**Committee for Risk Assessment**

**RAC**

# Opinion

proposing harmonised classification and labelling
at EU level of

# spirodiclofen (ISO); 3-(2,4-dichlorophenyl)-2-oxo-1-xaspiro[4.5]dec-3-en-4-yl 2,2-dimethylbutyrate

**EC Number: -**
**CAS Number: 148477-71-8**

CLH-O-0000001412-86-135/F

**Adopted**

**9 December 2016**

9 December 2016

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

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

**Chemical name:** **spirodiclofen (ISO); 3-(2,4-dichlorophenyl)-2-oxo-1-oxaspiro[4.5]dec-3-en-4-yl 2,2-dimethylbutyrate**

**EC Number:** **-**

**CAS Number:** **148477-71-8**

The proposal was submitted by the **Netherlands** and received by RAC on **28 August 2015.**

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

# PROCESS FOR ADOPTION OF THE OPINION

**The Netherlands** has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **20 October 2015**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **4 December 2015**.

## ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Nikolaos Spetseris**

Co-Rapporteur, appointed by RAC: **Christina Tsitsimpikou**

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **9 December 2016** by **consensus**.

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

| | Index No | International Chemical Identification | EC No | CAS No | Classification | | Labelling | | | Specific Conc. Limits, M-factors | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Hazard Class and Category Code(s) | Hazard statementCode(s) | Pictogram, Signal Word Code(s) | Hazard statement Code(s) | Suppl. Hazard statement Code(s) | | |
| Current Annex VI entry | No current Annex VI entry | | | | | | | | | | |
| Dossier submitters proposal | TBD | spirodiclofen (ISO); 3-(2,4-dichlorophenyl)-2-oxo-1-oxaspiro[4.5]dec-3-en-4-yl 2,2-dimethylbutyrate | - | 148477-71-8 | Carc. 1B<br>Repr. 2<br>Skin Sens. 1B<br>STOT RE 2<br>Aquatic Chronic 1 | H350<br>H361f<br>H317<br>H373<br>H410 | GHS07<br>GHS08<br>GHS09<br>Dgr | H350<br>H361f<br>H317<br>H373<br>H410 | | M=10 | |
| RAC opinion | TBD | spirodiclofen (ISO); 3-(2,4-dichlorophenyl)-2-oxo-1-oxaspiro[4.5]dec-3-en-4-yl 2,2-dimethylbutyrate | - | 148477-71-8 | Carc. 1B<br>Repr. 2<br>Skin Sens. 1B<br>STOT RE 2<br>Aquatic Chronic 1 | H350<br>H361f<br>H317<br>H373<br>H410 | GHS07<br>GHS08<br>GHS09<br>Dgr | H350<br>H361f<br>H317<br>H373<br>H410 | | M=10 | |
| Resulting Annex VI entry if agreed by COM | TBD | spirodiclofen (ISO); 3-(2,4-dichlorophenyl)-2-oxo-1-oxaspiro[4.5]dec-3-en-4-yl 2,2-dimethylbutyrate | - | 148477-71-8 | Carc. 1B<br>Repr. 2<br>STOT RE 2<br>Skin Sens. 1B<br>Aquatic Chronic 1 | H350<br>H361f<br>H373<br>H317<br>H410 | GHS08<br>GHS07<br>GHS09<br>Dgr | H350<br>H361f<br>H373<br>H317<br>H410 | | M=10 | |

# GROUNDS FOR ADOPTION OF THE OPINION

## RAC general comment

Spirodiclofen (ISO) is an active substance used in plant protection products approved under Regulation (EC) No 1107/2009. It is mainly used as an acaricide or insecticide on various crops and fruits. The draft assessment report (DAR) has been peer reviewed by EFSA (EFSA, 2009). The degree of purity is ≥ 96.5% with five major impurities among which N,N-dimethylacetamide (DMAC, EC 204-826-4) has an harmonised classification as Repr. 1B (H360D) in Annex VI of the CLP Regulation.

Spirodiclofen (ISO) has not previously been assessed for harmonised classification and has no entry in Annex VI of the CLP Regulation (CLP). The current opinion differs however, from the conclusions drawn by EFSA during their assessment of the hazard classification of this substance. The EFSA peer review report was first issued in 2007 and re-issued in July 2009 at the request of Commission. Carc. 2 (R40) was proposed by EFSA (2009) who compared the data at that time against the Dangerous Substances Directive (67/548/EC) criteria which are qualitatively different to those of Regulation (EC) No 1272/2008 on the classification, labelling and packaging of substances and mixtures (CLP). No classification for fertility effects was proposed since all reproductive and endocrine-mediated toxicity effects were concluded to be of low potency and not considered for risk assessment by EFSA.

Additional information (some generated since 2009), was made available to RAC for the development of the current opinion. This comprised the full reports of all key Guideline studies, additional mechanistic studies & reviews, revised historical control data and statistical analysis of the tumours.

## RAC evaluation of physical hazards

### Summary of the Dossier Submitter's proposal

The Dossier Submitter (DS) did not propose classification for physical hazards. The data on physico-chemical properties did not indicate any concerns and as such, spirodiclofen does not meet the criteria for classification.

### Comments received during public consultation

There were no comments regarding the classification for physico-chemical hazards.

### Assessment and comparison with the classification criteria

Tests applied according to methods EC A. 14, EC A. 10 and EC A. 16 showed that spirodiclofen is not explosive, is not highly flammable and does not undergo spontaneous combustion. In addition, the structural formula of spirodiclofen does not contain any of the chemical groups characteristic of oxidizing agents. Therefore, it is regarded as incapable of reacting exothermically with combustible materials. Therefore RAC is in agreement with the DS that **classification is not required for physico-chemical hazards**.

# HUMAN HEALTH HAZARD EVALUATION

## RAC evaluation of acute toxicity

### Summary of the Dossier Submitter's proposal

A total of three guideline studies, one for each acute toxicity endpoint, are discussed.

***Oral***

Spirodiclofen was tested for acute oral toxicity in Wistar rats, according to OECD test guideline (TG) 423, GLP-compliant study (Krottlinger, 1996a). No deaths were observed at the dose tested (2000 mg/kg bw). No treatment related clinical signs of toxicity or effects on body weight were observed. No pathological abnormalities were observed at necropsy.

No classification for acute oral was proposed by the DS, as the $LD_{50}$ was >2000 mg/kg bw for rats.

***Dermal***

Spirodiclofen was tested for acute dermal toxicity in the Wistar rats, according to OECD test guideline (TG) 402, GLP-compliant study (Krottlinger, 1996b). No deaths were observed at the single dose tested, 2000 mg/kg bw. No treatment related clinical signs of toxicity or effects on body weight were observed. No pathological abnormalities were recorded at necropsy.

No classification for acute dermal was proposed by the DS, as the $LD_{50}$ was >2000 mg/kg bw for both males and females.

***Inhalation***

In an OECD TG 403 (GLP-compliant study) acute inhalation study (Pauluhn, 1997), rats (5/sex/dose) were nose-only exposed to two doses(520 and 5030 mg/$m^3$) of spirodiclofen for 4 hours. No deaths were observed at the limit concentration dose of 5030 mg/$m^3$. Statistically significantly decreased rectal temperatures were measured in females of both dose groups. No other treatment related clinical signs of toxicity or effects on body weight were observed. No pathological abnormalities were recorded at necropsy.

No classification for acute inhalation was proposed by the DS, as the $LC_{50}$ was >5.03 mg/L for both male and female rats.

### Comments received during public consultation

There was one comment from a MSCA received during the public consultation supporting the DS's proposal not to classify spirodiclofen for acute toxicity.

### Assessment and comparison with the classification criteria

Comparison with CLP criteria

***Oral***

Taking into account that the oral $LD_{50}$ value in male and female rats as reported in Krottlinger (1996a) exceeds the value for which classification for acute oral toxicity is justified (2000 mg/kg bw), RAC agrees with the DS, that spirodiclofen should **not be classified for acute oral toxicity** according to the CLP criteria.

***Dermal***

Taking into account that the dermal $LD_{50}$ value in male and female rats as reported in Krottlinger (1996b) is above the threshold value for classification (2000 mg/kg bw), RAC agrees with the DS, that spirodiclofen should **not be classified for acute dermal toxicity** according to the CLP criteria.

***Inhalation***

Taking into account that the inhalation $LC_{50}$ value in male and female rats as reported in Pauluhn(1997), is above the threshold value for classification (5 mg/L/4h), RAC agrees with the DS, that spirodiclofen should **not be classified for acute inhalation toxicity** according to the CLP criteria.

## RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

### Summary of the Dossier Submitter's proposal

In the available acute toxicity studies (Krottlinger, 1996a; Krottlinger, 1996b and, Pauluhn, 1997), no specific organ effects were observed after single acute exposure via the oral, inhalation or dermal route. In addition, in an available GLP compliant acute oral neurotoxicity study in Wistar rats (Sheets *et al.*, 2000), no compound related effects were observed up to the highest tested dose of 2000 mg/kg bw.

Therefore, based on the acute toxicity studies no classification is proposed by the DS for specific target organ toxicity (single exposure).

### Comments received during public consultation

There was one comment received from a MSCA during public consultation supporting the DS's proposal not to classify spirodiclofen for specific target organ toxicity (single exposure) - STOT SE.

### Assessment and comparison with the classification criteria

According to CLP criteria, 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 single exposure are classified in STOT SE 1 or 2. Classification should be supported by evidence associating single exposure to the substance with a consistent and identifiable toxic effect. Classification as STOT SE 3 is reserved for transient target organ effects and is limited to substances that have narcotic effects or cause respiratory tract irritation.

In the acute toxicity studies there were no clinical signs of toxicity following oral (Krottlinger, 1996a) and dermal exposure (Krottlinger, 1996b) to spirodiclofen. The decreased core temperature of females following inhalation of spirodiclofen aerosol particles that was not accompanied by any other pathological findings (Pauluhn, 1997) is not regarded relevant for classification. In addition, no effects were observed in the neurotoxicity study (Sheets *et al.*, 2000).

Therefore, there was no clear evidence of specific toxic effects at any target organ or tissue and no signs of respiratory tract irritation or narcotic effects were observed. RAC concludes that **no classification for specific target organ toxicity (single exposure) is warranted**.

## RAC evaluation of skin corrosion/irritation

### Summary of the Dossier Submitter's proposal

In the available skin irritation study (Leuschner, 1997a) conducted in accordance to OECD TG 404 (GLP compliant), no local irritation effects were observed after topical cutaneous application of spirodiclofen to the skin of Himalayan rabbits for 4h under semi-occlusive conditions.

Therefore, no classification was proposed by the DS for skin irritation/corrosion.

### Comments received during public consultation

There was one comment received from a MSCA during public consultation supporting the DS's proposal not to classify spirodiclofen as irritating or corrosive to the skin.

### Assessment and comparison with the classification criteria

Spirodiclofen was tested in a guideline compliant rabbit skin irritation study (Leuschner, 1997a).

Considering that no cutaneous irritation was observed during the available skin irritation study, no classification is required for spirodiclofen under the CLP Regulation. Therefore, RAC agrees with the DS's proposal that spirodiclofen **should not be classified as a skin irritant.**

## RAC evaluation of serious eye damage/irritation

### Summary of the Dossier Submitter's proposal

In the available eye irritation study in accordance to OECD TG 405 (GLP compliant) (Leuschner, 1997b), no eye irritation effects were observed after ocular application of spirodiclofen to the eyes of Himalayan male rabbits. The cornea, iris and conjunctiva were not affected by instillation of the test compound (all scores were zero at all time points).

Therefore, the DS proposed no classification for eye irritation for spirodiclofen under the CLP regulation.

### Comments received during public consultation

There was one comment received from a MSCA during public consultation supporting the DS's proposal not to classify spirodiclofen as irritating to the eye.

### Assessment and comparison with the classification criteria

A substance which has the potential to induce reversible eye irritation shall be classified in Category 2 (irritating to eyes) if when applied to the eye of an animal, it produces:

- at least in 2 of 3 tested animals, a positive response of:

    - Corneal opacity ≥ 1 and/or

- Iritis ≥1 and/or
- Conjunctival redness ≥ 2 and/or
- Conjunctival oedema ≥ 2

- Calculated as the mean scores following grading at 24, 48, 72 hours after instillation of the test material, and which fully reverse within an observation period of 21 days.

Considering that no eye irritation was observed during the available eye irritation study, no classification is required for spirodiclofen under the CLP Regulation for serious eye damage/eye irritation. Therefore, RAC agrees with DS for **no classification for eye damage/irritation** since neither irreversible nor reversible effects were observed.

## RAC evaluation of respiratory sensitisation

### Summary of the Dossier Submitter's proposal

No classification is proposed by the DS due to lack of data.

### Comments received during public consultation

There was one comment received from a MSCA during public consultation supporting the DS's proposal not to classify spirodiclofen as a respiratory sensitiser.

### Assessment and comparison with the classification criteria

No assessment and comparison with the classification criteria is possible due to lack of data.

## RAC evaluation of skin sensitisation

### Summary of the Dossier Submitter's proposal

In a Guinea PigMaximisation Test (GPMT) according to OECD TG 406 (GLP compliant) (Stropp, 1996), spirodiclofen was found to be a skin sensitiser since a skin reaction was observed in 40% of the animals in thetest group after the first and second challenge (0% in control group) at an intradermal induction dose of 5%. Therefore the effects of the test substance were considered positive and the DS proposed to classify spirodiclofen as a Skin Sensitiser 1B; H317: May cause an allergic skin reaction.

