit test), occlusive, 24 h	> 2000 mg/kg bw	Anonymous 17 1993

Table 14: Summary table of animal study on acute dermal toxicity (from the REACH registration dossier; ECHA, 2019)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
Dermal OECD 402 GLP No deviations mentioned	Rat, Sprague Dawley, 5 M & 5F	Diuron (reported as Diuron Technical, >98% purity) in arachis oil	2000 mg/kg Semi-occlusive 24 h	>2000 mg/kg bw	Anonymous 2007 (retrieved from ECHA, 2019)

There are no human data on acute dermal toxicity of diuron available.

10.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

In the acute dermal toxicity study by Anonymous 5 (1983) no mortality occurred; transient clinical signs (apathy and reduced motility) were observed after application of 5000 mg/kg bw but not at the lower dose. In line with that, no adverse findings were obtained in the limit test by Anonymous 17 (1993) and study by Anonymous (2007) identified in and retrieved from the REACH registration dossier (ECHA, 2019).

10.2.2 Comparison with the CLP criteria

Table 15 presents the results of the valid toxicological studies in comparison with the CLP criteria for acute dermal toxicity. The dermal LD_{50} values was above 2000 mg/kg bodyweight for classifying acute toxicity hazard categories.

Table 15: Results of acute dermal toxicity in comparison with CLP criteria

Result of the toxicological studies	CLP criteria
LD_{50} > 5000 mg/kg (dermal) in rat LD_{50} > 2000 mg/kg (dermal) in rat (2 studies)	Cat. 4 (H312): 1000 < LD_{50} ≤ 2000 mg/kg (dermal) Cat. 3 (H311): 200 < LD_{50} ≤ 1000 mg/kg (dermal) Cat. 2 (H310): 50 < LD_{50} ≤ 200 mg/kg (dermal) Cat. 1 (H310): LD_{50} ≤ 50 mg/kg (dermal)

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

Based on the available evidence, diuron should not be classified for acute dermal toxicity.

10.3 Acute toxicity - inhalation route

Diuron proved to be of low acute inhalation toxicity. The studies are compiled in Table 16 (identified in the RAR) and Table 17 (identified in the REACH registration dossier).

Table 16: Summary table of animal studies on acute inhalation toxicity (from the RAR)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
Inhalation (nose-only) Similar to OECD 403 No mention of GLP Deviation: limited data reporting of study outcomes	Rat, Wistar Bor: WISW (SPF-Cpb), 10 M & 10 F	diuron (98.8 %), in ethanol-lutrol mixture (1:1), aerosol	0.073, 0.195, 0.223 mg/L (analytical verified, nominal concentrations: 0.25, 1.0, 1.5 mg/L, respectively) single 4-h exposure followed by 14-d post-treatment observation period	> 0.223 mg/L (4h)	Anonymous 5 1983
Inhalation (nose-only) US EPA F 81-3 GLP	Rat, Crl:CD®BR, 10 M & 10 F	diuron (99.0 %), dust atmosphere MMAD: 6-10 µm	0, 7.0, 7.1 mg/L (average conc.) single 4-h exposure followed by 14-d post-treatment observation period	> 7.1 mg/L (4h)	Anonymous 9 1987

Table 17: Summary table of animal study on acute inhalation toxicity (from the REACH registration dossier; ECHA, 2019)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
Inhalation (nose-only) OECD 403 GLP No deviations mentioned	Rat, Sprague-Dawley, 5 M &5	Diuron (reported as Diuron Technical; >98% purity), aerosol MMAD: 3.59 µm GSD: 2.56	5.05 mg/L (average conc.) Single 4-h exposure followed by 14-d post-treatment observation period	> 5.05 mg/L	Anonymous 2007 (retrieved from ECHA, 2019)

There are no relevant human data on acute inhalation toxicity of diuron available for evaluation. One case report with accidental occupational exposure to diuron via inhalation also included inhalation exposure to other substances along with limited information on the exposure conditions, thereby precluding the determination of acute inhalation toxicity in humans that is specific to diuron exposure.

10.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

Among the three rat acute inhalation toxicity studies, only the Anonymous (2007) study identified in the REACH registration dossier reported mortality in 1 out of 10 animals exposed to diuron (see below for details).

The first acute inhalation toxicity study conducted similarly to OECD Guideline 403 by Anonymous 5 (1983) only observed transient, non-specific behavioural effects in rats for up to 2 hours after nose-only exposure to 0.195 mg/L or 0.223 mg/L (reported to be the maximum concentration producible in air) of diuron as an aerosol. The animals recovered by the end of the study. No effects on body weight or organ lesions were found.

In the second acute inhalation toxicity study conducted in accordance with the U.S. EPA Pesticide Assessment Guideline F 81-3 from 1982 by Anonymous 9 (1987), CD®BR rats of both sexes were exposed to an average dust concentration of 7 mg/L diuron as dust (highest technically achievable concentration) for 4 hours followed by a 14-d post-treatment observation period. The study reported the difficulty of generating and maintaining an extremely high dust concentration as reflected in the inconsistent particle size distributions and low respirability of the dust atmosphere. Clinical signs like lethargy, laboured breathing, red nasal, ocular or oral discharge, stained fur or hair loss as well as transient body weight loss were seen. Most of the clinical signs were observed only up to 3 days after exposure; however, stained fur and perineum were observed up to 8 days after exposure and hair loss for the entire observation period.

Lastly, the acute inhalation toxicity study identified in the REACH registration dossier (Anonymous, 2007) , which was conducted in accordance with OECD Guideline 403 and under GLP compliance, exposed Sprague-Dawley rats of both sexes nose-only to an average concentration of 5.05 mg/L of diuron (reported to be the maximum achievable atmosphere concentration) for 4 hours followed by a 14-d post-exposure observation period. Clinical signs such as increased respiratory rate, hunched posture, pilo-erection and wet fur were observed in both sexes; males and females recovered by day 6 and day 11, respectively, post-exposure. One female died on day 1 post-exposure with abnormally dark lungs and accentuated lobular pattern of the liver found during necropsy. Abnormally dark lungs were also found in one surviving animal; otherwise, no abnormalities were detected in the other animals.

10.3.2 Comparison with the CLP criteria

Table 18 presents the results of the valid toxicological studies in comparison with the CLP criteria for acute inhalation toxicity. The inhalation LC_{50} values of diuron in dust or aerosol forms were all reported to be above the highest technically achievable concentrations of up to 7.1 mg/L, which is above the highest dose of 5.0 mg/L (dusts and mists) for classifying acute toxicity hazard categories.

Table 18: Results of acute inhalation toxicity in comparison with CLP criteria

Result of the toxicological studies	CLP criteria
LC_{50} > 0.223 mg/L (inhalation, aerosol) in rat LC_{50} > 5.05 mg/L (inhalation, aerosol) in rat LC_{50} > 7.1 mg/L (inhalation, dust) in rat	Cat. 4 (H332): 1.0 < LC_{50} ≤ 5.0 mg/L (dusts and mists) Cat. 3 (H331): 0.5 < LC_{50} ≤ 1.0 mg/L (dusts and mists) Cat. 2 (H330): 0.05< LC_{50} ≤ 0.5 mg/L (dusts and mists) Cat. 1 (H330): LC_{50} ≤ 0.05 mg/L (dusts and mists)

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Based on the available evidence, diuron should not be classified for acute inhalation toxicity

10.4 Skin corrosion/irritation

This endpoint is not addressed in this CLH report.

10.5 Serious eye damage/eye irritation

This endpoint is not addressed in this CLH report.

10.6 Respiratory sensitisation

This endpoint is not addressed in this CLH report.

10.7 Skin sensitisation

This endpoint is not addressed in this CLH report.

10.8 Germ cell mutagenicity

The genotoxicity of diuron was assessed in *in vitro* studies in bacteria and mammalian cells, *in vivo* studies in somatic cells and an *in vivo* study with germ cells. The valid *in vitro* mutagenicity/genotoxicity studies are compiled in

Table 19 (from the RAR) and Table 20 (additional studies identified from the REACH registration dossier; ECHA, 2019) and the valid *in vivo* mutagenicity/genotoxicity studies (evaluated both in RAR and REACH registration dossier) are given in Table 21.

Table 19: Summary table of mutagenicity/genotoxicity tests *in vitro* (from the RAR)

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Ames Test OECD 471 GLP Deviation: Missing strain to test for cross-linking mutagens (e.g. TA102, *E. coli* WP2 uvrA, or *E.coli* WP2 uvrA (pKM101))	diuron (98.7 % pure)	*Salmonella typhimurium* TA 98, TA 100, TA 1535, TA 1537, TA 1538 Concentrations: 20 - 1600 µg/plate Concentrations were chosen based on the results of the precipitation test (50 - 3200 µg/plate) followed by an initial toxicity test (strain TA 100, 20 - 1600 µg/plate, +/- S9-mix). Tested in the presence and absence of metabolic activation (liver S-9 mix).	**Negative +/- S9** Positive controls gave expected results non mutagenic in tested *Salmonella typhimurium* strains (+/- S9-mix)	Kamath, 1998
Ames Test U. S. EPA Pesticide Assessment Guidelines, Subdivision F, 84-2 GLP	diuron (98.2 % pure)	*Salmonella typhimurium* TA 97, TA 98, TA 100, TA 1535 Concentrations: - S9: 0.5 - 10 µg/plate +S9: 10 - 250 µg/plate	**Negative +/- S9** Positive controls gave expected results Results indicate that testing for mutagenicity was not performed up to cytotoxic levels.	Arce, 1984*

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
No signs of cytotoxicity in the test; only 4 instead of 5 strains of bacteria were used; missing strain to test for cross-linking mutagens		Concentrations were chosen based on the toxicity results (strain TA 1535, 10 - 5000 µg/plate, +/- S9-mix). Tested in the presence and absence of metabolic activation (liver S-9 mix).	Precipitation was seen at 5000 µg/plate non mutagenic in tested *Salmonella typhimurium* strains (+/- S9-mix)	
Ames test No OECD or EU guideline indicated No GLP Only 4 instead of 5 strains of bacteria were used; missing strain to test for cross-linking mutagens	diuron (98.8 % pure)	*Salmonella typhimurium* TA 98, TA 100, TA 1535, TA 1537 Concentrations: 125 - 2000 µg/plate Concentrations were chosen based on the toxicity results (20 - 12500 µg/plate, +/- S9-mix). Tested in the presence and absence of metabolic activation (liver S-9 mix).	**Negative +/- S9** Positive controls gave expected results Precipitation was seen at 12500 µg/plate non mutagenic in tested *Salmonella typhimurium* strains (+/- S9-mix)	Herbold, 1984a*
HPRT gene mutation OECD 476 GLP Deviation: only 100 metaphases counted instead of 300 as recommended in guideline	diuron (98.8 % pure)	Chinese hamster ovary (CHO)-K1 cells Concentrations: 180 - 310 µg/mL Concentrations were chosen based on the initial cytotoxicity test (100 - 1600 µg/mL, 5 h). Tested in the presence and absence of metabolic activation (liver S-9 mix).	**Negative +/- S9** Positive controls gave expected results non mutagenic in tested (CHO)-K1 cells (+/- S9-mix)	Shivaram, 1998*
HPRT gene mutation No guideline indicated No GLP	diuron (98.2 % pure)	Chinese hamster ovary (CHO)-K1 cells Concentrations: - S9: 0.01 – 1.25 mM +S9: 0.05 – 0.50 mM Concentrations were chosen based on the initial cytotoxicity test (+/- S9 mix) and solubility of compound, respectively. Tested in the presence and absence of metabolic activation (liver S-9 mix).	**Negative +/- S9** Positive controls gave expected results non mutagenic in tested (CHO)-K1 cells (+/- S9-mix)	Rickard, 1985*
Chromosome aberration *in vitro* OECD 473 GLP No deviations from	diuron (98.8 % pure)	Chinese hamster ovary (CHO)-K1 cells Concentrations: 90 - 360 µg/ml Concentrations were chosen based on the initial cytotoxicity	**Negative +/- S9** Positive controls gave expected results Initial cytotoxicity test revealed mitotic indexes at the highest concentration (350 µg/ml) tested	Shivaram, 1999

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
guideline		test (+/- S9-mix). Tested in the presence and absence of metabolic activation (liver S-9 mix).	of 54.8 % in the presence of S-9 mix and 59.5 % in the absence of S-9 mix. no significant increases in aberrant metaphases in tested (CHO)-K1 cells (+/- S9-mix)	
Cytogenetic study *in vitro* No guideline indicated GLP	diuron (98.3-98.7 % pure)	Human lymphocytes (2 donors, 1 F & 1 M) Concentrations: - S9: 62.5 – 750 mM +S9: 125 – 1500 mM Concentrations were chosen based on a previous study identifying mitotic indexes (30 – 5000 µg/mL, +/- S9-mix) Tested in the presence and absence of metabolic activation (liver S-9 mix).	**Positive +/- S9** Positive controls gave expected results Diuron produced a decrease in human lymphocyte mitotic index at concentrations up to 500 µg/mL (-S9) and up to 1000 µg/mL (+S9). There was a significant increase in the number of aberrations (+/- gaps) from 250 µg/mL (-S9) and from 500 µg/mL (+S9). Cytotoxicity was demonstrated from the same doses. Clastogenic effects at cytotoxic concentration range (+/- S9 mix)	Herbold, 1989
E.coli Pol-A1 assay (POL test) Non-guideline screening assay No GLP	diuron (98.8 % pure)	*Escherichia coli* (K 12)p 3478 (repair deficient), W 3110 (repair competent) Concentrations: 312.5 – 5000 µg/plate Tested in the presence and absence of metabolic activation (liver S-9 mix).	**"No test" (+/- S9)** Positive and negative controls gave expected results No inhibition zone (+/- S9) with Diuron in neither *Escherichia coli* strain (K 12)p 3478 nor W 3110 Not interpretable ("No test")	Herbold, 1984b*
Unscheduled DNA synthesis (UDS) No guideline indicated No GLP	diuron (98.2 % pure)	freshly isolated hepatocytes of male Crl:CD®(SD)BR rats Concentrations: 0.001 – 20 mM Concentrations were chosen based on the initial cytotoxicity test.	**Negative** Precipitate was observed at concentrations of 1 - 20 mM. Cytotoxicity was evident at concentrations ≥ 0.33 mM. no unscheduled DNA synthesis in the primary rat hepatocytes under the conditions tested. A seemingly positive result from 0.33 - 20 mM was discussed to be due to cytoplasmic response (cytotoxicity) instead of increased nuclear grain counts at the high concentrations tested. Under the conditions of the reported study the test substance is considered to be not mutagenic.	Arce, 1985*

*study is considered supplementary due to deviation from current guidelines

Table 20: Summary table of mutagenicity/genotoxicity tests *in vitro* (from the REACH registration dossier; ECHA, 2019)

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Ames test OECD 471 GLP No deviations reported	Diuron (98.8% purity)	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and *Escherichia coli* WP2 uvrA Tested in the presence and absence of metabolic activation (liver S-9 mix) Experiment I: 10- 5000 µg/plate (TA 98, TA100, TA1535, TA1537 with metabolic activation and *E.coli* WP2 uvrA with and without metabolic activation) 3.16, 10.0, 31.6, 100, 316, 1000, and 2500 µg/plate (TA 98, TA100, TA1535, TA1537 without metabolic activation). Experiment II: 5-5000 µg/plate (TA 98, TA1535, TA1537 with metabolic activation and *E.coli* WP2 uvrA with and without metabolic activation) 1.58-1580 µg/plate (TA 98, TA100, TA1535, TA1537 without metabolic activation). Experiment III: 500-5000 µg/plate (only tester strain TA100 with metabolic activation)	**Negative +/- S9** Positive controls provided valid results Non-mutagenic in all the 3 experiments with or without metabolic activation	Anonymous 2008 (retrieved from ECHA, 2019)
Ames test OECD 471 GLP No deviations reported	Diuron (98.8% purity)	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and *Escherichia coli* WP2 uvrA Tested in the presence and absence of metabolic activation (liver S-9 mix) Experiment I: 10- 5000 µg/plate (TA 98, TA100, TA1535, TA1537 with metabolic activation and *E.coli* WP2 uvrA with and without metabolic activation) 3.16, 10.0, 31.6, 100, 316,	**Negative +/- S9** Positive controls provided valid results Non-mutagenic in all the 3 experiments with or without metabolic activation	Anonymous 2008 (retrieved from ECHA, 2019)

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
		1000, and 2500 µg/plate (TA 98, TA100, TA1535, TA1537 without metabolic activation). Experiment II: 5-5000 µg/plate (TA 98, TA1535, TA1537 with metabolic activation and *E.coli* WP2 uvrA with and without metabolic activation) 1.58-1580 µg/plate (TA 98, TA100, TA1535, TA1537 without metabolic activation). Experiment III: 500-5000 µg/plate (only tester strain TA100 with metabolic activation)		
Ames test OECD 471 GLP Deviation: Only TA100 strain was tested	Diuron (98.8% purity)	*Salmonella typhimurium* TA100 Tested in the presence and absence of metabolic activation (liver S-9 mix) Experiment I : 3.16-5000 µg/plate Experiment II/ Toxicity experiment: 250- 5000 µg/plate	**Negative +/- S9** Positive controls provided valid results Non-mutagenic in TA100 with or without metabolic activation	Anonymous 2008 (retrieved from ECHA, 2019)
Ames test OECD 471 GLP Deviation: Only TA100 strain was tested	Diuron (98.8% purity)	*Salmonella typhimurium* TA100 Tested in the presence and absence of metabolic activation (liver S-9 mix) Experiment I : 3.16-5000 µg/plate Experiment II/ Toxicity experiment: 250- 5000 µg/plate	**Negative +/- S9** Positive controls provided valid results Non-mutagenic in TA100 with or without metabolic activation	Anonymous 2008 (retrieved from ECHA, 2019)

Table 21: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Chromosome aberration *in vivo* No OECD or EU guideline indicated, but compliance was claimed. GLP Deviation (from OECD 475): only 50 metaphases counted instead of 200 recommended in the guideline	diuron (98.2 % pure)	Rat, Crl:CD®(SD)BR 5 M & 5 F / dose single dose (gavage) Dose: 0, 50, 500 or 5000 mg/kg bw in corn oil for 6, 24, or 48 hours Positive control: 20 mg/kg bw cyclophosphamide (24 h) Bone marrow cells from both femurs were analysed for chromosomal aberration	**Negative** Positive control gave expected results Significant bone marrow cytotoxicity (depressed mitotic indices) and systemic toxicity were observed in animals dosed with the high dose A statistical difference between dosed and control animals was observed, but the findings were within the historical control for the laboratory non clastogenic in bone marrow cells	Anonymous 1, 1985/1997*
Chromosome aberration *in vivo* OECD 475 GLP Deviation (from OECD 475): only 50 metaphases counted instead of 200 recommended in the guideline	diuron (98.4 % pure)	Chinese Hamster 6 M & 6 F / dose, or 10 M & 10 F / 48 h single dose (gavage) Dose: 0, 500, 1670, 5000 mg/kg bw in cremophor RH 40 for 24 hours; 5000 mg/kg bw for 6 or 48 h. Positive control: 40 mg/kg bw cyclophosphamide (24 h) Dose selection was based on a preliminary experiment and was intended to reach a (cyto)toxic range. Bone marrow cells from both femurs were analysed for chromosomal aberration	**Negative** No chromosomal aberrations above negative control Positive control gave expected results. Under the conditions of the reported study the test substance is considered to be not mutagenic.	Anonymus 16, 1987a*
Mammalian Spermatogonial Chromosomal Aberration Test OECD 483 GLP Deviation (from OECD Guideline 483): only 100	diuron (98.4 % pure)	Mouse, NMRI 6 M / dose, or 12 M / 5000 mg/kg bw for 24 or 48 h. single dose (gavage) Dose: 0, 500, 1670, 5000 mg/kg bw in cremophor for 24 hours; 5000 mg/kg bw for 6 or 48 h.	**Negative** Positive control gave expected results All treated animals expressed toxic symptoms: apathy. All mean chromosome aberration rates were in the range of the negative control value. Under the conditions of the reported study the test substance is considered to	Anonymus 16, 1988*

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations	Reference
metaphases counted instead of 200 as recommended in guideline		Positive control: 7.5 mg/kg bw adriblastin (doxorubicin hydrochloride) Dose selection was based on a preliminary experiment and was intended to reach a (cyto)toxic range. Spermatogonial cells were analysed for chromosomal aberration	be not mutagenic.	
Micronucleus assay No OECD or EU guideline indicated No GLP	diuron (98.8 % pure)	Mouse, Bor:NMRI (SPF Han) 5 M & 5 F / dose Single dose (stomach tube) Dose: 0 (only 24 h), 2500 mg/kg bw in cremophor emulsion for 24, 48 or 72 hours Positive control: 30 mg/kg bw cyclophosphamide (24 h) bone marrow smears were used for counting of polychromatic erythrocytes and the incidence of micronuclei.	**Negative** Positive control gave expected results None of the treated mice showed effects after the oral administration of 2500 mg diuron/kg bw. Their behaviour, appearance and motoric activity remained unaffected. There were no substance-induced mortalities. No increased number of micronucleated erythrocytes in the bone marrow. Under the conditions of the reported study the test substance is considered to be not mutagenic.	Herbold, 1983*
Micronucleus assay No guideline No GLP Inadequate reporting of the study	diuron (no batch no. or purity reported)	Mouse, Swiss Albino 6 M & 6 F / dose Single dose (intraperitoneal) Dose: 0, 85, 170, 340 mg/kg bw in DMSO for 30, 48, or 72 hours. Positive control: 20 mg/kg bw cyclophosphamide (30, 48, or 72 h) Dose selection was based on pilot test, given that 340 mg/kg bw was the maximum tolerated dose bone marrow smears were used for counting of polychromatic erythrocytes and the incidence of micronuclei.	**Positive** Positive control gave expected results Increased numbers of polychromatic erythrocytes with micronuclei after treatment with 170 and 340 mg/kg bw of diuron compared to control after 30 and 48 hours (but not after 72 hours) equivalent to the number following treatment with the positive control. Dose relation could be shown. Under the conditions of the reported study the test substance is considered to be clastogenic. Both, the positive control and especially the negative control values are extremely low, possibly due to late reading of the test, so the result can be very hard to evaluate.	Agrawal et al., 1996*

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Micronucleus assay OECD 474 GLP Deviation (from OECD 474): only 1000 PCEs counted	diuron (98.1 % pure)	Mouse, Hsd/Win:NMRI 5 M & 5 F / dose Single dose (intraperitoneal) Dose: 0 (only 24 h), 700 mg/kg bw in cremophor emulsion for 16, 24, or 48 hours. Positive control: 20 mg/kg bw cyclophosphamide (24 h) Dose selection was based on pilot test, showing signs of intoxication. bone marrow smears were used for counting of polychromatic erythrocytes and the incidence of micronuclei.	**Negative** Positive control gave expected results Animals appeared clinically affected of the dose until sacrifice. No mortalities occurred, feeding behaviour was normal. No increase number of micronucleated cells per 1000 polychromatic erythrocytes was observed compared to negative control. No indication of a clastogenic effect under the conditions tested.	Anonymous 7, 1998
Sister chromatid exchange assay No OECD or EU guideline indicated GLP	diuron (98.4 % pure)	Chinese Hamster 6 M & 6 F / dose single doses (gavage) Dose: 0, 500, 1670, 5000 mg/kg bw in cremophor RH 40 for 24 hours Positive control: 15 mg/kg bw cyclophosphamide (24 h) Bone marrow cells from both femurs were analysed	**Negative** Positive control gave expected results All animals expressed toxic symptoms. 1 M and 1 F died within 24 hours after dose administration. 5 (of 6) animals per sex and group were evaluated. No increased number of SCEs. Under the conditions of this indicator test, the test substance is considered to be not mutagenic.	Anonymus 16, 1987b*
Unscheduled DNA Synthesis No OECD or EU guideline indicated GLP	diuron (no batch no. or purity reported)	Rat, BOR:WISW 6 M & 6 F / dose repeated dose (dietary), 7 days Dose: 0, 25, 250, 2500 ppm (0, 11, 104, 687 mg/kg bw) Positive control: 100 mg/kg bw MMS methyl methane sulphonate (18 h, gavage) Bladder urothelial cells were analysed.	**Positive** Positive control gave expected results The high dose animals consumed less feed than the other two dose groups. 11 mg/kg bw: (only) a slight, statistically insignificant rise in S-phases 104, 687 mg/kg bw: increased number of cells with induced UDS, reduced degree of DNA repair, increased proportion of S-phase cells; only cells with 3 silver grains were significantly increased and these are not necessarily indicators of genetic effect. Results of this study were considered to be equivocal.	Klein, 1986*

*study is considered supplementary due to deviation from current guidelines

No human data on germ cell mutagenicity were available for evaluation.

10.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

In vitro studies:
Diuron proved negative in an Ames test (Kamath 1998) that was conducted according to the state of the art. The negative result was confirmed by two similar studies in bacteria which were considered to be supplementary (Arce 1984, and Herbold 1984a). The Ames test by Kamath (1998) did not include a strain to test for cross-linking mutagens (e.g. TA102, *E. coli* WP2 uvrA, or *E.coli* WP2 uvrA (pKM101)); however, in the REACH registration dossier, 2 additional Ames tests (2008) with the inclusion of the missing strain (*E. coli* WP2 uvrA) were provided (ECHA, 2019). Both tests showed negative mutagenic potential of diuron.
Diuron did not cause gene mutations in the HPRT test in CHO cells (Shivaram 1998, Rickard 1985) even though both studies were considered supplementary only.
No evidence of an impact of diuron on DNA damage and repair was obtained in an UDS test in rat hepatocytes (Arce 1985), which might indicate direct interaction. A *POL* test in *E. coli* (Herbold 1984b) did not yield interpretable results due to lack of inhibition zones in all treatment groups. However, both studies must be considered supplementary from a today's view if strict criteria regarding GLP and compliance to guidelines are applied. Still, they are considered reliable.
In contrast to this clearly negative outcome, *in vitro* chromosome aberration assays revealed contradictory results and further clarification and testing *in vivo* was needed.
Shivaram (1999) did not find evidence of clastogenic activity of diuron up to the highest tested concentration of 360 µg/mL in CHO-K1 cells, neither with nor without metabolic activation at two different harvest times. In a previous study, however, Herbold (1989) reported a significant increase in chromosome aberrations in peripheral human lymphocytes. Positive results were noticed at concentrations of 250 and 500 µg/mL without and from 500 µg/mL onwards with activation.

In a published study by Federico et al. (2011), an increase in chromosome aberration was observed at low and high concentrations (0.45 or 4.5 to 90 µg/mL) in both CHO cells without metabolic activation and in the metabolically active Chinese hamster epithelial liver (CHEL) cells, respectively, supporting previous findings that diuron could be clastogenic *in vitro*.

In vivo studies:
Diuron was negative in a cytogenetic study in bone marrow cells of rats by Anonymous 1 (1985, revised 1997) following single oral application of up to 5000 mg/kg bw. Cytotoxicity to the bone marrow and systemic toxicity were observed in high dose animals providing evidence that the target tissue had been reached by the test substance. However, the study has to be considered supplementary due to an insufficient number of metaphases examined. Likewise, in a study in Chinese hamsters, Anonymus 16 (1987a) did not find evidence of chromosome aberrations in femur bone marrow of these animals following application of single oral doses up to a maximum dose level of 5000 mg/kg bw. Again, severe systemic toxicity (mortality) was seen in the highest dose group.

A third valid cytogenetic study for chromosome aberrations was performed also by Anonymus 16 (1988) utilizing spermatogonial cells of NMRI mice. No evidence of clastogenicity was obtained. No increase in the percentage of aberrant cells was observed up to the highest dose of 5000 mg/kg bw (single gavage application), i.e., a dose which proved already systemically toxic to the animals. It is the only valid study in germ cells. Two dominant lethal tests providing contradictory results (Anonymous 6 1985, Agrawal and Mehrotra 1997) were both found inadequate and were not considered any longer in risk assessment.

