**Committee for Risk Assessment**

**RAC**

# Opinion

proposing harmonised classification and labelling
at EU level of

# Spirotetramat (ISO)

**EC number: not allocated**
**CAS number: 203313-25-1**

CLH-O-0000001688-63-02/F

**Adopted**

**10 September 2013**

10 September 2013

CLH-O-0000001688-63-02/F

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

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

**Chemicals name: Spirotetramat (ISO)**

**EC number: not allocated**

**CAS number: 203313-25-1**

The proposal was submitted by **Austria** and received by the RAC on **25 September 2012.**

In this opinion, all classifications are given firstly in the form of CLP hazard classes and/or categories, the majority of which are consistent with the Globally Harmonised System (GHS) and secondly, according to the notation of 67/548/EEC, the Dangerous Substances Directive (DSD).

## PROCESS FOR ADOPTION OF THE OPINION

**Austria** 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 **25 September 2012**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **9 November 2012**.

### ADOPTION OF THE OPINION OF THE RAC

Rapporteur, appointed by RAC: **Elodie Pasquier**

Co-rapporteur, appointed by RAC: **Steve Dungey**

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

The RAC opinion on the proposed harmonised classification and labelling was reached on **10 September 2013** and the comments received are compiled in Annex 2.

The RAC Opinion was adopted by **consensus**.

## OPINION OF THE RAC

The RAC adopted the opinion that **Spirotetramat (ISO)** should be classified and labelled as follows:

### Classification and labelling in accordance with the CLP Regulation

| | Index No | International Chemical Identification | EC No | CAS No | Classification | | Labelling | | | Specific Conc. Limits, M-factors |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Hazard Class and Category Code(s) | Hazard statement Code(s) | Pictogram, Signal Word Code(s) | Hazard state-ment Code(s) | Suppl. Hazard statement Code(s) | |
| **Current Annex VI entry** | No current Annex VI entry | | | | | | | | | |
| **Dossier submitters proposal** | 607-711-00-0 | spirotetramat (ISO); (5s,8s)-3-(2,5-dimethylphenyl)-8-methoxy-2-oxo-1-azaspiro[4.5]dec-3-en-4-yl ethyl carbonate | - | 203313-25-1 | Repr. 2<br>Eye Irrit. 2<br>Skin Sens. 1<br>Aquatic Acute 1<br>Aquatic Chronic 1 | H361fd<br>H319<br>H317<br>H400<br>H410 | GHS07<br>GHS08<br>GHS09<br>Wng | H361fd<br>H319<br>H317<br>H410 | | M = 1<br>M = 1 |
| **RAC opinion** | | | - | 203313-25-1 | Repr. 2<br>STOT SE 3<br>Eye Irrit. 2<br>Skin Sens. 1A<br>Aquatic Acute 1<br>Aquatic Chronic 1 | H361fd<br>H335<br>H319<br>H317<br>H400<br>H410 | GHS07<br>GHS08<br>GHS09<br>Wng | H361fd<br>H335<br>H319<br>H317<br>H410 | | M = 1<br>M = 1 |
| **Resulting Annex VI entry if agreed by COM** | | | - | 203313-25-1 | Repr. 2<br>STOT SE 3<br>Eye Irrit. 2<br>Skin Sens. 1A<br>Aquatic Acute 1<br>Aquatic Chronic 1 | H361fd<br>H335<br>H319<br>H317<br>H400<br>H410 | GHS07<br>GHS08<br>GHS09<br>Wng | H361fd<br>H335<br>H319<br>H317<br>H410 | | M = 1<br>M = 1 |

**Classification and labelling in accordance with the DSD**

| | Index No | International Chemical Identification | EC No | CAS No | Classification | Labelling | Concentration Limits |
|---|---|---|---|---|---|---|---|
| **Current Annex VI entry** | No current Annex VI entry | | | | | | |
| **Dossier submitters proposal** | 607-711-00-0 | spirotetramat (ISO); (5s,8s)-3-(2,5-dimethylphenyl)-8-methoxy-2-oxo-1-azaspiro[4.5]dec-3-en-4-yl ethyl carbonate | - | 203313-25-1 | Repr. Cat. 3; R62–63<br>Xi; R36<br>R43<br>N; R50-53 | Xn; Xi; N<br>R: 36-43-50/53-62-63<br>S: 2-13-20/21-24/25-27/28-36/37/39-56-66-60-61 | |
| **RAC opinion** | | | - | 203313-25-1 | Repr. Cat. 3; R62-63<br>Xi; R36/37<br>R43<br>N; R50-53 | Xn; N<br>R: 36/37-43-50/53-62-63<br>S: (2-)36/37-60-61 | Xi; R43: C ≥ 0,1 %<br>N; R50-53: C ≥ 25 %<br>N; R51-53: 2,5 % ≤ C < 25 %<br>R52-53: 0,25 % ≤ C < 2,5 % |
| **Resulting Annex VI entry if agreed by COM** | | | - | 203313-25-1 | Repr. Cat. 3; R62-63<br>Xi; R36/37<br>R43<br>N; R50-53 | Xn; N<br>R: 36/37-43-50/53-62-63<br>S: (2-)36/37-60-61 | Xi; R43: C ≥ 0,1 %<br>N; R50-53: C ≥ 25 %<br>N; R51-53: 2,5 % ≤ C < 25 %<br>R52-53: 0,25 % ≤ C < 2,5 % |

# SCIENTIFIC GROUNDS FOR THE OPINION

## HUMAN HEALTH HAZARD ASSESSMENT

## RAC evaluation of physical hazards

### Summary of the Dossier submitter's proposal

No physical/chemical classification is proposed by the Dossier Submitter (DS) based on the following observations:

- Flammability: Spirotetramat is not a highly flammable solid in the sense of EC guideline A.10.
  Auto-flammability: No self-ignition temperature was observed up to the melting point or up to the maximum test temperature of 401 °C. Spirotetramat is not classified in Division 4.2 (a negative result was obtained in the test using a 10 cm cube sample at 140 °C).
- Flash point: The active substance is a solid; its melting point is > 40 °C; therefore, criteria regarding the flash point are not applicable.
- Explosive: Not explosive in the sense of EC guideline A.14.
- Oxidising: Spirotetramat has no oxidizing properties in the sense of EC guideline A.17.
  Oxidation or reduction properties: Technical Spirotetramat was found to be non-reactive with ammonium dihydrogen phosphate, metallic iron or an aqueous solution of 0.1N potassium permanganate in terms of significant temperature increase or evolution of gas. No significant temperature increase was observed for all applied test mixtures, i.e. the temperature increase was clearly below 5 °C in the course of the experiments and never exceeded ambient conditions.