### Comments received during public consultation

Three comments were received from different MSCAs during public consultation supporting the DS's proposal to classify spirodiclofen as a Skin Sensitiser 1B; H317: May cause an allergic skin reaction.

### Assessment and comparison with the classification criteria

There is one Guinea Pig Maximization Test (GPMT, OECD TG 406) available to assess the skin sensitisation properties of spirodiclofen. The study is a summarised in the Table below.

Table: Summary of the results of the GPMT study.

| **Dose/group** | **First challenge*** | | **Second challenge*** | |
|---|---|---|---|---|
| | **48h** | **72h** | **48h** | **72h** |
| 5% intradermal injection (physical saline 2% Chemophor EL); challenge concentration 50% topical induction and challenges | 4/10 animals | 1/10 animals | 1/10 animals | 4/10 animals |
| Control group | 0 | | 0 | |

**One animal showed a positive reaction in both challenge treatments.*

When the data available is derived only from animal studies (GPMT), substances shall be classified as skin sensitizers in accordance with the following criteria:

- If there are positive results from an appropriate animal test (adjuvant type GPMT)
  - Cat. 1A: ≥ 30% responding at ≤ 0.1% intradermal induction dose or ≥ 60% responding at > 0. 1 to ≤ 1% intradermal induction dose; or
  - Cat. 1B: ≥ 30% to 60% responding at > 0.1 to ≤ 1% intradermal induction dose or ≥ 30% responding at > 1% intradermal induction dose.

The positive response in the Guinea Pig Maximization Test was 40% with an intradermal induction dose of > 1%. The data are considered sufficient for sub-classification: the response of 40% animals with positive skin reactions is comparably low at such a high intradermal induction dose of 5%. Therefore, RAC concluded that classification of spirodiclofen for skin sensitisation as **Skin Sens. 1B (H317: May cause an allergic skin reaction)** is appropriate.

## RAC evaluation of specific target organ toxicity– repeated exposure (STOT RE)

### Summary of the Dossier Submitter's proposal

The evaluation of the specific target organ toxicity – repeated exposure (STOT RE) hazard point was based on two mouse (Leser & Romeike, 1998; Wahle, 2000), two rat (Krottlinger, 2000; Wirnitzer, 1998) and four dog (Wetzig, 2000;Wetzig, 2001a; Wetzig, 2001b; Wetzig, 2001c) studies by the oral route and one rat study by the dermal route. In addition, data from the combined chronic toxicity/carcinogenicity (Wirnitzer, 2000), 2-generation reproductive toxicity (Eiben, 2000), acute neurotoxicity (Wirnitzer, 1998) and subchronic immunotoxicity (Sheets, 2001) studies were also considered.

***Oral***

Mouse

In the mouse some histopathological changes were observed in the liver, the adrenal glands and Leydig cells in the testes along with increased organ weights (adrenals, liver, testes) and altered haematological parameters (MCHC, WBC, neutrophils, lymphocytes).

However, the effect levels were mostly above the guidance value with the exception of centrilobular hepatocellular hypertrophy at a dose of 15.3mg/kg bw/d in the 13 week study. However, these effects do not represent severe effects, as there is no evidence of marked organ damage or dysfunction, as specified in the Guidance on the Application of the CLP Criteria. The DS concluded that the mouse repeated dose toxicity studies were not sufficient to classify spirodiclofen for STOT RE.

Rat

In rats, haematological changes and changes of clinical chemical parameters (mostly related to liver) were mainly observed at the highest dose-level tested after 14 weeks of exposure (males: 851.4 mg/kg bw/d, females: 995.8 mg/kg bw/d). Further, changes in absolute organ weights were observed (reduced in liver and spleen, increased in adrenals) and histological changes including increased adrenal cortical vacuolation. Non-neoplastic effects in the 2-year rat carcinogenicity study were mainly observed at the highest dose level and included changes in organ weights, increased adrenal hypertrophy/vacuolation and focal Leydig cell hyperplasia.

The effect levels in the 4-week, 14-week and 2-year rat repeated dose toxicity studies were above the guidance values for STOT RE 2 classification. However, increased small cortical vacuolation of the adrenals (dose-dependent) was observed in male animals at lower dose levels but they were within the range of historical controls. Additionally, the results from the combined chronic toxicity/carcinogenicity study , the 2-generation reproduction toxicity study and the 13-week subchronic neurotoxicity screening study did not report severe effects below guidance values. Therefore the DS concluded that the rat studies are not sufficient to classify spirodiclofen for STOT RE.

Dog

In dogs, the available repeated dose toxicity studies performed in dogs indicated that the adrenal glands, the liver and haematological parameters are the main target organs. The 14-week study revealed a significant reduction of Hb and Ht levels of 20% at the highest dose level (83 mg/kg bw/d). In all dog studies except the 1 year study, liver necrosis was observed at doses relevant for STOT RE 2 classification.

Additional relevant toxicological effects were observed in some animals below the guidance value for STOT RE 2. However, according to 3.9.1.4 of the CLP Guidance, also generalised changes of a less severe nature involving several organs should be taken into account. For the effects affecting the adrenal glands in dogs, the DS concluded that the effects were not severe enough. By contrast, the reduction in Hb and Ht in the 14 week dog study was around 20% at the highest dose level (83mg/kg bw/d) which is below the guidance value for STOT RE 2 were considered relevant. In addition, liver necrosis was also observed in all dog studies at doses relevant for STOT RE 2 classification. The latter two effects are considered by the DS as generalised changes of a less severe nature involving several organs and are taken into account. As it cannot be excluded that the observed effects in dogs are not relevant to humans, the DS proposed classification for STOT RE in category 2.

***Dermal***

A single experimental dose was applied (i.e. 1000 mg/kg bw/d) to Wistar rats for 22 times in a 28 days study according to OECD TG 410 and GLP (Kröttlinger, 1999). This effect-level was above the upper limit for STOT RE 2 classification (600 mg/kg bw/d for a 28-day study) and the type and severity of the observed effects do not fulfil the criteria for classification for STOT RE. The DS proposed no classification for the dermal route.

## Comments received during public consultation

Two MSCAs commented on the STOT RE 2 proposal by the DS.

The first MSCA stated that the proposed classification for spirodiclofen as STOT RE 2 is mainly based on effects observed in dogs which were inconsistent. Haematological effects were observed in the 14-week dog study and consisted of a dose-related reduction of haemoglobin and haematocrit of about 20%. Such effects though were not reproduced in the other dog studies. In

the 4-week dog study a non-dose related and not quantified decrease of Hb and Ht was observed. No such effects were found in the 8-week and 1-year studies. Given the inconsistencies of these haematological effects among the dog studies and the absence of haematological effects in other tested species (mouse and rat), the relevance of the classification as STOT RE for these effects was questioned.

In response to this statement the DS noted that although the effects on the haematological system were not observed in the 8-week and 1-year dog study, effects were observed in the 4-week and 14-week studies. Dose-related effects on Hb and Ht levels and % erythrocytes were observed in the 14-week oral dog study, in which a 20% decline of these parameters was observed at the highest dose level of 82.8 mg/kg bw/d (i.e. below the upper limit of 100 mg/kg bw/d for STOT RE 2). Additionally, in the 4-week oral dog study, haematological parameters were affected and reduced erythrocytes, Hb and Ht were observed at ≥ 65.5 mg/kg bw/d. However, no quantitative information is available in this 4-week dog study.

Moreover, the liver was found to be a target organ in the 4-week, 8-week, 14-week and 1-year dog studies. Effects included increased organ weight and increased biochemical parameters. Furthermore, hepatocellular necrosis was observed at 284.5 mg/kg bw/d in the 4-week study (i.e. below the upper limit of 300 mg/kg bw/d for STOT RE 2), at 55.9 mg/kg bw/d in the 8-week study (i.e. below the upper limit of 150 mg/kg bw/d for STOT RE 2) and 82.8 mg/kg bw/d in the 14-week study (i.e. below the upper limit of 100 mg/kg bw/d for STOT RE 2).

The DS acknowledged that some effects would not fulfil the classification criteria for STOT RE. However, according to section 3.9.1.4 of the CLP Guidance "Assessment shall take into consideration not only significant changes in a single organ or biological system but also generalised changes of a less severe nature involving several organs". Furthermore, according to section 3.9.2.5.2 of the CLP Guidance, a reduction in Hb of ≥20% would fulfil the classification criteria. In addition, necrosis is also one of the effects which fulfil the classification criteria as mentioned in and illustrated in an example of the Guidance on the application of the CLP criteria.

In summary, the DS proposed classification as STOT RE 2 since it cannot be excluded that the observed effects in dogs are relevant for humans. Given that the effective dose levels are below the upper limit of STOT RE 2, classification as STOT RE 2 was proposed.

The second MSCA noted that although effects seen on several target organs (liver, prostate) fulfil the criteria for being classified as STOT RE 2, there is one effect in particular (adrenal) that warrants classification in category 1 due to the rather low dose levels at which the effect occurs.

The DS responded with a review of the adrenal effects in all the species tested.

Most of the adrenal effects were observed at effective dose levels above the upper limit for STOT RE 2 and therefore do not warrant classification. However at individual level, some of the adrenal effects were observed below the upper limit for STOT RE 2 classification and even below the upper limit for STOT RE 1 classification. Effects included increased adrenal weight, cytoplasmic vacuolation and mononuclear cell infiltration in the adrenal cortex. Although these effects clearly point towards the adrenal glands as a target organ, they were considered by the MSCA not severe enough to fulfil the classification criteria (i.e. no evidence of marked organ damage or dysfunction).

## Assessment and comparison with the classification criteria

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 (i.e. were observed in a 90-day repeated-dose study conducted in experimental animals below the guidance value range of 10 mg/kg bw/d or 20 mg/kg bw/d for oral and dermal exposure respectively).

Substances are classified in category 2 for target organ toxicity (repeated exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were observed in a 90-day repeated-dose study conducted in experimental animals within the guidance value ranges of 10-100 mg/kg bw/d for oral exposure or 20-200 mg/kg bw/d for dermal exposure.

***Oral***

Given that no human data are available and the effective dose levels in the repeated dose animal studies were above the upper limit for STOT RE 1 (i.e. 30 mg/kg bw/d for a 4-week study, 15mg/kg bw/d for a 8-week study, 10 mg/kg bw/d for a 90-day study, 2.5 mg/kg bw/d for a 1-year study), classification for STOR RE 1 is not considered.

Effect-levels in the mouse repeated dose toxicity studies (13-week and 18 month) were mostly above the upper limit for STOT RE 2 classification (100 mg/kg bw/d for a 90-day study, 25mg/kg bw/d for a 1-year study). However, in the 13-week study centrilobular hepatocellular hypertrophy was observed in 3 out of 10 male animals (grade 2: 3/3 animals) at a dose of 15.3mg/kg bw/d. These effects do not fulfil the classification criteria based on the observed severity (as there is no evidence of marked organ damage as described in the CLP guidance). Based on the mouse repeated dose toxicity studies, classification is not required.

Effect levels in the 4-week, 14-week and 2-year rat repeated dose toxicity studies were mostly above the upper limit for STOT RE 2 classification (300 mg/kg bw/d for a 4-week study, 100 mg/kg bw/d for a 90-day study, 12.5 mg/kg bw/d for a 2-year study). However, increased small cortical vacuolation of the adrenal glands (with increased grading) was observed in male animals at dose levels of 6.6 and 32.1 mg/kg bw/d and higher. However, according to the DS, this was within the range of historical controls (Hartmann, 2005). Based on the rat repeated dose toxicity studies, classification is not required.

Effect levels in the available dog repeated dose toxicity studies were around or below the upper limit for STOT RE 2 classification (300 mg/kg bw/d for a 4-week study, 150 mg/kg bw/d for an 8-week study, 100 mg/kg bw/d for a 90-day study, 25 mg/kg bw/d for a 1-year study). In most studies, the highest dose tested was below the upper limit for STOT RE 2 classification. At the highest dose level tested many parameters were effected. Most effects individually would not fulfil the classification criteria. However, according to 3.9.1.4 of the CLP Guidance also generalised changes of a less severe nature involving several organs should be taken into account. Further, the reduction in Hb and Ht in the 14 week dog study was around 20% at the highest dose level (83 mg/kg bw/d) which was below the upper limit for STOT RE 2. In the same study there was a reduction in the reticulocytes exceeding 50% observed in males only. In the 4-week study in dogs at 2000 ppm a reduction of Hb up to 9% in 50% of the animals was observed while at 1000 ppm the decrease reaches 12 % in all animals. No statistical effects on reticulocytes was observed. In addition, in the 4-, 8- and 14 –week dog studies liver necrosis was observed in doses relevant for STOT RE 2 classification. The adrenal effects were not considered since histopathological analysis did not reveal severe organ damage and/or dysfunction. As it cannot be excluded that the observed haematological and liver effects in dogs are relevant for evaluating potential effects of spirodiclofen in humans, the observed effects should be taken into account for potential classification for STOT RE. Together,the available information in dogs warrant classification for STOT RE in category 2.