In mice, a total of three micronucleus assays of different quality and reliability are available. In a supplementary study (no GLP), Herbold (1983) administered a single oral dose of 2500 mg/kg bw to male and female mice. The animals tolerated the application quite well and the percentage of micronucleated cells in bone marrow preparations was not increased as compared to the negative control group. In contrast, a positive result was reported by Agrawal et al. (1996) who administered diuron at doses of up to 340 mg/kg bw by the intraperitoneal route to male and female Swiss albino mice. This published study by Agrawal et al. was considered to be supplementary only because of inadequate reporting and possible methodical deficiencies. For instance, it was not reported whether slides were read blindly. In this study, a significant and dose-related increase in micronucleus frequency was reported from a dose level of 170 mg/kg bw onwards. In addition to serious doubts about study quality and reliability, the positive result was contravened and overwhelmed in a comparable micronucleus test also with intraperitoneal application that was performed two years later by Anonymous 7 (1998) even though in another mouse strain. This study was conducted under GLP conditions even though the number of polychromatic erythrocytes evaluated was lower than required by the current OECD Guideline 474. In this comprehensively documented study, a twofold higher dose of 700 mg diuron/kg bw was applied. No evidence of an increase in micronucleus incidence was observed at any dose or time point. This result is of particular importance since the same author had earlier reported the only positive result with diuron in an *in vitro* chromosome aberration study (Herbold 1989).

An *in vivo* sister chromatid exchange assay in Chinese hamster bone marrow by Anonymus 16 (1987b) was negative since no increase in SCE frequency was seen up to the highest tested (oral) dose of 5000 mg/kg bw. The study is considered acceptable although, as an "indicator test" for interaction with DNA, is of rather low relevance for risk assessment.

An *in vivo* UDS test by Klein (1986) is considered supplementary since batch and purity of the test substance were not given and since it was not performed under GLP. Female Wistar rats were fed diuron for one week at dietary concentrations of 25, 250, or 2500 ppm. Following sacrifice, urinary bladder cells were isolated and examined for occurrence of UDS by microscopic counting of silver grains in 50 cells per animal. At two upper dose levels, a dose-related and statistically significant increase in the induction of UDS and a higher percentage of cells in the S-phase was observed. Even though not conclusive, these findings might point to a mitogenic effect in bladder cells which would be well in line with the neoplastic effect in this organ as observed in the long-term study in rats.

10.8.2 Comparison with the CLP criteria

Table 22 presents the CLP criteria for germ cell mutagenicity. No human data are available; hence a classification in category 1A is not possible.

Table 22: Criteria for classification for germ cell mutagenicity

CLP criteria
The classification in **Category 1A** is based on positive evidence from human epidemiological studies. Substances to be regarded as if they induce heritable mutations in the germ cells of humans. The classification in **Category 1B** is based on: - positive result(s) from *in vivo* heritable germ cell mutagenicity tests in mammals; or - positive result(s) from *in vivo* somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. It is possible to derive this supporting evidence from mutagenicity/genotoxicity tests in germ cells in vivo, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells; or

CLP criteria
- positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cells of exposed people. The classification in **Category 2** is based on: - positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments, obtained from: - somatic cell mutagenicity tests in vivo, in mammals; or - other in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays. Note: Substances which are positive in in vitro mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, shall be considered for classification as Category 2 mutagens.

The valid *in vitro* tests, with one exception, were all negative. With regard to chromosome aberrations *in vitro*, results of different tests were contradictory. In such cases, clarification by suitable *in vivo* studies is needed. With diuron, three cytogenetic studies in rats, hamsters and mice did not find evidence of chromosome aberrations in bone marrow or spermatogonial cells, up to very high and already toxic dose levels.

In addition, two out of three mouse micronucleus assays were clearly negative. The only positive result was obtained in a study using the i.p. route. However, a second i.p. micronucleus test in which a higher dose was employed failed to reproduce the positive outcome. Thus, based on weight of evidence, the conclusion can be drawn that diuron was not clastogenic in the tissues investigated. Some uncertainty remains with regard to the representativeness of the test results for the urothelium – the primary target organ in the rat carcinogenicity study -, in particular with regard to tissue exposure.

A positive result in an *in vivo* UDS test in which rat bladder cells were investigated might be in line with the occurrence of bladder tumors in a long-term study in rats. The data suggest rather a stimulation of mitosis than a true genotoxic effect. This assumption is supported by the unequivocally negative outcome of the *in vitro* UDS test in rat hepatocytes.

On balance, diuron is considered to be of no genotoxic potential even though nearly all studies must be considered supplementary from a today's point of view. However, if all the information is taken into account, they are of sufficient extent and quality to support this final conclusion.

Overall, classification in category 1B or 2 is currently not triggered.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

No classification for mutagenicity was considered necessary as the criteria laid down in CLP regulation were not met.

10.9 Carcinogenicity

There is a current classification and labelling of diuron as "Carc. 2 – suspected of causing cancer" (H351). This is based on "translation" from previous classification as in Annex I of Dir 67/548/EEC with the risk phrase R40 assigned to diuron. Therefore, the carcinogenicity of diuron is addressed in the present CLH dossier.

Two studies on chronic toxicity and carcinogenicity of diuron in rats and mice are available which are summarised in Table 23.

Table 23: Summary table of animal studies on carcinogenicity

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr>
<td>2-year combined chronic toxicity / carcinogenicity study

Oral (dietary)

Rat, Wister (BOR:WISW)

50/sex/dose in main treatment group

Satellite group 10/sex/dose

US EPA Pesticides Assessment Guidelines F 83-1

GLP

Comparable to OECD 453, but not all parameters were included e.g. for clinical chemistry, or urinalysis analysis</td>
<td>Diuron (98.7 % pure)

Dose: 0, 25, 250 & 2500 ppm

equivalent to: Males: 0, 1.0, 10 or 111 mg/kg bw Females: 0, 1.7, 17 and 203 mg/kg bw (mean daily doses)

Exposure: 24 months for main treatment group

12 months for satellite group</td>
<td>Non-neoplastic findings:

1.0/1.7 mg/kg bw: ↑ MCV, MCH, reticulocytes, ↓ erythrocyte count, haemoglobin and haematocrit in females; marginally increased heaemosiderin deposits in spleen in males

10/17 mg/kg bw: Reduction in bodyweight and bodyweight gain in males, ↑ leucocyte count in males: ↑ in absolutes and relative liver and spleen weights; ↑ haemosiderin deposits in spleen in females

111/203 mg/kg bw: Impairment in food efficiency in both sexes, ↑ MCV, MCH, reticulocytes, ↓ erythrocyte count and haemoglobin in males; ↑ leucocyte count in females; about half of the males and about a quarter of the females exhibited hardness and/or thickening of the urinary bladder wall; urothelial hyperplasia: in M increased severity, in F total percentage of affected animals higher and evidence of an increase in severity

NOAEL: could not be established

LOAEL: 1.0 mg/kg bw/day in males, based on marginally increased haemosiderin deposits in spleen; 1.7 mg/kg bw/day in females, based on anaemia and increased spleen weight

neoplastic findings:

Significant increase of transitional epithelial carcinoma of urinary bladder in males and females at 111/203 mg/kg bw/d; increase of uterine adenocarcinoma in females at 203 mg/kg bw/d.
<table>
<tr><td>Sex</td><td colspan="4">Males</td><td colspan="4">Females</td></tr>
<tr><td>Dose (mg/kg bw/d)</td><td>0</td><td>1.0</td><td>10</td><td>111</td><td>0</td><td>1.7</td><td>17</td><td>203</td></tr>
<tr><td>Animals examined</td><td>50</td><td>50</td><td>50</td><td>50</td><td>48</td><td>50</td><td>50</td><td>50</td></tr>
<tr><td colspan="9">Urinary bladder</td></tr>
<tr><td>Transitional epithelial papilloma (b)</td><td>0</td><td>0</td><td>0</td><td>3
6 %</td><td>1
2 %</td><td>0</td><td>2
4 %</td><td>2
4 %</td></tr>
<tr><td>Transitional epithelial carcinoma (m)</td><td>1
2
%</td><td>0</td><td>1
2 %</td><td>33**
67 %</td><td>0</td><td>0</td><td>0</td><td>11**
22 %</td></tr>
<tr><td>Carcinoma in situ (m)</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>1
2 %</td><td>0</td></tr>
<tr><td colspan="9">Renal pelvis, Transitional epithelium</td></tr>
<tr><td>Papilloma (b)</td><td>0</td><td>0</td><td>0</td><td>1
2 %</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>Carcinoma (m)</td><td>0</td><td>0</td><td>0</td><td>2*
4 %</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td colspan="9">Uterus</td></tr>
</table>
</td>
<td>Anonymous 14, 1985</td>
</tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td></td><td>
<table>
<tr><td>Polyp (b)</td><td>-</td><td>-</td><td>-</td><td>-</td><td>7
15
%</td><td>7
14
%</td><td>6
12
%</td><td>3
6 %</td></tr>
<tr><td>Fibromyoma (b)</td><td>-</td><td>-</td><td>-</td><td>-</td><td>0</td><td>1
2 %</td><td>0</td><td>0</td></tr>
<tr><td>Leiomyosarcoma (m)</td><td>-</td><td>-</td><td>-</td><td>-</td><td>0</td><td>1
2 %</td><td>0</td><td>0</td></tr>
<tr><td>Endometrium sarcoma (m)</td><td>-</td><td>-</td><td>-</td><td>-</td><td>0</td><td>0</td><td>0</td><td>2
4 %</td></tr>
<tr><td>Adenocarcinoma (m)</td><td>-</td><td>-</td><td>-</td><td>-</td><td>5
10
%</td><td>5
10
%</td><td>5
10
%</td><td>10 #
20 %</td></tr>
<tr><td>Squamous epithelial carcinoma (m)</td><td>-</td><td>-</td><td>-</td><td>-</td><td>0</td><td>0</td><td>1
2 %</td><td>1
2 %</td></tr>
</table>
b=benign; m= malignant; statistically significant trend *for $p \leq 0.05$ and **for $p \leq 0.01$ (Cochrane Armitage linear trend test, two-sided) and # for $p \leq 0.05$ (Cochrane Armitage linear trend test, one-sided)

No valid detailed HCD available.</td><td></td></tr>
<tr><td>2-year combined chronic toxicity / carcinogenicity study

Oral (dietary)

Mouse, NMRI outbred (SPF, Bor strain)

50/sex/dose in main treatment group

Satellite group 10/sex/dose

OECD 453

not all parameters were included e.g. for clinical chemistry, or urinalysis analysis

GLP</td><td>diuron (98.7 % pure)

Dose: 0, 25, 250 & 2500 ppm

equivalent to: Males: 0, 5.4, 50.8 or 640 mg/kg bw Females: 0, 7.5, 77.5 and 867 mg/kg bw (mean daily doses)

Exposure: 24 months for main treatment group

12 months for satellite group

haematological and clinical chemistry analyses after 6, 12, 18 and 24 months</td><td>Non-neoplastic findings:

5.4/7.5 mg/kg bw: -

50.8/77.5 mg/kg bw: -

640/867 mg/kg bw: Reduction in bodyweight and bodyweight gain (by 7 % M, 12 % F), Impairment in food efficiency; (weak) hyperchromic and macrocytic anaemia (in F: ↑ MCHC, MHC, MCV, in M ↓ MCHC), increase in reticulocytes, haemosiderosis; increased absolute / relative liver weights (by 9 % / 12 %) in M, accompanied by histopathological findings and alterations in clinical chemistry parameters (in M ↑ ALAT, ↑ Bilirubin); increased incidence of epithelial hyperplasia in urinary bladder and pigmentation of renal tubules (top dose females only)

NOAEL: 50.8 mg/kg bw/day in males and 77.5 mg/kg bw/day in females

LOAEL: 640.1 mg/kg bw/day in males and 867 mg/kg bw/day in females, based on effects on red blood cells and the liver in both sexes, and on urothelial hyperplasia in females

neoplastic findings:

Increased incidence of mammary carcinoma and of ovarian luteoma in females at 867 mg/kg bw/d; higher incidence of liver cell tumours in male mice in all treated groups compared to control, but not statistically significant and no convincing dose-response</td><td>Anonymous 3, 1990</td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference

Sex	**Males**				**Females**			
Dose (ppm)	**0**	**25**	**250**	**2500**	**0**	**25**	**250**	**2500**
Liver								
Adenoma (b)	2/45 4 %	5/48 10 %	5/46 11 %	6/46 13 %	0/46	0/38	1/48 2 %	0/46
Carcinoma (m)	2/45 4 %	1/48 2 %	0/46	0/46	0/46	0/38	0/48	0/46
Adenoma and carcinoma combined	4/45 9 %	6/48 13 %	5/45 11 %	6/46 13 %	0/46	0/38	1/48 2 %	0/46
Mammary gland								
Adenocarcinoma (m)	-	-	-	-	2/39 5 %	1/32 3 %	1/44 2 %	6/39* 15 %
Anaplastic carcinoma (m)	-	-	-	-	0/39	1/32 3 %	0/44	0/39
Fem**ur**, Osteosarcoma (m)	0/45	0/47	0/47	0/46	0/46	0/38	0/47	1/46 2 %
Ovaries, Luteoma								
unilateral (b)	-	-	-	-	3/45	0/37	2/46	7/44*
bilateral (b)	-	-	-	-	0/45	1/37	0/46	0/44
Sum of uni and bilateral					3/45 7 %	1/37 3 %	2/46 4 %	7/44* 16 %

b=benign; m= malignant; * statistically significant trend $p \leq 0.05$ (Peto trend test); statistically evaluation by study director

No relevant HCD available.

There is one epidemiological study (performed in Swedish railroad workers) available, in which diuron was included. From this data (Axelson et al. 1974) followed by an update (Axelson et al. 1980), it cannot be concluded what the actual contribution of diuron (or any other compound) to the reported outcomes as increased tumour morbidity and mortality might have been.

No further long-term studies in rodents with diuron have been published. However, there is a number of investigations on mechanisms and human relevance of carcinogenic effects, in particular with regard to bladder tumours in rats.

Table 24: Summary table of other studies relevant for carcinogenicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
26 weeks toxicity study Non-guideline Oral (dietary) GLP	diuron (98.8 % pure)	Rat, Wister (BOR:WISW) 10 M / dose Dose: 0, 2500 ppm (equivalent to: ~ 0, 200 mg/kg bw) Administration in diet for 2, 4, 12 and 26 weeks with recovery	Enlarged/swollen spleens associated with a red-black colouration Increase in the incidence and severity of urothelial hyperplasia (after minimum 4 weeks exposure); metaplasia in urinary bladder of some animals	Anonymous 15, 1987
20 weeks toxicity study Oral (dietary) Not GLP Publication	diuron (97 % pure)	Rat, Wistar 15 M / dose Dose: 0, 60, 125, 500, 1250 and 2500 ppm (equivalent to: ~ 0, 4.9, 10.1, 40.5, 101, 203 mg/kg bw) Exposure: 20 weeks	Significant reductions in mean body weight and body weight gain at 203 mg/kg bw Significantly increased mean absolute and relative spleen weights at 101 & 203 mg/kg bw Significantly increased incidences of urinary bladder urothelial simple hyperplasia at 101 and 203 mg/kg bw compared to the other groups Dose–response influence on the rat urothelium of orally administrated diuron for 20-weeks, NOEL of 10.1 mg/kg bw, LOAEL of 40.5 mg/kg bw, 101 mg/kg bw ppm as effective as the 203 mg/kg bw ppm dose to induce necrotic and proliferative lesions	Cardoso et al., 2013
Toxicity study Oral (dietary) Not GLP Publication	diuron (97 % pure)	Rat, Wistar 10 M / dose (15, 30 weeks), 12 M / dose (25 weeks) Dose: 0 and 2500 ppm (~ 0, 135 mg/kg/day) for 15, 25 & 30 weeks and 2500 ppm for 15 weeks followed by another 15 weeks basal diet Positive control for urine acidification: 1000 ppm (~ 540 mg/kg/day) ammonium chloride (NH_4Cl)	Significantly increased incidences of simple hyperplasia in the urinary bladders at 135 mg/kg/day after 15, 25 and 30 weeks Precipitates and magnesium ammonium phosphate crystals were present in the urine of diuron-treated rats comparable to control animals Coadministration with NH_4Cl decreased urinary pH, accompanied by marked reduction of crystals and precipitates in the urine, but did not affect the incidence or severity of the urothelial lesions induced by diuron Data suggest that urinary solids do not play a role in the MOA of diuron in the development of the cytotoxicity or putative preneoplastic hyperplastic urothelial lesions	Da Rocha et al., 2010
Toxicity study Oral (dietary) Not GLP	diuron (98.6 % pure)	Rat, Wistar 10 M / dose (1, 3, 7 & 28 days), 3-5 M / dose (1 &	Absolute and relative bladder weights were increased at 295 mg/kg/day (statistically significant on study days 7 and 28)	Da Rocha et al., 2012

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Publication		8 weeks) Dose: 0 and 2500 ppm (~ 0, 295 mg/kg/day)	In urinary bladder: swollen superficial cells at 1 day after the start of treatment, clear evidence of necrosis and cell loss (exfoliation) by day 7, evidence of increased proliferation by 28 days	
Toxicity study Oral (dietary) Not GLP Publication	diuron (98.6 % pure)	Rat, Wistar 5 M / dose (8 weeks) Dose: 0 and 2500 ppm (~ 0, 295 mg/kg/day) urinary concentrations of diuron and its metabolites DCPU, 2-OH-DCPU, DCPMU and DCA were determined by HPLC/MS *in vitro*- experiments: DCPU, 2-OH-DCPU, DCPMU and DCA were tested for their relative cytotoxicity to rat (MYP3) and human urothelial cells (1T1)	*In vivo* findings in the urinary bladder were reported in da Rocha et al., 2012 (see above) Authors reported, that there was no differences in the urine volume or creatinine levels Concentrations of the metabolites in 24-h urine: DCPU (> 488 µM) > 2-OH-DCPU (125 µM) > DCA (35 µM) > DCPMU (13µM); unchanged diuron (54 µM) IC_{50} relative potency of diuron metabolites cytotoxicity to MYP3 rat cells lines was: DCPMU > DCPU > DCA > 2-OH-DCPU (IC_{50}s of 104, 185, 213 and 230 µM); IC_{50} 1T1 human cells line was: DCA > DCPU > DCPMU > 2-OH-DCPU (IC_{50}s of 72, 157, 224 and 329 µM)	Da Rocha et al., 2013
mammary two-stage carcinogenesis model in rat Not GLP Publication	diuron (> 98. % pure)	Rat, Sprague-Dawley, adult ovary-intact non-initiated controls: 10F non-initiated Diuron treated (161 mg/kg bw/d): 15 F initiated groups: 18 F / dose Dose: 0, 250, 1250 and 2500 ppm Diuron; corresponding to 0, 16.2, 81.4 and 160 mg/kg bw/d Exposure: 25 weeks chemical carcinogen initiator: 50 mg/kg bw 7,12-dimethyl-benz(a)anthracene (DMBA, gavage, prior to dietary diuron treatment)	Significant reduction in food consumption and body weight gain in all groups treated; significant increase in the relative liver and spleen weights In non-initiated/diuron treated (161 mg/kg) animals, no hyperplastic lesion or benign or malignant neoplasms were observed in the mammary gland. No additional increase in incidence, multiplicity or reduction in latency for mammary gland tumors in DMBA-initiated animals by Diuron treatment. For DMBA initiated animals, slight reduction in estradiol and progesterone levels in high dose animals vs. controls (21.1 vs. 29.7 µg/mL and 11.3 vs. 26.3 ng/mL) and slight increase in tumor mass (14.9 vs. 10.9 g).	Grassi et al., 2011a
mammary and urinary bladder two-stage carcinogenesis	diuron (purity not specified)	Mouse, Swiss, 4 weeks old 15 F / dose Dose: 0, 1250 and 2500 ppm, corresponding to 0, 86 and 142 mg/kg bw/d	Food consumption, body-weight gain and final body weight were decreased (not significantly); increase in relative liver and spleen weights at 142 and 160 mg/kg bw/d.	de Moura et al., 2009

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
model in mice Not GLP Publication		for initiated groups and 0 and 160 mg/kg bw/d for non-initiated groups Exposure: 13 weeks chemical carcinogen initiator: repeatedly i.g. injections during the first 6 weeks: 7,12-dimethylbenz(a)anthracene (DMBA, 5 X 1.5 mg/ mouse) and N-butyl-N-(4-hydroxy-butyl) nitrosamine (BBN, 8 X 7.5 mg/mouse)	No significant alteration in incidence or burden of hyperplastic lesions or neoplasms in the mammary gland from DMBA/BBN-initiated mice; no preneoplastic or neoplastic lesions in mammary glands from non-initiated/diuron treated (160 mg/kg bw/d) mice. Significant increase in BrdU labelling index in urothelium, the incidence of simple/nodular urothelial hyperplasia in the urinary bladder and preneoplastic urothelial lesions in both DMBA/BBN-initiated and non-initiated groups at 86 and 142 mg/kg bw/d Diuron compared to respective control groups. Two invasive transitional cell carcinomas in DMBA/BBN-initiated treated with 142 mg/kg bw/d Diuron. Non-initiated/diuron-treated mice developed hyperplasia at 160 mg/kg bw/d.	

10.9.1 Short summary and overall relevance of the provided information on carcinogenicity

In a combined chronic toxicity and carcinogenicity feeding study over two years in Wistar rats by Anonymous 14 (1985), the red blood cells and the urogenital system (i.e., bladder, kidneys, and uterus) proved the main target organs of toxicity. Haematological examinations revealed haemolytic (hyperchromic) anaemia in both sexes and at least in females at all dose levels. Effects on the spleen and bone marrow such as a higher spleen weight, spleen fibrosis, increased haemosiderin deposition and iron storage or activation of haematopoiesis were considered secondary effects and of partly regenerative nature. According to the arguments put forward by Hardisty (2006) or Everds (2006), the splenic findings were more adaptive than indicative of a clear toxic effect since, e.g., the haemosiderin storage would have resulted from increased red blood cell turnover. However, if seen at this lowest dose level, it must have been preceded by destruction of erythrocytes which is per se considered adverse.

Pre-neoplastic bladder lesions (urothelial hyperplasia) were observed in females from 17 mg/kg bw/d onwards but also in males at the top dose of 111 mg/kg bw/d. In renal pelvis, hyperplasia was common but appeared more pronounced at higher doses. In females, the highest degree of this finding was seen at the two upper dose levels but with a rather low incidence (8 %). In males, renal pelvis hyperplasia of all degrees was observed in all groups (from 6 % up to 62 %) but degree 3 was most frequently seen in the high dose group accounting for 36 %.

The highest tested dose of 111 mg/kg bw/day in males and 203 mg/kg bw/day in females proved carcinogenic. There was a strong and statistically significant increase in malignant bladder tumours, i.e., transitional epithelial carcinoma in both sexes. In addition, the incidences of (benign) transitional epithelial papilloma and of papilloma and carcinoma in the renal pelvis were increased in male rats. These tumors were seen only in the high dose group but never in the controls or in the

low or mid dose groups. For neoplasia in the renal pelvis, the number of affected animals was low (1 papilloma, 2 carcinomas) and the differences were not significant in the statistical test applied by the study author. Unfortunately, it is not clear, which statically test was used. For clarification, a Cochrane Armitage linear trend test was performed by the DS, resulting in a statistically significant trend for transitional epithelial carcinoma in the renal pelvis (Cochrane Armitage linear trend test, two-sided, p = 0.014). There was no statistically significant trend for transitional epithelial papilloma.

In females, the incidence of adenocarcinoma in the uterus of females was doubled as compared to the control, the low and mid dose groups. Follow-up statistics by the DS revealed a borderline statistically significant trend for uterus adenocarcinoma (Cochrane Armitage linear trend test, two-sided, p = 0.0712, one-sided, p = 0.0356). In addition, it was noted that squamous epithelial carcinoma of the uterus occurred only at the two upper dose levels even though only one female per group was affected. Sarcoma of the endometrium was reported in two high dose females but in no other group.

Some mechanistic information is available supporting a non-genotoxic mode of action of diuron for the induction of bladder tumors in rats:

In a 26-week feeding study (Anonymous 15 1987) (Table 24), metaplasia was found in the urinary bladder of some animals dosed 2500 ppm diuron (equivalent to ~ 200 mg/kg bw/d), that might progress to epithelial carcinoma at later time points provided that exposure would continue. According to the authors, the early appearance of urinary bladder epithelium hyperplasia, the early diameter increase of the urinary wall, the intensification of effects within a comparatively short time, and a trend towards reversibility of diuron-induced effects would have led to the conclusion that constant local irritation was the cause for the reported alterations. However, this hypothesis is not supported by any further evidence, as other organs in the urinary system such as kidneys and renal pelvis were affected as well. No urinalysis was performed which might have revealed the presence of physical sediments or pH differences that could support the local irritation theory. In fact, in a more recently published study (Da Rocha et al. 2010), precipitates and magnesium ammonium phosphate crystals were present in the urine of male Wistar rats treated with diuron (2500 ppm, equivalent to ~ 135 mg/kg bw/d) as well as in animals which were not treated and there was no significant difference between these groups. An extra group of animals was treated with 135 mg/kg bw/d diuron and ~ 540 mg/kg bw/d of NH_4Cl for 25 weeks, and there was a reduction in the urinary pH associated with a reduction in the presence of crystals and precipitates in the urine. However, this 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.

Cardoso et al. (2013) found histological and “ultrastructural” lesions (by means of electron microscopy) of bladder and kidney urothelium after feeding diuron to Wistar rats for 20 weeks at dose levels from 500 ppm (equivalent to ~ 40.5 mg/kg bw/d) onwards with hyperplasia and a higher cell proliferation index being the predominant effects. 125 ppm (equivalent to ~ 10.1 mg/kg bw/d) was the NOAEL in this study. For the occurrence of bladder carcinoma in the long-term study, a non-genotoxic mode of action with urothelial necrosis induced by direct cytotoxicity, regenerative cell proliferation and sustained urothelial hyperplasia was proposed that would increase the likelihood of neoplasia development.

Similar conclusions have been drawn by Da Rocha et al. (2012) who performed a study in which male Wistar rats received 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. The data supports the postulated mode of action for diuron-induced urinary

bladder tumors in rats, that it is based on cytotoxicity with prolonged, sustained, consequent regenerative proliferation, leading to urothelial hyperplasia and ultimately bladder tumours. In principle, 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 (Klein 1986, see also 10.8.1). Further examination of biological samples from the 20-week feeding experiment by microarray analysis (Ihlaseh et al. 2011, same animals & experimental design as described by Cardoso et al. 2013) revealed a clear difference in gene expression at the higher dose group (~ 101 und 230 mg/kg bw/d) in comparison with the lower dose group (~ 4.9, 10.1 mg/kg bw/d). The numbers of differentially expressed transcripts between each treatment group and control increased with diuron dose. In the 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. The most significant pathways associated with the low-dose group were involved in drug metabolism, lipid metabolism, and small molecule biochemistry. Transcripts that are associated with the higher level function of cell death were significantly altered for all doses with a dose-response relationship. These findings were consistent with the phenotypic histological response. The authors' conclusion is that the cellular and molecular pathways involved in chronic toxicity from diuron treatment may also be associated with a tumor response.

In the study of Da Rocha et al. (2013) the urine metabolites of diuron in rat were investigated. The metabolite with the highest concentration in the urine of male Wistar rats treated with the carcinogenic dose of diuron (~295 mg/kg/day) was DCPU (> 488 µM). DCPU was the only diuron metabolite with a urinary concentration higher than the rat urothelial cell *in vitro* IC_{50} (185 µM) indicating that it is the major contributor to the diuron-induced cytotoxic effects. The authors suspected that N-(3,4-dichlorophenyl)urea (DCPU) and, to a lesser extent, 4,5-dichloro-2-hydroxyphenyl urea (2-OH-DCPU), i.e., main urinary metabolites of diuron might be responsible for the lesions.

On balance, these additional investigations have clearly shown that diuron (and/or its metabolites) is cytotoxic to the urothelium leading to regenerative hyperplasia and ultimately bladder tumor formation. In the absence of genotoxicity *in vivo*, existence of a threshold may be assumed for this type of tumor. However, it should be noted that *in vitro* clastogenicity data was inconsistent and the in vivo micronucleus assay may not be representative for the urothelium, in particular with regard to exposure to diuron metabolites.

No mechanistic information on uterine tumors is available.