### Comments received during public consultation

No specific comments were received.

### Assessment and comparison with the classification criteria

RAC supports the proposal of DS not to classify Spirotetramat for physical hazards.

## RAC evaluation of acute toxicity

### Summary of the Dossier submitter's proposal

***Acute toxicity: oral***
No classification is proposed based on the absence of mortality or any treatment-related findings at the limit dose of 2000 mg/kg in an OECD TG 425 study in rats. In this study, the $LD_{50}$ of Spirotetramat in rat by the oral route exceeded 2000 mg/kg.

***Acute toxicity: inhalation***
Rats were exposed (nose-only) to 1.1 or 4.183 mg/L of Spirotetramat (as an aerosol) for four hours in an OECD TG 402 study. No mortality was observed in any dose group. In both groups, some animals experienced a transient effect on body weight gain. Clinical signs consisting of ungroomed hair-coat, piloerection, bradypnea, laboured breathing and serous nasal discharge were observed up to day 4 at 1.1 mg/L. These clinical signs were also observed at 4.183 mg/L up to day 7, together with breathing sounds, reddened nostrils, nasal discharge, red encrustations in the nostrils and nose/snout region, stridor, reduced motility and limp high-legged gait. Abnormal reflexes were also reported. From this study, the $LC_{50}$ of Spirotetramat in rat by inhalation exceeds 4.183 mg/L. No classification was proposed by the DS.

***Acute toxicity: dermal***
Rats were exposed to a limit dose of 2000 mg/kg in an OECD TG 403 study and no mortality, effect on body weight gain or gross pathological findings were observed. Clinical signs consisting of red stain (nose), wetness (urogenital area), yellow stain (urogenital area) and red zone (back) were

reported from day 1 to 3. From this study, the $LD_{50}$ of Spirotetramat in the rat by the dermal route exceeds 2000 mg/kg. No classification was proposed by the DS.

### Comments received during public consultation

No specific comments were received.

### Assessment and comparison with the classification criteria

RAC supports the proposal of DS not to classify Spirotetramat for physical hazards.

## RAC evaluation of acute toxicity

### Summary of the Dossier submitter's proposal

***Acute toxicity: oral***
The $LD_{50}$ of Spirotetramat in the rat is greater than 2000 mg/kg, below which classification for acute toxicity by the oral route applies according to the criteria of both the CLP Regulation and DSD.

***Acute toxicity: inhalation***
The available study provides no evidence that the $LC_{50}$ of Spiroteramat in rats is below the 5 mg/L trigger for classification for acute toxicity by inhalation for aerosols under the criteria of both the CLP Regulation and DSD.

***Acute toxicity: dermal***
The $LD_{50}$ of Spirotetramat in rat is greater than 2000 mg/kg, below which classification for acute toxicity by the dermal route applies according to the criteria of both the CLP Regulation and DSD.

RAC supports the proposal of the DS not to classify Spirotetramat for acute toxicity.

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

### Summary of the Dossier submitter's proposal

Spirotetramat was found to be of low acute toxicity to the rat by the oral route. No treatment-related findings were observed at the limit dose of 2000 mg/kg bw. Treatment-related acute dermal toxicity findings were limited to minor clinical signs (resolved by day 3) at the dose of 2000 mg/kg bw. Treatment-related findings in the acute inhalation toxicity study were limited to minor effects on bodyweight gain and clinical signs (resolved by day 8) at the doses of 1.1 and 4.183 mg/L.

The DS concluded that no specific, non-lethal, target organ toxicity after single exposure was observed in the acute toxicity studies. In addition, no human data are available that would support classification for this endpoint. No classification as STOT SE under the CLP Regulation was proposed by the DS.

### Comments received during public consultation

No specific comments were received.

### Assessment and comparison with the classification criteria

The criteria for STOT SE 3 for respiratory irritation states that "this evaluation is primarily based on human data" but that "useful information may be obtained from the single and repeated inhalation toxicity tests" in animals. Effects that are relevant to consider for respiratory irritation according to the CLP criteria are clinical signs such as dyspnea and rhinitis, and histopathology findings such as hyperaemia, oedema, minimal inflammation, thickened mucous layer, which are reversible effects.

In the absence of relevant human data for Spirotetramat, the acute inhalation study provides some relevant evidence of respiratory irritation. No histopathological findings were observed but it is noted that gross necropsy was performed 14 days after exposure and hence effects, if present, may have reversed. The only indication of respiratory irritation therefore comes from the clinical signs observed. Reversible breathing difficulties, nasal discharge and red incrustation in the nostrils and nose/snout region in the acute inhalation are interpreted as signs of acute respiratory tract irritation.
As the test substance is a white solid, it cannot be excluded that the mechanical effect of solid particles contributed to the irritation observed at high concentrations.
However, clinical signs indicating respiratory irritation are not only observed at the high dose of 4.183 mg/L, but also at 1.1 mg/L, and therefore mechanical irritation may not fully explain the observed clinical signs.