The results of the combined chronic toxicity/carcinogenicity studies and 2-generation rat study do not warrant classification for STOT RE.

The results of the neurotoxicity and immunotoxicity studies do not warrant classification for STOT RE.

***Dermal***

A single experimental dose was applied (i.e. 1000 mg/kg bw/d). This effect-level was above the upper limit for STOT RE 2 classification (600 mg/kg bw/d for a 28-day study) and the type and severity of the observed effects do not fulfil the criteria for classification for STOT RE.

Therefore, classification for the dermal route is not required.

In summary, based on the available studies, classification for specific organ toxicity – repeated exposure as STOT RE 2 (H373: May cause damage to organs, through prolonged or repeated exposure) is required. This classification applies to all routes as comparable effects after inhalation exposure cannot be excluded. As the classification is mainly based on the general effects on several organs, no specific target organ is proposed.

**Comparison with CLP criteria**

In the table below, all the effects relevant for STOT RE observed in the three dog studies are summarised. Effects reported in mouse and rats studies are not reported since they were all above guidance values and not considered severe. However, the liver and the blood are also target organs in those species.

Table: Summary of effects on STOT RE parameters in available repeated dose toxicity in dogs

| **Dose/target organs** | **≤ Guidance Value for STOT RE 2** |
|---|---|
| Dog 4-week, oral, 2m+2f, (0, 11.3, 65.5, 284.5 mg/kg bw/day)<br>(STOT RE 2 ≤ 300 mg/kg bw/day)* | |
| general | Effects on haematology, ↓ kidney weight, ↑ weights of uterus,<br>adrenals and brains, Leydig cell vacuolation, cytoplasmic vacuolation adrenal cortex. |
| hematology | ↓ Hb, Ht, Lymphocytes (%) |
| adrenals | ↑ar weight,<br>cytoplasmic cortex vacuolation 1/4, 1/4, 4/4, 4/4 (1,2,1,3) |
| liver | increased ALAT, increased enzyme activities in liver tissue (N-DEM, O-DEM, P450, ECOD, ALD, EH, Glu-T),<br>periportal single cell necrosis: 0/4, 0/4, 0/4, 4/4 (2,1,2,1) |
| clinical chemistry | ↓cholesterol, triglycerides |
| Dog 8-week, oral, 5m, (0, 2.9, 55.9 mg/kg bw/day)<br>(STOT RE 2 ≤ 150 mg/kg bw/day)* | |
| general | ↑ AP, ↑ organ weights of thyroid, adrenals, thymus and pancreas, ↓ prostate weight |
| hematology | no toxicologically relevant effects |
| adrenals | cytoplasmic cortex vacuolation 0/5, 4/5, 5/5<br>mononuclear cell infiltration adrenal cortex 0/5, 1/5, 3/5 |
| liver | Increased AP, increased organ weights of liver<br>hepatocellular single cell necrosis 0/5, 0/5, 3/5 |
| clinical chemistry | ↓cholesterol, triglycerides, ↑LH |
| Dog 14-week, oral, 4/sex/dose, (0, 8.0, 27.3, 82.8 mg/kg bw/day)<br>(STOT RE 2 ≤ 100 mg/kg bw/day)* | |
| general | Effects on haematological parameters, clinical biochemistry, changes relative prostate weight and histopathological changes in the adrenal gland |
| hematology | ~ 20% ↓ Hb, Ht, and ↓lymphocytes (%), |

| | |
|---|---|
| adrenals | ↑r adrenal weight (m, f)<br>vacuolisation zona fasciculata, cortex 0/4, 2/4, 3/4, 4/4 (f), 0/4, 0/4, 3/4, 4/4 (m)<br>mononuclear cell infiltration 0/4, 2/4, 0/4, 4/4 (f), 0/4, 1/4, 1/4, 4/4 (m) |
| liver | ↑liver microsomal enzymes<br>periportal single cell necrosis 0/4, 0/4, 1/4 (f) |
| clinical chemistry | various parameters affected at 27.3 mg/kg bw/day |
| organ weights | various organs affected at 27.3 mg/kg bw/day, thymus ↓r |
| Dog 1-year, oral, 4 sex/dose, (0, 0.57, 1.45, 4.54, 16.9 mg/kg bw/day)<br>(STOT RE 2 ≤ 25 mg/kg bw/day)* | |
| general | ↑ adrenal weight and adrenal vacuolation |
| hematology | no toxicologically relevant effects |
| adrenals | ≥ 0.57 mg/kg bw/day ↑ar adrenal weight (m)<br>≥ 4.54 ↑ar adrenal weight (f)<br>↑ vacuolation 1/4, 2/4, 0/4, 4/4, 4/4 (m)<br>↑ vacuolation 1/4, 1/4, 0/4, 3/4, 4/4 (f) |
| liver | cytoplasmic inclusion 0/4, 0/4, 1/4, 1/4, 2/4 (m),<br>pigment 0/4, 0/4, /4, 0/4, 1/4, 2,4 (f) |
| clinical chemistry<br>organ weights | ↑ cholesterol (m), ↓cholesterol (f)<br>various organs affected at 0.57 mg/kg bw/day |

* no general toxicity observed; a: absolute; r, relative; m, male; f, female.

In the oral repeated dose toxicity studies in the **mouse** (13-week & 18-month) there was no evidence for severe liver effects such as organ lesions or dysfunction. Therefore, RAC agrees with the DS that classification based on the mouse studies is not justified.

The effect levels in the 4-week, 14-week and 2-year **rat** repeated dose toxicity studies were mostly above the upper limit for STOT RE 2 classificationIncreased small cortical vacuolisation of the adrenals (with increased grading in function of the dose) was observed in male animals at dose levels of 6.6 and 32.1 mg/kg bw/d and higher in the 14-week study (below the guidance valuefor STOT RE 2 classification). However this was within the range of historical controls. Additionally, the results from the combined chronic toxicity/carcinogenicity, 2-generation reproductive toxicity, neurotoxicity and immunotoxicity studies did not warrant classification. therefore, based on the aforementioned rat studies, RAC agrees with the DS for classification for STOT RE is not justified.

In the available repeated dose toxicity studies in **dogs** (4-week, 8-week, 14-weekand 1-year) many parameters of various organ systems were affected including the haematological system, the liver and the adrenals. RAC is of the opinion and agrees with the DS that the observed adrenal effects in dogs (cytoplasmic vacuolisation and mononuclear cell infiltration adrenal cortex effects) are not severe and do not fulfil the CLP criteria for STOT RE classification.

Effects on the haematological system were not observed in the 8-week and 1-year dog studies. However, they were seen in the 4-week and 14-week dog studies. In both studies the effect-levels were below the upper limit for STOT RE 2. In the 4-week study reduced erythrocytes, Hb and Ht were observed. In the 14-week study though, a dose related effect on Hb and Ht levels and % erythrocytes was seen and at the highest dose level a 20 % decline of these parameters was observed which is considered a consistent and adverse effect in haematology (Guidance on the application of CLP criteria, Annex 3.9.2.7.3.(c)).

The liver was also identified as a target organ in dogs. Effects included increased organ weight and increased biochemical parameters. Also hepatocellular necrosis was observed although at effect-levels below the upper limit for STOT RE 2 classification.

In conclusion, RAC agrees with the DS's proposal to classify spirodiclofen as **STOT RE 2 (H373: May cause damage to organs through prolonged or repeated exposure)** based on the dog data. The classification is based on haematology and liver effects and apply to all routes of exposure with no specific organ specified.

## RAC evaluation of germ cell mutagenicity

### Summary of the Dossier Submitter's proposal

The evaluation of the germ cell mutagenicity hazard endpoint was based on three *in vitro* and one *in vivo* study.

- In the *in vitro* bacterial reverse mutation test according to OECD TG 471 (GLP compliant) spirodiclofen did not induce mutations in *S.typhimurium* (Herbold, 1996a).
- In the *in vitro* mammalian cell gene mutation test using the hprt assay according to OECD TG 476 (GLP compliant), spirodiclofen did not induce gene mutations (Brendler-Swaab, 1997).
- In the *in vitro* chromosome aberration study (OECD TG 473, GLP compliant) statistically significant increased values of aberrations were observed both in the absence and in the presence of S9 but these increased values were within the range of historical control values. Therefore, the biological relevance of this observation is considered low and spirodiclofen considered as not-clastogenic in mammalian cells *in vitro* (Herbold, 1996b).
- In the *in vivo* micronucleus test (bone marrow) (OECD TG 474, GLP compliant) spirodiclofen did not induce micronuclei in mouse bone marrow cells (Herbold, 1996c).

Based on the negative results of the available *in vitro* and *in vivo* mutagenicity tests the DS proposed no classification for mutagenicity for spirodiclofen.

### Comments received during public consultation

There was one comment received from a MSCA during public consultation supporting the DS's proposal not to classify spirodiclofen for mutagenicity.

### Assessment and comparison with the classification criteria

Considering that no positive responses were observed in the available *in vitro* and *in vivo* mutagenicity tests, RAC agrees that **no classification is warrented for spirodiclofen for mutagenicity** under the CLP Regulation.

## RAC evaluation of carcinogenicity

### Summary of the Dossier Submitter's proposal

The evaluation of the carcinogenicity hazard endpoint by the DS was based on two long-term oral studies on rodents.

In the mouse study (Wahle, 2000, OECD TG 451, GLP compliant), observed effects included decreased body weight, changes in haematological parameters and organ weights of adrenal glands, kidneys, liver and testes in the mid and high dosed groups. Histopathological examination showed effects on testes (increased incidence and severity of interstitial cell hypertrophy/hyperplasia), epididymides (increased incidence of aspermia), adrenals (vacuolation

and pigmentation, increased incidence and severity) and liver (hepatocytomegaly). Remarkable was the increased incidence/increased average severity of amyloid in several tissues in all dose groups (heart, liver, thyroids and parathyroids of males). Based on the observed adrenal pigmentation and vacuolation, hepatocytomegaly and amyloid in all dose groups, a NOAEL could not be established in this study and the LOAEL for chronic toxicity is 4.1 mg/kg bw/d. Spirodiclofen is considered carcinogenic for inducing liver tumours in the mouse. Hepatocellular tumours were observed at and above 610 mg/kg bw/d. A significant increased combined (benign and malignant) frequency of hepatocellular neoplasms over controls was observed. The incidence of hepatocellular carcinoma was however not significantly increased upon exposure to spirodiclofen. The NOAEL for neoplastic lesions is therefore 4.1 mg/kg bw/d.

In the rat study (Wirnitzer, 2000), substance-related effects were mainly observed in the two highest dose groups and included decreased body weight, changes in haematology and clinical biochemistry (AP, cholesterol, triglycerides) and organ weights of adrenal glands, liver, thymus, testes and ovaries. Histopathologically, effects were observed in testes (Leydig cell hyperplasia), adrenals (hypertrophy/vacuolation of cortex cells) and jejunum (vacuolation of enterocytes), whereas neoplastic lesions were observed in the highest dose group in testes (Leydig cell tumours) and uterus (adenocarcinoma). The NOAEL for chronic toxicity is set at 100 ppm, equivalent to 5.93 mg/kg bw/d. Spirodiclofen is considered carcinogenic for inducing (benign) Leydig cell tumours and uterus adenocarcinomas in the rat. These tumours were observed at and above 110.14 mg/kg bw/d; the NOAEL for neoplastic lesions is therefore 14.72 mg/kg bw/d.

***Potential mechanism and human relevance***

Based on the mutagenicity tests, the mechanism for the potential carcinogenic effect is probably nongenotoxic.

Spirodiclofen induced testicular and uterine carcinogenicity in rats. The same effect did not occur in mice and dogs, which might point towards a species-specificity for the rat. The observed uterus tumours in rats were of a malignant type. No information is available which might point towards a potential irrelevance of this tumour type for humans. The observed testes tumours (Leydig cell tumours) were of a benign type. Spirodiclofen was shown to induce enlargement of the testes, hypertrophy and hyperplasia, which might be further related to the observed testes tumour formation (Leydig cells tumour) in rats. It is known that some tumour types occur with a high spontaneous incidence or are not relevant for humans. Leydig cell adenomas are observed with a high spontaneous incidence in male F344 rats (according to section 3.6.2.2.6-of the CLP Guidance), but not in male Wistar rats which is the rat strain used in the combined chronic/carcinogenicity rat study. Leydig cell adenomas induced by dopamine antagonists or gonadotropin-releasing hormones (GnRH) are considered not relevant for humans (according to section 3.6.2.2.6-k of the CLP Guidance). Therefore, mechanistic studies were performed to identify the underlying mechanism of the observed effects on testes and adrenals. The mechanistic studies showed that spirodiclofen clearly interferes with steroid hormone synthesis in the adrenals and gonads. Additionally, it was noted that the effect on steroidogenesis is probably mediated by effects on general biochemical pathways (interference with the formation of mitochondrial and cytoplasmatic NADPH, which is an important co-substrate in several steps of the biosynthesis of steroid hormones), and that no androgenic, anti-androgenic, estrogenic or anti-estrogenic effects were noted in the mechanistic studies. Further, a direct effect of spirodiclofen on enzymes involved in the synthesis of steroid hormones in the testes (microsomal hydrogenases) could not be excluded. Considering all of the above data, it cannot be excluded that the Leydig cell tumour formation (including the underlying mechanism) is relevant for humans.