In a combined chronic toxicity and carcinogenicity study performed over two years in NMRI outbred mice (Anonymous 3 1990), the results of haematological tests and blood clinical chemistry revealed a few adverse effects at the maximum dietary concentration of 640/867 mg/kg bw like an increase in the reticulocyte count in both sexes or a lower red blood cell count in female mice. Higher mean MCV and MCH values which were also more pronounced in females suggesting a (rather weak) hyperchromic and macrocytic anaemia. In both sexes, leucocytosis was observed at the top dose level. An increase in ALAT activity and in the bilirubin concentration at the highest dose level in males indicated an effect on the liver. The latter parameter could also be linked to red blood cell toxicity although one would expect it then to occur also in females but this was apparently not the case. In contrast, haemosiderosis in spleen and liver in both sexes at the top dose level, as well as the higher spleen weight in males, were in fact most likely related to the diuron effect on red blood cells.

Liver injury in the 640/867 mg/kg bw groups became obvious not only by the rather minor clinical chemistry findings mentioned above but also by histopathological changes such as hypertrophy, increased rates of mitosis and single cell necrosis. Absolute und relative liver weights were increased (9 % and 12 %) in high dose males only.

Additional non-neoplastic histopathological findings in high dose females comprised epithelial hyperplasia in the urinary bladder, pigmentation of cortical renal tubules and fibrosis of bone marrow. Uterine horns tended to have a wider lumen.

Carcinogenic effects on diuron in mice were observed in females receiving 867 mg/kg bw/day. There was a statistically significant increased incidence of mammary gland adenocarcinoma (Peto test) and a shift in sex cord stromal tumors towards luteoma in ovaries was also observed. There are no relevant historical control data available. The increased incidence of ovarian luteoma was also statistically significant (Peto test by Anonymous 3 1990).

In the published literature, there is only little relevant information. Grassi et al. (2011a) did not find evidence of a promoting potential of diuron for mammary tumors in a two-stage carcinogenesis model in female Sprague Dawley rats which had been initiated before with 7,12-dimethylbenz(a)anthracene (DMBA). In a similar experiment in female Swiss mice, De Moura et al. (2009) identified diuron as "a promoting agent to the urinary bladder, but not for the mammary gland". However, both studies were of limited duration and my not be sensitive for mammary adenocarcinoma.

With regard to the ovarian luteoma, there is only information from a published study by Grassi et al. (2011b), reporting that dietary treatment of pregnant and nursing Sprague Dawley rats with 1250 ppm (equivalent to ~ 146 mg/kg bw) diuron from gestational day 12 to the end of lactation on postnatal day 21 caused reductions in ovary weight and the number of *corpora lutea* in their female offspring on postnatal day 75. According to the authors, this would rather point to developmental delay than to toxicity to the ovary. In the same study, there were no effects on the mammary gland.

Table 25: Compilation of factors to be taken into consideration in the hazard assessment

Species and strain	**Multi-site responses**	**Tumour type and background incidence**	**Progression of lesions to malignancy**	**Reduced tumour latency**	**Responses in single or both sexes**	**Confounding effect by excessive toxicity?**	**Route of exposure**	**MoA and relevance to humans**
Rat	Yes	Urinary bladder transitional epithelial carcinoma	Yes	Unclear	Both	Yes, local cytotoxicity and regeneration likely	Oral	Yes, but threshold possible
		Uterus adenocarinoma	Yes	Unclear	Single (F)	Unclear	Oral	Yes
		Renal pelvis	Yes	Unclear	Single (M)	Unclear	Oral	Yes
Mouse	Yes	Mammary gland adenocarinoma	Yes	Unclear	Single (F)	Unclear	Oral	Yes
		Ovary Luteoma	No	Unclear	Single (F)	Unclear	Oral	Yes

10.9.2 Comparison with the CLP criteria

Table 26 presents the CLP criteria for carcinogenicity. There is no epidemiological evidence of an increased tumor incidence in human which could be attributed to diuron exposure. Therefore, category 1A is not appropriate and, for classification and labelling of diuron for carcinogenicity, only data obtained in laboratory animals can be taken into consideration. As outlined above, diuron was not genotoxic.

Table 26: Criteria for classification of carcinogenicity

CLP criteria
A substance is classified in Category 1 (known or presumed human carcinogens) for carcinogenicity on the basis of epidemiological and/or animal data. A substance may be further distinguished as: Category 1A, known to have carcinogenic potential for humans, classification is largely based on human evidence, or Category 1B, presumed to have carcinogenic potential for humans, classification is largely based on animal evidence. The classification in Category 1A and 1B is based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived from: - human studies that establish a causal relationship between human exposure to a substance and the development of cancer (known human carcinogen); or - animal experiments for which there is sufficient (1) evidence to demonstrate animal carcinogenicity (presumed human carcinogen). In addition, on a case-by-case basis, scientific judgement may warrant a decision of presumed human carcinogenicity derived from studies showing limited evidence of carcinogenicity in humans together with limited evidence of carcinogenicity in experimental animals. The placing of a substance in Category 2 (suspected human carcinogens) is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived either from limited (1) evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies. […] 3.6.2.2.3. Strength of evidence involves the enumeration of tumours in human and animal studies and determination of their level of statistical significance. Sufficient human evidence demonstrates causality between human exposure and the development of cancer, whereas sufficient evidence in animals shows a causal relationship between the substance and an increased incidence of tumours. Limited evidence in humans is demonstrated by a positive association between exposure and cancer, but a causal relationship cannot be stated. Limited evidence in animals is provided when data suggest a carcinogenic effect, but are less than sufficient. The terms ‘sufficient’ and ‘limited’ have been used here as they have been defined by the International Agency for Research on Cancer (IARC) and read as follows: (a) Carcinogenicity in humans The evidence relevant to carcinogenicity from studies in humans is classified into one of the following categories: - sufficient evidence of carcinogenicity: a causal relationship has been established between exposure to the agent and human cancer. That is, a positive relationship has been observed between the exposure and cancer in studies in which chance, bias and confounding could be ruled out with reasonable confidence; - limited evidence of carcinogenicity: a positive association has been observed between exposure to the agent and cancer for which a causal interpretation is considered to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence. (b) Carcinogenicity in experimental animals Carcinogenicity in experimental animals can be evaluated using conventional bioassays, bioassays that employ genetically modified animals, and other in-vivo bioassays that focus on one or more of the critical stages of carcinogenesis. In the absence of data from conventional long-term bioassays or from assays with neoplasia as the end-point, consistently positive results in several models that address several stages in the multistage process of

CLP criteria
carcinogenesis should be considered in evaluating the degree of evidence of carcinogenicity in experimental animals. The evidence relevant to carcinogenicity in experimental animals is classified into one of the following categories: - sufficient evidence of carcinogenicity: a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence. A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites; - limited evidence of carcinogenicity: the data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs. 3.6.2.2.4. Additional considerations (as part of the weight of evidence approach (see 1.1.1)). Beyond the determination of the strength of evidence for carcinogenicity, a number of other factors need to be considered that influence the overall likelihood that a substance poses a carcinogenic hazard in humans. The full list of factors that influence this determination would be very lengthy, but some of the more important ones are considered here. 3.6.2.2.5. The factors can be viewed as either increasing or decreasing the level of concern for human carcinogenicity. The relative emphasis accorded to each factor depends upon the amount and coherence of evidence bearing on each. Generally there is a requirement for more complete information to decrease than to increase the level of concern. Additional considerations should be used in evaluating the tumour findings and the other factors in a case-by-case manner. 3.6.2.2.6. Some important factors which may be taken into consideration, when assessing the overall level of concern are: (a) tumour type and background incidence; (b) multi-site responses; (c) progression of lesions to malignancy; (d) reduced tumour latency; (e) whether responses are in single or both sexes; (f) whether responses are in a single species or several species; (g) structural similarity to a substance(s) for which there is good evidence of carcinogenicity; (h) routes of exposure; (i) comparison of absorption, distribution, metabolism and excretion between test animals and humans; (j) the possibility of a confounding effect of excessive toxicity at test doses; (k) mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity. Mutagenicity: it is recognised that genetic events are central in the overall process of cancer development. Therefore evidence of mutagenic activity in vivo may indicate that a substance has a potential for carcinogenic effects.

In two reliable long-term studies in rodents, a statistically significant increase in tumor frequency was observed in five different organs in two species. In four of these organs, the tumors of concern were malignant. Thus, there is a two-species, multi-site response with progression to malignancy. In the rat, both sexes were affected whereas in mice, due to the nature of the tumors, only effects in females were seen. The increase in all five tumor types was confined in both studies to the highest dose levels which were, however, clearly below a dose of 1000 mg/kg bw/day.

In the past, carcinogenicity of diuron was established on the basis of bladder tumors resulting in its current classification as Carc. 2. According to the available information, uterine adenocarcinomas in rats and luteoma in mouse ovaries had not been taken into consideration during the previous

assessment of carcinogenicity whereas the finding of mammary gland adenocarcinoma in rats had not been influential. For re-evaluation, however, all five tumor types should be taken into account.

There are studies available, providing mechanistic information on bladder tumors in rats. Information on mammary carcinogenesis in mice is scarce and, in principle, no efforts have been taken to address the uterine adenocarcinoma in rats and the benign ovarian tumors in mice. The latter three tumor types might suggest endocrine-mediated modes of action.

Even though the mechanistic data for bladder tumors and the occurrence of all tumors only at high dose levels suggest dose-dependency and, on balance, a low risk for man due to much lower exposure, there is no information that would exclude human relevance. None of the observed tumor types belongs to a category of rodent tumors which may be considered non-relevant to man.

10.9.3 Conclusion on classification and labelling for carcinogenicity

Based on the criteria given in the 2017 "Guidance on the Application of the CLP Criteria, Version 5.0", there is sufficient evidence of carcinogenicity of diuron in animals. A causal relationship has been established for bladder tumors in rats and cannot be reasonably excluded for uterine adenocarcinoma in rats and for mammary gland adenocarcinoma as well as for (benign) luteoma in mice. Malignant tumors of different types were observed in two independent studies in two species. Following is stated in the guidance: "*Thus, if a substance causes tumours at multiple sites and/or in more than one species then this usually provides evidence of carcinogenicity. Typically such a tumour profile would lead to classification in category 1B*."

Because of these considerations, classification of diuron as a carcinogen of the Category 1B (H350) is proposed.

10.10 Reproductive toxicity

This endpoint is not addressed in this CLH report.

10.11 Specific target organ toxicity-single exposure

This endpoint is not addressed in this CLH report.

10.12 Specific target organ toxicity-repeated exposure

There is a current classification and labelling of diuron for specific target organ toxicity (STOT RE 2, H373). This is based on "translation" from previous classification as in Annex I of Directive 67/548/EEC with the risk phrase "Harmful" (Xn, R48/22). It should be noticed that in the 2005 EFSA Conclusion on diuron, in addition, R48/23 had been proposed to indicate a hazard also of inhalative exposure. Hence, the specific target organ toxicity after repeated exposure of diuron is addressed in the present CLH dossier.

Several studies on short-term toxicity of diuron in rats, dogs and mice are available which are summarised in Table 27.

Table 27: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
Oral subacute study Rat, Sprague-Dawley 10 M / dose No Guideline No GLP	diuron (purity not reported) in corn oil (5 ml/kg) Oral (gavage) Dose: 0, 125 and 250 mg/kg bw Exposure: 4 weeks	No apparent effects or mortalities; statistical significant dose-response increase in liver, testes and spleen weight at 250 mg/kg bw; statistical significant decrease in the total red blood cells count, increased mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) at both diuron doses. Degeneration and necrosis of both liver and kidneys parenchymal cells were associated with high levels of lipid peroxides and low levels of antioxidants, indicating the systemic and target-organ toxicity of diuron. NOAEL: 250 mg/kg bw/day in male rats based on effects indicating macrocytic hypochromic anaemia. LOAEL: -	Yahia et al. 2012[#]
Oral subacute study Rat, Albino 10 F / dose No Guideline No GLP	diuron (technical grade) Oral (dietary) Dose: 0, 35 and 70 ppm (equivalent to: ~ 4.1 and 8.2 mg/kg bw)Exposure: 30 days	Significant and dose-related increase in absolute and relative liver weight at both diuron doses. Significant and dose-related increase in activity of GGT, GOT and GPT in both liver and serum samples. Results indicate diuron hepatotoxicity. NOAEL: 8.2 mg/kg bw/day in female rats LOAEL: -	Anthony et al. 1990[#]
90-day feeding study	diuron (99.5 % pure)	Decreased body weight, body weight gain and food consumption; depression in red blood cell count and	Anonymous 10, 2004

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
Rat, Wistar 20/sex/dose OECD 408 GLP No deviations	Oral (dietary) Dose: 0, 100, 250 and 2500 ppm equivalent to: Males: 0, 6.7, 17.0 and 176 mg/kg bw Females: 0, 8.7, 23.3 and 214 mg/kg bw (mean daily doses) Exposure: 90 days, recovery period of 90 days (10/sex/dose)	haemoglobin, increased sulfhaemoglobin and reticulocytes, increased bilirubin, increased extramedullary haematopoiesis in spleen; spleen weight and relative liver weight increased; hyperplasia of transitional epithelium in kidneys and urinary bladder; effects partly reversible NOAEL: 6.7 mg/kg bw/day in males, NOAEL could not be established in females LOAEL: 17.0 mg/kg bw/day in males, 8.7 mg/kg bw/day in females, based on spleen findings (higher organ weight, extramedullary haematopoiesis, congestion, pigmentation) and haematological changes including sulfhaemoglobin formation, lower erythrocyte count, decreased haemoglobin, or higher reticulocyte count. (Details are given below) *LOAEL in M ~ STOT RE 2 (> 10 mg/kg/d, ≤ 100 mg/kg/d)* *LOAEL in F ~ STOT RE 1 (≤ 10 mg/kg/*d)	
6-month feedings study with special attention to blood effects Rat, Wistar 10/sex/dose No guideline GLP	diuron (98.8 % pure) Oral (dietary) Dose: 0, 4, 10 and 25 ppm equivalent to: Males: 0, 0.3, 0.7 and 1.6 mg/kg bw Females: 0, 0.3, 0.8 and 1.8 mg/kg bw Exposure: 26 weeks	Decrease in haemoglobin, increase in reticulocytes at 1.8 mg/kg bw/d in females and (transiently only) in males at 1.6 mg/kg bw/d; increase in ferrous pigmentation of the spleen in both sexes. NOAEL: 0.7 / 0.8 mg/kg bw in males/females LOAEL: 1.6 / 1.8 mg/kg bw in males/females *LOAEL ~ STOT RE 1 (≤ 5 mg/kg/*d)	Anonymous 15, 1986
6-month feedings study with special attention to urothelial lesions Rat, Wistar 10 M / dose No guideline GLP	diuron (98.8 % pure) Oral (dietary) Dose: 0, 2500 ppm (equivalent to: ~ 200 mg/kg bw) Exposure: 2, 4, 12 and 26 weeks with recovery (4 + 4, 26 + 8)	Enlarged/swollen spleens associated with a red-black colouration Increase in the incidence and severity of urothelial hyperplasia (after minimum 4 weeks exposure); metaplasia in urinary bladder of some animals (after 26 weeks) at approx. 200 mg/kg bw/d NOAEL / LOAEL: not derived	Anonymous 15, 1987
6-month feedings study Mouse, NMRI 10/sex/dose	diuron (98.5 % pure) dissolved in 1 % groundnut oil Oral (dietary)	No consistent effects up to top dose Reduced haemoglobin in males after 4 weeks at 78.5 mg/kg bw/d (MCH ↓ by 4.2 %, MCHC ↓ by 6.4 %), but increased at low (MCH ↑ by 4.1 %) and mid dose (MCH ↑ by 4.1 % and 3.0 %), in females at 100 mg/kg bw/d reduced MCH (by	Anonymous 2, 1988

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
No guideline GLP	Dose: 0, 5, 25 and 250 ppm equivalent to: Males: 0, 1.6, 7.5, and 78.5 mg/kg bw Females: 0, 2.0, 10, and 100 mg/kg bw (mean daily doses) Exposure: 1, 3 and 6 months	2.3 %); no consistent dose-related changes after 3 or 6 months of feeding; effect was transient and/or compensated Increased erythrocyte count (↑ by 5.3 – 6.4 %), haemoglobin concentration (↑ by 6 %) and haematocrit (↑ by 5.3 – 6.9 %) in female after 4 weeks with no clear dose-related response; no pathological in the haematology parameters after 3 or 6 months NOAEL: 78.5 / 100 mg/kg bw/day in males / females LOAEL: -	
1-year feeding study Dog, Beagle (Bor:Beag) 6/sex/dose No guideline indicated GLP	diuron (98.2 – 98.5 % pure) Oral (dietary) Dose: 0, 50, 300 and 1800 ppm equivalent to: 0, 1.8, 11 and 64 mg/kg bw (males and females combined) Exposure: 1 year	Slightly reduced food intake (↓ by 4 – 6 % in M, 8 – 16 % in F) and lower body weight (↓ by 6 % in M, 9 % in F at high dose); decreased erythrocyte counts and haemoglobin, increased MCV, Heinz bodies, reticulocytes, leucocytes and thrombocytes (details are given below), reactive fat-deficient bone marrow with siderin content, iron-containing pigment in liver (11 of 12 dogs at high dose), spleen (8 dogs at high dose, 9 at mid dose) and kidney (10 at high dose, 6 at mid dose). Results indicate diuron induces hypochromic anaemia in dogs. NOAEL: 1.8 mg/kg bw/day in males & females LOAEL: 11 mg/kg bw/day in males & females, based on alterations in haematological parameters	Anonymous 8, 1985
3-week inhalation toxicity study Rat, Wistar 10/sex/dose Based on OECD 412 GLP	diuron (98.9 % pure) in a 1:1 mixture of poly-ethylene glycol E 400 and ethanol Inhalation, head/nose exposure Dose: 0, 6.6, 47.6 and 311 mg/m^3 Exposure: 6 hours per day, 5 days per week, 3 weeks	NOAEL: 6.6 mg Diuron aerosol /m³ corresponding to 1.8 mg/kg bw/day in males & females LOAEL: 47.6 mg Diuron aerosol /m³ corresponding to 14 mg/kg bw/day in males & females, due to significantly decreased red blood cell parameters and increased reticulocytes and Heinz' body formation in both mid and high dose females and in high dose males (details given below) and enlarged and congested spleens in mid and high dose males and females	Anonymous 12, 1986a
Subacute inhalation toxicity study Rat, Wistar 5/sex/dose Based on OECD 412	diuron (98.4 % pure) in a 1:1 mixture of poly-ethylene glycol E 400 and ethanol Inhalation, head/nose exposure	NOAEL: 4.1 mg Diuron aerosol /m³ corresponding to 1.1 mg/kg bw/day in females and 37.4 mg Diuron aerosol /m³ corresponding to 11 mg/kg bw/day in male rats LOAEL: 37.4 mg/m³ corresponding to 11 mg/kg bw/day in females based on significantly increased levels of reticulocytes and Heinz' bodies and dark and enlarged spleens; 268.1 mg/m³ corresponding to 72 mg/kg bw/day in male rats, due to significant changes in many haematological parameters	Anonymous 12, 1986b

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
GLP	Dose: 0, 4.1, 37.4 and 286.1 mg/m^3 Exposure: 6 hours per day, 5 days per week, 4 or 8 weeks	and dark, enlarged spleens in the high dose group for both sexes both after 4 weeks and after 8 weeks exposure. Details are given below *LOAEL ~ STOT RE 1 (≤ 0.06 mg/l/6h/d (~60 mg/m³/6h/d)*	
3-week dermal toxicity study Rabbit, New Zealand White 5/sex/dose OECD 410 GLP No deviation	diuron (96.8 % pure) in deionised water Dermal, occlusive Dose: 0, 50, 500 and 1200 mg/kg bw Exposure: 6 hours per day, 5 days per week, 3 weeks	No significant effects of dosing with respect to clinical observations, body weights or body weight gains, food efficiency, gross pathology or histopathology, absolute or relative organ weights, haematology, including white blood cell differential count, or any clinical chemistry parameter. NOAEL: 1200 mg/kg bw/day in males & females LOAEL: - *No classification for STOT RE*	Anonymous 11, 1992
90-day dermal toxicity study Rat, Sprague-Dawley 12/sex/dose OECD 411 GLP Deviation: Application only 5 days per week	diuron (98.5 % pure) in cottonseed oil Dermal, occlusive Dose: 0, 250, 500 and 1000 mg/kg bw Exposure: 6 hours per day, 5 days per week, 13 weeks	Decreased erythrocyte counts (↓ by 13 – 16 % in M, 22 – 24 % in F), haemoglobin (↓ by 7.8 – 9.9 % in M, 14 – 16 % in F), increased mean (red blood) cell volume (MCV, ↑ by 5.8 – 9.1 % in M, 13. – 16 % in F); statistical significance was not reported NOAEL: - LOAEL: 250 mg/kg bw/day in males & females since haematological effects suggesting anaemia were seen at all dose levels In the study report itself it was claimed that no effects had been observed up to the highest dose level of 1000 mg/kg bw/day. *LOAEL ~ STOT RE 2 (> 200 mg/kg/d, ≤ 600 mg/kg/*d)	Anonymous 17, 1996*

[#] study is considered supplementary since methods and clinical signs were not reported extensively, as well as only one gender was exposed to only two doses of diuron
* study is considered supplementary due to deviation from current guidelines

No human data on specific target organ toxicity after repeated exposure of diuron were available for evaluation.

10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

Main targets of diuron were the red blood cells and the excretory organs kidney and bladder. Rats appeared more vulnerable than dogs and, with regard to haematological effects (haemolytic anaemia), much more sensitive than mice.

In the only valid 90-day feeding study in Wistar rats (Anonymous 10 2004), red blood cells, the urogenital tract, the spleen, bone marrow and liver were identified the target organs, along with an high dose effect on body weight and body weight gain, food consumption and efficiency. Most pathological organ findings as well as the increase in bilirubin at the top dose level are indicative of anaemia. Whereas the lowest dose of 100 ppm may be considered the NOAEL for males (equivalent to a mean daily intake of 6.7 mg/kg bw), there were still treatment-related adverse findings in females at the same dietary concentration. This LOAEL (corresponding to 8.7 mg/kg bw/day) is based on spleen findings (higher organ weight, extramedullary haematopoiesis, congestion and pigmentation) and haematological changes including sulfhaemoglobin formation, lower erythrocyte count, decreased haemoglobin, or higher reticulocyte count. In Table 28 the mean organ weights at the 90-day sacrifice are given. A dose dependent significant increase in absolute and relative spleen weights can be seen in females, as well as significant increase in absolute and relative spleen weights and relative liver weights for high dose male rats. No effects on the weights of other organs were noted.

Table 28: Mean organ weights at the 90-day sacrifice (Anonymous 10 2004)

Dose (mg/kg bw)	Males				Females			
	0	**6.7**	**17.0**	**176**	**0**	**8.7**	**23.3**	**214**
Number of animals	10	10	10	10	10	10	10	10
Absolute liver weight (g)	11.841	12.340	11.952	12.255	6.999	6.791	7.434	7.388
Relative liver to body weights (%)	2.663	2.572	2.675	3.092^{+}	2.832	2.872	2.972	3.315^{+}
Absolute spleen weights (g)	0.851	0.958	0.978	1.686^{+}	0.555	0.675^{+}	0.752^{+}	1.563^{+}
Relative spleen to body weights (%)	0.192	0.201	0.219	0.424^{+}	0.226	0.290^{+}	0.300^{+}	0.702^{+}

$^{+}$ Significant differences between control and treated groups ($p < 0.05$), calculated by Dunn's test or by the Jonckheere-Terpstra trend test

In Table 29 the results of haematological examinations are given. Generally, a decrease in red blood cell parameters in a dose-related manner is shown. All the haematological findings proved reversible during the recovery period although there were still very few significant differences left, mostly on days 111/112 but with a tendency to weaken and eventually to disappear.

Table 29: Haematological examinations – means after ~ 45, 90, 110 and 145 days (Anonymous 10 2004)

Dose (mg/kg bw)	Days	Males				Females			
		0	**6.7**	**17.0**	**176**	**0**	**8.7**	**23.3**	**214**
White blood cells (x 10^3/µL)	47/48	12.08	11.62	12.30	14.10	8.27	9.31	9.55	11.13$^+$
	92/93	**9.78**	**1043**	**9.60**	**14.16$^+$**	**7.59**	**7.66**	**7.71**	**9.62**
	111/112	10.20	8.91	1.65	11.44	6.84	6.91	6.19	6.64
	145/146	11.40	9.96	1.87	11.26	7.98	8.82	8.28	9.30
Red blood cells (x 10^6/µL)	47/48	8.42	8.14	7.89$^+$	7.20$^+$	8.40	8.05	7.56$^+$	6.71$^+$
	92/93	**8.72**	**8.63**	**8.36**	**7.36$^+$**	**8.47**	**7.98$^+$**	**7.51$^+$**	**6.57$^+$**
	111/112	8.82	8.61	8.50	8.34$^+$	8.50	8.58	8.43	8.34
	145/146	8.95	9.09	8.75	9.17	8.75	9.05	8.72	8.78
Haemoglobin conc. (g/dL)	47/48	15.1	14.7	14.4	14.1$^+$	15.5	15.4	14.4$^+$	14.1$^+$
	92/93	**15.3**	**15.2**	**14.9**	**14.1$^+$**	**16.0**	**15.2$^+$**	**14.2$^+$**	**13.6$^+$**
	111/112	15.1	14.4$^+$	14.6	15.1	15.2	15.3	15.5	16.6$^+$
	145/146	16.1	15.9	15.6	16.1	16.5	16.6	16.3	16.3

Dose (mg/kg bw)	Days	Males				Females			
		0	**6.7**	**17.0**	**176**	**0**	**8.7**	**23.3**	**214**
Haematocrit (%)	47/48	47.1	46.5	46.5	46.2	47.7	47.8	45.4^{+}	45.4^{+}
	92/93	**48.5**	**48.4**	**47.6**	**46.1^{+}**	**48.5**	**47.1**	**45.1^{+}**	**44.5^{+}**
	111/112	48.4	46.6	47.2	49.1	47.9	48.4	49.2	52.0^{+}
	145/146	48.8	48.8	47.8	49.3	50.2	50.8	49.3	50.0
Reticulocyte count (x 10^3/µL)	47/48	162.6	223.1	258.8^{+}	455.3^{+}	226.9	241.3	284.7	651.3^{+}
	92/93	**131.3**	**153.3^{+}**	**206.7^{+}**	**451.4^{+}**	**152.2**	**216.3^{+}**	**366.7^{+}**	**686.1^{+}**
	111/112	174.4	159.4	157.8	142.7	182.7	160.1	151.2	75.0^{+}
	145/146	154.8	163.8	153.5	170.5	154.3	178.9	188.7	251.4^{+}
Mean cell volume (fl)	47/48	56.0	57.2	58.9^{+}	64.4^{+}	56.8	59.3+	60.1+	67.7^{+}
	92/93	**55.6**	**56.1**	**57.0**	**62.6^{+}**	**57.4**	**59.0**	**60.1^{+}**	**67.8^{+}**
	111/112	54.9	54.2	55.5	58.9^{+}	56.4	56.5	58.4	62.3^{+}
	145/146	54.6	53.8	54.6	53.8	57.6	56.1	56.6	56.9
Mean corpusc. haemoglobin (pg)	47/48	17.9	18.1	18.3	19.6^{+}	18.5	19.1	19.1	21.0^{+}
	92/93	**17.5**	**17.6**	**17.9**	**19.1^{+}**	**18.9**	**19.1**	**19.0**	**20.8^{+}**
	111/112	17.1	16.8	17.2	18.1^{+}	17.9	17.9	18.5	19.9^{+}
	145/146	18.0	17.6	17.8	17.6	18.9	18.4	18.7	18.6
Mean corpuscular haemoglobin conc. (g/dL)	47/48	32.0	31.7	31.1^{+}	30.5^{+}	32.6	32.2	31.7^{+}	31.1^{+}
	92/93	**31.5**	**31.3**	**31.4**	**30.6^{+}**	**32.9**	**32.3**	**31.5^{+}**	**30.6^{+}**
	111/112	31.2	31.0	31.0	30.7	31.8	31.7	31.6	31.9
	145/146	32.9	32.6	32.6	32.8	32.9	32.7	33.0	32.6
Red blood cell distribution width (%)	47/48	12.2	12.6	13.2^{+}	12.8	11.8	11.6	11.5	13.5+
	92/93	**12.0**	**11.8**	**12.2**	**12.6^{+}**	**10.7**	**10.9**	**11.9+**	**13.8+**
	111/112	12.2	11.9	11.8	11.1^{+}	11.3	10.7	11.2	11.1
	145/146	12.1	12.1	11.8	11.6	11.3	10.7	11.4	11.7^{+}
Methaemoglobin (%)	57/58	0.6	0.6	0.5	0.8^{+}	0.6	0.7	0.7	0.9^{+}
	92/93	**0.5**	**0.5**	**0.6**	**0.8^{+}**	**0.5**	**0.6**	**0.7^{+}**	**0.8^{+}**
	111/112	0.5	0.6	0.6	0.6	0.5	0.6	0.6	0.5
	145/146	0.6	0.6	0.6	0.5	0.6	0.6	0.6	0.6
Sulfhaemoglobin(mmol/L)	57/58	0.000	0.000	0.005^{+}	0.050^{+}	0.000	0.011	0.024^{+}	0.057^{+}
	92/93	**0.000**	**0.001**	**0.008^{+}**	**0.051^{+}**	**0.000**	**0.011**	**0.023^{+}**	**0.056^{+}**
	111/112	0.000	0.000	0.000	0.005^{+}	0.000	0.000	0.002	0.004^{+}
	145/146	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Animals in which sulf-haemoglobin was detected	**92/93**	**0/9**	**4/9**	**9/9**	**10/10**	**0/9**	**7/7**	**8/8**	**9/9**
PCE/NCE ratio	**92/93**	**1.314**	**1.61**	**1.189**	**3.043^{+}**	**1.712**	**2.118**	**3.211**	**3.391**
	183/184	1.003	1.155	1.756	1.236	0.828	0.892	1.398	1.183

$^{+}$Significant differences between control and treated groups ($p < 0.05$), calculated by Dunnett's or Dunn's test

The male NOAEL and the failure to establish the NOAEL in females, as well as the findings themselves, confirmed what was seen in a previous long-term study in Wistar rats (see sub-section 2.6.5 below). In addition, there were histopathological lesions (mucosal hyperplasia) in the kidneys and in the urinary bladder but these findings were of minor degree and were, in both sexes, confined to the upper two dose levels. Most but not all of the toxic effects proved to be reversible during the 3-month recovery period. Similar effects were already observed in published oral 28-day studies of limited relevance and reliability (Yahia et al. 2012, Antony et al. 1990).