On this basis, RAC proposes to classify Spirotetramat with STOT SE 3 – H335 according to the CLP Regulation and Xi; R37 according to DSD.

## RAC evaluation of skin corrosion/irritation

### Summary of the Dossier submitter's proposal

In an OECD TG 404 study, Spirotetramat (as a powder, moistened with water) was applied to the skin of three rabbits for 4 hours under semi-occlusive conditions. No irritation was observed at any time point in any animal (scores of 0). The DS concluded that Spirotetramat is not irritating to the rabbit skin.

### Comments received during public consultation

No specific comments were received.

### Assessment and comparison with the classification criteria

In the absence of any sign of irritation in a guideline-compliant study, Spirotetramat does not fulfil the criteria for skin irritation under the CLP Regulation or DSD, either in terms of severity of scores or in terms of irreversibility. RAC supports the proposal of the DS not to classify Spirotetramat for skin irritation/corrosion.

## RAC evaluation of eye corrosion/irritation

### Summary of the Dossier submitter's proposal

In an OECD TG 405 study, Spirotetramat was instilled into the conjunctival sac of three rabbits. Corneal opacity (grade 1) was observed in all eyes from 24 hours to 6 days after instillation and in two eyes until 7 days after instillation. No corneal opacity was observed on day 8. Iritis (grade 1) was noted in two eyes at 24 and 48 hours, in three eyes at 72 hours and days 4 and 5, and in one eye on day 6 after instillation. No iritis was observed on day 7.
Conjunctival redness (grade 1) was noted in all eyes from 1 to 72 hours after instillation and in two eyes until 4 days after instillation. Conjunctival chemosis (grade 1) was noted in one eye at 24 and 48 hours after instillation. All conjunctival irritation was resolved by day 5.
The DS concluded that the effects observed on corneal opacity, iritis, conjunctival redness and conjunctival chemosis warrants classification of Spirotetramat as Eye Irrit. 2 – H319 according to the CLP Regulation and Xi; R36 according to DSD.

### Comments received during public consultation

Three MSCA agreed with the proposed classification as Eye Irrit. 2 – H319 without further comments.

### Assessment and comparison with the classification criteria

RAC supports classification as Eye Irrit. 2 – H319 according to the CLP Regulation on the basis of three animals with corneal opacity scores of 1, and three animals with iritis scores of 1 over three consecutive days, and as Xi; R36 according to the DSD on the basis of three animals with iritis scores of 1 over three consecutive days.

As all effects were reversible before the end of the observation period, a more stringent classification is not justified.

## RAC evaluation of respiratory sensitisation

### Summary of the Dossier submitter's proposal

Based on the data from the acute inhalation studies, the DS concluded that Spirotetramat is not a respiratory sensitiser.

### Comments received during public consultation

One MSCA agreed with the proposal not to classify for respiratory irritation without including any further comments.

### Assessment and comparison with the classification criteria

In the absence of any relevant data to compare with existing criteria, RAC considers that a conclusion on classification for respiratory sensitisation is not possible.

## RAC evaluation of skin sensitisation

### Summary of the Dossier submitter's proposal

The skin sensitising potential of Spirotetramat was tested in three different studies conducted according to OECD test guidelines.

A Guinea pig maximisation test (GPMT) was conducted with Spirotetramat in polyethylene glycol 400, using an intradermal induction concentration of 5%, a topical induction concentration of 50% and a challenge topical concentration of 25%. 95% of animals (18/19) exhibited dermal reactions (grade 1-3) while no reactions were observed in the control group.

A Buehler Guinea pig test was conducted with Spirotetramat in polyethylene glycol 400, using a topical induction concentration of 71% and a challenge topical concentration of 71%. No reactions were observed either in the test group or in the control group.

A Local Lymph Node Assay (LLNA) was conducted with Spirotetramat in dimethylformamide, using a topical concentration of 10, 5, 2.5 or 1%. The stimulation index (SI) values of Spirotetramat were 5.9, 5.4, 4.3 and 3.4 while SI values for the positive control (isoeugenol) were 3.4, 1.8, 1.3 and 0.8% at concentrations of 5, 2.5, 1 and 0.5%, respectively.

The sensitisation potential of Spirotetramat was supported by two cases of Type IV hypersensitivity that were reported in Spirotetramat manufacturing plant personnel. No further details are given in the Report on medical surveillance of manufacturing plant personnel.

Based on the positive GPMT and LLNA, the DS concluded that Spirotetramat should be classified as Skin Sens. 1A – H317 according to the CLP Regulation and Xi; R43 according to DSD.

### Comments received during public consultation

Three MSCAs agreed with the proposed classification as Skin Sens. 1A – H 317 without further comments. Two additional MSCAs also agreed with classification in category 1 but one commented that available data do not enable sub-categorisation as 1A and the other that the rationale supporting sub-categorisation in 1A should be clarified.

### Assessment and comparison with the classification criteria

On the basis of the positive results observed in the GPMT and in the LLNA, the RAC considers that Spirotetramat should be classified as Skin Sens. 1 – H317 according to the CLP Regulation and Xi; R43 according to DSD.

The skin sensitisation potency of Spirotetramat, as evaluated in the LLNA, indicates that a sub-categorisation in 1A is justified. The GPMT results indicate that Spirotetramat has at least a moderate potency but the study did not use experimental conditions that could identify a high potency. The GPMT results are therefore not inconsistent with the high potency identified in the LLNA.

RAC therefore agrees with the DS that Spirotetramat should be classified as Skin Sens. 1A – H317 according to the CLP Regulation.

RAC also concludes that the results justify a specific concentration limit of 0.1% for Xi; R43 according to the DSD.