In mice, a significantly increased frequency of hepatocellular adenomas over controls was observed in males. Further, a dose-related increase (not statistically significant) of malignant

hepatic tumour types (carcinomas) was observed in male animals. The combined frequency of hepatocellular neoplasms (adenomas and carcinomas) was also significantly increased. Furthermore, liver hypertrophy was observed in the 13-week and chronic study (liver hepatocytomegaly) which might be related to the observed hepatic neoplasms. It is known that some tumour types occur with a high spontaneous tumour incidence or are not relevant for humans. Liver tumours are observed with a high spontaneous incidence in B6C3F1mice (according to section 3.6.2.2.6-of the CLP Guidance). However, this is not the case for CD-1 mice which is the mouse strain used in the combined chronic/carcinogenicitymouse study. Further, liver tumours in rodents conclusively linked to peroxisome proliferation are considered not relevant for humans (according to section 3.6.2.2.6-k of the CLP Guidance). This specific mechanism (peroxisome proliferation) is not operating for spirodiclofen-induced liver tumours in mice. A potential mechanism for the hepatocellular adenomas and carcinomas is discussed by the notifier as follows: "a potential mechanism for the hepatocellular adenomas and carcinomas found in mice might be an induction of CYP450-dependent liver enzymes, which might subsequently result in liver hypertrophy, hyperplasia and hepatic tumour formation as it is also shown for organochlorine pesticides according to the notifier. Tumour induction by organochlorine pesticides was not observed in other species, including humans. Spirodiclofen induced liver tumours in mice are, therefore, deemed as mouse specific and not of relevance to humans. " P450 induction was observed in the 4-, 8-, 14-week, 1-year dog studies and the 4-week rat studies, however not in any of the available mouse studies. Further, no information is available which demonstrates that spirodiclofen-mediated CYP450-induction and subsequent liver tumour formation might not be relevant for humans. Therefore, a potential irrelevance for humans is not clearly demonstrated for the spirodiclofen induced liver tumours.

## Comments received during public consultation

Three MSCAs commented and supported the DS's proposal to classify spirodiclofen as Carc.1B (H350: May cause cancer).

The first MSCA noted that in the mouse study (CD-1), spirodiclofen induced a significantly increase of hepatocellular adenomas in males. Furthermore, a dose-related increase (not statistically significant) of malignant hepatocellular tumour (carcinomas) was observed in male animals. The combined frequency of adenomas and carcinomas was also significantly increased. As a potential irrelevance for humans is not clearly demonstrated, these effects should be taken into account for classification of spirodiclofen for carcinogenicity in humans. In rat (Wistar), spirodiclofen induced neoplastic effects in testes and uterus. The tumours in the testes (Leydig cell tumours) are benign, while the tumours in the uterus (uterus adenocarcinomas) are malignant. The mechanistic studies showed that spirodiclofen clearly interferes with steroid hormone synthesis in the adrenals and gonads. For both tumour types, it cannot be excluded that they could be relevant for humans, and should be taken into account for the classification of spirodiclofen.

In conclusion, there was a combination of benign and malignant neoplasms of potential relevance to humans in two species and therefore, there is sufficient evidence for classification of spirodiclofen as a Carc.1B.

The second MSCA noted that since the available long-term oral carcinogenicity studies showed that spirodiclofen induced adenocarcinomas in the uterus and benign Leydig cell tumours in rats, and hepatocellular carcinomas and adenomas in mice, there is evidence matching the criteria for classification of spirodiclofen as a Category 1B carcinogen. In addition, 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 two or more species of animals.

The third MSCA agreed with the proposed classification but identified two important points for further evaluation. Firstly, there is a new publication (Yoshida *et al.,* 2015) providing information on the mode of action of spirodiclofen which was not included in the CLH report. The study focused on predictive modes of action of pesticides in uterine adenocarcinoma development in rats. The DS evaluated the publication, a short summary is included below.

Yoshida *et al.*(2015) evaluated pesticides for potential uterine carcinogenicity and attempted to predict their mechanism using parameters from mechanistic and toxicity studies. Five pathways for uterine carcinogenesis in rodents were presented (of which the first three appear to be accepted as the major pathways): 1) estrogenic activity, 2) increased serum 17beta-estradiol (E2) to progesterone (P4) ratio, 3) modulation of oestrogen metabolism to produce 4-hydroxyestradiol via P450 induction, 4) inhibition of oestrogen excretion and 5) increased aromatase *in situ* in the tumour.

Their evaluation of a total of 300 pesticides revealed that seven chemicals increased uterine tumour formation in rats, and the pathways of 4 chemicals (including spirodiclofen) could be predicted based on various mechanistic studies. The mode of action of spirodiclofen was predicted to be increased serum 17beta-estradiol (E2) to progesterone (P4) ratio given that mechanistic studies showed that E2-levels did not change while P4-levels decreased.

The second point the MSCA focused on the historical control data (HCD). In the oncogenicity study in CD-1 mice (Wahle, 2000) significantly increased incidences of hepatocellular adenoma and a significantly increased combined frequency of hepatocellular adenomas and carcinomas were found in males of the mid- and high-dose group. A dose-related but not significant increase of hepatocellular carcinomas was found in male mice.

In the combined chronic toxicity and carcinogenicity study in Wistar rats (Wimitzer*et al.,* 2000) increased incidences in benign Leydig cell tumours and malignant uterus adenocarcinomas (both not statistically significant) were found in the high-dose group. An occurrence of thyroid C-cell adenoma and carcinoma in female Wistar rats was considered to be irrelevant based on HCD. It is also well known though that Leydig cell tumours and hepatocellular adenomas occur spontaneously and with a high variability in certain rat and mice strains (Section 3.6.2.3.2 insee Guidance on the application of the CLP criteria), although not in the strains used in the specific studies. An inquiry by the commenting MSCA on publicly available HCD revealed (although certain limitations regarding differences in laboratories, animal specification, time window broadness and time window distance existed) that the combined multiplicity of data indicates a relatively high spontaneous occurrence and variability in incidences of hepatocellular tumours in CD-1 mice and of Leydig cell tumours in Wistar rats, the strains used in the carcinogenicity studies of the CLH report. Based on this HCD according to the MSCA all tumour types except the malignant uterus adenocarcinoma could be considered to lie within the HCD.

The DS addressed the HCD using the Guidance on the Application of the CLP criteria (November 2015): "*The historical data must be from the same animal strain/species, and ideally, be from the same laboratory to minimise any potential confounding due to variations in laboratory conditions, study conditions, animal suppliers, husbandry etc. It is also known that tumour incidences in control animals can change over time, due to factors such as genetic drift, changes in diagnostic criteria for pathological changes/tumour types, and husbandry factors (including the standard diet used), so the historical data should be contemporary to the study being evaluated (e.g. within a period of up to around 5 years of the study). Historical data older than this should be used with caution and acknowledgement of its lower relevance and reliability (RIVM, 2005; Fung et al., 1996; Greim et al., 2003)*". The DS concluded that the HCD claimed by the MSCA and not numerically presented in the PC, are not relevant and the afore mentioned tumours should be part of the carcinogenicity evaluation.

## Assessment and comparison with the classification criteria

Based on the available mutagenicity tests, RAC agrees that the mechanism(s) for the carcinogenic effect of spirodiclofen is probably non-genotoxic.

***Dose Selection***

In the 18 months mouse study, doses were selected based principally upon the toxicological profile which emerged in the mouse over the course of a subchronic study conducted with the test chemical (Leser 1998). Based on a relatively weak toxicological response of the mouse through 7000 ppm (limit dose), it was estimated that the low and high doses chosen of 25 and 7000 ppm would constitute a no-observed effect level and a maximum tolerated dose (limit dose), respectively, with the intermediate dosage of 3500 ppm serving to establish possible dose response relationships.

In the 2 years rat study, the dose levels were selected based on results of a 14 weeks study followed by a 4 week recovery period with substance administration in food (0, 100, 500, 2500 and 12500 ppm). Lower body weights (2500 ppm males, and above both sexes) and reduced food intake (2500 ppm first 4 days, 12500 ppm both sexes) were determined. During recovery the body weight gain and food intake (females) increased. Thrombocyte counts were reversibly decreased (males 12500 ppm). Functional liver effects (500 ppm and above) were derived from increased plasma enzyme activities of aspartate and alanine aminotransferase (12500 ppm, both sexes), reduced plasma concentrations of cholesterol (2500 ppm males, 12500 ppm both sexes) and triglycerides (500 ppm females, 2500 and 12500 ppm, both sexes) as well as decreased plasma protein concentration (both sexes 12500 ppm). Histologically, hepatocellular glycogen content was reduced in four females at highest dose. After the recovery period activities of aspartate aminotransferase were still slightly increased and cholesterol, triglyceride and protein concentrations were reduced in plasma of males. Secondary effects on organs and tissues of the immune system were seen at 2500 and 12500 ppm: decreased peripheral blood leucocytes (12500 ppm), cell counts (12500 ppm) and immunoglobulins as well as a shift (12500 ppm) and decreases in subclass composition. Absolute and relative spleen (females) and thymus weights (males) were reduced at 12500 ppm. Adrenal weights were increased (12500 ppm, both sexes); histologically, the incidence of cytoplasmic cortical vacuolation (500 ppm females, 2500 ppm and above, both sexes) was increased. This was reversible within the recovery period. Mucosal epithelial cells of the small intestine (mainly the jejunum) were vacuolated (2500 ppm and above, both sexes) and plasma alkaline phosphatase activity was increased during treatment (2500 and 12500 ppm, both sexes) and at the end of the recovery.

On the basis of these results the following dose levels were selected for the present combined chronic toxicity/carcinogenicity study: 0, 50,100, 350 and 2500 ppm

***Historical Control Data (HCD)***

Historical control data in mice from the literature suggest a rate of 0%-9.6% in male controls (n=499) and 0%-2.7% in females (n=497) in nominal 18-month studies. Data from five in-house studies conducted 1989-1998 show a rate for the combined hepatocellular neoplasms in controls of 4%-14% in male controls (n=250) and 0%-2% in female controls (n=250). While the male control numbers in the mouse carcinogenicity study are historically low, at 2% hepatocellular neoplasms occurrance, the female values are consistent with historical data. Male frequencies at 3,500 and 7,000 ppm (16% and 20% respectively), and corresponding female values of 10% and 6% are above the range seen in either in-house or literature historical data.

HCD references were provided during PC on hepatocellular adenomas in mice (Maita *et al.,* 1988; Chandra and Frith, 1992; Giknis and Clifford, 2000; Giknis and Clifford, 2001; Giknis and Clifford,

2005; Forster *et al.,* 2014) and on Leydig cell tumours in Wistar rats (Bomhard and Rinke, 1994; Eiben and Bomhard, 1999; Walsh and Poteracki, 1994; Poteracki and Walsh, 1998; Giknis and Clifford, 2003). They are shown in the two following tables for CD1 mice and Wistar rats, respectively.

Table: HCD for tumours in CD-1 mice (1988-2000)

| **Study HCD** | **% liver adenomas** | **% liver carcinomas** | **% combined liver tumours** | **ODD study** | **Dose (ppm)** | **% liver adenomas** | **% liver carcinomas** | **% combined liver tumours** |
|---|---|---|---|---|---|---|---|---|
| Males | | | | | | | | |
| Maita *et al.,* 1988 | 26 | 9.1 | 35.4 | Wahle, 2000 | 3500 | 10 | 6 | 16 |
| Chandra and Frith, 1992 | 11 | 5.7 | 16.7 | | 7000 | **12*** | **10** | **22** |
| Giknis and Clifford, 2000 | 10.46 | 5.29 | 15.8 | | | | | |
| Females | | | | | | | | |
| Maita *et al.,* 1988 | 5.17 | 0.9 | 6.07 | Wahle, 2000 | 3500 | **6** | **4** | **10** |
| Chandra and Frith, 1992 | 1.8 | 0.7 | 2.48 | | 7000 | 2 | **4** | **6** |
| Giknis and Clifford, 2000 | 0.99 | 0.66 | 1.64 | | | | | |

**Bold shows values outside the HCD range.*

The table below presents HCD for tumours in Wistar rats.

Table: HCD for tumours in Wistar rats

| **Study HCD** | **% Benign Leydig cell tumours** | **Uterus adenocarcinomas** | **ODD study** | **Dose (ppm)** | **% Benign Leydig cell tumours** | **Uterus adenocarcinomas** |
|---|---|---|---|---|---|---|
| Bomhard and Rinke, 1994 | 2.1-16.3 (7.0) | 0.0-16.3 (7.8) | Wirnitzer et al.2000 | 350 | 8 | 4 |
| Eiben and Bomhard, 1999 (Bayer AG rats) | 7.0 | 6.5 | | | | |
| Walsh and Poteracki, 1994 | 3.9 | 1.6 | | 2500 | **20** | **28** |
| Giknis and Clifford, 2003 | No reference | 2.3 | | | | |

The analysis of Historical Control Data shows:

1. High variation and inconsistencies;

2. Incidences of liver adenomas, carcinomas and combined tumours in the mouse carcinogenicity study are higher than the HCD in some cases (especially in females). A combination of benign and malignant hepatocellular tumours were observed in the CD-1 mice in both sexes.