In a special study to investigate the toxicity of diuron to the kidneys and the bladder, dietary administration of 2500 ppm diuron to male rats for 2, 4, 8, 12, or 26 weeks did not cause mortality but growth was retarded (Anonymous 15 1987). Enlarged/swollen spleens were found associated with a red-black colouration. After a minimum of 4 weeks administration, diuron caused an increase in the incidence and severity of urothelial hyperplasia, the incidence and severity of which tended to increase with longer duration of diuron administration. After 26 weeks, metaplasia was found in the urinary bladder of some animals which is expected to progress to epithelial carcinomas at later time points provided that exposure would continue. Thus, these findings are in line with those obtained in the long-term study with diuron in rats (see sub-section 10.9) and may be considered to precede them. There were indications of a regression of these effects following the cessation of diuron administration, however, recovery was by far not complete and in particular strong lesions apparently did not heal. The kidney was also confirmed as a target organ of diuron. After 26 weeks of treatment, the cells in the proximal renal tubules exhibited an increase in finely granulated brownish pigments, thought to be lipofuscin.

Another six-month feeding study in Wistar rats was performed by Anonymous 15 (1986) in which the focus was laid on the occurrence mainly of haematological effects at rather low doses. At the maximum dose of 25 ppm, diuron reduced the haemoglobin concentration and increased the number of reticulocytes in Wistar rats. In line with that, there was also an increased amount of iron in the spleens at that dose whereas the original finding of an increase in extramedullary haematopoiesis was doubted in a re-evaluation (Hardisty 2006). The incidences of gross morphological findings in the urinary bladder were also greater in treated groups but no clear dose-response was discernible and these findings were not corroborated by histopathological examination. The next lower dose of 10 ppm (equivalent to mean daily doses of 0.7 or 0.8 mg/kg bw in males or females, respectively) was considered the NOAEL in both sexes. Even though the argument of Hardisty (2006) that the splenic findings would rather reflect the increased red blood cell turnover is reasonable, the haematological findings themselves are considered adverse by the DS.

There is only one study available in mice. It was apparently performed to compare differences in species sensitivity to haematological effects of diuron. NMRI mice fed diuron at 5, 25 or 250 ppm for one, 3 or 6 months did not exhibit any effects (Anonymous 2 1988). The only exception might have been the detection of lower MCH and MCHC values in high dose males after 4 weeks. However, this effect was transient and/or compensated and cannot be considered adverse. Thus, the maximum dose of 250 ppm (corresponding to 78.5 mg/kg bw/day in males and 100 mg/kg bw/day in females) was considered the NOAEL proving that the mouse is much less sensitive than the rat, mainly with regard to haematological effects but also to organ toxicity.

The main finding in the one-year feeding study in Beagle dogs by Anonymous 8 (1985) was hypochromic anaemia as proven by alterations in haematological parameters (details are given in Table 30). Most of the clinical chemistry and pathological findings (affecting mainly the liver, the spleen, and the bone marrow) were assumed to reflect this process. In both sexes, the NOAEL was 50 ppm, corresponding to 1.8 mg/kg bw/day.

Table 30: Haematological examinations in dogs, mean values (sexes combined) (Anonymous 8, 1985)

Parameter	weeks	Dose (mg/kg bw)			
		0	1.8	11	64
Erythrocytes (x 10^{12}/L)	-2	6.203	6.083	6.229	6.340
	6	6.379	6.118	5.957^{+}	5.490^{++}
	13	6.374	5.915	5.863^{++}	5.522^{++}
	26	6.810	6.677	6.477	5.765^{++}
	39	6.741	6.572	6.270	5.663^{++}
	52	6.777	6.745	6.636	5.938^{++}
Haemoglobin (g/L)	-2	139.3	137.8	141.0	147.0
	6	149.2	142.8	139.6	129.9^{+}
	13	148.4	139.6	139.3^{+}	132.5^{+}
	26	153.4	149.6	144.9	132.3^{++}
	39	158.7	152.2	150.3	137.8^{++}
	52	160.3	160.2	158.1	142.6^{++}
Haematocrit (L/L)	-2	0.4393	0.4397	0.4472	0.4608
	6	0.4482	0.4276	0.4254	0.4177
	13	0.4880	0.4555	0.4627	0.4550
	26	0.4865	0.4795	0.4745	0.4417
	39	0.4859	0.4748	0.4636	0.4387^{+}
	52	0.4558	0.4559	0.4551	0.4257
MCV (fL)	-2	71.7	71.8	71.5	72.5
	6	69.3	69.0	70.3	74.8^{++}
	13	75.3	75.8	77.4^{+}	80.8^{++}
	26	71.7	71.8	73.3^{+}	76.5^{++}
	39	72.3	72.6	74.2^{+}	77.5^{++}
	52	67.9	68.3	69.2	72.3^{++}
MCH (pg)	-2	22.86	22.73	22.67	23.22
	6	23.08	23.01	23.12	23.32
	13	22.97	23.26	23.41	23.65
	26	22.57	22.34	22.41	22.95
	39	23.37	22.98	23.72	24.09
	52	23.70	23.79	23.80	24.01
MCHC (g/L erythrocytes)	-2	315.3	312.3	314.3	317.3
	6	331.9	331.5	327.1^{+}	309.9^{++}
	13	302.8	306.3	299.6	289.8^{++}
	26	318.1	313.1	307.7^{++}	301.8^{++}
	39	324.8	318.7	322.0	312.2^{++}
	52	350.3	350.2	345.9^{+}	333.8^{++}
Thrombocytes (x 10^{9}/L)	-2	297.5	288.8	297.3	295.6
	6	238.2	240.2	298.3^{++}	480.7^{++}
	13	257.8	265.6	315.5^{++}	455.7^{++}
	26	264.4	280.3	312.8^{+}	419.8^{++}
	39	261.2	262.0	323.8^{++}	425.0^{++}
	52	237.8	229.8	268.3	359.4^{++}
Leucocytes (x 10^{9}/L)	-2	16.01	16.02	14.76	16.08
	6	11.63	12.46	10.31	13.67
	13	13.47	13.70	11.99	16.64
	26	13.44	14.12	13.67	16.49+

Parameter	weeks	Dose (mg/kg bw)			
		0	1.8	11	64
	39	13.74	13.00	11.68	16.77
	52	13.76	13.86	12.10	17.35++
Reticulocytes (‰)	-2	7.1	7.8	6.6	7.0
	6	4.7	4.9	6.8	40.9++
	13	7.2	5.4	8.0	47.2++
	26	4.6	4.9	6.3	44.3++
	39	7.7	5.2	7.1	37.8++
	52	10.4	6.9	10.0	38.3++
Heinz' bodies (‰)	-2	-	-	-	-
	6	-	-	-	-
	13	1.3	1.4	3.7	204.2++
	26	0.5	0.9	1.9	203.8++
	39	1.4	1.3	4.2	409.2++
	52	2.8	2.2	4.9	427.4++

+ $p \leq 0.05$; ++ $p \leq 0.01$

Subacute inhalative toxicity of diuron in rats was tested in two subacute studies by Anonymous 12 (1986a, 1986b) which differed with regard to exposure duration and the analytically determined concentrations. In both studies, the animals were exposed (head/nose only) to aerosols of the test substance and exhibited significant changes in many haematological parameters including increased levels of reticulocytes and Heinz' bodies and dark, enlarged spleens. In the first study with a duration of 3 weeks, these effects were seen in both sexes at the intermediate and high concentrations resulting in a NOAEC of 6.6 mg/m³. A systemic intake of 1.8 mg/kg bw/day was calculated to correspond to this concentration. Results of the haematological examinations are given in Table 31.

Table 31: Haematological examinations in subacute inhalation toxicity study in rat (Anonymous 12, 1986a)

Measured mean concentration (mg/m³)	Males					Females				
	Air	0	6.6	47.6	311	Air	0	6.6	47.6	311
Erythrocytes TERA/l	7.55	7.58	7.94	8.07+	7.01+	7.00	7.25	7.38	6.72	6.30++
Mean corpuscular (cell) volume fl	61	61	61	60+	66++	59	60	59	61	67++
Reticulocytes ‰	22	27	22	24	65++	26	24	25	43++	94++
Heinz bodies ‰	1	2	5++	6++	151++	3	2	4	48++	298++
Mean corpuscular (cell) haemoglobin pg/E	19.3	19.3	19.1	18.8++	20.7++	18.8	19.3	18.8	19.4	21.2++

+ $p \leq 0.05$; ++ $p \leq 0.01$

In the second study with longer duration, female rats appeared a bit more sensitive since, this time, effects were seen in males only at the highest concentration but in females also at the mid dose level. Accordingly, the NOAEC for females was 4.1 mg/m³ (corresponding to a mean daily intake of 1.1 mg/kg bw/day) whereas it was higher in males (37.4 mg/m³, equivalent to 11 mg/kg bw/day).

There were no clear and consistent differences in the strength of effects after 4- or 8-week exposure in this second study. Data is presented in Table 32.

Table 32: Haematological examinations in subacute inhalation toxicity study in rat following 4/8 weeks of exposure (Anonymous 12, 1986b)

Measured mean concentration (mg/m³)	**Males (4 wk/8wk exposure)**				**Females (4 wk/8wk exposure)**			
	0	4.1	37.4	268.1	0	4.1	37.4	268.1
Erythrocytes TERA/L	7.82/ 8.77	7.56/ 8.53	7.18++/ 8.43	6.67++/ 7.43++	6.95/ 7.70	6.64/ 7.80	6.31+/ 6.60++	5.62++/ 6.25++
Leucocytes GIGA/L	5.6/ 5.0	5.2/ 5.2	5.7/ 6.0	6.9/ 7.2+	3.8/ 4.2	3.6/ 4.3	3.4/ 4.2	5.1/ 4.5
Haemoglobin g/L	152/ 157	145/ 152	142+/ 153	137++/ 144++	134/ 141	130/ 142	125/ 130	119++/ 127+
Mean erythrocyte (cell) volume fl	60/ 53	58/ 52	59/ 53	64/ 57++	59/ 53	59/ 54	62/ 59+	65+/ 61++
Heinz bodies ‰	0/ 0	0/ 0	5++/ 0	13++/ 3+	1/ 0	0/ 1	1/ 5+	8++/ 17++
Reticulocytes ‰	9/ 15	9/ 21	8/ 9+	42++/ 34++	20/ 13	19/ 11	25/ 42+	64++/ 71++
Haematocrit l/L	0.47/ 0.46	0.44/ 0.44	0.43++/ 0.45	0.43++/ 0.43	0.41/ 0.42	0.39/ 0.42	0.39/ 0.39	0.36+/ 0.38
Mean cell haemoglobin pg	19.4/ 18.0	19.2/ 17.8	19.7/ 18.2	20.6/ 19.4+	19.3/ 18.4	19.6/ 18.2	19.7/ 19.8+	21.2++/ 20.4++
Thrombocytes GIGA/L	763/ 957	830/ 1061	911++/ 964	931++/ 1073	772/ 987	840/ 982	876/ 931	938+/ 970

+ $p \leq 0.05$; ++ $p \leq 0.01$

In a subchronic (13 weeks) dermal study by Anonymous 17 (1996) in rats, no NOAEL could be set since haematological effects suggesting anaemia (lower erythrocyte count, lower haemoglobin, higher mean erythrocyte volume) were consistently seen in both sexes at all dose levels even though there was no further decrease or increase with dose. Apart from the effects on red blood cells, there were no adverse systemic or local findings up to the top dose level. In contrast, a subacute (3 weeks) dermal study in rabbits (Anonymous 11 1992) did not reveal any evidence of a local or systemic effect of treatment at any dose level.

10.12.2 Comparison with the CLP criteria

A number of studies in rats and dogs indicated that repeated administration of diuron may cause haemolytic anaemia. Haematological findings included reduced erythrocyte count and haemoglobin content, reduced haematocrit and an increase in Heinz bodies which are formed in erythrocytes by precipitation of oxidised haemoglobin. In line with haemolysis, accumulation of ferrous pigment due to storage of iron originating from haemoglobin was observed in the liver, kidney and spleen. Moreover, the serum bilirubin (i.e., a breakdown product of haemoglobin) was increased. As a compensatory response to haemolysis, the number of reticulocytes went higher and there was evidence of an increase in extramedullary haematopoiesis in the spleen. The findings of enlarged spleens may also result from an increased workload on the splenic reticuloendothelial system to

remove damaged erythrocytes. Haemolysis is a finding that may trigger classification and labelling. Table 33 presents the CLP criteria for specific target organ toxicity.

Table 33: Criteria for classification of specific target organ toxicity

CLP criteria
Category 1 (H372): - Substances that have produced significant toxicity in humans or - that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of: reliable and good quality evidence from human cases or epidemiological studies; or observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations. Equivalent guidance values for 28-day and 90-day studies: Oral, rat: 28-day: ≤ 30 mg/kg bw/d; 90-day: ≤ 10 mg/kg bw/d Dermal, rat: 28-day: ≤ 60 mg/kg bw/d; 90-day: ≤ 20 mg/kg bw/d Inhalation, rat: 28-day: ≤ 0.06 mg/l/6h/d (dusts and mists); 90-day: ≤ 0.02 mg/l/6h/d (dusts and mists) **Category 2 (H373):** Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following repeated exposure. Substances are classified in category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations. In exceptional cases human evidence can also be used to place a substance in Category 2. Equivalent guidance values for 28-day and 90-day studies: Oral, rat: 28-day: ≤ 300 mg/kg bw/d; 90-day: ≤ 100 mg/kg bw/d Dermal, rat: 28-day: ≤ 600 mg/kg bw/d; 90-day: ≤ 200 mg/kg bw/d Inhalation, rat: 28-day: ≤ 0.6 mg/l/6h/d (dusts and mists); 90-day: ≤ 0.2 mg/l/6h/d (dusts and mists)

The usual approach for STOT RE classification is to consider the subchronic studies since the "Guidance values mainly relate to studies of this type. For oral studies in the rat, the "guidance dose" for category 1 is below or equal to 10 mg/kg bw/day. This criterion is clearly fulfilled in the oral diuron studies and is supported by the inhalation studies. Accordingly, category 1 might be appropriate if the main focus of assessment was on the effect doses.

On the other hand, there were no deaths or severe clinical signs due to anaemia, perhaps due to the fact that the effects, even though occurring at low doses, were rather weak in terms of the magnitude of changes. A good part of the related findings might be more adaptive than adverse (as outlined by Anonymous 4 2001, Hardisty 2006, or Everds 2006). The guidance also suggests taking into account severity of effects. Thus, on balance, it can be proposed to keep the current classification and labelling of diuron for haematological effects as STOT RE 2 (H373).

A second toxic effect was observed in feeding studies in rats that might trigger classification and labelling, i.e., the histopathological lesions in the bladder and kidneys. In the 90-day study on rats (Anonymous 10 2004), the NOAEL for these findings was the lowest dose of 6.7 or 8.7 mg/kg bw/day (100 ppm) in males and females, respectively, whereas LOAELs of 17 or 23.3 mg/kg bw/day (250 ppm) were established. No such effects were noted in the subchronic (i.e., one-year) study in dogs. In the long-term study in rats, preneoplastic bladder lesions were observed in females from 250 ppm (17 mg/kg bw/day) onwards but were confined to the top dose level of 2500 ppm

(111 mg/kg bw/day) in males. Thus, these effects also support a need for classification and labelling as STOT RE 2.

10.12.3 Conclusion on classification and labelling for STOT RE

Based on the criteria given in the 2017 “Guidance on the Application of the CLP Criteria, Version 5.0”, there is sufficient evidence of specific target organ toxicity after repeated exposure to diuron in animals. Altogether, classification of diuron with STOT RE 2 (H373) is proposed with the indication of blood and bladder as target organs of concern.

10.13 Aspiration hazard

This endpoint is not addressed in this CLH report.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 Rapid degradability of organic substances

Table 34: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
OECD 111, EEC C.7	pH 5 at 25°C : DT50: 313 d pH 7 at 25°C : no degradation within 30 d pH 9 at 25°C : no degradation within 30 d	Reliability 1	Williams (1995)
OECD 111, US EPA N-161-1	diuron is stable to hydrolysis at pH 5, pH 7 and pH 9 and 25°C	Reliability 1	Hawkins (1988)
OECD 309	aerobic mineralisation in surface water (60 d): DT_{50} of 491 d and a DT_{90} of 1630 d were extrapolated for the low dose experiments applying SFO kinetics.	Reliability 1	Swales (2016)
OECD 316	Degradation rates of 0.2754 h^{-1} and 0.3077 h^{-1} were determined in degradation experiments	Reliability 1	Hellpointner (1991)
OECD 316	DT_{50} of 9.0 d was derived equivalent to a DT_{50} of 43 d under natural sunlight conditions	Reliability 2	Hawkins (1989)

11.1.1 Ready biodegradability

No study is available. The active substance diuron is considered as not ‘readily biodegradable’ by default.

11.1.2 BOD_5/COD

No study is available.

11.1.3 Hydrolysis

Study 1

Author: Williams, M.D.
Title: Hydrolysis of ^{14}C-diuron as a function of pH at 25 ° and 50 °C
Date: 02.11.1995
Doc ID: 42580
Guidelines: US EPA Guideline, Subdivision N, 161-1, EEC Directive, C.7, Hydrolysis
GLP: Yes
Validity: Acceptable

Material and methods

A hydrolysis study was performed with ^{14}C-diuron labelled in the benzene ring. Hydrolysis was studied at a nominal test concentration of 1.0 µg/L in sterile aqueous buffer solution at pH 4, pH 5, pH 7 and pH 9 and 25±1 °C and 50±1 °C in the dark. The duration of the study was 30 days.

The ^{14}C-acitivity was determined by LSC and the test substance and its hydrolysis products were quantified with HPLC.

Results

Total mass balance of the samples ranged from 97.9 to 106.5 %. The DT_{50} values derived for diuron following SFO kinetics and the observed degradation products are presented in table below

Table 34: Hydrolysis DT50 values of diuron

pH	Temp (°C)	DT_{50} (d)	Degradation products
4	25	798	None <5 %
	50	25.7	52.5 % 3,4-dichloraniline on day 29 7.41 % & 7.11 % degradation A on day 29 and 30
5	25	313	None <5 %
	50	55.6	34.3 % 3,4-dichloraniline on day 28
7	25	insignificant. degradation*	None <5 %
	50	insignificant. degradation **	9.06 & 9.7 % 3,4-dichloraniline on day 29 and 30
9	25	insignificant. degradation***	None <5 %
	50	109	20.8 % degradate D on day 28

* no decrease of diuron (101.6 % at study end)
**decrease of diuron from 100 to 95.1 %
*** no decrease of diuron (101.4 % at study end)

Conclusion

The study was re-evaluated according to the actual OECD 111 guideline and is still considered acceptable. Under temperatures representative to natural environmental conditions best (25 °C), diuron is stable to hydrolysis at pH 4, pH 5, pH 7 and pH 9.

Study 2

Author: Hawkins, D.R.; Kirkpatrick, D.; Shaw, D.
Title: The Hydrolytic Stability of ^{14}C-diuron
Date: 21.04.1988
Doc ID: AMR-908-87
Guidelines: US EPA Guideline, Subdivision N, 161-1
GLP: Yes
Validity: Acceptable

Material and methods

A hydrolysis study was performed with ^{14}C-diuron labelled in the benzene ring. Hydrolysis was studied at a nominal test concentration of 10 ppm in sterile aqueous buffer solution at pH 5, pH 7 and pH 9 and 25±1 °C in the dark. The duration of the study was 30 days.
The ^{14}C-acitivity was determined by LSC and the test substance and its hydrolysis products were quantified with TLC and HPLC.
The ^{14}C-acitivity was determined by LSC and the test substance and its hydrolysis products were quantified with TLC. Besides, selected samples were analysed with HPLC.

Results

Total recoveries of the applied radioactivity were greater than 97 % at all times. Unaltered diuron accounted for more than 96 % applied radioactivity at all times and all pH values. Thus, diuron is considered stable to hydrolysis at 25±1 °C at pH 5, pH 7 and pH 9. No degradation products in concentrations >2 % were found.

Conclusion

The study was re-evaluated according to the actual OECD 111 guideline and is still considered acceptable although no measurements were performed at pH 4. At 25 °C, diuron is stable to hydrolysis at pH 5, pH 7 and pH 9.

11.1.4 Other convincing scientific evidence

11.1.4.1 Field investigations and monitoring data (if relevant for C&L)

No study is available.

11.1.4.2 Inherent and enhanced ready biodegradability tests

No study is available.

11.1.4.3 Water, water-sediment and soil degradation data (including simulation studies)

Study 1

Author:	Swales, S.
Title:	[^{14}C]-diuron:aerobic mineralisation in surface water and suspended sediment
Date:	23.03.2016
Doc ID:	3200983 (714-001)
Guidelines:	OECD 309
GLP:	Yes
Validity:	Acceptable

Material and method

The mineralisation and degradation of ^{14}C-diuron (>99 % purity, 12.2 MBq/mg) was assessed in the laboratory in a natural water system with 0.02 g/L suspended sediment.
Water and sediment were collected on 5th October 2015 from a Lake at Studley Royal, Ripon UK, where no pesticides were applied in the immediate vicinity in the last 5 years. The water was collected from 0-5 cm through a 100 µm sieve straight into a clean plastic container. The sediment from top 5 cm and passed through a 2 mm sieve into a clean container. The characteristics of the surface water system are presented in table below.

Table 35: Characteristics of the surface water system

Parameter [unit]		**Value**
Water		
pH on the 5th of October	n.a.	8.74
Total organic carbon (TOC) [mg/L]		5.7
Dissolved organic carbon (DOC) [mg/L]		3.8
Biological oxygen demand (BOD_5) [mg O_2/L]		2.51
Total nitrogen [%]		0.000375
Total phosphorus [mg/L PO_4^{3-}]		1.24
Suspended solids [mg/L]		1.8
Hardness as $CaCO_3$ [mg/L]		138
Alkalinity [mg $CaCO_3$/L]		126
Nitrite [mg/L NO_2-N]		
Nitrate [mg /L NO_3-N]		
Sediment		
UK particle Size Distribution (PSD)	Sand (2000 – 63 µm) [%]	53
	Silt (63 – 2 µm) [%]	33
	Clay (< 2 µm) [%]	14
	Textural Class	Sandy loam
USDA	Sand (2000 – 50 µm) [%]	57
	Silt (50 – 2 µm) [%]	29
	Clay (< 2 µm) [%]	14
	Textural Class	Sandy loam
Organic carbon (OC) [%]		2.0
Organic Matter (OM) [%]		3.4
pH on the 5th of Oct	n.a.	8.55
pH on the 16th of Oct	H_2O	7.2
	0.01M $CaCl_2$	6.7

A preliminary test was conducted at the highest proposed test concentration (95 µg/L) using test water containing amended sediment (0.02 g/L). Test vessels were incubated for up to 7 days and samples were removed and analysed at 0 DAT and 7 DAT, in order to determine an approximate degradation rate and establish sampling times for the main test. No degradation of diuron was observed in the 7 day period and the main test sampling times were selected accordingly.

In the main test, surface water containing 0.02 g/L suspended sediment (100 mL) was dispensed into test vessels (250 mL, ca. 6 cm diameter amber glass reagent bottles). Sterilised water samples were dispensed into sterilised vessels in a laminar flow cabinet to maintain sterility. Additional incubation groups were set up for the sodium [^{14}C]-benzoate reference samples which were used to demonstrate that the microbial population was viable in the test system. Control groups contained test water and reference standard or test water, reference standard and acetonitrile (86 µL) to assess the impact of the organic solvent on the test system.

The study also included two untreated blank control vessels in which oxygen content and pH were measured without contaminating the probes used in their measurement. One of the replicates was treated with acetonitrile (86 µL) to provide a comparison of water qualities following treatment of the test samples (A, B and C) with acetonitrile. All test vessels were maintained in the dark in a temperature controlled room at 20 ± 2 °C.

Test vessels were treated with ^{14}C-diuron three days after collection of the test system (5 October 2015). Test vessels were gently shaken after application to distribute the test item in the water. The test item [^{14}C]diuron was supplied as an ethanol solution. The solvent was removed under nitrogen and the test item was

reconstituted in acetonitrile (4 mL) to form a stock solution. The concentration was determined by LSC as 1.19 mg/mL. The stock solution was diluted with acetonitrile to prepare the application solution. The concentration of the application solution was 0.111 mg/mL (determined by LSC). The nominal application rates for the test item (95 µg/L and 10 µg/L) and sodium [^{14}C]-benzoate (10 µg/L) were achieved by applying selected volumes of application solutions drop wise to the water surface of the test vessels.
Dissolved oxygen and pH were measured in the blank controls whenever test samples were removed for analysis.
Duplicate test vessels were removed for analysis from non-sterile groups treated with diuron at 0, 7, 14, 21, 28, 46 and 60 day after treatment (DAT). The sterilised samples were removed at 60 DAT. Corresponding traps were collected for sampled vessels or replenished with fresh solutions every four weeks as necessary. Traps connected to test vessels of reference substance were collected and replenished accordingly at 4, 8, 12, 14, 18, 21, 28, 35 and 60 DAT.
For characterisation of low dose samples, a weighed subsample of the test water was concentrated (centrifugal evaporation) to dryness before being reconstituted in acetonitrile:water (1:1 v:v) and transferred to a pre-weighed vial. Following centrifugation (2000 rpm, 5 minutes) the vial and contents were re-weighed. Aliquots of these solutions were submitted to LSC and HPLC analyses for the low dose (10 µg/L) samples, whereas measurements were conducted directly in aliquots of the test system water for the high dose (96 µg/L) and sterile samples.
The nature of the radioactivity trapped in the sodium hydroxide traps was not investigated as the levels of trapped volatiles was ≤ 2 % AR but assumed to be $^{14}CO_2$.
HPLC was conducted with a C18 column (Ace 5 C18 (5 µm, 250 x 4.6 mm) using a gradient of 1 % trifluoroacetic acid in water and tetrahydrofuran at a flow rate of 1 mL/min:
TLC was conducted with solvents chloroform, methanol and acetic acid (90:8:2 v/v/v). Following chromatography, radiolabelled compounds were detected by preparation of a radioluminogram of the TLC plate using a Fuji BAS 1500 Bio-image analyser and non radiolabelled compounds were visualised under UV light at 254 nm. Chromatograms were evaluated from the radioluminograms using the associated software (Tina version 2.09 g), with Rf values being used to determine the identity of each region.
The presence of diuron in the samples was confirmed by LC-MS/MS comparison to non radiolabelled reference standards. In addition, it was also confirmed by LC-MS/MS that the peak obtained in the test samples was due solely to diuron.
The limit of detection (LOD) for chromatographic analysis by HPLC was estimated based on the smallest metabolite with values of 1.1 % AR for 10 µg/L samples and 0.5 % AR for 96 µg/L samples. No limit of detection for TLC was determined as it was used as confirmatory method.