## RAC evaluation of repeated dose toxicity (DSD) and specific target organ toxicity (CLP) – repeated exposure (STOT RE)

### Summary of the Dossier submitter's proposal

The insecticidal mode of action (lipid biosynthesis inhibition) was not reflected in the results of the short-term toxicological studies in rodents and dogs. Rats, mice and dogs did not exhibit changes in plasma lipid parameters such as plasma triglycerides and plasma cholesterol. The thyroid and thymus glands were the target organs in oral (sub)chronic toxicity studies in the dog. (Sub)chronic (90-day and 1 year) exposure of dogs to Spirotetramat was characterised by statistically significant reductions in circulating thyroid hormones (T4) at ≥1200 ppm (32 mg/kg bw/day) and 600 ppm (20 mg/kg bw/day) respectively; decreased T3 was observed at the highest dose tested (2500 ppm in the 90-day study, and 1800 ppm in the 1 year study, respectively). Despite reductions in thyroid hormone levels at some time points, no changes in thyroid weight or compensating increases in thyrotropin (TSH) were seen at ≤ 600 ppm. After exposure of dogs to Spirotetramat for 1 year, thymus involution and dilated brain were observed in males at 600 and 1800 ppm.

In rats, the testis was the target organ following subchronic oral treatment at a high dose. Abnormal spermatozoa and hypospermia in the epididymis, decreased testicular weight, and testicular degeneration and vacuolation in males were observed after 90 days of exposure at 10000 ppm (616 mg/kg bw/day). These effects proved to be reversible in most animals after cessation of treatment. Other effects in subchronically treated rats were limited to reductions in terminal body weight in 10000 ppm male rats and an increased incidence of accumulation of alveolar macrophages in both sexes at 10000 ppm. It is noteworthy that the thyroid and thymus were unaffected in rats at all doses, while testicular histopathology was not observed in dogs.

Chronic toxicity was tested in rats following oral exposure of Spirotetramat for one year. Target organs in rats were the kidney (both sexes) at mid and high doses and the liver (females) at the highest dose only. These results are consistent with the excretory and/or detoxification roles of the kidney and liver. Consistent with the subchronic study, the lung demonstrated treatment-related presence of alveolar macrophages in mid and high dose males and high dose females. Exfoliated germ cells/debris in the epididymis and abnormal spermatozoa were observed in high dose males and these findings were considered toxicologically relevant. In the last month of the one year chronic toxicity study, 10 rats/sex/dose were evaluated in weekly, open field, Functional Observational Battery (FOB) assessments that included evaluation of motor activity and responses to sensory stimuli. Treatment-related effects in the FOB assessments were not observed.

Unlike the rat, no adverse effects of any kind were observed in mice tested orally up to the limit dose. Results from a comparative *in vitro* metabolism study using hepatocytes from male rats, mice, and humans revealed species differences in the metabolism of Spirotetramat. Specifically,

mouse hepatocytes were better able to metabolise BYI 08330-enol via glucuronidation compared to rat and human liver cells. Based on these findings, it was hypothesised that potentially lower levels of the enol metabolite in mice *in vivo* might account for the lack of testicular toxicity observed in this species.
Subchronic exposure of rats by the dermal route yielded no evidence of systemic toxicity when Spirotetramat was tested up to 1000 mg/kg bw/day. This result may in part be a reflection of the low dermal absorption (approximately 10%) in rats.

The DS concluded that Spirotetramat did not induce significant toxic effects at doses relevant for classification.

## Comments received during public consultation

No specific comments were received.

## Assessment and comparison with the classification criteria

By the oral route, no toxicity was observed in mice after exposure to Spirotetramat for 28 days up to 1415 mg/kg bw/day, for 90 days up to 1305/1515 mg/kg bw/day or for 18 months up to 1022/1319 mg/kg bw/day.

In rats, the main target organs were the testis and epididymides, lung, kidney and liver. All effects were, however, observed at doses exceeding the criteria for classification, except for effects reported in the lung in the two-year study. In this latter study, an increased incidence in accumulation of alveolar macrophages was observed from 250 ppm in males. It is noted that this effect was not observed in males at the highest dose and no increase was noted in females at any dose. However, an increased incidence in interstitial pneumonia was reported in the high dose males and in the females from 250 ppm with a dose-related increase in incidence and severity; the findings reflected an inflammatory response in the lung, occurring with a dose-related increase in incidence in both males and females. Besides, an increased incidence of accumulation of macrophages was also reported in the 90-day and the 1-year study, therefore its relationship to treatment is not in doubt. It is however noted that:

- 250 ppm (corresponding to 12 mg/kg bw/day in males and 17 mg/kg bw/day in females) is at the upper limit dose of 12.5 mg/kg bw/day relevant for classification under CLP and exceeds the limit dose of 6.5 mg/kg bw/day relevant for classification under DSD;
- the severity of both findings at 250 ppm was not marked (severity of 1.2 and 1.1 for accumulation of macrophages and 1.7 and 1.4 for interstitial pneumonia, in males and females, respectively) and was similar to that seen in the controls. It is therefore considered that it does not provide clear evidence of marked organ dysfunction at this dose.

On this basis, RAC considers that the effect of Spirotetramat on the lung in the rat does not justify classification for repeated dose toxicity.

The dog is the most sensitive species tested, with the main effects observed on thyroid hormone T4 (and in some studies on T3) and on the thymus, these being consistently reported in a 28-day, a 90-day and a 1-year study. The following effects were observed at doses relevant for classification according to CLP:

- A marked effect on body weight in males (-33%) and females (-29%) was observed at the high dose (104/127 mg/kg bw/day, respectively) in the 28-day study. A 17% decrease in body weight was also observed in females at 2500 ppm (72 mg/kg bw/day) but not in males in the 90-day study. Such effects were not found at doses relevant for classification in the 1-year study. In all cases, decreases in body weights were accompanied by decreases in food consumption. No clinical signs indicating a poor general health status were observed (only some animals were reported as thin). Body weight reduction may therefore be secondary to palatability problems due to the presence of high levels of the substance in the diet and may well not be an indication of morbidity associated with toxicity of the substance. It is not therefore considered that classification is justified.
- A decrease of 17% in the RBC count, 17% in the haemoglobin concentration and 16% in haematocrit in high dose females (2500 ppm or 71 mg/kg bw/day) in the 90-day study was observed. No effect on haematology parameters were however reported in the two

other dog studies making the interpretation of this finding uncertain and it was not considered sufficient to justify classification.