In the available mouse carcinogenicity study, non-neoplastic findings included effects in the liver (hepatocytomegaly). The size of hepatocytes was significantly increased over control at dose levels of 3500 and 7000 ppm males. The change was essentially not seen in females (M: 2, 6, 17*, 21*; F: 0, 0, 0, 1). This finding correlates well with liver relative and absolute weights in males.

In addition, hypertrophy and/or hyperplasia of testicular interstitial cell was noted with significantly increased frequency in both the 3500 and 7000 ppm (male) groups (M: 6, 6, 26*, 31*). Some of the control and 25 ppm testes showed hyperplasia only. The lesion in the 3500 and 7000 ppm groups consisted of both greatly increased cell size and increased numbers of cells; thus, the dual diagnosis of hypertrophy and hyperplasia. Average severity increased with dose. The enlarged foamy cells in the two higher groups were sometimes accompanied by small numbers of inflammatory cells or occasional giant cells, but there was no significant increase in the frequency of inflammatory changes. Tubular degeneration of the testis was slightly, but not significantly increased in the high dose. The frequency of the background lesion of abnormal spermatozoa in the epididymis was directly related to the testicular changes (M: 15, 15, 15, 26*).

As far as neoplastic lesions are concerned, the combined frequency of hepatocellular neoplasm (hepatocellular adenoma and carcinoma) was significantly increased over controls in 3500 and 7000 ppm males and in 3500 ppm females. Data were corrected for one animal with both types of tumor. (M: 1, 1, 8*,10*; F: 0, 0, 5*, 3).

In the 2 years rat study in male rats a positive trend for Leydig cell adenomas was observed (Trend Test p=0.0010). The frequency of benign Leydig cell tumors was markedly increased at 2500 ppm. Concurrently, also the incidence of focal Leydig cell hyperplasia was significantly increased in males of the high dose group (Trend Test p=<0.0005+). The average severity per group was slightly increased in affected high dose males. The pooled incidence, i.e. the number of males with Leydig cell adenomas and/or focal Leydig cell hyperplasias showed a highly significant increased incidence exclusively in the 2500 ppm-group. The majority of Leydig cell adenomas and focal hyperplasias were found in males at the termination of the study suggesting a late onset of these alterations. Diffuse Leydig cell hyperplasia was only seen in two males (-/-/-/1/1). According to the registrant, the Leydig cell is a target structure of spirodiclofen known from previous toxicity studies with mice and beagle dogs where Leydig cell hypertrophy and/or vacuolation was diagnosed at high dose levels. Spirodiclofen is assumed to exert an influence on steroid biosynthesis or metabolism. No indications for lesions influenced by the test compound were found in the epididymides, prostate, and seminal vesicles/coagulations glands.

In the ovaries of female rats, the frequency of females with metastases of carcinomas increased (1/-/-/1/5). These tumors originated from uterine adenocarcinomas. The incidence of primary ovarian neoplasms themselves was not influenced by the treatment.

In addition, in the uterus adenocarcinomas 8/14 tumors graded as malignant neoplasms probably fatal were metastatic to multiple organs an sites such as: lungs, forestomach, glandular stomach, liver pancreas, ovaries, kidneys, colon, lymphnotes etc. One adenocarcinoma was metastatic only to liver. Compared to controls and rats of the other groups, the number of females with metastases of carcinomas in the spleen (primary tumor uterus) was increased after 2500 ppm (incidence: -/1/1/-/3). In the 350 ppm dose, all uterus adenocarinomas were metastatic.

A positive trend was observed in the presence of corpora lutea. The incidences were 28/28/33/39*/32 (Trend Test, p=0.038). The average grading was similar in all groups including

controls. The Exact Fisher Test was only significant in females at 350 ppm and not in the high dose females. However, since dose correlation and similar average severity were absent, an influence by the treatment can be ruled out. A significant negative trend was calculated for the incidence of ovarian atrophy (19/24/17/13/13, p=0.0154). The Exact Fisher Test revealed no significant decrease. Because the missing of a dose correlation and the great variance of this finding in aged rats, the slight decrease is regarded as incidental,

In the uterus, adenocarcinomas were increased at 2500 ppm (Trend Test: p=0.0088) when compared to controls and females dosed up to and including 350 ppm. This correlates with the increased gross incidence of uterine nodules in this group.

The majority of the adenocarcinomas (11 out of 14) was found in females which died or had to be sacrificed before the termination of the study. As already mentioned, many of the adenocarcinomas in these high dose females had metastasized in various organs namely of the abdominal cavity by invasion and also into the lungs (5 cases) and the bone marrow (two cases). The high prevalence of metastatic uterine adenocarcinomas at 2500 ppm might have contributed to the slightly increased overall mortality in females of this group (mortality: 21/18/19/15/24). The incidence of other epithelial or mesenchymal neoplasms of the uterus was not affected as was the incidence of possibly pre-neoplastic focal glandular hyperplasia.

In females, the incidence of c-cell adenomas of the thyroid gland was 2/2/3/5/6, being statistically significant in the Trend Test (p=0.0319+). The incidences of focal and diffuse c-cell hyperplasia were not influenced by the dosing with the test compound.

In males, c-cell adenomas (incidence: 4/5/5/4/7) were distributed evenly among the groups as were focal and diffuse c-cell hyperplasia. A significantly increased incidence of colloidal alteration was observed in high dose males (Trend Test p=0.0047). The incidence was 23/23/28/28/35*. In the Exact Fisher Test significance was achieved only for high dose males. There was no evidence of an influence on hyperplastic or neoplastic lesions of the follicular epithelium in both sexes.

In conclusion, spirodiclofen was found to lead to an increase in the incidences of tumors in the testes and the uterus in rats after the administration of a very high dose. The no-effect-level for neoplastic lesions was considered at 350 ppm for both sexes.

***Stastitical Analysis***

A statistical analysis of the tumor incidences in mice and rats, respectively is shown in the tables below.

Table: statistical analysis of the tumor incidences in mice

| CD-1 mouse | | | | | | |
|---|---|---|---|---|---|---|
| Dose | Male | | | Female | | |
| | Liver hepatocellular adenomas | Liver hepatocellular carcinomas | Combined tumours | Liver hepatocellular adenomas | Liver hepatocellular carcinomas | Combined tumours |
| Control (0 ppm) | 0/50 | 1/50 | 1/50 | 0/50 | 0/50 | 0/50 |
| 25 ppm | 0/50 | 1/50 | 1/50 | 0/50 | 0/50 | 0/50 |
| 3500 ppm | **5/50 (p=0.02)** | 3/50 (p=0.31) | **8/50 (p=0.01)** | 3/50 (p=0.08) | 2/50 (p=0.16) | **5/50 (p=0.02)** |
| 7000 ppm | **6/50 (p=0.01)** | 5/50 (p=0.09) | **11/50 (p=0.002)** | 1/50 (p=0.31) | 2/50 (p=0.16) | 3/50 (p=0.08) |

| Table: statistical analysis of the tumor incidences in rats | | | | |
|---|---|---|---|---|
| Wistar rat | | | | |
| Dose | | Male | Female | |
| | | Benign Leydig cell tumours | Uterus adenocarcinomas | Thyroid C-Cell adenoma |
| Control (0 ppm) | Except deaths | 2/31 | 2/29 | 1/29 |
| | Only deaths | 0/19 | 2/21 | 1/21 |
| | *Total* | *2/50* | *4/50* | *2/50* |
| 50 ppm | Except deaths | 1/30 (p=0.57) | 3/32 (p=0.72) | 2/32 (p=0.61) |
| | Only deaths | 0/20 | 2/18 (p=0.87) | 0/18 (p=0.35) |
| | *Total* | *1/50 (p=0.56)* | *5/50 (p=0.73)* | *2/50 (p=1)* |
| 100 ppm | Except deaths | 0/36 | 2/31 (p=0.94) | 3/31 (p=0.33) |
| | Only deaths | 0/14 | 1/19 (p=0.61) | 0/19 (p=0.34) |
| | *Total* | *0/50* | *3/50 (p=0.70)* | *3/50 (p=0.65)* |
| 350 ppm | Except deaths | 4/31 (p=0.39) | 0/35 (p=0.11) | 4/35 (p=0.24) |
| | Only deaths | 0/18 | 2/15 (p=0.72) | 1/15 (p=0.80) |
| | *Total* | *4/50 (p=0.40)* | *3/50 (p=0.40)* | *5/50 (p=0.24)* |
| 2500 ppm | Except deaths | 9/41(p=0.07) | 3/26 (p=0.55) | 4/26 (p=0.12) |
| | Only deaths | 1/9 (p=0.16) | **11/24 (p=0.007)** | 2/24 (p=0.63) |
| | *Total* | ***10/50 (p=0.014)*** | ***14/50 (p=0.009)*** | *6/50 (p=0.14)* |

***Bold*** *denotes statistical differences with the controls; P values have been calculated by RAC; Data in italics have been calculated by RAC.*

A combination of benign and malignant hepatocellular tumours were observed in the CD-1 mice in both sexes at 2 doses in a statistically significant manner and a dose-dependent way in the males. In Wistar rat, statistical significant benign Leydig cell tumours in the male at the highest dose and statistical significant metastatic uterus adenocarcinomas in the female at the highest dose, too, were observed.

In the following table a summary of carcinogenicity related neoplastic and non-neoplastic findings are presented for all three species tested for spirodiclofen.

Table: summary of carcinogenicity related neoplatic and non-neoplastic findings is mice, rats and dogs

| | **Mouse 18 month carcinogenicity study, 7000 ppm** | | **Rat 108 week carcinogenicity study, 2500 ppm** | | **Dog, 52 week, repeated dose toxicity study, 600 ppm** | |
|---|---|---|---|---|---|---|
| | **female** | **male** | **female** | **male** | **female** | **male** |
| **Epididymides** | | Aspermia | | | | 28 % ↑ wt |
| **Leydig Cells** | | – | | benign tumors | | vacuolation 4/4 (grade 1,2,1,1) hypertrophy 1/4 (2) focal tubular degeneration 1/4 (2) |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Liver** | Hepatocellular adenomas and carcinomas, 14 % ↑ wt, hepatocytomegaly 1/50 grade 2.0 | Hepatocellular adenomas and carcinomas, 18 % ↑ wt, hepatocytomegaly 21/50 grade 1.9 | 4 % ↓ wt, no neoplastic lesions | 9 % ↓ wt, no neoplastic lesions | 4 % ↓ wt, no neoplastic lesions | 26 % ↓ wt, no neoplastic lesions |
| **Ovaries** | 48 % ↓ wt, no neoplastic lesions | | 31 % ↑ wt, 5/50 carcinomas metastatic from uterus adenocarcinomas | | vacuolation 1/4 (1), no neoplastic lesions | |
| **Testes** | | ↑20 % Degeneration - grade 3.8, 19 % ↑ wt, Hypertrophy/hyperplasia/Interstitial cell 31/50 - grade 2.5 | | 6 % ↑ wt, no neoplastic lesions | | 30 % ↑ wt, no neoplastic lesions |
| **Uterus** | – | | adenocarcinomas | | 150 ppm, mononuclear infiltration 3/4 (2), no neoplastic lesions | |

### *General Toxicity*

Additionally, RAC analysed the tumor incidences in mice and rats in relation to general toxicity issues. The results are shown in the table below.

Table: Overview of effects on carcinogenicity and general toxicity parameters in available repeated dose toxicity and carcinogenicity studies

| **Study** | | **males** | **females** |
|---|---|---|---|
| Leser, Romeike (1998) | **13-wk oral mouse repeated dose toxicity study** 0, 100, 1000, 10000 ppm | **≥ 1000 ppm**<br>non significant ↓ in body weight<br>**≥ 10000 ppm**<br>non significant ↓ in body weight, no mortality, no clinical signs,<br>no food & water consumtion effects | **≥ 1000ppm**<br>no effects<br>**≥ 10000 ppm**<br>non significant decrease in body weight, no mortality, no clinical signs, no food & water consumtion effects |
| Wahle (2000) | **18-month mouse carcinogenicity study** 0, 25, 3500, 7000 ppm | **≥ 3500 ppm**<br>no mortality, ↓ bw (statistically not consistent)<br>↑ food consumption<br>**hepatocellular adenoma** 5/50<br>**hepatocellular carcinoma 3**/50<br>**hepatocellular combined adenoma/carcinoma** 8/50<br>**≥ 7000 ppm**<br>no mortality, ↓ bw (statistically not consistent)<br>increased food consumption<br>**hepatocellular adenoma** 6/50<br>**hepatocellular carcinoma** 5/50 | **≥ 3500 ppm**<br>no mortality, ↓ bw (statistically not consistent)<br>terminal body weight was significantly ↓<br>**hepatocellular adenoma** 3/50<br>**hepatocellular carcinoma** 2/50<br>**hepatocellular combined adenoma/carcinoma** 5/50<br>**≥ 7000 ppm**<br>no mortality, ↓ (statistically not consistent)<br>body weight, terminal body weight was significantly ↓<br>**hepatocellular adenoma** 1/50<br>**hepatocellular carcinoma** 2/50 |

| | | | |
|---|---|---|---|
| | | **hepatocellular combined adenoma/carcinoma** 11/50 | **hepatocellular combined adenoma/carcinoma** 3/50 |
| Wirnitzer, Romeike (1998) | **14-week oral rat repeated dose toxicity study** 0, 100, 500, 2500, 12500 ppm | **≥ 2500 ppm**<br>no mortality, no clinical signs, ↓ bw statistically<br>not significant, no food & water consumtion effects<br><br>**≥ 12500 ppm**<br>no mortality, no clinical signs,<br>↓ bw statistically significant,<br>↓ food consumption(significant),<br>↓ water consumtion (non significant) | **≥ 2500 ppm**<br>no mortality, no clinical signs, ↓ bw statistically<br>not significant, no food & water consumtion effects<br><br>**≥ 12500 ppm**<br>no mortality, no clinical signs, ↓ bw statistically<br>significant, ↓ food consumption significantly ,<br>↓ water consumtion (non significant) |
| Wirnitzer (2000) | **108-week rat carcinogenicity study** 0, 50, 100, 350, 2500 ppm | **≥ 2500 ppm**<br>no mortality, no clinical signs, ↓ bw (up to 11%)<br>↑food consumption<br>**Liver Leydig cell tumors** 10/50 | **≥ 2500 ppm**<br>no mortality, no clinical signs, ↓ bw (up to 8%)<br>↑food consumption<br>**Uterus adenocarcinomas** 14/50 |

* HCD and statistical analysis can be found in tables above.