To obtain degradation rates for diuron, SFO was fitted to the residues measured in the low and the high dosed samples using the kinetic program CAKE v.2.

Results

During the incubation, water parameters (pH and oxygen content) were measured in control vessels. No systematic differences between the groups (low dose, high dose and sterile) were encountered. During incubation the oxygen content of the water ranged from 7.61 to 8.62 mg/L demonstrating aerobic conditions and the pH of the water was measured to be in the range of 8.08 to 8.76.
The mass balances and the radioactive distribution of the low and high test concentrations in the viable samples are provided in table below.

Table 36: Recovery and distribution of radioactivity (% of AR) in lake water containing suspended sediment after application of [^{14}C]diuron (mean values of replicates)

Low concentration (10 µg/L)						
Time (d)	**Diuron**	**Unknown metabolites**	**Unresolved background**	**total NaOH traps**	**Vessel wash**	**Mass balance**
0	96.5	nd	0.3	-	1.4	98.3
7	96.3	nd	1.0	0.1	1.9	99.3
14	95.1	nd	0.2	0.2	1.5	97.0
21	93.9	nd	1.0	0.3	1.4	96.5
28	93.5	nd	0.4	0.4	2.2	96.5
46	92.0	nd	1.3	1.2	2.3	96.8
60	88.2	0.5	1.0	2.00	3.2	94.8
High concentration (96 µg/L)						
Time (d)	**Diuron**	**Unknown metabolites**	**Unresolved background**	**total NaOH traps**	**Vessel wash**	**Mass balance**
0	96.1	nd	0.9	-	1.0	97.9
7	95.7	nd	1.1	0.1	1.8	98.7
14	96.4	nd	0.1	0.2	1.2	97.9
21	94.0	nd	0.3	0.2	1.2	95.7
28	93.8	nd	0.9	0.2	2.4	97.3
46	94.2	0.7	0.6	0.3	1.3	97.1
60	94.9	0.4	0.4	0.4	1.1	97.2

Mean mass balance of the high and low test concentrations were in the range 94.8 to 99.3 % AR in the viable test vessels.
In the low dose samples, diuron decreased from 96.5 % to 88.2 % after 60 d of incubation. In the high dose samples, decrease of diuron was insignificant with 96.1 % diuron at day 0 and 94.9 % diuron at day 60. Degradation products in water accounted for < 1 % AR. Besides, 2 % and 0.4 % CO_2 was formed in the low and high dose samples, respectively. Non-extractable residues in the suspended sediment were not determined.
In the sterile vessels, a mass balance of 102 % AR was achieved while no degradation of diuron was observed (99.8 % AR at day 60).

The degradation rates for diuron in the low and the high concentration experiments are presented in table below.

Table 37: Degradation rates of diuron in lake water containing suspended sediment applying SFO

Experiment	**Degradation rate constant**	**Prob.>t**	**Chi^2 (%)**	**Visual fit**	**DT_{50}**	**DT_{90}**
Low concentration (10 µg/L)	0.001411	1.29E-005	1.01	good	491	1630
High concentration (96 µg/L)	2.82E-004	0.07123	1.01	good	2460	8160

A statistically measurable degradation rate could only be determined for the low dose experiments. For the high dose experiment, the t-test returned a probability <0.05, thus there is no significant difference of the degradation rate to zero

Conclusion
The study was evaluated against the requirements of OECD 309 and is considered acceptable.
The degradation of diuron was investigated in lake water from a Lake at Studley Royal, Ripon UK with 0.02 g/L suspended sediment under aerobic conditions at 20 °C. In the low dose experiment with 10 µg/L diuron, the test substance decreased from 96.5 % to 88.2 % after 60 d of incubation. A DT_{50} of 491 d and a DT_{90} of

1630 d was extrapolated for the low dose experiments applying SFO kinetics. In the high dose experiments, decrease of diuron was insignificant with 96.1 % diuron at day 0 and 94.9 % diuron at day 60. No degradation rates could be obtained for this experiment.
Degradation products in water accounted for < 1 % AR in both experiments.

11.1.4.4 Photochemical degradation

Study 1

Author:	Hellpointer, E.
Title:	Determination of the quantum yield and assessment of the environmental half-life of the direct photodegradation of diuron in water
Date:	19.3.1991
Doc ID:	PF 3486
Guidelines:	Phototransformation of Chemicals in Water, Part A, UBA, 1989
GLP:	Yes
Validity:	Acceptable

Material and method

The quantum yield of direct photodegradation of diuron (99.7 % purity) in pure water was determined using the ECETOC method. Therefore, the degradation of diuron was determined using polychromatographic light in 5 nm steps for the wavelengths from 295 to 400 nm and in 10 nm steps from 401 nm on. The degradation experiment was conducted in a merry-go-round irradiation apparatus fitted with a mercury immersion lamp. The intensity of the light acting on the test solution was measured by means of the chemical actinometer uranyloxalate. The concentration of diuron in the samples was determined by reversed phase-HPLC and the respective UV-signal was evaluated using external acetonitrile standards. The quantum yield was calculated using the computer program QUANT.

Results

The UV-absorption spectrum of diuron in highly pure water shows a maximum at 211 nm and a maximum at 248 nm. The absorption of diuron ends with ε = 730 L/mole cm at 295 nm to ε = 20 L/mole cm in the environmentally relevant range of wavelengths. Degradation rates of 0.2754 h^{-1} and 0.3077 h^{-1} were determined in the two degradation experiments with diuron. In both experiments accelerated degradation took place with increasing duration of exposure. This is explained by the secondary photodegradation mechanisms induced by exposure of the primary photoproducts of diuron, in the course of which diuron molecules may be attacked and degraded additionally. Quantum yields of 2.54 and 2.33 10^{-2} were calculated for the two experiments resulting in a mean quantum yield of 0.0243.

Conclusion

The study was re-evaluated according to the actual OECD 316 guideline where the determination of the quantum yield is described as an additional option. The study is still considered acceptable, although the degradation rates of diuron were not determined in buffered solution but in pure water without buffer.

Study 2

Author:	Hawkins, D.R. ; Kirkpatrick, D. ; Shaw, D. ; Mobbs, J.
Title:	The photodegradation of ^{14}C-diuron in water
Date:	30.08.1989
Doc ID:	AMR-909-87
Guidelines:	US EPA Guideline, Subdivision N, 161-2
GLP:	Yes
Validity:	partly acceptable

Material and method

The photolysis of ^{14}C- diuron has been studied in an aqueous solution buffered at pH 7.0. The initial concentration of diuron in the solution was 10 ppm. A xenon arc simulated sunlight source including a filter for light of wavelength < 290 nm was used for continuous irradiation for 15 days. One day of irradiation was determined to be equivalent to 4.8 days of natural sunlight assuming a 12-hour day length. At a latitude of 52°N Irradiated and dark control solutions were maintained at 25 ± °C under sterile conditions. The test vessels were connected to a series of traps to collect volatile compounds.
The ^{14}C-acitivity was determined by LSC and the test substance and its hydrolysis products were quantified with TLC. Since under irradiated conditions, diuron degraded to several polar compounds, three different solvent systems B, D and F of increasing polarity were used for further separation of the fractions. Besides, selected samples were analysed with HPLC. For HPLC analysis, the samples were extracted 3 x 2 mL dichloromethane for ca. 15 seconds shaking each. The combined extracts were dried under nitrogen at ambient temperature and re-dissolved in 1 mL acetonitrile.

Results

The total recovery of the irradiated test solutions and the dark controls were between 86.5 – 100.4 %. 16.4 % volatiles radioactivity was found after 15 days which accounted mostly for CO_2.
Under irradiated conditions, diuron decreased from 98.8 to 33.2 %. Several radioactive fractions above 5 % but not above 10 % at study end were found in the irradiated samples which were not elucidated any further. None of the observed peaks corresponded to 3,4-dichloroaniline, DCPU, DCPMU, TCAB or TCAOB.
No degradation of diuron under dark conditions was observed.

For diuron an experimental DT_{50} of 9.0 d was derived equivalent to a DT_{50} of 43 d under natural sunlight conditions assuming 12 h sun per day.

Conclusion

The study was re-evaluated according to the actual OECD 316 guideline. The study still fulfills most of the requirements of the current guideline. However, the total recovery was below 90 % in the two irradiated samples at day 11 and in two out of four irradiated samples at day 15. Besides, several unknown compounds were found above 5 % at the end of study while 33.2 % diuron was still present. Thus, these compounds might reach concentrations > 10 % if irradiation would be continued and should be elucidated. Also the lacking separation of the Polars using TLC system B and fraction F9 and F10 is considered unsatisfactory and the interpretation of fraction F9 and F10 is ambiguous.

Considering the available information on the abiotic and biotic degradation of diuron in aquatic systems, it can be concluded that the substance is not ultimately degraded, to > 70 % within 28 days (equivalent to a half-life < 16 days). Consequently, according to CLP criteria, diuron is considered to be not rapidly degradable for the purpose of classification and labelling.

11.2 Environmental transformation of metals or inorganic metals compounds

This endpoint is not addressed in this CLH report.

11.2.1 Summary of data/information on environmental transformation

This endpoint is not addressed in this CLH report.

11.3 Environmental fate and other relevant information

This endpoint is not addressed in this CLH report.

11.4 Bioaccumulation

Table 38: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
OECD 117, EC A.8	log Po/w = 2.87 (25 °C) log Po/w = 2.85 (pH 6.4, 19 ± 1 °C)	Reliability 1	Madsen and Yousuf (1995)

11.4.1 Estimated bioaccumulation

11.4.2 Measured partition coefficient and bioaccumulation test data

As the log Pow value of Diuron (log Pow = 2.87) and its major metabolites DCPMU (log Pow = 2.59), mCPDMU (log Pow = 1.79) and DCPU (log Pow = 2.23) are below the trigger of 4, there is no potential of bioaccumulation of the substance according to CLP criteria. No experimental study characterising the bioconcentration potential in fish is available.

11.5 Acute aquatic hazard

Table 39: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results	Remarks	Reference
OECD No. 203, EC Guideline Annex V - Method C.1.	*Oncorhynchus mykiss*	diuron (98.2% purity)	Acute 96 hr (static), LC_{50}=14.7 mg a.s./L (nom)	Reliability 1	Study F1 (1993) DOM93008 REACH registration dossier
FIFRA Guideline 72-1, OPPTS 850.1075	*Pimephales promelas*	diuron	Acute 96 hr (static), LC_{50}=14.2 mg a.s./L (nom)	Reliability 2	Study F2 (1983) US EPA
OECD No. 202 (2004)	*Daphnia magna*	diuron (99.4% purity)	Acute 48 hr (static), EC_{50}>5.0 mg a.s./L (nom)	Reliability 1	Zawadsky, C. (2016) S15-00747
FIFRA Guideline 72-3, OPPTS 850.1035	*Americamysis bahia* Saltwater mysid	diuron (99.0% purity)	Acute 96 hr (static), LC_{50}=1.1 mg a.s./L (nom)	Reliability 2	Boeri, R.L. (1987) HLO 725-87
OECD No. 202 (2004)	*Daphnia magna*	diuron (98.6% purity)	Acute 48 hr (static), EC_{50}=22.6 mg a.s./L (nom)	Reliability 1	Weyers, A. (2008) REACH registration dossier
OECD No 202 (Draft)	*Daphnia magna*	diuron (98.8% purity)	Acute 48 hr (static), EC_{50}=1.4 mg a.s./L (nom)	Reliability 2	Heimbach, F. (1983) REACH registration dossier
OECD No. 201 (1984)	*Desmodesmus subspicatus*	diuron (98.5% purity)	96 h (static) EyC50=0.0079 mg a.s./L (ini.) ErC50=0.022 mg a.s./L (ini.) NOEC =0.0032 mg a.s./L (ini.)	Reliability 1	Heimbach, F. (1991) REACH registration dossier

OECD No. 201 (2011)	*Synechoccus leopoliensis*	diuron (99.9% purity)	72 h (static) EyC_{50}=0.00281 mg a.s./L (nom.) ErC_{50}=0.0078 mg a.s./L (nom.) NOEC =0.000632 mg a.s./L (nom.)	**Key study** Reliability 1	Wenzel, A. (2015) ASA-001/4-10/C/1
OECD No. 201 (2011)	*Navicula pelliculosa*	diuron (99.4% purity)	72 h (static) EyC_{50}=0.0112 mg a.s./L (nom.) ErC_{50}=0.026 mg a.s./L (nom.) NOEC =0.00428 mg a.s./L (nom.)	Reliability 1	Falk, S. (2016) S15-01978
OECD No. 201 (1984)	*Anabaena flos-aquae*	diuron (98.6% purity)	72 h (static) EyC_{50}=0.0178 mg a.s./L (nom.) ErC_{50}=0.02437 mg a.s./L (nom.) NOEC =0.01 mg a.s./L (nom.)	Reliability 1	Memmert, U. (1998) 694574
OECD 221	*Lemna gibba*	diuron (98.7% purity)	7 d (static) EyC_{50}=0.01106 mg a.s./L (mm) ErC_{50}=0.02323 mg a.s./L (mm) (frond number) NOEC=0.00321 mg a.s./L (mm)	Reliability 1	Dorgerloh, M. (1999) DOM99054 REACH registration dossier

11.5.1 Acute (short-term) toxicity to fish

Study F1

Author: X

Title: DIURON TECHN. - ACUTE TOXICITY TO RAINBOW TROUT (ONCORHYNCHUS MYKISS) IN A STATIC TEST

Date: 1993

Doc ID: DOM 93008; E 2800692-7 (994-08005)

Guidelines: US EPA Guideline, Subdivision E, 72-1, OECD No. 203, EEC Directive, Part V, 5.1.1

GLP: Yes

Validity: Yes

Material and methods

Specifications: Batch No. Pt. 232956014
Test concentrations:
1. part: 0.45, 1.00, 2.15, 4.65 and 10.00 mg test substance/L + negative control and solvent (acetone) control
2. part: 21.5, 46.4 and 100 mg test substance/L + negative control and solvent (acetone) control Sampling: 3 - 4, 24, 48, 72, 96 h
Measurement parameter: Intoxication symptoms, mortality

Measurements: LC_{50} (96 h), NOEC, LOEC, LLC, NOEC
Species: Rainbow Trout - *Oncorhynchus mykiss* (lot 7/92). Obtained from G. Müller, 3413 Moringen
Type: Fish Acute Toxicity Test, Static
Test design:
Part one: 7 groups often fish
Part two: 5 groups of ten fish
Statistical analysis: During the peer review, the two parts of the test were reanalyzed by combining the data of both tests and using ToxRatProfessional 3.2.1.
Conditions: 16/8 h light/dark, no feeding 48 h prior to test and during test, Part 1 fish weight and length were 2.7 ± 0.7 g* and 6.5 ± 0.6 cm, respectively. Part 2 fish were similarly 2.8 ± 0.6 g and 6.8 ± 0.5 cm, respectively. Water was prepared by adding salt to demineralised water. pH : 7.0 - 7.6; DO : 10.1 - 11.6 mg/L. T: 12 ±1 °C
* mean ± SD
Analytical verification of the test item was performed at day 0 and day 4 and the measured concentrations were always within ± 20 % of the nominal values.

Observations

Test substance concentrations during testing were higher than 80 % of the nominal, hence all reported values are related to the nominal concentrations. The pH and DO were constant throughout the experiment.
Less than 3 % mortality was observed in the 14-days acclimation period prior to test initiation. Although the nominal acetone concentration (part 1 = 0.3 ml/L; part 2 = 2.2 ml/L) was higher than recommended in the test guideline (100 mg/L), there were no mortalities or symptoms of intoxication in the control and the solvent control.

Conclusion

The acute toxicity study on rainbow trout (*Oncorhynchus mykiss*) was conducted using diuron at different concentrations up to 100 mg a.s./L and a 96 h LC_{50} of 14.7 mg a.s./L was derived.
The study was conducted according to the EPA guideline 72-1 which is comparable to the currently valid OECD 203 guideline and is considered acceptable during EU review. The study is valid and relevant for classification and labelling.

Study F2

Author:	X
Title:	Toxicity, Bioconcentration, and Metabolism of Five Herbicides in Freshwater Fish
Date:	1983
Doc ID:	PB83-263681
Guidelines:	None
GLP:	No
Validity:	Acceptable

Material and methods

Test water was taken from lake superior. Several chemical parameters (dissolved oxygen, pH, hardness, acidity, and alkalinity) were monitored throughout the tests by standard analytical methods (American Public Health Association, 1975). Water was not filtered or sterilized. A portion of the water was heated (>30°C) before being distributed to the test systems. Temperature control was maintained by proportional mixing of heated and unheated lake water in constant head reservoirs. Electronic sensors monitored the temperatures in the reservoirs and added heated water when necessary to maintain a desired water temperature of 25°C.

Lighting for the acute and embryo-larval toxicity tests was supplied by two 40-watt fluorescent bulbs centered above the exposure chambers. The test organism was fathead minnow.
Acute tests were run in 17.5 L glass aquaria (20 cm x 35 cm x 25 cm) containing 6 L of water. Water was delivered through proportional diluter systems (Mount and Brungs. 1967} with five toxicant concentrations and a control. Each toxicant concentration and control was run in 10 duplicate. The diluters delivered 0.5 L to each test tank 4 to 8 times per hour depending upon the toxicant used. Twenty 30-day-old fathead minnows were placed into each aquarium. Observations for mortality were made several times during the first 24 hours and twice daily for the remaining 7 days of the test. Fish were not fed during the test. Fish were considered dead, and removed from the aquaria if opercular movements had ceased. Water temperatures were measured two or more times weekly. All tests were run at a nominal water temperature of 25°C.
Test concentrations were measured with a Beckman double-beam UV-visible spectrometer at a wave length of 250 nm.

Observations
Diuron was tested at six duplicated contro1 and exposure concentrations (means of 5.54, 7.94, 11.14, 16.42, and 24.20 mg/L). The mean water temperature was 24.3°C with a range from 22.3 to 25.6°C.
The LC50(48h) of 19.9 (19.5-20.4) mg/L and the LC50(96h) of 14.2 (13.4-15.0) mg/L were determined.

Conclusion
The study was conducted in compliance with the relevant guidelines in Europe. The resulting LC50 is 14.2 mg/L for *Pimephales promelas*. The study is acceptable and relevant for classification and labelling

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

Study 1

Author:	Zawadsky, C.
Title:	DIURON TC: TOXICITY TO THE WATER FLEA DAPHNIA MAGNA STRAUS UNDER LABORATORY CONDITIONS (ACUTE IMMOBILISATION TEST - STATIC)
Date:	2016
Doc ID:	S15-00747 (822-002)
Guidelines:	OECD No. 202 (2004)
GLP:	Yes
Validity:	Yes

Material and methods

The acute toxicity of diuron on the immobilisation of *D. magna* was determined in a static 48 h test design. Test concentrations were selected based on the results of a preliminary test. Next to a control and solvent control (acetone, 0.1 mg/L in the solvent control as well as in the tested diuron concentrations), following nominal concentrations were tested 0.313, 0.625, 1.25, 2.50 and 5.00 mg a.s./L. The test consisted of four replicate test vessels with five individuals each. Each were established and maintained under the same conditions.
The number of immobilised *D. magna* in each replicate test vessel was recorded at 24 and 48 h of exposure. Immobilisation was defined as those organisms not able to swim within 15 seconds after gentle agitation of the test vessel. Biological observations and observations of the physical characteristics of each replicate test solution were also made and recorded at 0, 24 and 48 h.
The content of diuron in the samples was determined by analysing with HPLC-MS/MS. The analytical method was validated with regard to specificity, linearity, accuracy (recovery), precision and limit of

quantification. Samples from control, solvent control, 0.313, 0.625, 1.25, 2.50 and 5.00 mg a.s./L taken at test start t = 0 h from fresh test solutions and after 48 hours from aged test solutions were analysed.

Observations

Since no immobilisation higher than 50 % was found in any of the test concentrations the EC_{50} was empirically estimated to be greater than the highest treatment level. No statistical evaluation was indicated since no immobilisation was observed up to the solubility limit of 5.00 mg a.s./L.
The measured initial content of Diuron was between 79 and 100 % of nominal with a mean measured content of 87 % of nominal. The measured content after 48h was between 87 and 142 % of nominal with a mean measured content of 97.25 % of nominal. Since the mean measured content of Diuron in the samples was between 80 and 120 % of nominal the toxicological endpoints were evaluated by using nominal concentrations.
At the end of the study no immobilised daphnids were found up to and including the highest test concentration of 5 mg Diuron/L. The rate of immobilisation was 0 % in the 5.00 mg/L test item treatment group after 48 hours of exposure.

Conclusion

In a static acute immobilisation test with the water flea *Daphnia magna* the effects of Diuron were assessed over a test period of 48 h. No effects occurred in any of the treatment groups. Thus, the NOEC was determined to be ≥ 5 mg a.s./L, EC10, EC20 and EC50-values are estimated to be greater than the highest test concentration of 5.00 mg a.s./L. The study is valid and relevant for classification and labelling.

Study 2

Author:	Boeri, R.L.
Title:	STATIC ACUTE TOXICITY OF HASKEL SAMPLE NUMBER 16, 035 TO THE MYSID, MYSIDOPSIS BAHIA
Date:	1987
Doc ID:	HLO 725-87 (994-08010)
Guidelines:	FIFRA Guideline 72-3
GLP:	Yes
Validity:	Not assessed

Material and methods

Test substance: Diuron, Content of as 99.0% (nominal);
Specifications: CAS No 330-54-1, Medical Research no. 4581-443. Haskell no 16,035.
Test organism: Twenty (age unknown) mysids, *Mysidopsis bahia*, were randomly distributed among two replicates of each treatment within 30 min. of test material addition.
Test concentrations: 0, 0.6, 1.0, 1.6, 2.6, 4.3 and 7.1 mgas/L (nominal).
Test media: The experiment was performed in natural, filtered (5 micron) Atlantic ocean water. The test compound was reported totally dissolved.
Test design: 1 l. per replicate in a static system.
Food: Each 24 hr.
Conditions: Temperature: 22, Light (fluorescent)/Dark: 16/8, Salinity: 20-21 ppt, pH : 7.6-7.9, oxygen : 6.2-7.2 ppm.
Measured: Survival and the occurrence of sublethal effects (loss of equilibrium, erratic swimming, loss of reflex, excitability, discoloration, change in behaviour) were determined visually and recorded after 0, 24, 48, 72, and 96 hr.
Statistical analysis: The LC_{50} were calculated by Probit, moving average, and a nonlinear interpolation technique.

Observations

All test containers were initially clear and remained clear throughout the test. Measured diuron water concentrations were not reported.
The survival in controls was 100%, and the mysids had an average weight of 0.120 mg (0.036-0.226 mg) and an average length of 5 mm (all mysids measured 5 mm). It is unclear whether this is the initial or final size.
The LC_{50}'s for survival were 2.8 mg/L (2.6-4.3 mg/L), 1.7 mg/L (1.5-1.9 mg/L). 1.4 mg/L (1.2-1.5 mg/L), and 1.1 mg/L (0.9-1.3 mg/L) for 24, 48, 72 and 96-hr respectively. Mysids exposed to 1.6 mg/L exhibited less of reflex during the period from 48-hr until mortality occurred. No other sublethal effects were observed.

Conclusion

The acute static toxicity study on *Mysidopsis bahia* was conducted using diuron and a 96 h LC_{50} of 1.1 mg a.s/L (nominal) was derived. The study was conducted in compliance with the relevant guidelines and is considered acceptable during EU review. However, no analytical verification was reported.

Study 3

Author:	Weyers, A.
Title:	Daphnia sp., Acute Immobilisation Test with Preventol A
Date:	2008
Guidelines:	OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test)
Report No.	2008/0103/03
GLP:	Yes
Validity:	Yes

Material and methods

Test substance: Diuron, Content of as 98.6%;
Test organism: *Daphnia magna*, age < 24 h
Test concentrations: 0, 8, 16, 32 mg as/L (nominal).
Test media: M4 medium
Test design: 2 replicates per concentration and control with 10 organisms. Test vessels: 50 mL glass beakers with 20 mL of test medium
Conditions: Temperature: 18-22°C, Light (fluorescent)/Dark: 16/8, pH: 7.7-7.8, oxygen: 8.4 – 8.5 mg/L (48 h).
Measured: Mobility (normal mobility behavior and the loss of locomotory actions of the neonates), observed at 24 and 48 hours.
Analytical verification of test concentrations: Yes. Recovery rates ranged from 100.9 – 101.1% of nominal values at 0 hours, and from 99.2 – 100.2% of nominal values at 48 hours, respectively.

Observations

Analysis of the immobilisation rates gave the following results:

Time [h]	**EC0 [mg/L]**	**EC100 [mg/L]**	**EC50** (geometric mean of EC0/EC100 [mg/L])
24	16	> 32	> 32
48	16	32	22.6

Highest test concentration resulting in 0% immobilisation (EC 0 48h): 16 mg/L

Lowest test concentration resulting in 100% immobilisation (EC 100 48h): 32 mg/L

Conclusion

A short-term acute toxicity study was conducted in accordance with the prevailing OECD testing guidelines (OECD 202), with GLP. Based on the nominal concentrations, the 48h EC50 was calculated to be 22.6 mg/L diuron. The study is considered acceptable for classification purposes.

Study 4

Author:	Heimbach, F.
Title:	Acute Toxicity of Diuron to Water Fleas
Date:	1983
Report no.	HB/DM7
Guidelines:	OECD Guide-line 202 (Proposal, September 1982)
GLP:	No
Validity:	Yes

Material and methods

Test substance: Diuron, Content of as 98.8%;
Test organism: *Daphnia magna*, age < 24 h
Test concentrations: 1, 1.8, 3.2, 5.6, 10 mg as/L (nominal).
Test media: freshwater, reconstituted
Test design: 3 replicates per concentration with 10 organisms. 9 replicates with 10 organisms in the control.
Test vessels fill volume: 50 mL test medium
Conditions: Temperature: 20.7°C, pH : 7.91-8.1, oxygen : 100.2 – 100.5%, conducted in the dark.
Measured: Mobility (normal mobility behavior and the loss of locomotory actions of the neonates), observed at 24 and 48 hours.
Analytical verification of test concentrations: No.
Statistics: Probit analysis

Observations

Effect data:

	EC_{50}[1]	95 % c.l.	EC_0[1]	EC_{100}[1]
24 h [mg/L]	n.d.	n.d.	n.d.	> 10.0 (n)
48 h [mg/L]	1.4 (n)	1.2 – 1.6	n.d.	3.2 (n)

[1]effect data are based on nominal (n) concentrations
n.d.: not determined

Conclusion

A short-term acute toxicity test was conducted in accordance with the prevailing OECD testing guideline (OECD 202), however the test concentrations were not confirmed by chemical analysis. Based on the nominal concentrations, the 48h EC50 was calculated to be 1.4 mg/L diuron. Due to missing chemical analysis the results of this non-GLP study are only considered as reliable with restrictions. The study is considered as supporting information for classification purposes.