- In the 1-year dog study, significant decreases in T4 levels were reported in males and females from 600 ppm (corresponding to 20 and 19 mg/kg bw/day, respectively). A reduction in T4 and as well as sometimes in T3 was identified in studies of shorter durations at high doses. It was not associated with effect on thyroid weight in any study. Histological findings were restricted to two out of four males with reductions in the size of the peripheral thyroid follicles at the high dose of 1800 ppm (48 mg/kg bw/day), which were above classification cut-offs, in the 1-year study. No functional, morphological or histological effect related to the perturbation in T4 levels was identified at a dose relevant for classification.
- In the 1-year study, males of the 600 ppm group (20 mg/kg bw/day) had reduced thymus size, non-significantly lowered thymus weight and 1 animal (out of 4) with thymus involution graded as mild. Considering the low incidence and severity of this lesion it is not considered sufficient to indicate a significant toxic effect triggering classification.
- In the 1-year study, gross necropsy revealed dilated brain at 600 ppm in males and females, which was confirmed following histopathological examination of brain dilatation in one male (mild) and one female (moderate). It was however not accompanied by any clear histopathological alterations. Such an effect was not reported in females at the high dose or in studies of shorter duration. In addition, brain ventricular dilatation is occasionally reported to occur spontaneously in the strain of dogs used in the test and this finding is considered to be of unclear toxicological significance.

RAC therefore agreed with the DS that classification is not justified for repeated toxicity under neither CLP nor DSD.

## RAC evaluation of germ cell mutagenicity

### Summary of the Dossier submitter's proposal

*In vitro* assays for point mutations and chromosomal aberrations were negative in the presence of Spirotetramat. A weak positive finding was noted in a single *in vitro* chromosomal aberration test, but at cytotoxic concentrations only. Negative results were observed in one *in vitro* unscheduled DNA synthesis assay also using rat hepatocytes.

*In vivo* assays for point mutations and chromosomal aberrations were negative in the presence of Spirotetramat. Negative findings in two *in vivo* chromosomal aberration studies and one *in vivo* unscheduled DNA synthesis assay using rat hepatocytes do not suggest a genotoxic concern for Spirotetramat and no classification is proposed by DS.

**Table 5 – Summary of mutagenicity tests**

| Type of Study | Test system | Dose | Results | Reference |
|---|---|---|---|---|
| In vitro | | | | |
| Salmonella/microsome test | *Salmonella typhimurium* (TA98, TA100, TA102, TA1535, TA1537) | 0 - 5000 µg/plate | Negative | Herbold, 2006 |
| Salmonella/microsome test | *Salmonella typhimurium* (TA98, TA100, TA102, TA1535, TA1537) | 0 - 5000 µg/plate | Negative | Herbold, 2002 |

| | | | | |
|---|---|---|---|---|
| *In vitro* Chromosome aberration test | Chinese hamster V79 cells | 0 - 750 µg/ml | Weakly positive at cytotoxic concentrations only. | Herbold, 2002 |
| Mammalian cell gene mutation | Chinese hamster V79 cells | 0 - 140 µg/ml | Negative | Herbold, 2002 |
| In vivo | | | | |
| Mouse Micronucleus Test | bone marrow of male mice | 500mg/kg | Negative | Herbold, 2002 |
| Chromosomal aberration assay in mice | bone marrow of male mice | 500mg/kg | Negative | Herbold, 2003 |
| Unscheduled DNA synthesis – rat liver | liver of male rats | 2000 mg/kg | Negative | Brendler-Schwaab, 2003 |

### Comments received during public consultation

One MSCA agreed with the no classification being proposed for mutagenicity without further comments.

### Assessment and comparison with the classification criteria

Based on the three negative *in vivo* studies (micronucleus test, chromosomal aberration test and UDS test) Spirotetramat is considered to be non-mutagenic *in vivo*.
RAC therefore agreed that a classification for mutagenicity was not warranted.

## RAC evaluation of carcinogenicity

### Summary of the Dossier submitter's proposal

Carcinogenicity was tested in rats and mice following application of Spirotetramat for two years or 18 months, respectively. Based on type, incidence and organ distribution of neoplastic lesions in treated and control rats and mice, there was no indication for an oncogenic effect of Spirotetramat in either of the species tested. The DS concluded that no oncogenic effect was observed in studies conducted with Spirotetramat, neither in rat nor in mouse carcinogenicity studies and proposed no classification for carcinogenicity (according to both CLP and DSD).

### Comments received during public consultation

One MSCA agreed with the no classification being proposed for carcinogenicity without further comments.

### Assessment and comparison with the classification criteria

RAC agreed that:

- no carcinogenic effect was observed in a rat 2-year study;
- the unclear evidence of carcinogenicity provided in the mouse 18-month study based on the observation of two lipomatous tumours is not sufficient to justify a classification for carcinogenicity.

RAC agreed with the DS that Spirotetramat should not be classified for carcinogenicity.