In the 18 month mouse study there is no mortality in the two highest dose groups (3500 and 7000 ppm). There are some clinical signs, increased food consumption and decreased body weight in both male and female mice. However, there is no quantifiable data in the CLH report and the studies are not available to the rapporteurs since there are all industry studies. In the rat study, in the highest dose group (2500 ppm) there was a decrease of up to 11% in male and up to 8% in female body weight. No mortality and no clinical signs were observed but there was a slight increase in food consumption.

In addition, in the available repeated dose toxicity studies RAC noted the following:

1. In the 13-week mouse study at the 10000 ppm dose no mortality, no clinical signs and no food & water consumption effects were observed. There was a decrease in the body weight of both male and female mice but not in a significant way.
2. In the 14-week rat study at the 2500 ppm dose there was no mortality, no clinical signs, no food & water consumption effects and a non significant decrease in the body weight of both male and female rats.

Therefore, the rapporteurs do not believe that the relevant doses are at a MTD level in the carcinogenicity studies.

In conclusion, based on the HCD and statistical analysis RAC believes that the observed tumors should be considered for classification.

The hormonal and cholesterol disrupting properties of spirodiclofen could be responsible for the adrenal, testicular and uterine effects observed. Chronic stimulation of the pituitary hormone production could lead to chronic stimulation of testicular Leydig cells and endometrial uterine cells resulting in hypertrophy, hyperplasia and eventual tumor formation. This is further supported by the fact that spirodiclofen does not exhibit mutagenic properties. Therefore, the mode of action appears to be relevant to humans and should be considered in the weight of evidence for the classification of spirodiclofen.

The available data cannot be considered as 'limited evidence', as they include an expected combination of benign and malignant metastatic neoplasms in two or more species. Classification in category 2 is thus not supported.

Overall, RAC concludes there is sufficient evidence for carcinogenic effects of spirodiclofen, and agrees with the DS to classify it as **Carc. 1B (H350: May cause cancer)**.

## RAC evaluation of reproductive toxicity

### Summary of the Dossier Submitter's proposal

The evaluation of reproductive toxicity of spirodiclofen by the DS was based on a two-generation study on Wistar rats (OECD TG 416, GLP compliant), a developmental toxicity study on Himalayan rabbits and one in Wistar rats (OECD TG 414, GLP compliant). In addition, several repeated dose toxicity studies were used for the effects on reproductive organs.

In the 2-generation study with rats, the F0 male animals showed in all dose groups decreased bodyweight, increased brain weight and decreased liver weight. In females, effects were observed in the mid and high dose groups and included decreased body weight and increased severity of adrenal vacuolation. F1 pups showed in the mid and high dose groups decreased body weight at birth and during lactation and at the same dose groups changed relative and absolute weights of brain and spleen. In the F1 animals in clinical chemistry observations in all dose groups, dose related decreases in cholesterol and triglycerides were seen. Furthermore, in the high dose group, F1 animals showed changes in weights of brain, adrenals, liver, kidneys, ovaries and uterus. In the high dose group, decreases were also observed in the number of spermatids in the testes and in the number of sperms in the epididymis. Testes atrophy was observed with incidences of 0/1/1/4 for the control, low, mid and high dose group. Since testes atrophy was only observed in the F1-males and not in the F0-males it is probable that this observed effect may not be the result of exposure of adult animals (an effect on sexual function and fertility) but instead it may be the result of an exposure during the prenatal or pre-adult period (developmental effect). However, the findings of testes atrophy in the high dose groups were within the ranges for historical controls. In the repeated dose toxicity studies in rats and at similar doses, no such effects were observed and at the high dose an increase in testes weight was observed which which might have been due to compensation effect. Thus, in the 2-generation study no effects on sexual function are considered for classification.

In the repeated dose toxicity and carcinogenicity studies changes on reproductive organs and on sexual function/fertility were observed. Effects on testes were observed in all studied species (mouse, rat, dog), though most pronounced in dogs since effects occurred at lower dose levels. These included increased testis weight (absolute and relative), hyperplasia, hypertrophy and vacuolisation of testis, but also oligo- and aspermia (in 4-and 14-week dog studies, 18-month mouse study). Further, changes of weight of uterus/oviduct and ovaries were observed in female animals as summarised in the table below.

Table: Overview of effects on sexual function/fertility parameters and reproductive organs in available repeated dose toxicity, carcinogenicity and reproductive toxicity studies

| Study | | males | females |
|---|---|---|---|
| Leser, Romeike (1998) | **13-wk oral mouse repeated dose toxicity study** 0, 100, 1000, 10000 ppm | **≥ 1000 ppm**<br>slight ↓bw, 8 % ↑ r (dose-related) testes weights<br>Hypertrophy/activation of Leydig cells (testes)<br>1/10, 1/10, **9/10**, 10/10<br>Average Severity (1)<br>**≥ 10000 ppm**<br>12% ↑ r testes weights<br>Hypertrophy/activation of Leydig cells (testes)<br>1/10, 1/10, 9/10, **10/10**<br>Average Severity (2.3)<br>Vacuolation of Leydig cells 7/10<br>Average Severity (1.1) | **≥ 1000 ppm**<br>no effects<br>**≥ 10000 ppm**<br>slight ↓bw, 10% ↑ weight ovaries |

| | | | |
|---|---|---|---|
| Wahle (2000) | **18-month mouse carcinogenicity study**<br>0, 25, 3500, 7000 ppm | **≥ 3500 ppm**<br>no mortality, ↓ bw (statistically not consistent)<br>↑ food consumption<br>↑ar testis weight<br>Hypertrophy/hyperplasia interstitial cells testis<br><br>**≥ 7000 ppm**<br>**Epididymides**<br>Aspermia: 15/50, 15/50, 15/50, 26/50, ↑ s<br>average severity: 4.3, 4.2, 4.8, 4.7<br>**Testes**<br>23% ↑r testes weight<br>Hypertrophy/hyperplasia of interstitial cells<br>6/50, 6/50, 26/50, 31/50<br>Average Severity: 1.2, 1.3, 1.8, 2.5 | **≥ 3500 ppm**<br>no mortality, ↓ bw (statistically not consistent)<br>terminal body weight ↓ significantly<br><br>**≥ 7000 ppm**<br>no mortality, ↓ weight (statistically not consistent)<br>body weight, terminal body weight was significantly ↓<br>**Ovaries**<br>38% ↓ r ovaries wt |
| Krottlinger, GeiB (2000) | **4-wk oral rat**<br>f, 0,100, 500, 5000 ppm | – | **≥ 5000 ppm**<br>no mortality, no bw change, ↓17% r weight ovaries |
| Wirnitzer, Romeike - 1998 | **14-week oral rat**<br>0, 100, 500, 2500, 12500 ppm | **≥ 12500 ppm:** 10% ↑r testes weight, no mortality,<br>no clinical signs, ↓ s bw m, ↓ water consumption<br>↓ s food consumption | **≥ 12500 ppm:**<br>↓ s bw f |
| Wirnitzer 2000 | **108-week rat carcinogenicity study**<br>0, 50, 100, 350, 2500 ppm | **≥ 350 ppm:** no effects<br><br>**≥ 2500 ppm:** no mortalities, no clinical signs<br>↓s bw, ↑ food consumption<br>↑$^{r}$ testis weight<br>Focal Leydig cell hyperplasia<br>4/31, 4/30, 4/36, 6/31, 19/41 ↑$^{s}$ | **≥ 350 ppm:** 33% ↑ar ovaries weight<br><br>**≥ 2500 ppm:** no mortalities, no clinical signs,<br>↓s bw, ↑ food consumption |
| Wetzig, Romeike, Sander (2001) | **4-week oral dog**<br>0, 400, 2000, 10000 ppm | **≥ 2000 ppm**: no general toxicity effects<br>Leydig cell vacuolation 2/2 (1,1)<br><br>**≥ 10000 ppm:** no general toxicity effects<br>Leydig cell vacuolation 2/2 (3,1)<br>Leydig cell hypertrophy/activation 1/2 (3)<br>Immature testes/prostate, 1/2 (2)<br>Massive oligospermia, slight spermic debris 1/2 (5) | **≥ 2000 ppm:** no general toxicity effects<br>33% ↑ar weight uterus<br><br>**≥ 10000 ppm:** no general toxicity effects<br>43 % ↑ar weight ovaries<br>18 % ↑ar weight uterus |
| Wetzig, Hartmann (2001b) | **8-week oral dog**<br>0, 100, 2000 ppm | **≥ 100 ppm:** no general toxicity effects<br>↓ar wt prostate (dr), 13 % ↓r wt prostate<br>Degeneration germinal epithelium 1/5 (2)<br><br>**≥ 2000 ppm:** no general toxicity effects<br>Hypertrophy and vacuolization of Leydig cells (testes) 5/5 (3,2,3,2,2) | - |

| | | | |
|---|---|---|---|
| | | Degeneration germinal epithelium 4/5 (2,1,1,1) | |
| Wetzig, Hartmann (2001a) | **14-week oral dog**<br>0, 200, 630, 2000 ppm | **≥ 200 ppm**:<br>52% ↓r weight prostate<br><br>**≥ 630 ppm:** ↓ bw<br>Testes<br>Vacuolization Leydig cells, 2/4 (2,3)<br>Hypertrophy Leydig cells, 2/4 (2,2)<br>Epididymides<br>Aspermia, 1/4<br>Oligospermia, 2/4 (2,2)<br>Immature prostate, 1/4 (4)<br><br>**≥ 2000 ppm:** ↓ bw<br>Testes<br>Degeneration germinal epithelium, 2/4<br>Vacuolization Leydig cells, 4/4 (3,2,2,3)<br>Hypertrophy Leydig cells, 3/4 (3,3,4)<br>Epididymides<br>Aspermia, 2/4<br>Immature prostate, 4/4 (4,3,3,4) | **≥ 200 ppm**:<br>↓ r weight uterus<br><br>**≥ 630 ppm:** ↓ bw<br>↓ r weight uterus<br><br>**≥ 2000 ppm:** ↓ bw<br>48% ↓ r weight uterus<br>15% ↓ r weight ovaries |
| Wetzig, Ruh-Fehlert (2001) | **52-week oral dog**<br>0, 20, 50, 150, 600 ppm | **≥ 20 ppm:** no general toxicity effects<br><br>↑ ar testes weight<br><br>**≥ 50 ppm:** no general toxicity effects<br>↑ ar testes weight, ↑ ar epididymis weight<br><br>**≥ 150 ppm:** no general toxicity effects<br>↑ ar testes weight, ↑ ar epididymis weight<br>Focal tubular degeneration testes, 1/4 (1)<br><br>**≥ 600 ppm:** no general toxicity effects<br>30% ↑ r testes wt, 17% ↑ r epididymis wt<br>19% ↑ ar prostate weight<br>Vacuolization Leydig cells, 4/4 (1,2,1,1)<br>Hypertrophy Leydig cells, 1/4 (2)<br>Focal tubular degeneration testes, 1/4 (2) | **≥ 20 ppm:** no general toxicity effects<br>↓ ar uterus/oviduct weight<br><br>29% ↓ r uterus/oviduct weight |
| Krottlinger, Sander (1999) | 4-wk dermal rat | – | – |
| Eiben (2000) | **2-generation study rat**<br>0, 70, 350 & 1750 ppm | **F0:** ↓ bw dose related<br>**≥ 70 ppm:** ↓ bw<br>↑sr prostate weight<br>↓srepididymides weight, ↓sr seminal vesicles | **F0:** ↓ bw dose related<br>**≥ 70 ppm:**<br>– |

| | | | |
|---|---|---|---|
| | | **≥ 350 ppm:** ↓ s bw<br>↑sr prostate weight<br>↓sr epididymides weight, ↓sr seminal vesicles | **≥ 350 ppm:** bw |
| | | **≥ 1750 ppm:**↓ s bw<br>↑sr testes weight<br>**Testes** (diminished in size)<br>0/25, 1/25, 1/25, **4/25**<br>**Epididymides** (diminished in size)<br>0/25, 1/25, 1/25, **4/25** | **≥ 1750 ppm:**↓ s bw<br>– |
| | | **F1:**↓ bw dose related<br>**≥ 350 ppm:** ↓ bw<br>**≥ 1750 ppm:** ↓ s bw, ↑s food consumption<br>**Mating/fertility/gestation***<br>spermatids per mg testis: -23%<br>sperms per mg epididymides: -18%<br>**Testes***<br>atrophy, diffuse: 0/25, 1/25, 1/25, 4/25**<br>**Epididymides***<br>Oligospermia: 0/25, 1/25, 1/25, 4/25<br>Atrophy: 0/25, 1/25, 1/25, 4/25<br>**Prostate***<br>Atrophy: 0/25, 0/25, 0/25, 3/25 | **F1**<br>**≥ 350 ppm:**<br>–<br>**≥ 1750 ppm:** ↓ s bw<br>↑ ar uterus & ovaries weight |

* Effects were observed in four specific animals where there was a sever decrease in body weight.
** The testes atrophy was within the HCD range.
a: absolute, r: relative, s: statistically significant, bw: body weight

The observed effects on testes (enlargement, hypertrophy, hyperplasia), uterus (enlargement) but also adrenals (enlargement, vacuolation) may be secondary to the effects or spirodiclofen on steroidogenesis i.e. an adaptive response. However, at the high dose levels clear effects on spermatogenesis (oligospermia, aspermia) were observed. Thus, the DS proposed that the adaptative effects (enlargement, hypertrophy, hyperplasia and vacuolation) should not be considered for classification in contrast to oligospermia and aspermia.