11.5.3 Acute (short-term) toxicity to algae or other aquatic plants

Study 1

Author: Heimbach, F

Title: Growth Inhibition of Green Algae (Scenedesmus subspicatus)

Date: 1991

Report no HBF/A1 88

Guidelines: OECD No. 201 (1984)

GLP: Yes

Validity: Yes

Material and methods

Test type: static

Water media type: freshwater

Exposure duration: 96 h

Test temperature: 23 ± 2 °C

pH: 7.71 – 9.09

Test concentrations: Initial concentrations of test substance Control, 0.0001, 0.00032, 0.00056, 0.001, 0.0018, 0.0032, 0.0056, 0.01 and 0.032 mg diuron/L. The average of mean measured concentrations in the main test was 96.7 % of the nominal

Initial cells density: 1.0 x 104 cells/mL

Replicates: Test concentrations 3, control 6

Photoperiod: continuous

Light intensity: 8000 lux

Statistics: Probit analysis, Dunnett's test

Observation

The following inhibition of the growth rate and biomass was observed:

Test concentration	**Percent inhibition**			
	Growth rate		**Biomass**	
	72 h	**96 h**	**72 h**	**96 h**
Control	-	-	-	-
0.00010	-2.2	0.1	-12.1	-5.5
0.00032	-1.2	1.9	1.3	3.7
0.00056	1.5	1.2	-1.1	3.5
0.0010	0.6	-1.2	-6.4	-4.6
0.0018	10.7	2.1	17.3	17.1
0.0032	0.2	1.3	2.2	5.2
0.0056	18.2	11.5	51.1	47.3
0.010	26.6	23.7	57.6	64.8
0.032	59.5	62.7	81.5	90.7

Conclusion

A short-term acute toxicity test, in accordance with the testing guideline OECD 201 (1984) and GLP, is available for this substance. Based on measured concentrations, the 72h and 96h EC50 (growth rate) were calculated to be 22 µg/L test item, while the 72h and 96h EC50 (biomass) were calculated to be 9 µg/L and 7.9 µg/L, respectively. The NOEC (growth rate) was calculated to be 3.2 µg/L. The study is acceptable for classification purpose.

Study 2

Author:	Wenzel, A.
Title:	FRESHWATER CYANOBACTERIA, GROWTH INHIBITION TEST (OECD 201). DIURON (TECHNICAL): EFFECTS ON SYNECHOCOCCUS LEOPOLIENSIS
Date:	2015
Doc ID:	ASA-001/4-10/C/1 (823-002)
Guidelines:	OECD No. 201 (2011)
GLP:	Yes
Validity:	Yes

Material and methods

The acute toxicity of diuron upon the growth of a freshwater cyanobacteria *(Synechococcus leopoliensis*) at nominal test concentrations of 0.100, 0.200, 0.632, 2.00, 6.32 and 20.0 µg a.s./L was observed over a period of 72 hours in a static test system. Four replicate flasks at each test concentration and eight control replicate flasks (only algal growth medium) were included in the test. Except for day one and the highest test concentration, the cell concentrations were determined by measurements of chlorophyll fluorescence using a microplate reader (Synergy MX-Multi-Detections-Reader, Biotek Instruments Inc., 74177 Bad Friedrichshall, Germany) on the second and third day of the test. After 24 hours the cells were counted microscopically using a Fuchs-Rosenthal counting chamber (Mean cell number of five one square millimetre areas (depth 0.2 mm) x 5000 = cell counts/mL). Analytical samples were taken from all test item concentrations and from the control after 0, 24, 48 and 72 hours. All test item concentrations of 0 and 72 hours were analysed by high pressure liquid chromatography/mass spectrometry (HPLC-MS/MS) to verify the actual test concentrations of diuron using a validated analytical method.

Observations

Analytical samples indicate that diuron was still present at measurable levels after 72 h. The nominal concentrations had been verified by chemical analysis, except for the lowest concentration. With exception of the lowest tested diuron concentration the mean measured concentrations at test initiation were between 89.5 % and 107 % of nominal diuron concentrations, and between 93.5 % and 106 % of nominal in the 72 hour samples.

The average specific growth rate was estimated for each replicate flask during the experimental period using daily cell counts (see Table below). Statistically significant inhibitory effects for the test item were observed at a nominal concentration of 2.00 µg a.s./L and above after 72 hours for growth rate and yield. The highest test item concentration caused nearly a complete inhibition of growth rate and yield (96.0 and 99.8 %). The NOEC value was determined to be 0.632 µg a.s./L for yield and growth rate. The EC50 values for yield and growth rate were determined to be 2.81 µg a.s./L and 7.88 µg a.s./L, respectively. The nominal EC_{10} and EC_{20} value for growth rate were 3.70 and 4.80 µg a.s./L, respectively. The nominal EC_{10} and EC_{20} value for yield were 0.895 and 1.33 µg a.s/L, respectively.

Table 40: Inhibition of growth rates and yield of diuron determined from daily cell counts at 0, 24, 48 and 72 h

Nominal Concentration of test item	Inhibition of growth rate [%]	Inhibition of yield [%]
[µg/L]	**0-72 h**	**0-72 h**
Control[a]	-	-
0.100	-2.1	-12.0
0.200	1.8	9.5
0.632	0.6	3.1
2.00	8.3*	33.9*
6.32	34.3*	82.5*
20.0	96.0*	99.8*

[a] One control replicate was identified as outlier on day 2 and 3 (Outlier-test after Dixon & Hartley). The replicate was omitted from evaluation.

* Significantly different compared to control based on Williams t-test (growth rate) and Welch-t-test (yield), significance level 0.05, one-sided smaller

Table 41: Toxicity endpoints for growth rate and yield of *Synechococcus leopoliensis* after exposure to diuron

Parameter	EC_{10} [µg a.s./L]	EC_{20} [µg a.s./L]	EC_{50} [µg a.s./L]	NOEC [µg a.s./L]
72 h yield (95 % lower confidence interval – 95 % upper confidence interval)	0.895 (0.472 – 1.26)	1.33 (0.835 – 1.74)	2.81 (2.24 – 3.54)	0.632
72 h growth rate (95 % lower confidence interval – 95 % upper confidence interval)	3.70 (3.36 - 4.00)	4.80 (4.49 - 5.08)	7.88 (7.56 – 8.26)	0.632

Conclusion

In a static growth inhibition test with the cyanobacteria *Synechococcus leopoliensis* the effects of diuron were assessed over a test period of 72 h. Based on nominal concentrations the 72-hour EC_{50} values for growth rate and yield of *Synechococcus leopoliensis* were estimated to be 7.88 and 2.81 µg a.s./L, respectively. The 72 h NOEC values were estimated to be 0.632 µg a.s./L, for growth rate and yield.
The nominal EC_{10} and EC_{20} value for growth rate were 3.70 and 4.80 µg a.s./L, respectively. The nominal EC_{10} and EC_{20} value for yield were 0.895 and 1.33 µg a.s/L, respectively.
The study was classified as valid as the following validity criteria were met: cell numbers in the pooled controls were increased by a factor of 144 (validity criterion ≥ 16), the coefficient of variation of average growth in control cultures was 3.2 % (validity criterion: ≤ 10 %) and the mean coefficient of variation for the section-by-section specific growth rates in the control was 30 % (validity criterion: ≤ 35 %). Synechococcus leopoliensis is explicitly mentioned as test species in OECD 201. The study is valid and relevant for classification and labelling.

Study 3

Author:	Falk, S.
Title:	DIURON TC: TOXICITY TO THE DIATOM NAVICULA PELLICULOSA UNDER LABORATORY CONDITIONS
Date:	2016
Doc ID:	S15-01978, (823-003)
Guidelines:	OECD No. 201 (2011)
GLP:	Yes
Validity:	Yes

Material and methods

The toxicity of diuron upon the growth of a freshwater diatom *(Navicula pelliculosa*) at nominal test concentrations of 0.417, 1.34, 4.28, 13.7, 43.8 and 140 µg a.s./L was observed over a period of 72 hours in a static test system. Three replicate flasks at each test concentration, six control replicate flasks (only algal growth medium) and six solvent replicates were included in the test. All stock and test solutions were prepared with test medium containing algae cells with an intended cell density of 1×10^4 algae per mL. The control group contained only algal growth medium. Cell concentrations were measured by fluorescence detection at 24, 48 and 72 h. Analytical samples were taken from all test item concentrations and from the control after 0, 24, 48 and 72 hours. All test item concentrations of 0 and 72 hours were analysed by high pressure liquid chromatography/mass spectrometry (HPLC-MS/MS) to verify the actual test concentrations of diuron using a validated analytical method.

Observation

Analytical samples indicate that diuron was still present at measurable levels after 72 h. The mean measured concentrations at test initiation were between 86 % and 103 % of nominal diuron concentrations, and between 88 % and 103 % of nominal in the aged samples (after 72 h). As the content of diuron was in the range of 80 and 120 % of nominal at test start and end, all toxicological endpoints were evaluated based on the nominal test item concentrations.

The study was considered to be valid as the following validity criteria were met: cell numbers in the pooled controls were increased by a factor of 23.4 (validity criterion ≥ 16), the coefficient of variation of average growth in pooled replicate control cultures was 5.8 % (validity criterion: ≤ 10 %) and the mean coefficient of variation for the section-by-section specific growth rates in the control was 32.9 % (validity criterion: ≤ 35 %).

The average specific growth rate was estimated for each replicate flask during the experimental period using daily cell counts. Statistically significant inhibitory effects for the test item were observed at a nominal concentration of 13.7 µg a.s./L and above after 72 hours for growth rate and yield. The highest test item concentration caused a complete inhibition of growth rate and yield. The NOEC value was determined to be 4.28 µg a.s./L for yield and growth rate. The EC_{50} values for yield and growth rate were determined to be 11.2 µg a.s./L and 26.0 µg a.s./L, respectively. The nominal ErC_{10} and ErC_{20}-value were 7.08 and 11.1 µg test item/L, respectively. The nominal EyC_{10} and EyC_{20}-value were 3.29 and 5.17 µg a.s./L, respectively. The overall LOEC was determined to be at 13.7 µg a.s./L and the overall NOEC was determined to be at 4.28 µg a.s./L (nominal).

Conclusion

In a static growth inhibition test with the diatom *Navicula pelliculosa* the effects of diuron were assessed over a test period of 72 h. Since no statistically significant differences were observed between the control and solvent control after 72 h, both controls were pooled and all values were evaluated based on the pooled controls. Based on nominal concentrations the 72-hour EC_{50} values for growth rate and yield of *Navicula pelliculosa* were estimated to be 26.0 and 11.2 µg a.s./L, respectively. The nominal EC_{10} and EC_{20} value for growth rate were 7.08 and 11.1 µg a.s./L, respectively. The nominal EC_{10} and EC_{20}-value for yield were 3.29

and 5.17 µg a.s./L, respectively. The overall 72 h LOEC was determined to be at 13.7 µg/L and the overall NOEC was determined to be at 4.28 µg/L (nominal).

The study meets the control validity criteria and the requirements of the OECD guideline 201 (March 2001, adopted: 23 March 2006, Annex 5 corrected: 28 July 2011). The study is valid and relevant for classification and labelling.

Study 4

Author:	Memmert, U.
Title:	TOXICITY OF DIURON TECHN. TO ANABAENA FLOS-AQUAE (CYANOPHYTA) IN A 72-HOUR ALGAL GROWTH INHIBITATION TEST
Date:	1998
Doc ID:	694574 (994-08021)
Guidelines:	OECD No. 201, EEC Directive, Annex Part C.3
GLP:	Yes
Validity:	Yes

Material and methods

Substance: diuron technical (98.6 % purity)
Concentrations: 1.0, 3.2, 10, 32, and 100 µg/L and control
Sampling: 72 h
Measurement parameter: biomass (b), growth rate (r)
Measurements: NOEC, LOEC, E_bC_{50}, E_bC_{10}, E_bC_{90}, E_rC_{50}, E_rC_{10}, E_rC_{90}
Species: *Anabaena flos-aquae* strain No. B 30.87
Type: 72 h alga growth inhibition test
Test design: Three replicate concentrations and six controls
Statistical analysis: Method adopted from OECD Guideline 201.
Conditions: 50 mL of 1 x 10^4 cells per mL in 50 mL Erlenmeyer flasks at 24 °C and 7600 lux

Observations

The measured concentrations of diuron ranged from 98 – 104 % at test start and from 95 -103 % at the end of the tests. Therefore, all endpoints are based on nominal values. The pH increased during the test from 7.9 - 8.1 at the start to 8.1 - 9.2 after 72 hours. The EC_{50} of *Anabaena flos-aquae* for diuron were calculated to be 30.9 µg/L for growth rate and 23.2 µg/L for biomass. The algal toxicity study was statistically re-evaluated in order to and to comply with OECD 201 (2011) and to fulfil the current data requirements (EC 283/2013).

Conclusion

Based on the re-evaluation the 72 h E_rC_{10}, E_rC_{20} and E_rC_{50} values for growth rate, were determined to be 12.16, 15.44 and 24.37 µg a.s./L, respectively. The 72 h E_yC_{10}, E_yC_{20} and E_yC_{50} values for yield were determined to be 10.39, 12.52 and 17.88 µg a.s./L, respectively. The NOEC for growth and yield was determined to be 10.0 µg a.s./L. All reported biological results are related to the nominal concentrations of the test item diuron, since the analytical results demonstrated that the measured concentrations of diuron during the 72 h test period were between 95 and 104 % of nominal concentration. The study was conducted in compliance with the relevant guideline OECD 201 (2011) and the current validity criteria were all met. The study is valid and relevant for classification and labelling.

Study 5

Author:	Dorgerloh, M.
Title:	DIURON - TOXICITY (7 DAYS) TO LEMNA GIBBA G3
Date:	1999
Doc ID:	DOM 99054 (994-08029)
Guidelines:	OECD-Draft "Lemna Growth Inhibition Test, June 1998"
GLP:	Yes
Validity:	Yes

Material and methods

Test substance: diuron (purity 98.7 %)
Concentrations (nominal): Control, 721, 240, 80.1, 26.7, 8.9, and 2.97 µg a.s./L
Sampling: 0, 2, 5 and 7 days
Measurement parameter: growth rate r (frond area 7 d); frond number (0, 2, 5, 7 d), and biomass (dry weight 7 d)
Measurements: EC50, LOEC, NOEC, TEC
Species: Lemna gibba G3 from laboratory stock culture. The original culture was obtained from Dr. Janet Slovin, Horticulture Crops Quality Laboratory, U.S. Department of Agriculture, Beltsville, M.D. USA.
Type: Seven-day toxicity test
Test design: 3 x 12 fronds for each of the 6 test concentrations were exposed in a chronic multigeneration test for 7 days under static test conditions. Solvent and test medium controls were included.
Sampling: 0, 2, 5 and 7 days
Measurement parameter: growth rate r (frond area 7 d); frond number (0, 2, 5, 7 d), and biomass (dry weight 7 d)
Measurements: EC50, LOEC, NOEC, TEC
Species: Lemna gibba G3 from laboratory stock culture. The original culture was obtained from Dr. Janet Slovin, Horticulture Crops Quality Laboratory, U.S. Department of Agriculture, Beltsville, M.D. USA.
Type: Seven-day toxicity test
Test design: 3 x 12 fronds for each of the 6 test concentrations were exposed in a chronic multigeneration test for 7 days under static test conditions. Solvent and test medium controls were included.
Statistical analysis: A t-test was performed in order to determine if controls can be pooled. A Chi-square test was conducted to assess normal distribution of the data set. Bartlett's test for homogeneity of variances was used. Kruskal Wallis, Dunn's multiple comparison test as well as an ANOVA and Bonferroni's test were used depending on the data.
Conditions: Incubated in 10 cm diameter glass dishes (400 mL), at 7852 lux, 24 ± 2 °C, and pH of 5.5 - 5.6 at day 0.
The following deviation from the guideline was noted in the report: "Modifications of the test medium to reach a sufficient doubling time during the test duration of 7 days". Actually a modified Steinberg medium was used, where the draft OECD-guideline for *Lemna gibba* recommend a 20X AAP growth medium.

Observations

The pH increased to a range between 5.5 and 6.1. The pH at day 7 was negatively correlated with the diuron concentration. Chlorosis was noted at the two highest concentrations of diuron.
105 - 119 fronds were reached after a 7 day cultivation in the control, corresponding to approximately a 9-fold increase in biomass in 7 days. Stability of diuron under test conditions was confirmed through analytical measurements at start and end of the test. The EC_{50} (0 - 7 d) was calculated to 18.3 µg a.s./L
The *Lemna gibba* growth inhibition study was statistically re-evaluated in order to fulfil current data requirements (EC 283/2013). The re-evaluation was done on mean measured concentrations and the doubling time of frond number was 2.2 d. Therefore, the validity criteria were met according to OECD 221 (2006) were met (validity criterion: < 60 h). The measured concentrations in day 0 ranged from 111.6 –

120.2 µg/L and on day 7 from 101.7 – 114.6 µg/L.

Conclusion

Based on the results regarding growth rate (frond number), the re-evaluated 7 day E_rC_{10}, E_rC_{20} and E_rC_{50} were determined to be 6.65, 10.21, and 23.23 µg a.s./L, respectively, and the 7 day NOEC was determined to be < 3.21 µg a.s./L. For yield (frond number), the 7 day E_rC_{10}, E_rC_{20} and E_rC_{50} were determined to be 2.35, 4.00, and 11.06 µg a.s./L, respectively, and the 7 day NOEC was determined to be < 3.21µg a.s./L.
Based on the results regarding growth rate (frond area), the 7 day E_rC_{10}, E_rC_{20} and E_rC_{50} were determined to be 4.70, 7.44, and 17.9 µg a.s./L (mean measured concentrations), respectively, and the 7 day NOEC was determined to be < 3.21µg a.s./L (mean measured concentration). For yield (frond area), the 7 day E_rC_{10}, E_rC_{20} and E_rC_{50} were determined to 1.55, 2.67, and 7.57 µg a.s./L (mean measured concentrations), respectively, and the 7 day NOEC was determined to be < 3.21µg a.s./L (mean measured concentration).
The study is valid and relevant for classification and labelling.

11.5.4 Acute (short-term) toxicity to other aquatic organisms

All information on acute toxicity is taken from the RAR and list of endpoints for diuron, March 2018.

11.6 Long-term aquatic hazard

Table 42: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results[1]	Remarks	Reference
OECD No. 204	*Oncorhynchus mykiss* Rainbow Trout	diuron (98.2% purity)	28 d (semistatic) NOEC=0.41 mg a.s./L (mm)	Reliability 1	Study F3 (1993) DOM93009 REACH registration dossier
FIFRA Guideline 72-4, OPPTS 850.1400 OECD No. 210	*Cyprinodon variegatus*	diuron (96.8% purity)	38 d ELS (flow through) mortality NOEC=1.7 mg a.s./L (mm)	Reliability 1	Study F4 (1992) HLO866-91
FIFRA Guideline 72-4, OPPTS 850.1300	*Pimephales promelas*	diuron (98.6% purity)	60 d ELS (static) NOEC=0.033 mg a.s./L (nom.)	Reliability 2	Study F5 (1983) PB83-263681
OECD No. 234 (2011)	*Danio rerio*	diuron (98.7% purity)	63 d ELS (flow through) Post hatch survival NOEC=0.00119 mg a.s./L (mm)	Reliability 1	Study F6 (2018) REACH registration dossier
OECD No. 211 (1998)	*Daphnia magna*	diuron (98.7% purity)	21 d (semistatic) Reproduction NOEC=0.096 mg a.s./L (mm)	Reliability 1	Heimbach, F. (1996) HBF/rDm58 REACH registration dossier
OECD No. 219 (2004)	*Chironomus riparius*	diuron (99.4% purity)	28 d (static water/sediment system) Development NOEC=1.55 mg a.s./L (mm)	Reliability 1	Gonsior, G. (2016) S15-01907
OECD No. 201 (1984)	*Desmodesmus subspicatus*	diuron (98.5% purity)	96 h (static) EyC50=0.0079	Reliability 1	Heimbach, F. (1991) REACH registration

			mg a.s./L (ini.) ErC50=0.022 mg a.s./L (ini.) NOEC =0.0032 mg a.s./L (ini.)		dossier
OECD No. 201 (2011)	*Synechoccus leopoliensis*	diuron (99.9% purity)	72 h (static) EyC_{50}=0.00281 mg a.s./L (nom.) ErC_{50}=0.00788 mg a.s./L (nom.) ErC_{10}=0.0037 mg a.s./L (nom.) NOEC =0.000632 mg a.s./L (nom.)	**Key study** Reliability 1	Wenzel, A. (2015) ASA-001/4-10/C/1
OECD No. 201 (2011)	*Navicula pelliculosa*	diuron (99.4% purity)	72 h (static) EyC_{50}=0.0112 mg a.s./L (nom.) ErC_{50}=0.026 mg a.s./L (nom.) NOEC =0.00428 mg a.s./L (nom.)	Reliability 1	Falk, S. (2016) S15-01978
OECD No. 239 (2014)	*Ceratophyllum demersum*	diuron (99.9% purity)	14 d (static) dry weight EyC_{50}=0.0138 mg a.s./L (mm) ErC_{50}=0.0311 mg a.s./L (mm) **ErC_{10}=0.000267 mg a.s./L (mm)** **NOErC =0.000463 mg a.s./L (mm)**	**Key study** Reliability 1	Wenzel, A. (2016) LDG-001/4-12/B
OECD No. 239 (2014)	*Chara globularis*	diuron (99.9% purity)	14 d (static) shoot length EyC_{50}=0.0207 mg a.s./L (mm) ErC_{50}=0.0809 mg a.s./L (mm) ErC_{10}=0.00311 mg a.s./L (mm) NOErC =0.00326 mg a.s./L (mm)	Reliability 1	Wenzel, A. (2016) LDG-001/4-12/D
OECD No. 239 (2014)	*Elodea canadiensis*	diuron (99.9% purity)	14 d (static) fresh weight EyC_{50}=0.00818 mg a.s./L (mm) ErC_{50}=0.0166 mg a.s./L (mm) ErC_{10}=0.000278 mg a.s./L (mm) NOErC =0.00586 mg a.s./L (mm)	Reliability 1	Wenzel, A. (2016) LDG-001/4-12/C

[1] Indicate if the results are based on the measured or on the nominal concentration

11.6.1 Chronic toxicity to fish

Study F3

Author:	x
Title:	DIURON TECHN. - PROLONGED TOXICITY (28 DAYS) TO RAINBOW TROUT IN A SEMI-STATIC TEST
Date:	1993
Doc ID:	DOM 93009, E 287 0691 - 3 (994-08007)
Guidelines:	OECD No. 204
GLP:	Yes
Validity:	Yes

Material and methods

Fish was held and observed according to OECD guideline 204 for at least 14 days prior to testing.
Test substance: Diuron Techn. (98.2% purity)
Specification: Batch no. Pt. 232956014
Test concentrations: 0. 23, 0.50, 1.08, 2.32, 5.00 mg test substance/L, negative and solvent control.
Sampling: 28 d
Measurement parameter: Mortality, body weight, and body length.
Measurements: LC_{50}(28 days), LLC, LOEC, NOEC
Species: Rainbow trout *Oncorhynchus mykiss* from G. Müller, D-3412 Moringen, FRG.
Type: Fish prolonged toxicity test 28 days, semi-static test
Test design: Five + two groups of ten fish
Statistical analysis: ANOVA for body weight, length data and condition factor. Duncan's test for differences to control. The LC_{50} with 95% C.L. was calculated by one of three methods, i.e. moing average, binomial probability, probit or non-linear interpolation.
Conditions: Fish were held in 40 Laquaria.
During the test fish were fed at a rate of 2.2% FW of the estimated body weight. Food was a commercial fish diet (Brutfutter FB 50, Kronen Fischkraftfutter, D-4231 Wesel, FRG. The water was prepared by adding salt stock solution to demineralized water. Hardness: 40-60 mg $CaCO_3$/L, Light/dark - 16/8 hs, Temperature: 12 ± 1 °C, DO: 8.8-12.0 mg/L, pH: 7.0-7.7. The test media were changed weekly.
Test water was analysed daily. Fish were observed daily for signs of intoxication and mortality. Statistical analysis was analysis of variance performed on body weight and length data.

Observations

There were neither mortalities nor symptoms of intoxication in the control and solvent control groups. Concentrations lower than 0.79 mg/L revealed no signs of intoxication. Signs of intoxication began at 0.79 mg/L and 1.97 mg/L at day 6, and at 4.45 mg/L they began at day 1. At 0.79 mg/L the only fish that died did it at day 8. No fish died at 1.97 mg/L. At 4.45 mg/L a total of 6 fish died during the four weeks-the first died at day 5, whereas the other died at day 21 to 22. At 1.97 mg/L signs of intoxication were slight the first 10 days and then they became increasingly severe for the rest of the test period.
Less than 3% mortality was noted during the observation period prior to testing. Feed was not analysed for any unwanted contaminants.
All reported results are related to measured concentrations of the substance, because the deviations from the nominal concentration were mostly greater than 20%.
The DO was higher than 80% during most of the test period, the three lowest values measured were 8.8 and 9.0 mg/L at day 27 in the 0.41 mg//L and the 0.15 mg/L treatments, respectively, and 8.8 mg/L at day 28 in the 0.79 mg/L treatment

Conclusion

The prolonged toxicity study on rainbow trout (*Oncorhynchus mykiss*) was conducted using technical diuron and a 28 day NOEC of 0.41 mg a.s./L was derived. As the analytical verification showed deviations of more than 20 % to nominal, the results were related to measured concentrations.

The study was conducted in compliance with the relevant guidelines which are still the currently valid guidelines. The study is valid and relevant for classification and labelling.

Study F4

Author:	x.
Title:	EARLY LIFE STAGE TOXICITY OF DPX-14740-166 (DIURON) TO THE SHEEPSHEAD MINNOW, CYPRINODON VARIEGATUS
Date:	1992
Doc ID:	HLO 866-91, 91136-DU (994-08008)
Guidelines:	US EPA Guideline, Subdivision E, 72-4
GLP:	Yes
Validity:	Yes

Material and methods

Test substance: DPX-14740-166 (diuron; 96.8 % purity) LOT# 2507
Concentrations: Nominal: 0.48, 0.90, 1.5, 3.0, and 6.0 mg/L + controls. Measured: 0.44, 1.0, 1.7, 3.6 and 7.1 mg/L, controls were < 0.12 mg/L (HPLC-UV).
Sampling: Daily
Measurement parameter: Mortality. Sub-lethal effects: Length and weight of surviving fish
Measurements: LOEL, NOEL, MATC (geometric mean of LOEL and NOEL)
Species: Sheepshead minnow, *Cyprinodon variegatus* embryos less than 24 hours old. They were obtained from Multi Aquaculture Systems, Amagansett, New York.
Type: 38 days early life stage toxicity test
Test design: Flow through conditions, with five test concentrations plus water control and solvent control. 80 embryos were equally distributed over two replicates of each treatment. Hatching was complete after six days of exposure, and fish were reduced to 20 per replicate and released into the test vessels (15 L in 23 L volume glass aquaria).
Statistical analysis: Shapiro-Wilk's test for normal distribution of data. Treatment and control means comparison by ANOVA and Bonferonni's test.
Conditions: The test was performed in filtered, UV-irradiated, natural seawater at 30 ± 1 °C. Salinity: 20 ppt, pH: 7.5, 16 hour light (10 $\mu Es^{-1}m^{-2}$) and 8 hour dark, aeration was initiated after two days because dissolved oxygen began to drop, DO was always > 75 %. Fish were fed twice daily with *Artemia salina* nauplii.