# RAC evaluation of reproductive toxicity

## Summary of the Dossier submitter's proposal

***Fertility***

In addition to testicular histopathology observed following subchronic and chronic exposure of male rats to Spirotetramat, evidence of male reproductive toxicity was provided in the 2-generation reproductive toxicity study. Abnormal sperm cells were reported in F1-generation male rats treated with 6000 ppm Spirotetramat in the diet (equivalent to 419 mg/kg bw/day), and decreased reproductive performance was also observed in one of these males. Similar results were obtained in the 1-generation reproductive toxicity range-finding study, in which decreased sperm motility and progression and increased abnormal sperm cells in the epididymides were observed in $F_1$ males at ≥6000 ppm (400 mg/kg bw/day). The highest dose of 10000 ppm, equivalent to 538 mg/kg bw/day, was associated with complete infertility in the parental generation animals. There were no implantation sites noted in the females due to treatment-related effects on sperm cells of males at this dose (increased numbers of abnormal sperms, reduced epididymal sperm counts, reduction in both motility and progression of epididymal sperm cells). Absolute and relative weights of the cauda epididymis were decreased in parental males. Histopathology showed abnormal sperm cells of minimal to moderate severity in the epididymis and the cauda epididymis.
Toxicity in the offspring was limited to decreased body weights in both studies, observed in F1 and F2 pups, respectively, of both sexes during lactation at 6000 ppm (400 and 419.3 mg/kg bw/day, respectively). Decreased body weights were also observed in parental animals at the same dose. Development of the sexual organs of offspring (balano-preputial separation, vaginal opening) was unaffected in both studies.

In February 2008, the notifier of the substance prepared a position paper (Temerowski M., 2008. High dose reproductive effects in male rats and their relevance to humans). In this paper it was stated that the effects on testicular spermatogenesis were attributed to Spirotetramat-enol, which is the main metabolite in the rat. Spirotetramat-enol is further metabolised by oxidation reactions to Spirotetramat desmethyl-enol (DME), Spirotetramat-enol-alcohol and Spirotetramat-ketohydroxy. Oxidation products accounted for approximately 14% of total metabolites. Conjugation was not detected. In the mouse liver cells, conjugation of Spirotetramat-enol with glucuronic acid accounted for approximately 30%. In human liver cells, conjugation to Spirotetramat-enol-glucuronic acid was 6%. The *in vitro* conjugation rate is dependent on the concentration used and declined in mice liver cells from 30% to 9% and in human liver cells from 6% to 2% at concentrations of 19 µg/g and 190 µg/g Spirotetramat, respectively. Glucuronidation of the Spirotetramat-enol in mice leads to much lower systemic levels of free Spirotetramat-enol when compared to the rat. The conjugation enables the mouse to utilise separate active transport systems in the kidneys, thus avoiding saturation of the elimination process. The notifier hypothesised that the utilisation of different transport systems renders the mouse less sensitive to Spirotetramat-mediated testicular toxicity when compared to the rat. The position paper argued that based on the metabolic similarity between mice and humans, it is likely that humans are also less sensitive to Spirotetramat-mediated testicular toxicity than rats.

The DS however did not agree with the conclusions of the position paper from the notifier. For human liver cells the ability to conjugate Spirotetramat-enol with glucuronic acid is five-fold lower than for the mouse liver cells (depending on the concentration, 6% and 2% in human liver cells; and 30% and 9% in mice liver cells, at 19 and 190 µg/g Spirotetramat, respectively). Therefore the DS considered that an argument based on the similarity in the metabolic pathways between mice and humans cannot be sustained. Since for human liver cells conjugation is only 2% at high doses, it cannot be assumed that humans are less sensitive to Spirotetramat than rats.

In a mechanistic study designed to explore the time of onset of testicular toxicity of Spirotetramat in rats (Kennel, 2005), decreased epididymal sperm counts were recorded after ≥10 days of treatment with 1000 mg/kg bw/day by gavage. Therefore, the author of the study concluded that repeated dosing is necessary to produce male reproductive toxicity in rats; however, the author did not consider the relatively long period of spermatogenesis (about 60 days in man, 55 in rat).

Hence, a single high dose at one critical point in development may be required, but the effects would not be seen until later.

In a second mechanistic study, male rats were treated by gavage with the enol metabolite of Spirotetramat for 21 days at a dose of 800 mg/kg bw/day (Tinwell, 2006). Spermatotoxicity, abnormal sperm, and Sertoli cell vacuolation were observed in the testes-epididymides of treated animals. Therefore, the author concluded that male reproductive toxicity in rats is likely to be due to the enol metabolite of Spirotetramat.

From the above mechanistic investigations, the DS concluded that classification for reproductive toxicity in category 2 according to the CLP Regulation for fertility would be appropriate.

***Developmental toxicity***

In the developmental toxicity study in rats, toxicity to the offspring was observed in the presence of maternal toxicity, including decreased food consumption and body weight or body weight gain, at 1000 mg/kg bw/day. Reduced fetal weight and increased incidences of skeletal malformations and skeletal deviations were observed at 1000 mg/kg bw/day. Malformations at the high dose included one case of supernumerary lumbar vertebra, one case of cleft palate and one case of co-arctation of aortic arch. One case of atrial septal defect of the heart and microphthalmia were observed in the control, low and high dose groups, but not at the mid-dose.

Four cases of dysplastic forelimb bones (1.5%) and three cases of malformed sacral vertebral arches with pelvic shift (1.1%) were observed at the high dose. Historical control data of the performing laboratory (Bayer HealthCare AG) for dysplastic forelimb bones in studies conducted in the years 1999 to 2004 showed 26 affected animals out of 1975 animals, (1.3 %) [range 0.4 – 4.3% due to one study conducted in the year 2000, were 10 animals out of 232 were affected (4.3%)]. An incidence of 1.5 % in the study with Spirotetramat is therefore outside the concurrent control (0.4%) and the historical control data (1.3%). Statistically significantly increased incidences of sacral vertebral alterations (1.1 %) were observed at a dose of 1000 mg/kg bw/day Spirotetramat in comparison with the concurrent controls (0.0 %). The incidence in historical controls in studies conducted from 1999 to 2006 showed 2 affected animals out of 6554 animals, i.e. 0.03 % (range 0 – 0.4%). Statistically significantly increased incidences of wavy ribs were observed at all doses compared to concurrent and historical control values. Statistically significantly increased numbers of fetuses with $14^{th}$ ribs were observed at a dose of 1000 mg/kg bw/day Spirotetramat. The NOAELs for both maternal and developmental toxicity in this study were set at 140 mg/kg bw/day.