In the developmental toxicity studies on rabbits, in the high dose group, one female showed liver lobulation. In foetuses of the highest dose group, an increased incidence in liver lobulation was observed which was outside the range of historical controls, whereas the litter incidence for this type of effects was within the range of historical controls. It could therefore not fully excluded that the observed effect of liver lobulation was treatment related. However, no information on the severity of liver lobulation was presented in the study report.

Two neurodevelopmental toxicity studies in rats were presented. The first study showed negative results with the exception of some equivocal results for the water maze test. In a follow-up study in which parts of the developmental neurotoxicity study were repeated (and included two types of water maze tests) no neurotoxic effects were observed.

In the teratogenicity study with rats, no toxicologically relevant effects were observed.

## Comments received during public consultation

Two MSCA's commented and agreed with the proposal by the DS for the classification of spirodiclofen for the effects on sexual function and fertility – Repr.2(H361f: Suspected of damaging fertility).

The second MSCA added the following reasoning: in the 2-generation study with rats, weights of adrenals, ovaries and uterus in the F1 animals had changed. In the high dose group, decreases were observed in the number of spermatids in the testes and in the number of sperms in the epididymidis. In addition, effects on the reproductive organs were observed in the repeated dose toxicity and carcinogenicity studies. Effects on testes were observed in all studied species (i.e. mouse, rat, dog), though most pronounced in dogs. These effects included increased testis weight (absolute + relative), hyperplasia, hypertrophy and vacuolation of testis, but also oligo- and aspermia (in 4- and 14-week dog studies, 18-month mouse study). Further, changes of weight of uterus/oviduct and ovaries were observed in female animals.

## Assessment and comparison with the classification criteria

### *Effects on fertility and sexual function*

In the 2-generation study (Eiben, 2000) in rats there were effects on sexual function parameters such as decreased number of spermatids in testes, testes atrophy and decreased number of sperms in the epididymis. The effects were seen at doses with general toxicity and the testes atrophy was within HCD (Table 53 of the CLH report) excluding the evidence of clear substance related effects. Moreover, in the 14 weeks toxicity study in rats at high dose levels an increase in testes weight was observed, which could be a compensation effect for the spirodiclofen induced hormonal disruption/depletion.

RAC notes that in the repeated dose toxicity and carcinogenicity studies there were clear effects on the sexual function/fertility parameters as well as on the reproductive organs in all tested species although more pronounced in dogs since the effects were observed at lower doses than the ones in mice and rats and clearly at levels where there is no general toxicity. RAC concluded that although it could be argued that some of the observed effects are adaptive, the oligospermia and aspermia seen in dogs are **not** secondary effects and could be relevant for the evaluation of possible fertility effects of spirodiclofen in humans. It is noted that the aspermia observed in the 4 week dog study was massive but occurred in one dog with relative testes weight 4.6% less than the average of the control and testing animals and with a relative prostate weight reduction of 56%. Morever, the aspermia observed in the 18 month carcinogenicity study in mice increased significantly both in frequency and severity but only at the high dose. Therefore, aspermia was observed in two species but the significance of the incidence is limited. In addition, the Leydig cell and degeneration of the germinal epithelium effects as well as the uterus/oviduct/ovaries effects observed in dogs are consistent and not in general toxicity doses and should also be considered for classification purposes. Thus, the afore mentioned observed effects in dogs should be taken into account for potential classification for effects on sexual function and fertility. Similarly, leydig cell hypertrophy/activation was observed at the mid dose in mice where there were no signs of general toxicity effects. At the high dose, leydig cell vacuolation (Leser 1998) and hypertrophy/hyperplasia of interstitial cells (Wahle 2000) were seen. In rats, at the high dose in the carcinogenicity study focal leydig cell hyperplasia was observed (Wirnitzer 2000).

A table summarising the sexual function/fertility/reproductive organ effects is presented hereafter:

| Effect/target reproductive organ | Mouse | Rat | Dog |
|---|---|---|---|
| Aspermia/Oligospermia | + | - | ++ |
| Testes | + | + | ++ |
| Epididymides | + | - | ++ |
| Prostate | - | - | ++ |
| Ovaries/corpora lutea | + | + | ++ |
| Uterus | - | ++ | ++ |

+: slight/moderate; ++: pronounced; -: not observed

Therefore, RAC concludes that although there is only one adverse effect observed (aspermia/oligospermia), in combination with the effects on reproductive organs (more pronounced in dogs) there is enough evidence for classification as Repr.2; H361f: Suspected of damaging fertility.

***Developmental toxicity***

RAC considers the results of the rabbit study as being equivocal. In the highest dose group foetuses show incidence for liver lobulation outside the HCD for the specific strain but in the litter the incidences lie within the HCD. In addition there is no data regarding the severity of the effect but based on the HCD for foetuses and litters it is more likely that the severity of this type of effect is low. Thus, it is not clear whether the effect of liver lobulation is treatment related and therefore RAC believes that it should not be considered for classification. In the rat developmental toxicity study, no substance related effects were observed and therefore RAC agrees that no classification is required for developmental toxicity.

RAC also agrees with the DS that no classification is required regarding effects on or via lactation. There is lack of data on the concentration of spirodiclofen in milk and therefore no conclusion can be drawn whether the effects observed during lactation are due to the transfer of spirodiclofen to the offspring via milk.

***Conclusions on classification and labelling***

In conclusion, RAC agrees with the DS, that spirodiclofen should be classified for effects on sexual function and fertility as **Repr. 2 (H361f: Suspected of damaging fertility)**.

Spirodiclofen may also contain the impurity N,N-dimethylacetamide. DMAC has a harmonised classification for reproductive toxicity as Repr.1B (H360D: May damage the unborn child) with a generic concentration limit (GCL) of 0.3%. Therefore, the presence of DMAC above the GCL could result in an additional classification of spirodiclofen for developmental toxicity as Repr.1B (H360D: May damage the unborn child).

## RAC evaluation of aspiration toxicity

### Summary of the Dossier Submitter's proposal

No classification is proposed by the DS due to lack of data.

### Comments received during public consultation

There was one comment received from a MSCA during public consultation supporting the DS's proposal not to classify spirodiclofen with regard to aspiration toxicity/hazard.

### Assessment and comparison with the classification criteria

No assessment and comparison with the classification criteria is possible due to lack of data.

## ENVIRONMENTAL HAZARD EVALUATION

## RAC evaluation of aquatic hazards (acute and chronic)

### Summary of the Dossier Submitter's proposal

#### *Degradation*

A summary of the relevant studies included in the CLH report for the degradation of spirodiclofen is shown in the table below.

Table: degradation studies

| Method | Results | Remarks | Reference |
|---|---|---|---|
| **Spirodiclofen** | | | |
| EPA guideline and SETAC, GLP study | *$DT_{50}$ for hydrolysis at 20 °C<br>pH 4: 124 days<br>pH 7: 53.4 days<br>pH 9: 2.2 days | Hydrolytically unstable<br>Major hydrolysis product: Spirodiclofen-enol | Babczinsky (2000)[a] |
| UBA (1992) guideline GLP study | $DT_{50}$ at 50 °N 80 days (no clouds)<br>$DT_{50}$ at 50 °N 110 days (cloudiness) in June | Photodegradation not expected | Hellpointer (1998)[a] |
| EPA guideline GLP study | $DT_{50}$ at 40 °N 123 days (midsummer) | Photodegradation not expected | Stupp and Brumhard (2000)[a] |
| BBA IV and SETAC guideline GLP study<br><br>Water/sediment aerobic | $DT_{50}$s for Honniger pond system<br><br>Water[(A)]: 0.3 day<br>Sediment: 4.4 days<br>System: 4.2 days<br><br>$DT_{50}$s for Anglerweiher pit system<br><br>Water: 1.1 day<br>Sediment: 2.5 days<br>System: 2.3 days | (A) Calculated using measured values in the water on day 0 and 1, assuming first order decline | Riegner (1999)[a] |
| EPA (Pesticide Assessment Guidelines Subdivision N, Series 162-3) guideline, GLP study<br><br>Water/sediment anaerobic<br><br>Water/sediment anaerobic | $DT_{50}$<br>Water: -<br>Sediment: 9.8 days<br>System: 10 days | | Wujcik *et al.* (2000)[a] |

*$DT_{50}$ values at 20 °C at pHs 4, 7 and 9 were calculated by the RMS; [a] As summarised in the DAR vol. 3-B8, April 2004.

Spirodiclofen hydrolyses in the pH range 4-9 in the absence of light under sterile conditions, with first-order $DT_{50}$s at 20°C of 124, 53.4 and 2.2 days at pHs 4, 7 and 9 respectively as calculated by the rapporteur member state (RMS) during the pesticide review based on the original data.

The hydrolytic stability of spirodiclofen decreases as temperature and pH increase. The main hydrolysis product was spirodiclofen-enol, the total amount of other products at any point did not exceed 2% of the applied radioactivity (AR). Spirodiclofen-enol is hydrolytically stable.

Spirodiclofen is not considered to be directly photodegradable in two photodegradation studies. Environmental photolysis half lives in pure water were estimated to be 54, 64, 80 and 110 days at 30, 40, 50 and 60 °N, respectively (clouds not considered), or 110 days at 50 °N (cloudiness taken into consideration).

No study on ready biodegradability was submitted.

In two aerobic water/sediment systems (pond and pit), the $DT_{50}s_{,water}$ of spirodiclofen were 0.3 and 1.1 days, respectively, while the whole system $DT_{50}$s resulted in 4.2 and 2.3 days. In both systems, spirodiclofen was detected in the sediments at levels of 58-68% AR on day 1. This indicates that spirodiclofen disappears rapidly from the water phase. Mineralisation was at a comparable low level in both systems, 2.1% and 2.6% after 110 days. The major metabolite was spirodiclofen-enol which reached maximum levels of 84% (days 14-59) and 30% (day 110) in water and sediment, respectively.

The total levels of other degradates in any system were < 5.0% AR. The $DT_{50}$s of 186 and 393 days were calculated for the disappearance of spirodiclofen-enol from the pond and pit system, respectively. In the pit system, no degradation of spirodiclofen-enol was apparent until the end of the study (day 110).

In an anaerobic water/sediment study, spirodiclofen was almost quantitatively lost from the water phase at the first sampling, with a maximum level occurring in sediment of 91.9% AR on day 0. Spirodiclofen dissipated from sediment and overall system with a $DT_{50}$ of 9.8 and 10 days, respectively. Maximum levels of the major metabolite spirodiclofen-enol were 71-80% AR (water) and 82-94% AR (whole system) between days 34 and 365. Levels of non-extractable radioactivity in sediment (< 3.6% AR), production of $CO_2$ (< 1.0% AR) and organic volatiles (< 0.5% AR) were low throughout the study.

Based on the findings from the aerobic and anaerobic water/sediment test, spirodiclofen appears to be susceptible for primary degradation ($DT_{50}$s < 16 days) but not for ultimate mineralisation ($CO_2$ production). Considering the low levels of mineralisation in the simulation studies, spirodiclofen is considered not rapidly degradable (degradation of > 70% degradation within 28 days) by the DS for purposes of classification and labelling.

***Bioaccumulation***

The CLH report contains a BCF study with bluegill sunfish (*Lepomis macrochirus*) according to OECD TG 305 and EPA 72-6 guidelines; the highest BCF derived for spirodiclofen was 491 L/kg for the whole fish, and 323 L/kg after 5% lipid normalisation. Spirodiclofen therefore does not fulfil the criteria for bioaccumulation according to Regulation EC 1272/2008, since the BCF is < 500 L/kg.