Observations

Hatching (number of embryos hatched, time to hatch and mortality of embryos at day 6) was not influenced by any of the tested concentrations.
At least 72.5 % of the control and solvent control embryos hatched in each replicate with a mean hatch of at least 75 %. Control survival was at least 95 % after 32 days post hatch (test day 38). Sublethal effects (lethargy in all surviving fish) were noted in the highest tested concentration at day 7 and 8 post hatch, and from day 13 to end of test at 3.6 mg/L measured concentration. Mortality was complete on day nine at the highest concentration, i.e. 7.1 mg/L measured concentration.
Mean measured test concentrations were used for calculations. The measurements of length and weight did

not differ from the control. However, measurements from the two highest concentrations were not included because the mortality was significantly different from control. It should, however be noted that the surviving fish at 3.6 mg/L was ca. 70 % shorter and 95 % lighter than the control fish.
Exposure of embryos, larvae and juvenile fish resulted in a lowest observed effect level (LOEL) of 3.6 mg a.s./L, a no observed effect level of 1.7 mg a.s./L, and a maximum acceptable toxicant concentration of 2.5 mg a.s./L. The most sensitive measured parameter was the mortality of Sheepshead minnows from day 11 to 32 post hatch, and the sublethal effects.

Conclusion

The study was re-evaluated considering the actual OECD TG 210 (2013) using ToxRat version 3.2. Based on the most sensitive parameter (post-hatch survival) the NOEC was determined to be 1.70 mg a.s./L. Reliable estimation of EC_{10} and EC_{20} values for hatchability, post-hatch survival, fresh weight and fish length was not possible due to a lack in the dose response.
The fish early life stage toxicity study on diuron was conducted using the saltwater species *Cyprinodon variegatus*. The recommendations of EPA guideline concerning the temperature of the test medium were not met. The test was conducted in a range of 29°C and 31°C while EPA recommends a temperature of 25+/-2°C. The NOEC based on survival of the hatched embryos was determined to be 1.7 mg a.s./L after 32 days of exposure. The results were related to measured concentrations.
The study was considered valid and acceptable in the diuron DAR (2005) and is still considered valid, scientifically acceptable and appropriate for the assessment of chronic fish toxicity in compliance to OECD 210 (2013). The study is valid and relevant for classification and labelling.

Study F5

Author:	x
Title:	Toxicity, Bioconcentration, and Metabolism of Five Herbicides in Freshwater Fish
Date:	1983
Doc ID:	PB83-263681
Guidelines:	None
GLP:	No
Validity:	Acceptable

Material and methods

Test water was taken from lake superior. Several chemical parameters (dissolved oxygen, pH, hardness, acidity, and alkalinity) were monitored throughout the tests by standard analytical methods (American Public Health Association, 1975). Water was not filtered or sterilized. A portion of the water was heated (>30°C) before being distributed to the test systems. Temperature control was maintained by proportional mixing of heated and unheated lake water in constant head reservoirs. Electronic sensors monitored the temperatures in the reservoirs and added heated water when necessary to maintain a desired water temperature of 25°C. Lighting for the acute and embryo-larval toxicity tests was supplied by two 40-watt fluorescent bulbs centered above the exposure chambers. The test organism was fathead minnow.

Early life-stage tests were run using the same basic diluter and exposure system as in the acute tests. The system was modified to permit incubation of eggs in oscillating 130 mL specimen bottles with 200 m mesh nylon screen bottoms. The bottles were oscillated by a rocker arm assembly at a rate of about 6 times per minute, causing the eggs to move a vertical distance of about 5 cm. Modifications were also made within the aquaria to reduce the area and confine the fish. A glass and stainless steel mesh (200 ~m) barrier reduced the volume of water to 4.5 L. The bottom color of the aquaria was changed from black to white, permitting easier Observation of fry. Fathead minnow eggs <24 hours old were placed into the incubation jars (SO eggs per jar; 2 jars per treatment replicate). Dead eggs were removed daily, and upon completion of hatch the

survivors were counted and 15 fry from each incubation jar were released into the aquarium (30 per aquarium). Feeding was begun the day after natching and continued to the end of the test. Finely granulated dry fish food (Tetramin and newly hatched brine shrimp were fed for the first 30 to 45 days after hatching and supplements of frozen adult brine shrimp were added to the diet for the last 15 to 30 days of the test).
Equal volumes of food were provided to each aquarium. Several parameters were measured in the early life-stage tests. These included egg hatchability, occurrence of abnormal and dead fry at time of transfer from egg cups into the aquaria, fry survival at end of exposure period, and wet weight and length at end of exposure period. Observations were also made a behaviour and development of the fry throughout the exposures. Fathead minnow eggs (83 to 104 total per incubation chamber) less than 24 hrs old were exposed to diuron (98.6 % purity) at six duplicated control and exposure concentrations means of 2.6, 6.1, 14.5, 33.4, and 78.0 g/L).

Observation

The mean water temperature was 25.0°C with a range from 23.8 to 27.0°C. Hatching success for controls averaged 67.9%, and ranged from 66.1 to 77.9% for the five exposure levels. Hatching success was not affected by diuron exposure. The mean percent of abnormal including dead fry at time of transfer was 2.2% for controls, and ranged from 0.6 to 15.0% for the five diuron exposures. The highest concentration of 78.0 ~g·L-1 resulted in a significant increase ($p<0.01$) in percent abnormal fry at hatch. Survival of transferred fry (30 per aquarium} averaged 24 for controls, and ranged from 8 to 28 for the five diuron exposures. The highest concentration (78.0µg/L) resulted in a significant reduction ($p<0.05$) in survival through 60 days of exposure after hatching.
Wet weight at 60 days averaged 0.568 g for controls, and ranged from 0.496 g to 0.568 g for the five diuron exposures. Fry length at 60 days averaged 32.2 mm for controls, and ranged from 29.1 to 32.4 mm for the five exposures. Neither wet weight nor 1ength were significantly affected ($p>0.05$).

Table 43: Effects during the test

	Mean Diuron Concentration ± s.d. (µg/L)					
Parameter	**0.0 ± 0.0**	**2.6 ± 0.7**	**6.1 ± 1.6**	**14.5 ± 2.0**	**33.4 ± 4.8**	**78.0 ± 8.1**
Mean percent hatch[a]	67.9	77.9	75.0	71.8	67.9	66.1
Mean percect abnormal and dead[b]	2.2	0.6	1.3	3.7	8.2	15.0**
Mean number of surviors at 60 days post-hatch[c]	24.5	26.5	28.0	21.5	22.5	7.5*
Mean wet weight at 60 days post-hatch (g)	0.568	0.568	0.563	0.619	0.563	0.496
Mean total lenght at 60 days post-hatch (mm)	32.2	32.2	32.4	32.3	31.0	29.1

[a] Live fry/total eggs after 5 days.
[b] Abnormal (deformed) + dead fry/total fry at t1me of transfer from egg eups (5 days after in1tia1 exposure of eggs).
[c] Based on 30 fry transferred to duplieate exposure ehambers.

* Signicantly different from controls ($p<0.05$).
**Significantly different from controls ($p<0.01$)

Conclusion

The NOEC for fathead minnows exposed to diuron was between 33.4 and 78.0 µg/L. This estimate was based on the two parameters that were significantly affected at 78.0 µg/L. These were abnormal fry at time of transfer and survival through 60 days.
The study is acceptable and relevant for classification and labelling.

Study F6

There is a new fish sexual development test (2018) for diuron (98.7 % purity) with *Danio rerio* from REACH portal available.

Author:	x
Title:	Fish sexual development test with Preventol A6
Date:	2018
Guidelines:	OECD No. 234 (2011)
GLP:	Yes
Validity:	Yes

Material and methods

Test substance: diuron, concentration of the active substance: 98.7 % (nominal)
Test concentrations: Dilution water control, solvent control and 0.001, 0.00316, 0.010, 0.0316 and 0.1 mg/L (nominal). The results are based on measured concentrations of 0, 0.00119, 0.00326, 0.01132, 0.03251, and 0.10544 mg/L, deviating less than 20 % from nominal during the whole experiment.
Test organism: *Danio rerio*
Study design: flow through conditions
Solvent: Acetone
Study duration: 63 d

Observation

The NOEC for post hatch survival during early life stages was 0.00119 mg as/L (mean measured).

Conclusion

The NOEC for post hatch survival of zebrafish was 0.00119 mg as/L (mean measured).
The study is valid and relevant for classification and labelling.

11.6.2 Chronic toxicity to aquatic invertebrates

Study 1

Author:	Heimbach, F., 1996
Title:	INFLUENCE OF DIURON TECHNICAL ON THE REPRODUCTION RATE OF WATER FLEAS
Date:	1996
Doc ID:	HBF/rDM 58 (994-08015)
Guidelines:	OECD No. 202, EPA FIFRA Guideline 72-4
GLP:	Yes
Validity:	Yes

Material and methods

The purpose was to study the effects of diuron on the survival (mobility), growth and reproduction of *Daphnia magna*. The test was performed in water (semi-static system). Mobility, growth and reproductive capacity were recorded for 21 days.
Test substance: diuron, concentration of the active substance: 98.7 % (nominal). Specifications: Batch no 232 455 681,
Test concentrations: Dilution water control and 0.032, 0.056, 0.10, 0.18, 0.32, 0.56, 1.0 and 1.8 mg/L (nominal). The results are based on measured concentrations of 0, 0.033, 0.054, 0.096, 0.17, 0.32, 0.56. 0.97, and 1.75 mg/L, deviating less than 20 % from nominal during the whole experiment.
Test organism: First instars of *Daphnia magna* (strain form Bundesgesundheitsamt in Berlin, Germany) less than 24 h old. Genotype no 2/Type B. Instars were collected from adults 14 - 21 days old where only parents of the same age (± 12 hours) were used.
Study design: Static renewal conditions.
Test system: Test vessel of 250 mL were filled with approximately 200 mL test solution (non-aerated) to a depth of 8 cm. The solution was renewed every 24 h during the week and 72 h over the weekend. Test vessels were randomly assigned.
Replication: The replication was ten per concentration with each one individual for growth and reproduction and three vessels with five individuals for mortality.
Food: 1x 10^8 *Scenedesmus subspicatus* cells/L daily from Mondays to Thursdays. On Friday a threefold amount of algae was fed at a similar daily rate. In the pre-test culture they were occasionally fed commercial ornamental fish food (Trade name: TetraMin). Test media: "M7-medium" similar to "M4-medium"
Conditions: Temperature: 20 °C; Conductivity: 580 µS/cm; pH: 8.1 - 8.2; Oxygen: 8.3 - 9.6 mg O_2/L (102 ¬ 94 %); Light (ca. 700 lux)/Dark: 16/8 h; Test duration: 21 d.
Measured: The number of offspring (neonates) was monitored daily on days 7 - 12, thereafter on a Monday-Wednesday-Friday schedule, with exception of Saturday and Sunday on the first and the third week. Size (dry weight and length) of adults were also measured.
Statistical analysis: For testing of normality Kolmogorow-Smirnow-Test and for homogeneity of variance Bartlett's test were used. For normal distributed data Dunnett's test was performed. For the number of offspring per parent, the data were tested using Mann-Whitney U-test, since the variance was not homogeneous.

Observations

The maximum mortality recorded was 7 % at the highest concentration levels. Growth was significantly reduced at concentrations of 0.056 mg a.s./L (8 %) and 0.17 mg a.s./L (22 %), for length and dry weight respectively. Reproduction was significantly (19 %) reduced at 0.97 mg a.s./L.

Conclusion

The chronic *Daphnia magna* study from Heimbach (1996) was statistically re-evaluated in order to fulfil current data requirements (EU 283/2013). The study is still valid according to OECD 211 (2012) as the adult mortality did not exceed 20 % at the end of the study (each control and solvent control: 7.5 %) and the mean number of living offspring produced per parent animal surviving at the end of the test was ≥ 60 (control: 65.8, solvent control: 66.3). Reliable estimation of EC_{10} and EC_{20} values was not possible due to a lack in the dose response.
Based on the results regarding immobility as well as offspring numbers, the NOEC was determined to be ≥ 1.75 and 0.56 mg a.s./L, respectively.
The most sensitive endpoint in the test was growth (dry weight) with a NOEC of 0.096 mg/L mean measured concentration. The study is valid and relevant for classification and labelling

Study 2

Author: Gonsior, G.

Title: DIURON TC: ASSESSMENT OF SIDE EFFECTS ON THE LARVAE OF THE MIDGE, CHIRONOMUS RIPARIUS WITH THE LABORATORY TEST

METHOD - SPIKED WATER TEST

Date: 2016

Doc ID: S15-01907 (828-001)

Guidelines: OECD No. 219 (2004)

GLP: Yes

Validity: Yes

Material and methods

A 3.5 mg/L primary stock solution was prepared by dissolving 150 mg of active substance into 3 mL acetone. Test solutions were prepared by diluting stock solution in acetone followed by application of 30 µL to the water phase of the respective vessels. The concentration of acetone was the same in all test concentrations and the solvent control, i.e. 100 µg/L. A 28-day static toxicity study with *Chironomus riparius* exposed to diuron was conducted. Twenty midge larvae (2 days old) per nominal concentrations of 0.313, 0.625, 1.25, 2.50 and 5.00 mg a.s./L, a negative (dilution water) control and a solvent control (acetone) were tested. 600-mL glass beakers were used as test vessels. The sediment (1.5-cm layer) and overlying water (300 mL) were added to the test vessels two days prior to test initiation. The overlying water was gently aerated during the 28-day period. Four replicate vessels were established for the control, solvent control and each treatment level. Three additional replicate vessels were established to determine exposure concentrations of active substance in the overlying water, pore water and sediment. Test vessels were examined at least three times per week until test termination (day 28). Observations of midge emergence and abnormal behaviour were made. During the period of expected emergence (typically starting at day 10 and lasting until day 28), a daily check of emerged midges was made. The sex and number of adult midges that emerged were recorded daily.

The analysis of samples was performed for control, solvent control and all test item concentration at day 0 and after 28 days for control and the two highest test item concentrations 2.50 and 5.00 mg a.s./L.

The LOEC and NOEC were estimated for arcsine transformed emergence ratio and development rate. The NOEC and LOEC were determined by using a multiple comparison method. Where the data showed homogeneity of variance (Levene's test) and normal distribution (Shapiro-Wilk's test) a Williams test (α = 0.05; one sided smaller) was used for emergence ratio (ERarc) and development rate. The $EC_{10,\ 20,\ 50}$ for emergence ratio and development rate was evaluated by Probit analysis using linear maximum likelihood regression. Concentration dependent differences in sex of the emerged midges were tested by a Chi^2 Test (α = 0.05; one sided, greater). ToxRat Professional 2.10 was used for calculation.

Observations

The measured concentrations of diuron in the overlying water, pore water and sediment and the quantity of the respective sample were used to determine the total amount of diuron in the test vessels. The measured contents of diuron determined in the overlying water, pore water and sediment were summed up and compared to the nominal content of diuron.
The initially measured test item concentrations per test vessel were between 68 - 80 % of the nominal concentrations, with a mean measured initial concentration of 74 % of nominal. Since the mean measured initial concentration was below 80 - 120 % of nominal values, the biological endpoints were evaluated using nominal and initially measured test item concentrations.
The mean measured concentration at the tested concentration 2.5 mg/L (nominal) can be calculated by the geomean of initial measured (74 %) and measured at test termination (50 %). The mean of both values is 62 % and corresponds to a concentration of 1.55 mg/L.
No symptoms of toxicity were observed at the larvae, pupae and emerged midges during the study. The arcsine transformed emergence ratio was not statistically significantly decreased up to 5.00 mg a.s./L (nominal) compared to the solvent control. The NOEC for the arcsine transformed emergence ratio was therefore determined at 5.00 mg a.s./L (nominal) and 3.95 mg a.s./L (initially measured).

The development rate, as an indicator of an emergence delay, showed no significant differences to the control up to and including 2.50 mg/L (nominal). The highest tested diuron concentration caused 22 % inhibition of the development rate compared to the solvent control. The NOEC for development rate was therefore determined at 2.50 mg/L (nominal), corresponding to 1.85 mg a.s./L initially measured. The LOEC for development rate was determined at 5.00 mg a.s./L (nominal) and 3.95 mg a.s./L (initially measured)

The study was classified as valid as the following validity criteria were met: the emergence in the controls was 84 % and 89 % at the end of the test (validity criteria: ≥ 70 %); emergence of adults occured between 12 – 22 days in both controls after insertion into the vessels (validity criteria: 12 - 23 days); oxygen concentration was ≥ 79 % of air saturation value (validity criteria: ≥ 60 %). Furthermore, the pH of overlying water was determined to be 7.88 – 8.46 in all vessels (validity criteria: 6 – 9) and the water temperature differed not more than ± 1.0 °C (i.e. 19.6 – 20.3 °C).
Conclusion

The effects of diuron on *Chironomus riparius* were determined in a static 28-day chronic toxicity study according to OECD 219. Based on the nominal concentration the 28-day NOEC for the most sensitive endpoints (development rate) was determined to be 2.50 mg a.s./L (corresponding to 1.85 mg a.s./L of initially and 1.55 mg a.s./L mean measured concentration in overlying water, pore water and sediment). The EC_{10} and EC_{20} values were 4.04 mg a.s./L and > 5.00 mg a.s./L, respectively (corresponding to 2.99 and > 3.95 mg a.s./L initially measured concentrations of overlying water, pore water and sediment, respectively).
Since the mean measured initial concentrations and the two highest concentrations at test termination were below 80 % of the nominal concentrations, the NOEC, EC_{10} and EC_{20} have to be evaluated on the basis of mean measured concentrations. The study is valid and relevant for classification and labelling.

11.6.3 Chronic toxicity to algae or other aquatic plants

Study 1

Author:	Heimbach, F.
Title:	Growth Inhibition of Green Algae (Scenedesmus subspicatus)
Date:	1991
Report no	HBF/A1 88
Guidelines:	OECD No. 201 (1984)
GLP:	Yes
Validity:	Yes

Material and methods

Test type: static

Water media type: freshwater

Exposure duration: 96 h

Test temperature: 23 ± 2 °C

pH: 7.71 – 9.09

Test concentrations: Initial concentrations of test substance Control, 0.0001, 0.00032, 0.00056, 0.001, 0.0018, 0.0032, 0.0056, 0.01 and 0.032 mg diuron/L. The average of mean measured concentrations in the main test was 96.7 % of the nominal

Initial cells density: 1.0 x 104 cells/mL

Replicates: Test concentrations 3, control 6

Photoperiod: continuous

Light intensity: 8000 lux

Statistics: Probit analysis, Dunnett's test

Observation

The following inhibition of the growth rate and biomass was observed:

Test concentration	Percent inhibition			
	Growth rate		Biomass	
	72 h	96 h	72 h	96 h
Control	-	-	-	-
0.00010	-2.2	0.1	-12.1	-5.5
0.00032	-1.2	1.9	1.3	3.7
0.00056	1.5	1.2	-1.1	3.5
0.0010	0.6	-1.2	-6.4	-4.6
0.0018	10.7	2.1	17.3	17.1
0.0032	0.2	1.3	2.2	5.2
0.0056	18.2	11.5	51.1	47.3
0.010	26.6	23.7	57.6	64.8
0.032	59.5	62.7	81.5	90.7

Conclusion

A short-term acute toxicity test, in accordance with the testing guideline OECD 201 (1984) and GLP, is available for this substance. Based on measured concentrations, the 72h and 96h EC50 (growth rate) were calculated to be 22 µg/L test item, while the 72h and 96h EC50 (biomass) were calculated to be 9 µg/L and 7.9 µg/L, respectively. The NOEC (growth rate) was calculated to be 3.2 µg/L. The study is acceptable for classification purpose.

Study 2

Author: Wenzel, A.

Title: FRESHWATER CYANOBACTERIA, GROWTH INHIBITION TEST (OECD 201). DIURON (TECHNICAL): EFFECTS ON SYNECHOCOCCUS LEOPOLIENSIS

Date: 2015

Doc ID: ASA-001/4-10/C/1 (823-002)

Guidelines: OECD No. 201 (2011)

GLP: Yes

Validity: Yes

Material and methods

The acute toxicity of diuron (99.9 % purity) upon the growth of a freshwater cyanobacteria *(Synechococcus leopoliensis*) at nominal test concentrations of 0.100, 0.200, 0.632, 2.00, 6.32 and 20.0 µg a.s./L was observed over a period of 72 hours in a static test system. Four replicate flasks at each test concentration and eight control replicate flasks (only algal growth medium) were included in the test. Except for day one and

the highest test concentration, the cell concentrations were determined by measurements of chlorophyll fluorescence using a microplate reader (Synergy MX-Multi-Detections-Reader, Biotek Instruments Inc., 74177 Bad Friedrichshall, Germany) on the second and third day of the test. After 24 hours the cells were counted microscopically using a Fuchs-Rosenthal counting chamber (Mean cell number of five one square millimetre areas (depth 0.2 mm) x 5000 = cell counts/mL). Analytical samples were taken from all test item concentrations and from the control after 0, 24, 48 and 72 hours. All test item concentrations of 0 and 72 hours were analysed by high pressure liquid chromatography/mass spectrometry (HPLC-MS/MS) to verify the actual test concentrations of diuron using a validated analytical method.

Observations

Analytical samples indicate that diuron was still present at measurable levels after 72 h. The nominal concentrations had been verified by chemical analysis, except for the lowest concentration. With exception of the lowest tested diuron concentration the mean measured concentrations at test initiation were between 89.5 % and 107 % of nominal diuron concentrations, and between 93.5 % and 106 % of nominal in the 72 hour samples.

The study was classified as valid as the following validity criteria were met: cell numbers in the pooled controls were increased by a factor of 144 (validity criterion ≥ 16), the coefficient of variation of average growth in control cultures was 3.2 % (validity criterion: ≤ 10 %) and the mean coefficient of variation for the section-by-section specific growth rates in the control was 30 % (validity criterion: ≤ 35 %).

The average specific growth rate was estimated for each replicate flask during the experimental period using daily cell counts (see Table below). Statistically significant inhibitory effects for the test item were observed at a nominal concentration of 2.00 µg a.s./L and above after 72 hours for growth rate and yield. The highest test item concentration caused nearly a complete inhibition of growth rate and yield (96.0 and 99.8 %). The NOEC value was determined to be 0.632 µg a.s./L for yield and growth rate. The EC50 values for yield and growth rate were determined to be 2.81 µg a.s./L and 7.88 µg a.s./L, respectively. The nominal EC_{10} and EC_{20} value for growth rate were 3.70 and 4.80 µg a.s./L, respectively. The nominal EC_{10} and EC_{20} value for yield were 0.895 and 1.33 µg a.s/L, respectively.

Table 44: Inhibition of growth rates and yield of diuron determined from daily cell counts at 0, 24, 48 and 72 h

Nominal Concentration of test item	**Inhibition of growth rate [%]**	**Inhibition of yield [%]**
[µg/L]	**0-72 h**	**0-72 h**
Control[a]	-	-
0.100	-2.1	-12.0
0.200	1.8	9.5
0.632	0.6	3.1
2.00	8.3*	33.9*
6.32	34.3*	82.5*
20.0	96.0*	99.8*

[a] One control replicate was identified as outlier on day 2 and 3 (Outlier-test after Dixon & Hartley). The replicate was omitted from evaluation.

* Significantly different compared to control based on Williams t-test (growth rate) and Welch-t-test (yield), significance level 0.05, one-sided smaller

Table 45: Toxicity endpoints for growth rate and yield of *Synechococcus leopoliensis* after exposure to diuron

Parameter	EC_{10} [µg a.s./L]	EC_{20} [µg a.s./L]	EC_{50} [µg a.s./L]	NOEC [µg a.s./L]
72 h yield (95 % lower confidence interval – 95 % upper confidence interval)	0.895 (0.472 – 1.26)	1.33 (0.835 – 1.74)	2.81 (2.24 – 3.54)	0.632
72 h growth rate (95 % lower confidence interval – 95 % upper confidence interval)	3.70 (3.36 - 4.00)	4.80 (4.49 - 5.08)	7.88 (7.56 – 8.26)	0.632

Conclusion

In a static growth inhibition test with the cyanobacteria *Synechococcus leopoliensis* the effects of diuron were assessed over a test period of 72 h. Based on nominal concentrations the 72-hour EC_{50} values for growth rate and yield of *Synechococcus leopoliensis* were estimated to be 7.88 and 2.81 µg a.s./L, respectively. The 72 h NOEC values were estimated to be 0.632 µg a.s./L, for growth rate and yield.
The nominal EC_{10} and EC_{20} value for growth rate were 3.70 and 4.80 µg a.s./L, respectively. The nominal EC_{10} and EC_{20} value for yield were 0.895 and 1.33 µg a.s/L, respectively.
The study meets the control validity criteria and the requirements of the OECD guideline 201 (March 2001, adopted: 23 March 2006, Annex 5 corrected: 28 July 2011). Synechococcus leopoliensis is explicitly mentioned as test species in OECD 201. The study is valid and relevant for classification and labelling.

Study 3

Author: Falk, S.

Title: DIURON TC: TOXICITY TO THE DIATOM NAVICULA PELLICULOSA UNDER LABORATORY CONDITIONS

Date: 2016

Doc ID: S15-01978, (823-003)

Guidelines: OECD No. 201 (2011)

GLP: Yes

Validity: Yes

Material and methods

The toxicity of diuron (99.4 % purity) upon the growth of a freshwater diatom *(Navicula pelliculosa*) at nominal test concentrations of 0.417, 1.34, 4.28, 13.7, 43.8 and 140 µg a.s./L was observed over a period of 72 hours in a static test system. Three replicate flasks at each test concentration, six control replicate flasks (only algal growth medium) and six solvent replicates were included in the test. All stock and test solutions were prepared with test medium containing algae cells with an intended cell density of 1×10^4 algae per mL. The control group contained only algal growth medium. Cell concentrations were measured by fluorescence detection at 24, 48 and 72 h. Analytical samples were taken from all test item concentrations and from the control after 0, 24, 48 and 72 hours. All test item concentrations of 0 and 72 hours were analysed by high pressure liquid chromatography/mass spectrometry (HPLC-MS/MS) to verify the actual test concentrations of diuron using a validated analytical method.

Observation

Analytical samples indicate that diuron was still present at measurable levels after 72 h. The mean measured concentrations at test initiation were between 86 % and 103 % of nominal diuron concentrations, and between 88 % and 103 % of nominal in the aged samples (after 72 h). As the content of diuron was in the

range of 80 and 120 % of nominal at test start and end, all toxicological endpoints were evaluated based on the nominal test item concentrations.

The study was considered to be valid as the following validity criteria were met: cell numbers in the pooled controls were increased by a factor of 23.4 (validity criterion ≥ 16), the coefficient of variation of average growth in pooled replicate control cultures was 5.8 % (validity criterion: ≤ 10 %) and the mean coefficient of variation for the section-by-section specific growth rates in the control was 32.9 % (validity criterion: ≤ 35 %).

The average specific growth rate was estimated for each replicate flask during the experimental period using daily cell counts. Statistically significant inhibitory effects for the test item were observed at a nominal concentration of 13.7 µg a.s./L and above after 72 hours for growth rate and yield. The highest test item concentration caused a complete inhibition of growth rate and yield. The NOEC value was determined to be 4.28 µg a.s./L for yield and growth rate. The EC_{50} values for yield and growth rate were determined to be 11.2 µg a.s./L and 26.0 µg a.s./L, respectively. The nominal ErC_{10} and ErC_{20}-value were 7.08 and 11.1 µg test item/L, respectively. The nominal EyC_{10} and EyC_{20}-value were 3.29 and 5.17 µg a.s./L, respectively. The overall LOEC was determined to be at 13.7 µg a.s./L and the overall NOEC was determined to be at 4.28 µg a.s./L (nominal).

Conclusion

In a static growth inhibition test with the diatom *Navicula pelliculosa* the effects of diuron were assessed over a test period of 72 h. Since no statistically significant differences were observed between the control and solvent control after 72 h, both controls were pooled and all values were evaluated based on the pooled controls. Based on nominal concentrations the 72-hour EC_{50} values for growth rate and yield of *Navicula pelliculosa* were estimated to be 26.0 and 11.2 µg a.s./L, respectively. The nominal EC_{10} and EC_{20} value for growth rate were 7.08 and 11.1 µg a.s./L, respectively. The nominal EC_{10} and EC_{20}-value for yield were 3.29 and 5.17 µg a.s./L, respectively. The overall 72 h LOEC was determined to be at 13.7 µg/L and the overall NOEC was determined to be at 4.28 µg/L (nominal).

The study meets the control validity criteria and the requirements of the OECD guideline 201 (March 2001, adopted: 23 March 2006, Annex 5 corrected: 28 July 2011). The study is valid and relevant for classification and labelling.