In the developmental toxicity study in rabbits, offspring toxicity was not observed at any dose up to 160 mg/kg bw/day. However, maternal toxicity was observed at ≥40 mg/kg bw/day, including a dose-dependent increase in abortions and clinical signs of toxicity in affected animals (severely reduced food consumption, body weight loss, alopecia, altered appearance of faeces, discoloured urination). Gross pathology revealed treatment-related fluid/gaseous contents in the caecum, mottled gall bladder and discolouration of the liver. The maternal NOAEL was set at 10 mg/kg bw/day.

The DS therefore concluded that Repr. 2 for developmental effects would be appropriate.

## Comments received during public consultation

***Fertility***

Two MSCA supported the proposal to classify as Repr. 2 for fertility without further comment. Two other MSCAs requested a clear rationale why category 2 is more appropriate than category 1B, although one of them expressed their support for a category 2. One MSCA supported classification as Repr. 1B for fertility due to robust findings of testis toxicity in rats and the absence of information that casts doubts on the human relevance of this finding.

One industry comment recognised the testicular toxicity in rats but considered this to be a high dose phenomenon unlikely to occur in humans on the basis of risk assessment considerations. Interspecies differences in toxicokinetics and metabolism were also pointed out and it was proposed that reproductive effects occur in rats at doses which were above those resulting in

saturation of elimination mechanisms, which is not expected in humans, leading them to conclude that no classification for fertility is warranted.

***Developmental toxicity***
Three MSCA supported the proposal to classify as Repr. 2 for developmental effects. Another MSCA requested a clear rationale why category 2 would be more appropriate than category 1B for reproductive toxicity in general (not specifying developmental toxicity). One other MSCA requested further discussion on the incidence of malformations and deviations with regards to historical control data to decide whether it was sufficient for classification in category 2.

Industry considered that classification of Spirotetramat as a reproductive toxicant is not justified and commented more specifically on the historical control data relating to the findings in rats.

## Assessment and comparison with the classification criteria

***Fertility***
Spirotetramat has no effect on male reproductive organs and spermatogenesis in the mouse or the dog, although it is noted that a lower range of doses has been tested in the dog. Available data provide clear and consistent evidence that Spirotetramat alters spermatogenesis in the rat. Fertility was ultimately affected in all male rats exposed to 645 mg/kg bw/day in the range-finding reproductive toxicity study as well as in one F1 animal exposed to 487 mg/kg bw/day in the two-generation study.

Considering the occurrence of other toxic effects together with toxicity on the reproductive system and their potential relationship, RAC noted that effects on the reproductive system were generally observed at doses inducing a decrease in body weight compared to controls. However, decreases in body weight were generally limited (always equal to or less than 10%) and restricted to 2% in high dose parental males that were infertile in the range-finding reproductive study. The most sensitive effect was on sperm count and morphology. Reproductive organ weight was not systematically affected (i.e., in the 1-year rat study, 2-year rat study, and F1 generation of the range-finding reproductive toxicity study). Effects on sperm count and morphology cannot be explained by the limited decreases in body weight and are not considered to be secondary to other toxic effects.

It was commented during the public consultation that effects on spermatogenesis were observed in rats at doses where, due to the saturation of active transport mechanisms in the kidney elimination of Spirotetramat may be restricted. The existence of a conjugation pathway for Spirotetramat in mice and to a lesser extent in humans may prevent saturation of the elimination mechanisms and consequent exposure to high systemic levels of Spirotetramat metabolites in humans.

No information is available on the mechanism of toxicity of Spirotetramat on the reproductive system and the relevance of the observed effects for humans was not challenged based on mechanistic considerations.

RAC indeed noted that effects on the male reproductive system were observed only at doses that may exceed saturation of elimination. Toxicokinetic data *in vivo* shows that excretion into urine in rats was lower after a single dose of 1000 mg/kg (27% excreted in urine after 24h) than after a single dose of 2 mg/kg (88-95% excreted in urine after 24h). This indicates that saturation of active transport mechanisms occurs at the high dose and physiologically based pharmacokinetic (PBPK) simulations (assuming Spirotetramat enters the systemic simulation as an enol) suggests that repeated daily doses of ≥ 300 mg/kg may lead to non-linear elimination kinetics in rats.

On the other hand, the following uncertainties were identified:
- It cannot be excluded that mechanistic elements other that toxicokinetics may explain the species specific sensitivity;
- The existence of a glucuronidation pathway has been identified in mice in an *in vitro* study and such a pathway is not present in rats. Although, it is not known whether this would result in lower *in vivo* systemic levels of Spirotetramat and its metabolites at high doses in

the mice, it may indicate that elimination patways other than direct renal excretion of Spirotetramat exist in the mouse and in humans;
- *In vitro* data shows that the glucuronidation pathway is five-fold lower in humans than in mouse hepatocytes;
- In human hepatocytes, due to limited metabolism of the enol metabolite into further metabolites and limited conjugation, the level of enol metabolite formed *in vitro* is greater than in mouse or rat hepatocytes. This metabolite is likely to be involved in the reproductive toxicity of Spirotetramat;
- DME is formed in significant amounts in rats and this leads to lower levels of enol than in humans. DME and enol both have an affinity for OAT transporters and DME is likely to compete with enol for excretion into urine. But considering the lower level of the enol due to it being metabolised into DME, it is not known whether this difference in metabolism will lead to differences in systemic level of enol between rats and humans;
- It is noted that only one donor was used as the source for human hepatocytes in the *in vitro* metabolism study and the interpretation of the quantitative species differences is therefore not robust;
- Overall, the most appropriate model for humans between rats and mice is not established with certainty.