***Aquatic Toxicity***

The DS included in the CLH report aquatic toxicity studies for spirodiclofen and its major metabolite spirodiclofen-enol for all trophic levels: fish, invertebrates and algae. The studies for spirodiclofen are summarised in the table shown below. The studies for spirodiclofen-enol are included in the section "Supplemental information - In depth analyses by RAC" in the Background document (Annex 1).

Table: Summary of the information on aquatic toxicity for spirodiclofen

| Method | Results | Remarks | Reference |
|---|---|---|---|
| Acute fish spirodiclofen, 96h<br>OECD TG 203, EPA guideline | $LC_{50}$ ≥ 0.035 mg/L | 96h flow-through, limit test<br>*Oncorhynchus mykiss*<br>Measured concentrations | Dorgerloh (1999-a) |
| Acute fish spirodiclofen, 96h<br>OECD TG 203, EPA guideline | $LC_{50}$ ≥ 0.0455 mg/L | 96h flow-through, limit test<br>*Lepomis macrochirus*<br>Measured concentrations | Dorgerloh (1999-b) |
| Acute invertebrate spirodiclofen, 48h<br>OECD TG 202, EPA 72-2 | $EC_{50}$ ≥ 0.0508 mg/L | 48h flow-through, limit test<br>*Daphnia magna*<br>Measured concentrations | Heimbach (1998-a) |
| Algae inhibition, spirodiclofen, 96h<br>OECD TG 201, EPA 540/9-86-134 | $E_rC_{50}$ ≥ 29.2 µg/L (0.0292 mg/L)<br>$E_bC_{50}$ ≥ 29.2 µg/L (0.0292 mg/L)<br>NOEC ≥ 29.2 µg/L (0.0292 mg/L) | 96h static, limit test<br>*Pseudokirchneriella subcapitata*<br>Values based on geometrical mean measured concentrations | Anderson (1998)[b] |
| Chronic fish, spirodiclofen, 97d<br>OECD TG 210, FIFRA 72-4, early life stage | NOEC = 1.95 µg/L (0.00195 mg/L) | 97 days flow-through test<br>*Oncorhynchus mykiss*<br>NOEC based on fish growth<br>Measured concentration | Dorgerloh (2000)[b] |
| Chronic invertebrate, spirodiclofen, 21d<br>OECD TG 202 (II), EPA 72-4. | NOEC = 24.8 µg/L (0.0248 mg/L) | 21d flow-through test<br>*Daphnia magna*<br>NOEC is based on growth and reproductive effects<br>Mean measured concentration | Heimbach (1998-b)[b] |
| Chronic invertebrate, spirodiclofen, 21d<br>EPA 72-4 | NOEC = 11.1 µg/L (0.0111 mg/L) | 21d flow-through test<br>*Daphnia magna*<br>NOEC is based on reproduction<br>Mean measured concentration | Hall and Lam (2001)[b]. |

The acute and chronic aquatic toxicity hazard endpoints for spirodiclofen were studied for all three trophic levels.

Fish

The acute toxicity of spirodiclofen in fish was tested in two different species: *Oncorhynchus mykiss* and *Lepomis macrochirus*. In both studies the concentration of spirodiclofen was at or near its water solubility in the test medium (solubility in water 0.05 mg/L at pH 4 and 22 °C). Water quality parameters were within acceptable levels.

*Short term toxicity to fish*

A 96h acute toxicity flow-through limit test with technical spirodiclofen (97.6% pure) in acidified methanol (2.5% acetic acid) test medium with rainbow trout (*Oncorhynchus mykiss*), according

to OECD TG 203 and EPA 72-1 guidelines was conducted and in compliance with GLP. No mortalities occurred during the test and no sub-lethal effects were observed at the mean measured limit test concentration of 0.035 mg/L, which was at or near the limit of solubility of the compound in the test medium. No mortalities or other effects were observed in the control and solvent control. Thus, the $LC_{50}$ ≥ 35.1 µg/L (≥ 0.035 mg/L), is based on mean measured concentrations.

In a 96h acute toxicity flow-through limit test with technical spirodiclofen (97.6% pure) in acidified methanol (2.5% acetic acid) test medium in bluegill sunfish (*Lepomis macrochirus*), according to OECD TG 203 and EPA 72-1 guidelines and in compliance with GLP. No mortalities occurred during the test and no sub-lethal effects were observed at the mean measured limit test concentration of 0.0455 mg/L, which was at or near the limit of solubility of the compound in the test medium. No mortalities or other effects were observed in the control and solvent control. The $LC_{50}$ of ≥ 45.5 µg/L (≥ 0.0455 mg/L) is based on mean measured dissolved test concentration.

*Long term toxicity to fish*

A 97 days fish early life stage flow-through study was undertaken with eggs, larvae and juveniles of rainbow trout (*Oncorhynchus mykiss*). At day 61 post-hatch, fish length was significantly reduced at all tested concentrations (1.09, 1.95 and 3.81 µg/L) while dry fish weight was reduced by 11% only at the highest concentration (not statistically significant). The biological NOEC based on fish growth was 1.95 µg/L, measured concentration, since the reductions in fish length at 1.09 and 1.95 µg/L were small (< 5%) i.e. within the range of control variability and not accompanied by a simultaneous significant reduction in weight.

Aquatic invertebrates

*Short-term toxicity to aquatic invertebrates*

An acute toxicity flow-through limit test with technical spirodiclofen (97.8% pure) in acidified methanol test medium in *Daphnia magna* was conducted according to OECD TG 202 and EPA 72-2 guidelines and in compliance with GLP. No immobilities in the blank control and one (2.5%) in the solvent control were observed. Immobilities in the test concentrations were 0, 0, 1 (2.5%), 1 (2.5%), and 1 (2.5%) at 5.6, 10, 18, 32, and 56 µg/L nominal. No sub-lethal effects or changes in behaviour were observed during the study. The 48h $EC_{50}$ was set at ≥ 50.8 µg/L, i.e. the mean measured highest test concentration.

*Long-term toxicity to aquatic invertebrates*

The chronic toxicity of [dihydrofuranone-3-$^{14}$C]-spirodiclofen (chemical purity ≥ 97.8%, radiochemical purity ≥ 99%) to *Daphnia magna* was assessed in a 21-days flow-through study. The study was in accordance with GLP and OECD TG 202 (II) and EPA 72-4 with a few deviations from the protocols which are acceptable. Based on survival, growth and reproductive effects, the NOEC was identified at 24.8 µg/L, mean measured concentrations and the LOEC at 49.3 µg/L.

A second chronic toxicity study of spirodiclofen (purity 97.8%) on survival and reproduction of *Daphnia magna* was performed according to EPA 72-4. Survival observed among the treated parents was 88-100% and was not lower than in the controls. Sub lethal effects (pale coloration, abnormal position and unhatched neonates) were not dose related. Reproduction appeared to be the most sensitive endpoint. There were no significant differences in the time to first brood between the solvent control and the treatment groups and for this endpoint the resulting NOEC was 32.7 µg/L, measured concentration. The mean number of neonates per adult was significantly affected in the 20.2 µg/L and 32.7 µg/L (measured) concentrations. The mean number of neonates per adult in the pooled controls and in the 4.39, 6.65, 11.1, 20.2 and 32.7 µg/L concentrations were 6.15, 6.01, 5.55, 5.47, 4.47 and 2.78, respectively. For this endpoint the NOEC was therefore 11.1 µg/L, mean measured concentrations. For the effect on dry weight

and length of the exposed adult daphnids the NOEC was 20.2 µg/L, mean measured concentrations.

Algae and aquatic plants

A static algal growth inhibition test with technical spirodiclofen (97.8% pure) in acidified methanol test medium was conducted as a limit test (Anderson, 1998, amendment 2002). Test duration was 96 hours, the green algae species was *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*); it was performed according to OECD TG 201 (1984) and EPA 540/9-86-134 (1986) guideline and in compliance with GLP. No growth inhibition was observed. However, the test substance concentrations decreased during the study period, due to the instability of the test item under alkaline conditions. The geometrical mean measured test concentration of the highest nominal concentration at 60 µg/L was calculated to be 29.2 µg/L. Consequently, the $EC_{50}$ and NOEC values were based on geometrical mean measured concentrations; EbC50 and ErC50 ≥ 29.2 µg/L, NOEbC and NOErC ≥ 29.2 µg/L.

Other Aquatic Organisms (including sediment)

The chronic toxicity of spirodiclofen (purity 97.5%) to *Chironomus riparius* (< 3 day old, 1 instar larvae) was assessed in a 28 day water/sediment system under static conditions using the BBA method and in accordance with GLP. Emergence was the most sensitive parameter. Total number of emerged midges was dose-related reduced and at higher concentrations, no emergence occurred. $EC_{50}$ and NOEC for emergence were reported to be 0.094 and 0.032 mg/L, respectively.

## Comments received during public consultation

Three MSCAs supported the classification proposed for environmental hazards as Aquatic Chronic 1 (H410) with an M factor of 10.

## Assessment and comparison with the classification criteria

### *Degradation*

No ready biodegradation study or $BOD_5$/COD data is available. Spirodiclofen hydrolyses rapidly at pH 9 but not at pHs 4 and 7, thus not fulfilling the criteria for fast primary degradation.

In two water/sediment simulation studies, pond and pit, spirodiclofen half-lives in water were 0.3 and 1.1 days, and 4.2 and 2.3 days in the whole systems. However mineralisation was low in both systems, up to 2.6%, thus not fulfilling the criterion for rapid degradation. Furthermore, based on the information provided in the CLH report on the primary degradant spirodicolfen-enol it cannot be satisfactorily demonstrated that it does not fulfil the criteria for classification as hazardous to the aquatic environment. Overall, RAC considers spirodiclofen as not rapidly degradable for classification purposes based on the criteria in the CLP Regulation, and the indication of the CLP guidance, Annex II.4.

### *Acute aquatic hazards*

RAC notes that spirodiclofen is an insecticide and acaricide, its target organisms being sucking insects and mites, and that no acute toxicity on these thropic levels have been included in the CLH report. RAC would have appreciated the presence of these studies as confirmation of the lack of acute effects.

Spirodiclofen is a poorly water-soluble substance, 0.05 mg/L at pH 4 and 22 °C. Information on acute aquatic toxicity is available for all three trophic levels. No effects on aquatic organisms were observed at test concentrations between 0.0292 mg/L and 0.0508 mg/L, which are near the spirodiclofen limit of water solubility at pH 4. The available data are based on mean measured

concentrations in the test media and the L(E)$C_{50}$s values are above the water solubility at pH 4 (i.e. 0.05 mg/L). The available data show that the criteria for classification for acute aquatic hazards according to Annex I, Table 4.1.0 (a) of the CLP Regulation are not applicable to spirodiclofen. RAC notes that spirodiclofen solubility increases with increasing pH, from 0.05 mg/L at pH 4 to 0.19 mg/L at pH 7. As the tests were conducted at a pH higher than 4, spirodiclofen may have not been tested at the limit of its water solubility. However, due to the lack of effects observed at the tested concentrations and the absence of data at higher concentrations, RAC agrees with the DS's proposal **not to classify the substance for acute aquatic hazards**.

***Aquatic Chronic hazards***

Spirodiclofen is considered not rapidly degradable in the environment and does not fulfil the criterion for bioaccumulation, BCF < 500. Chronic aquatic toxicity data are available for all three trophic levels. The lowest NOEC of 0.00195 mg/L was obtained in fish (*Oncorhynchus mykiss*). This value is below the classification threshold value of 0.1 mg/L, therefore spirodiclofen does fulfil the criteria for classification as Chronic Category 1. As the lowest NOEC value of 0.00195 mg/L falls within the range 0.001< NOEC < 0.01 mg/L, an M-factor of 10 is applicable. In conclusion, RAC agrees with the DS proposal for classification of spirodiclofen as **Aquatic Chronic Category 1 (H410: Very toxic to aquatic life with long lasting effects)** with an **M-factor of 10**.

## RAC evaluation of hazards to the ozone layer

### Summary of the Dossier Submitter's proposal

No classification is proposed by the DS due to lack of data.

### Comments received during public consultation

No comments were received.

### Assessment and comparison with the classification criteria

No data.

## Additional references


Cambien F, Ducimetiere P, Richard J. Total serum cholesterol and cancer mortality in a middle-aged male population. Am J Epidemiol. 1980;112:388–394

Casey PJ, Solski PA, Der CJ, et al. p21ras is modified by a farnesyl isoprenoid. Proc Natl AcadSci (USA) 1989;86(21):8323–8327.

Delgado-Coello B, Briones-Orta Ma, Macías-Silva M, Mas-Oliva J.Cholesterol: Recapitulation Of its Active Role During Liver Regeneration Liver Int. 2011;31(9):1271-84

Dessì S, Batetta B, Pulisci D, Spano O, Anchisi C, Tessitore L, Costelli P, Baccino FM, Aroasio E, Pani P. Cholesterol content in tumor tissues is inversely associated with high-density lipoprotein cholesterol in serum in patients with gastrointestinal cancer. Cancer. 1994;73(2):253–258.

Saito N, Sairenchi T, Irie F, Iso H, Iimura K, Watanabe H, et al. Low serum cholesterol levels are associated with elevated mortality from liver cancer in Japan: the Ibaraki Prefectural health study. Tohoku J Exp Med 2013; 229: 203-11.

## ANNEXES:

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

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