Study 4

Author:	Wenzel, A.
Title:	MACROPHYTE GROWTH INHIBITION TEST - WATER-SEDIMENT CERATOPHYLLUM DEMERSUM TOXICITY TEST WITH DIURON EXPOSURE VIA THE WATER PHASE
Date:	2016
Doc ID:	LDG-001/4-12/B (851-008)
Guidelines:	OECD No. 239 (2014)
GLP:	Yes
Validity:	Yes

Material and methods

The toxicity of diuron (99.9 % purity) on the growth of the aquatic macrophyte *Ceratophyllum demersum* at nominal test concentrations of 1.00, 3.16, 10.0, 31.6 and 100 µg a.s./L was observed over a period of 14 days in a static test system. The test item was spiked to the water. Plants were grown in a static water-sediment system using artificial sterilised sediment overlaid with SMART AND BARKO medium. Five replicate test vessels were used for each treatment group and 10 replicates for the control group. Each vessel included three plants, which were healthy and without side shoots. Twenty additional plants of similar test population

size were selected and harvested at test initiation for shoot length, shoot wet weight and shoot dry weight measurement. These measurements were used to determine gain in biomass over the exposure period. The artificial sediment was prepared according to OECD Guideline No. 219 by mixing the following components (based on dry weight): 5 % sphagnum peat, 20 % kaolin clay and 75 % quartz sand (fine sand with more than 50 % of the particles between 50 and 200 microns) with 200 mg ammonium chloride and sodium phosphate per kg sediment. The constituents of the sediment were mixed homogenously. Water/nutrient solution (approx. 30 % w/w) was mixed thoroughly into this sediment.

On day 14 plants were harvested from each treatment group for assessment of shoot length, plant fresh weight, plant dry weight. Additionally the main shoot length and length of lateral branches were measured to calculate the total shoot length. Measurements were determined *in-situ* by using a ruler on days 0, 7 and 14 during the test.

Test item concentrations in the definitive test were verified by analysis of diuron at all concentration levels by analysing the overlying water at test start and test end and wet sediment at test termination on day 14.

All data were subjected to ANOVA. A test for normality of the data was carried out by calculating the Shapiro-Wilk's statistic. When treatment-effects were detected, a Williams' t-test, Dunnett's t-test or Welch t-test was performed to derive the No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC). The EC_{10}, EC_{20} and EC_{50} values were calculated by linear regression and Probit analysis modified for continuous data. All statistical analyses were conducted by the computer program ToxRat Professional®.

Observation

The measured concentration of the test item based on the diuron content in the test vessels at test start ranged between 86.6 and 121 % of nominal in the overlying water. The initial mean of the test item was 114 %. As the content of diuron was not between 80 and 120 % of nominal at test start all toxicological endpoints were evaluated using geometric mean measured concentrations of the test item. After 14 days, diuron concentrations in the water ranged between 24.8 and 77.3 % of nominal. In the sediment, concentrations of diuron were detectable for all concentrations, except the lowest concentration of 1.00 µg a.s./L. The total recovery (mass balance) of diuron in the whole test system (media, sediment, pore water) ranged between 40.2 and 98 % for the various treatments at the end of the test.
The control plants showed uniform growth over the test period of 14 days, with strongly growing side shoots. The study was considered to be valid as the following validity criteria were met: the total shoot length increased by a factor of 2.9 and fresh weight by a factor of 2.2 compared to the control treatment at day 14 (validity criterion: factor 2). The mean coefficient of variation for yield based on measurements of shoot fresh weight in the control cultures was 25.3 % at day 14 (validity criterion ≤ 35 %).
After 14 days the mean control total shoot length was 11.82 cm and 10.96, 10.96, 10.96, 13.5 and 13.37 cm in the test item geometric mean measured concentrations of 0.463, 2.10, 8.33, 28.1 and 91.4 µg a.s./L. No statistically significant inhibition was determined up to and including the highest tested concentration after 14 days of exposure for yield and growth rate. Therefore, the NOEC values after 14 days of exposure were determined to be ≥ 91.4 µg a.s./L with a corresponding LOEC value of > 91.4 µg a.s./L for yield and growth rate. For the parameter total shoot length the 14-day EC_{50} for growth rate and yield were determined to be > 91.4 µg a.s./L as well.
After 14 days the mean control fresh weight was 1930.7 mg. This compared to 1794.3, 1765.9, 1694.3, 1722.1 and 1645.4 mg exposed to geometric mean measured concentrations of 0.463, 2.10, 8.33, 28.1 and 91.4 µg a.s./L, respectively. Statistical comparison to the control identified no significant reduction in 14-day fresh weight (yield and growth rate) at all concentrations. Therefore, a 14-day NOEC of ≥ 91.4 µg a.s/L for yield and growth rate was determined. For the parameter fresh weight the 14-day EC_{50} for growth rate and yield were determined to be > 91.4 µg a.s./L as well.
Yield and Growth rate based on plant dry weight is presented in Table below. After 14 days the mean control dry weight was 220.1 mg. This compared to 206.5, 182.0, 169.0, 157.9 and 140.3 mg in the test item geometric mean measured concentrations of 0.463, 2.10, 8.33, 28.1 and 91.4 µg a.s./L. Statistical comparison to the control identified a significant reduction in 14-day dry weight for yield and growth rate at the concentrations ≥ 2.10 µg a.s./L which resulted in a 14-day NOEC of 0.463 µg a.s./L with a corresponding

14-day LOEC of 2.10 µg a.s./L. For the parameter dry weight the 14 day EC_{50} for yield was determined to be 13.8 µg a.s./L and the 14 day EC_{50} for growth rate 31.1 µg a.s./L.

Table 46: Mean yield and growth rate based on total plant dry weight of *Ceratophyllum demersum* after exposure to diuron

Geometric mean measured concentration [µg a.s./L]	Day 14 [mg]	Total plant dry weight [mg]			
		Yield [mg][1]	14-d inhibition of yield [%]	Growth rate [1/day]	14-d inhibition of growth rate [%]
Control	220.1	112.7	0.0	0.051	0.0
0.463	206.5	99.1	8.6	0.046	12.1
2.10	182.0	74.6*	26.8	0.037	33.8*
8.33	169.0	61.6*	36.5	0.032	45.3*
28.1	157.9	50.5*	46.2	0.027	55.2*
91.4	140.3	32.9*	62.6	0.019	70.8*

[1] based on 20 additional plants, representative of those used in the test (dry weight at test initiation = 85.9 mg)
* Significantly reduced compared to the control, based on Bonferroni-U Exact test

Table 47: Toxicity endpoints for growth rate and yield of *Ceratophyllum demersum* after exposure to diuron

Parameter	EC_{10} [µg a.s./L]	EC_{20} [µg a.s./L]	EC_{50} [µg a.s./L]	NOEC [µg a.s./L]
14 d yield (based on shoot length) (95 % confidence intervals)	n.d.	n.d.	> 91.4 (n.a.)	≥ 91.4
14 d yield (based on fresh weight) (95 % confidence intervals)	n.d.	2.26 (n.d.)	> 91.4 (n.a.)	≥ 91.4
14 d yield (based on dry weight) (95 % confidence intervals)	0.140*	0.678 (0.126 – 1.64)	13.8 (7.57 – 27.5)	0.463
14 d growth rate (based on shoot length) (95 % confidence intervals)	n.d	n.d.	> 91.4 (n.d.)	≥ 91.4
14 d growth rate (based on fresh weight) (95 % confidence intervals)	0.143* (n.d)	19.9 (n.d.)	> 91.4 (n.a.)	≥ 91.4
14 d growth rate (based on dry weight) (95 % confidence intervals)	0.267*	1.37 (0.343 – 2.94)	31.1 (17.8 – 67.9)	0.463

n.d. = not determined
* results are extrapolated

Following exposure to diuron, dry weight was found to be more sensitive than fresh weight and total shoot length for EC_{50} as indicated in the table above.

Conclusion

In a static growth inhibition test with the submerged rooted macrophyte *Ceratophyllum demersum* the intrinsic toxicity of diuron was assessed in a water-sediment test system over a 14 days period following the OECD Test Guideline 239. The test was evaluated using the geometric mean measured concentrations.
Dry weight was the most sensitive growth rate and yield parameter with an E_rC_{50} value of 31.1 µg a.s./L and an E_yC_{50} value of 13.8 µg a.s./L, respectively. E_rC_{50} and E_yC_{50} values of fresh weight and total shoot length could not be determined, since the observed effects were below 50 % compared to the control.
The lowest NOEC values for growth rate as well as yield were determined for dry weight. The NOEC for both parameters was 0.463 µg a.s./L. The E_rC_{10} for growth rate and dry weight was 0.267 µg a.s./L.
The study meets the control validity criteria and the requirements of the OECD 239 guideline (Sept 2014).. Considering the standardised test procedure, the quality of the test and the representativeness of the

worldwide occurring aquatic plant *C. demersum* for the trophic level of aquatic plants, the study is valid and relevant for classification and labelling.

Study 5

Author:	Wenzel, A.
Title:	MACROPHYTE GROWTH INHIBITION TEST - WATER-SEDIMENT CHARA GLOBULARIS TOXICITY TEST WITH DIURON EXPOSURE VIA THE WATER PHASE
Date:	2016
Doc ID:	LDG-001/4-12/D (851-009)
Guidelines:	OECD No. 239 (2014)
GLP:	Yes
Validity:	Yes

Materials and methods

The toxicity of diuron (99.9 % purity) on the growth of the aquatic macrophyte *Chara globularis* at nominal test concentrations of 1.00, 3.16, 10.0, 31.6 and 100 µg a.s./L was observed over a period of 14 days in a static test system. The test item was spiked to the water. Plants were grown in a static water-sediment system using artificial sterilised sediment overlaid with SMART AND BARKO medium. Five replicate test vessels were used for each treatment group and 10 replicates for the control group. Each vessel included three plants, which were healthy and without side shoots. Twenty additional plants of similar test population size were selected and harvested at test initiation for shoot length, shoot wet weight and shoot dry weight measurements. These measurements were used to determine gain in biomass over the exposure period. The artificial sediment was prepared according to OECD Guideline No. 219 by mixing the following components (based on dry weight): 5 % sphagnum peat, 20 % kaolin clay and 75 % quartz sand (fine sand with more than 50 % of the particles between 50 and 200 microns) with 200 mg ammonium chloride and sodium phosphate per kg sediment. The constituents of the sediment were mixed homogenously. Water/nutrient solution (approx. 30 % w/w) was mixed thoroughly into this sediment.
On day 14 plants were harvested from each treatment group for assessment of shoot length, plant fresh weight, plant dry weight. Additionally the main shoot length and length of lateral branches were measured to calculate the total shoot length. Measurements were determined *in-situ* by using a ruler on days 0, 7 and 14 during the test.
Test item concentrations in the definitive test were verified by analysis of diuron at all concentration levels by analysing the overlying water and wet sediment at test start and test end. The pore water was additionally measured of all test item concentrations at test termination.
All data were subjected to ANOVA. A test for normality of the data was carried out by calculating the Shapiro-Wilk's statistic. When treatment-effects were detected, a Williams' t-test or Welch t-test was performed to derive the No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC). The EC_{10}, EC_{20} and EC_{50} values were calculated by linear regression and probit analysis modified for continuous data. All statistical analyses were conducted by the computer program ToxRat Professional®.

Observation

The measured concentration of the test item based on the diuron content in the test vessels at test start and ranged between 110 and 157 % of nominal in the overlying water. As the content of diuron was ≥ 120 % of nominal at test start all toxicological endpoints were evaluated using geometric mean measured concentrations of the test item. After 14 days, diuron concentrations in the water phase ranged between 62.6 and 108 % of nominal. In the sediment, concentrations of diuron were detectable for all concentrations, except the lowest concentration (1.00 µg a.s./L). In the pore water samples diuron concentrations were below

the LOQ in all treatments. The total recovery (mass balance) of diuron in the whole test system (media, sediment, pore water) ranged between 62.7 and 74.3 % for the various treatments at the end of the test. The geometric mean measured diuron concentrations were 0.828, 3.26, 11.0, 40.7 and 124 µg a.s./L.
The control plants showed uniform growth over the test period of 14 days, with strongly growing side shoots and without visual symptoms of chlorosis. The study was considered to be valid as the following validity criteria were met: the total shoot length increased by a factor of 7.0 and of fresh weight of 7.3 compared to the control treatment at day 14 (validity criterion: factor > 2). The mean coefficient of variation for yield based on measurements of shoot fresh weight in the control cultures was 11.4 % at day 14 (validity criterion ≤ 35 %).

After 14 days the mean control total shoot length was 30.85 cm and 30.79, 26.95, 19.39, 14.15 and 11.48 cm in the geometric mean measured concentrations of 0.828, 3.26, 11.0, 40.7 and 124 µg a.s./L. Statistically significant inhibition was determined at the mean measured concentration of 3.26 µg a.s./L and above/all higher test concentrations after 14 days of exposure for yield. Therefore, a 14-day NOEC of 0.828 µg a.s./L for yield based on shoot length was determined. At 3.26 µg a.s./L 7.5 % inhibition of growth rate was significantly different from control, due to the low variability of the control replicates. However, since effects below 10 % are not considered to be ecotoxicologically relevant, the LOEC was set to 11.0 µg a.s./L and consequently, the NOEC to 3.26 µg a.s./L. For the parameter total shoot length the 14-day EC_{50} for growth rate and yield were determined to be 80.9 and 20.7 µg a.s./L, respectively.
After 14 days the mean control fresh weight was 233.6 mg. This compared to 258.0, 210.9, 179.2, 111.0 and 36.6 mg exposed to geometric mean measured concentrations of 0.828, 3.26, 11.0, 40.7 and 124 µg a.s./L, respectively. Statistical comparison to the control identified significant reduction in 14-day fresh weight for yield at test item concentrations of 11.0 - 124 µg a.s./L. Therefore, a 14-day NOEC of 3.26 µg a.s/L for yield was determined. For growth rate the NOEC based on fresh weight was suggested to be 3.26 µg a.s./L. However, no statistical significant difference compared to the control was determined for the highest concentration. For the parameter fresh weight the 14-day EC_{50} for growth rate and yield were determined to be 52.0 and 25.6 µg a.s./L, respectively.
After 14 days the mean control dry weight was 45.8 mg. This compared to 52.3, 44.2, 38.1, 23.6 and 8.3 mg in the geometric mean measured concentrations of 0.828, 3.26, 11.0, 40.7 and 124 µg a.s./L. Statistical comparison to the control identified a significant reduction in 14-day dry weight for yield at 11.0 µg a.s./L which resulted in a 14-day NOEC of 3.26 µg a.s./L with a corresponding 14-day LOEC of 11.0 µg a.s./L.
The NOEC for growth rate was set to be 11.0 µg a.s./L. For growth rate based on dry weight a LOEC of 40.7 µg a.s./L was calculated. The effects observed at the highest treatment level were not significantly different from the controls due to the high variability of the replicates. Since the dry weight of the plants decreased during the test period and the weights were very low, the data are not considered useful for the statistical NOEC assessment.
For the parameter dry weight the 14 day EC_{50} for yield was determined to be 29.2 µg a.s./L and the 14 day EC_{50} for growth rate 48.2 µg a.s./L.

The 14-day E_yC_{10}, E_yC_{20} and E_yC_{50} and NOEC values determined for this test as well as the 14-day E_rC_{10}, E_rC_{20} and E_rC_{50} and NOEC values are presented in Table below.

Table 48: Toxicity endpoints for growth rate and yield of *Chara globularis* after exposure to diuron

Parameter	EC_{10} [µg a.s./L]	EC_{20} [µg a.s./L]	EC_{50} [µg a.s./L]	NOEC [µg a.s./L]
14 d yield (based on shoot length) (95 % confidence intervals)	1.43 (0.597 – 2.49)	3.58 (1.96 – 5.36)	20.7 (15.3 – 29.1)	0.828
14 d yield (based on fresh weight) (95 % confidence intervals)	3.81 (1.46 – 6.40)	7.33 (3.75 – 10.8)	25.6 (18.6 – 36.6)	3.26
14 d yield (based on dry weight) (95 % confidence intervals)	6.19 (1.92 – 10.4)	10.5 (4.66 – 15.8)	29.2 (20.5 – 43.2)	3.26
14 d growth rate (based on shoot length) (95 % confidence intervals)	3.11 (1.09 – 5.65)	9.52 (5.11 – 14.3)	80.9 (52.9 – 157.6)	3.26*
14 d growth rate (based on fresh weight) (95 % confidence intervals)	10.1 (4.75 – 15.1)	17.7 (10.7 – 23.8)	52.0 (40.7 – 71.2)	3.26*
14 d growth rate (based on dry weight) (95 % confidence intervals)	12.9 (3.74 – 20.5)	20.3 (8.90 – 28.7)	48.2 (35.6 – 72.2)	11.0*

n.d. = not determined
* results on expert judgement

Conclusion

In a static growth inhibition test with the submerged rooted macrophyte *Chara globularis* the intrinsic toxicity of diuron was assessed in a water-sediment test system over a 14 days period following the OECD Test Guideline 239. The test was evaluated using the geometric mean measured concentrations.
Overall, the results indicate that the observed parameters of total shoot length, fresh weight and dry weight are in a close range regarding their observed effect concentrations. Dry weight was the most sensitive growth rate parameter with an E_rC_{50} value of 48.2 µg a.s./L. For fresh weight and total shoot length E_rC_{50} values of 52.0 and 80.9 µg a.s./L were determined for the test item diuron.
Total shoot length was the most sensitive yield parameter with an E_yC_{50} value of 20.7 µg a.s./L. For fresh weight and dry weight E_yC_{50} values of 25.6 and 29.2 mg a.s./L was determined for the test item diuron. For growth rate a NOEC value of 3.26 µg a.s./L was determined for total shoot length and fresh weight. The E_rC_{10} for growth rate and shoot length was 3.11 µg a.s./L. The NOEC for growth rate of dry weight was 11.0 µg a.s./L. For yield a NOEC value of 3.26 µg a.s./L was determined for total shoot length and fresh weight. The NOEC for yield of dry weight was 0.828 µg a.s./L.

The study meets the control validity criteria and the requirements of the OECD 239 guideline (Sept 2014).

The study is valid and relevant for classification and labelling.

Study 6

Author: Wenzel, A.

Title: MACROPHYTE GROWTH INHIBITION TEST - WATER-SEDIMENT ELODEA CANDENSIS TOXICITY TEST WITH DIURON EXPOSURE VIA THE WATER PHASE

Date: 2016

Doc ID: LDG-001/4-12/C (851-010)

Guidelines: OECD No. 239 (2014)

GLP: Yes

Validity: Yes

Materials and methods

The toxicity of diuron (99.9 % purity) on the growth of the aquatic macrophyte *Elodea canadensis* at

nominal test concentrations of 1.00, 3.16, 10.0, 31.6 and 100 µg a.s./L was observed over a period of 14 days in a static test system. The test item was spiked to the water. Plants were grown in a static water-sediment system using artificial sterilised sediment overlaid with SMART AND BARKO medium. Five replicate test vessels were used for each treatment group and 10 replicates for the control group. Each vessel included three plants, which were healthy and without side shoots. Twenty additional plants of similar test population size were selected and harvested at test initiation for shoot length, shoot wet weight and shoot dry weight measurements. These measurements were used to determine gain in biomass over the exposure period. The artificial sediment was prepared according to OECD Guideline No. 219 by mixing the following components (based on dry weight): 5 % sphagnum peat, 20 % kaolin clay and 75 % quartz sand (fine sand with more than 50 % of the particles between 50 and 200 microns) with 200 mg ammonium chloride and sodium phosphate per kg sediment. The constituents of the sediment were mixed homogenously. Water/nutrient solution (approx. 30 % w/w) was mixed thoroughly into this sediment.
On day 14 plants were harvested from each treatment group for assessment of shoot length, plant fresh weight, plant dry weight. Additionally the main shoot length and length of lateral branches were measured to calculate the total shoot length. Measurements were determined in-situ by using a ruler on days 0, 7 and 14 during the test.
All data were subjected to ANOVA. A test for normality of the data was carried out by calculating the Shapiro-Wilk's statistic. When treatment-effects were detected, a Williams' t-test or Welch t-test was performed to derive the No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC). The EC_{10}, EC_{20} and EC_{50} values were calculated by linear regression and probit analysis modified for continuous data. All statistical analyses were conducted by the computer program ToxRat Professional®.

Observation

The measured concentration of the test item based on the diuron content in the test vessels at test start ranged between 51 and 92 % of nominal in the overlying water. As the content of diuron was < 80 % of nominal at test start all toxicological endpoints were evaluated using geometric mean measured concentrations of the test item. After 14 days, diuron concentrations in the water ranged between 23 and 53.7 % of nominal. In the sediment, concentrations of diuron were detectable for all concentrations, except the two lowest concentrations (1.00 and 3.16 µg a.s./L). The total recovery (mass balance) of diuron in the whole test system (media, sediment, pore water) ranged between 58.8 and 101 % for the various treatments at the end of the test. The mean measured diuron concentrations were 0.342, 1.72, 5.86, 21.2 and 65.5 µg a.s./L.
The control plants showed uniform growth over the test period of 14 days, with strongly growing side shoots and without visual symptoms of chlorosis. The study was considered to be valid as the following validity criteria were met: the total shoot length increased by a factor of 3.1 and fresh weight increased by a factor of 2.6 compared to the control treatment at day 14 (validity criterion: factor > 2). The mean coefficient of variation for yield based on measurements of shoot fresh weight in the control cultures was 34.7 % at day 14 (validity criterion ≤ 35 %).

After 14 days the mean control total shoot length was 15.21 cm. This compared to 13.98, 11.96, 12.75, 10.47 and 8.37 cm in the test item geometric mean measured concentrations of 0.342, 1.72, 5.86, 21.2 and 65.5 µg a.s./L. A statistically significant inhibition was determined up to and including the two highest mean measured concentrations of 21.2 and 65.5 µg a.s./L after 14 days of exposure for yield and growth rate. Therefore, the NOEC values after 14 days of exposure were determined to be 5.86 µg a.s./L with a corresponding LOEC value of 21.2 µg a.s./L for yield and growth rate. For the parameter total shoot length the 14-day EC_{50} for growth rate and yield were determined to be 32.9 and 17.1 µg a.s./L, respectively.

After 14 days the mean control fresh weight was 257.8 mg. This compared to 223.9, 199.6, 219.5, 166.3 and 112.3 mg exposed to geometric mean measured concentrations of 0.342, 1.72, 5.86, 21.2 and 65.5 µg a.s./L., respectively. Statistical comparison to the control identified significant reduction in 14-day fresh weight (yield and growth rate) at test item concentrations of 21.2 and 65.5 µg a.s./L. Therefore, a 14-day NOEC of 5.86 µg a.s/L for yield and growth rate was determined. For the parameter fresh weight the 14-day EC_{50} for growth rate and yield were determined to be 16.6 and 8.18 µg a.s./L, respectively.

After 14 days the mean control dry weight was 42.1 mg. This compared to 38.7, 29.6, 35.7, 27.7 and 15.9 mg in the test item geometric mean measured concentrations of 0.342, 1.72, 5.86, 21.2 and 65.5 µg a.s./L.

Statistical comparison to the control identified a significant reduction in 14-day dry weight for yield and growth rate at the concentrations ≥ 21.2 µg a.s./L which resulted in a 14-day NOEC of 5.86 µg a.s./L with a corresponding 14-day LOEC of 21.2 µg a.s./L. For the parameter dry weight the 14 day EC_{50} for yield was determined to be 32.4 µg a.s./L and the 14 day EC_{50} for growth rate 17.8 µg a.s./L.
The 14-day E_yC_{10}, E_yC_{20} and E_yC_{50} and NOEC values determined for this test as well as the 14-day E_rC_{10}, E_rC_{20} and E_rC_{50} and NOEC values are presented in Table below.

Table 49: Toxicity endpoints for growth rate and yield of *Elodea canadensis* after exposure to diuron

Parameter	EC_{10} [µg a.s./L]	EC_{20} [µg a.s./L]	EC_{50} [µg a.s./L]	NOEC [µg a.s./L]]
14 d yield (based on shoot length) (95 % confidence intervals)	0.313* (n.d.)	1.24 (n.d.)	17.1 (6.67 – 78.4)	5.86
14 d yield (based on fresh weight) (95 % confidence intervals)	n.d.	0.371 (n.d.)	8.18 (1.79 – 55.3)	5.86
14 d yield (based on dry weight) (95 % confidence intervals)	0.7 (n.d.)	2.13 (n.d.)	17.8 (4.83 – 207.1)	5.86
14 d growth rate (based on shoot length) (95 % confidence intervals)	2.33 (n.d.)	5.77 (1.56 – 10.5)	32.9 (19.9 – 68.8)	5.86
14 d growth rate (based on fresh weight) (95 % confidence intervals)	0.278* (n.d.)	1.13 (n.d.)	16.6 (5.56 – 113.0)	5.86
14 d growth rate (based on dry weight) (95 % confidence intervals)	8.44 (0.512 – 16.0)	13.4 (1.98 – 22.2)	32.4 (18.1 – 59.9)	5.86

n.d. = not determined
* results are extrapolated

Conclusion

In a static growth inhibition test with the submerged rooted macrophyte *Elodea canadensis* the intrinsic toxicity of diuron was assessed in a water-sediment test system over a 14 days period following the OECD Test Guideline 239. The test was evaluated using the geometric mean measured concentrations.
Fresh weight was the most sensitive growth rate parameter with an E_rC_{50} value of 16.6 µg a.s./L. For dry weight and total shoot length E_rC_{50} values of 32.4 and 32.9 µg a.s./L was determined for the test item diuron. Fresh weight was the most sensitive yield parameter with an E_yC_{50} value of 8.18 µg a.s./L. For dry weight and total shoot length E_yC_{50} values of 17.8 and 17.1 µg a.s./L was determined for the test item diuron. A NOEC value of 5.86 µg a.s./L for growth rate as well as yield was determined for all observed endpoints (total shoot length, fresh weight, dry weight). The E_rC_{10} for growth rate and fresh weight was 0.278 µg a.s./L.
The study meets the control validity criteria and the requirements of the OECD 239 guideline (Sept 2014). The study is valid and relevant for classification and labelling

11.6.4 Chronic toxicity to other aquatic organisms

No relevant data

11.7 Comparison with the CLP criteria

11.7.1 Acute aquatic hazard

Acute aquatic toxicity data on diuron are available for fish, invertebrates, algae and aquatic plants. The lowest reliable acute endpoint is the 72 hours ErC_{50} = 0.00788 mg a.s./L for algae *Synechoccus leopoliensis.* Diuron can be classified as Aquatic Acute 1, with an M-factor 100 (0.001<L(E)C_{50} <=0.01 mg/L) based on the acute toxicity to algae.

11.7.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

Diuron fulfils the criteria for classification as Aquatic Chronic 1 since its chronic toxicity to aquatic species from all trophic level is below 0.1 mg/L (algae and aquatic plants as the most sensitive species *Synechoccus leopoliensis* 72 hours NOEC= 0.000632 mg/L and *Ceratophyllum demersum* 14 days ErC_{10}= 0.000267 mg/L, fish *Pimephales promelas* (fathead minnow) NOEC (60 days)= 0.033 mg/L and the invertebrate *Daphnia magna* 21 day NOEC= 0.096 mg/L) and combined with that the substance is not rapidly biodegradable. The M-factor for the Aquatic Chronic 1 classification is 100 (0.0001<NOEC=<0.001 mg/L.

Diuron has no potential for bioaccumulation since its log Pow value of 2.87 is below the trigger of 4 and no experimental study characterising the bioconcentration potential in fish is available.

Diuron is not rapidly degradable as it is stable to hydrolysis and the aerobic mineralisation in surface water (60 d) resulted in an extrapolated DT_{50} of 491 d (> 16 d).

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Hazard statement codes: *Hazardous to the aquatic environment*

Aquatic Acute 1; H400, M-factor 100

Aquatic Chronic 1; H410, M-factor 100

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

This endpoint is not addressed in this CLH report.

13 ADDITIONAL LABELLING

None

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15 ANNEXES

Diuron_RAR_08_Volume_3CA_B-6_2018-07-05

Diuron_RAR_10_Volume_3CA_B-8_2018-03-16