In conclusion, RAC considered that while the available data provided clear evidence of male reproductive toxicity of Spirotetramat in rats that is not secondary to other toxic effects, the absence of similar effects in mice and to some extent in dogs (although only a lower dose range was tested in dogs) suggests that this may be a species-specific mode of action. Effects on spermatogenesis were observed in rats at doses that may exceed doses above which the elimination rate is limited by saturation of active transport mechanisms. Metabolism in mouse and human hepatocytes also involves a conjugation pathway with glucuronic acid that is very limited in rats (up to 0.8% of metabolites *in vivo*). Such a pathway may prevent saturation of elimination mechanisms and exposure to high systemic levels of Spirotetramat metabolites.

Uncertainties exist in relation to the *in vivo* impact of the existence of the glucuronidation pathway in mice and the absence of formation of DME on the systemic level of Spirotetramat and its metabolites as well as in relation to the impact of the quantitative differences in glucuronidation between humans and mice. Besides, it is not known with certainty whether the sensitivity of rats can be explained by mechanistic factors and not (or not only) by toxicokinetic differences. On this basis, the relevance for humans cannot be excluded and a classification of Spirotetramat for fertility is justified.

However, the existence of a glucuronidation pathway in humans that is not present in rats indicates that humans may eliminate the Spirotetramat metabolites more efficiently and introduces doubt as to the relevance for humans of the effect observed in the rats. Therefore, on balance and having weighed all the available evidence, RAC concluded that classification as Repr. 2 according to the CLP Regulation and Repr. Cat. 3; R62 according to DSD was justified for fertility.

***SCL assessment***

The most sensitive effects on the rat reproductive function were observed as follows:
- from 373 mg/kg bw/day in the rat two-year study, 16% of animals showed degeneration of latter stage spermatids versus none in the controls
- from 6000 ppm in F1 males in the range-finding reproductive toxicity study (equivalent to 320 mg/kg bw/day in premating P-generation males, intake in F1 males not stated in the DAR) showed an increase in the number of abnormal sperm presenting as amorphous sperm head to 20.2 versus 2.8 in controls.

In both studies, these doses therefore exceed the effective dose with a 10% effect level above the background ($ED_{10}$). According to the CLP guidance, $ED_{10}$ is the relevant parameter for a preliminary assessment of potency. Since the $ED_{10}$ value of Spirotetramat is between 4 and 400 mg/kg bw/day, it is considered of medium potency.

Toxicokinetic differences may be a relevant modifying factor to consider when concluding on potency. However, the uncertainties regarding the metabolite involved in the reproductive

toxicity of Spirotetramat and their comparative *in vivo* kinetics between species, do not allow interpretation in the context of a potency assessment. It can be concluded that Spirotetramat induces effects on the male reproductive function with a moderate potency and the setting of specific concentration limits is therefore not justified.

***Developmental toxicity***
In the rabbit, an increased incidence of distinct liver lobulation was observed at the highest dose where severe maternal toxicity was observed (maternal deaths). Due to the unknown toxicological significance of this variation and its incidence being comparable to the historical control range, RAC did not consider this finding as relevant to justify classification for development.

In rats, the following findings were noted:

- Elevated incidence of retardation of ossification was observed, in particular in the phalanges. These findings were observed in the supplemental study in the absence of any maternal toxicity or foetal weight retardation, therefore a relationship with general growth retardation was not observed up to 140 mg/kg bw/day. However, in both studies, only a weak dose-response relationship was observed and incidences were within the historical control values; there is therefore only slight evidence that Spirotetramat may delay skeletal ossification.
- An increased incidence of wavy ribs was observed from 20 mg/kg bw/day in the initial study but this was not reproduced up to 140 mg/kg bw/day in the supplementary study and therefore only the high incidence observed at 1000 mg/kg bw/day was attributed to treatment. At this dose, this finding was present together with decreased maternal and foetal weight and may have been associated with general growth retardation. This finding is most often considered as reversible (Solecki, 2001) and as a variation. The increased incidence of two other skeletal variations, i.e. supernumerary ribs and enlarged fontanelles, was reported at this dose in the presence of general growth retardation. As variations, these findings are not considered sufficient to justify a classification for development.
- Dumbbell shaped thoracic vertebral bodies and pelvic shift in sacral vertebrae were observed at 1000 mg/kg bw/day in the presence of maternal toxicity. However, RAC considers that the decrease in maternal and foetal body weights observed may not explain these malformations and that this provides some evidence of developmental toxicity of spirotretramat in the rat.

Based on this rationale, RAC considered that a classification as Repr. 2 according to the CLP Regulation and Repr. Cat. 3; R63 according to DSD was justified for developmental toxicity of Spirotetramat.

## RAC evaluation of aspiration toxicity

### Summary of the Dossier submitter's proposal

The DS concluded that no classification for aspiration toxicity was warranted for Spirotetramat but did not provide any further details.

### Comments received during public consultation

No specific comments were received.

The viscosity of Spirotetramat was not reported in the CLH report, but as the substance is not a hydrocarbon, this is not relevant in this case.

### Assessment and comparison with the classification criteria

The substance does not fulfil the criteria for human evidence of an aspiration hazard, nor is it a hydrocarbon with a kinematic viscosity of 20.5 $mm^2/s$ or less. No classification was therefore proposed by the RAC.

# ENVIRONMENTAL HAZARD ASSESSMENT

## RAC evaluation of environmental hazards

### Summary of Dossier submitter's proposal

The DS proposed to classify the substance as Aquatic Acute 1 (H400) and Aquatic Chronic 1 (H410) in accordance with the CLP Regulation, with an M-factor of 1 for both. The corresponding classification according to the DSD is N; R50/53. The proposal is based on short-term marine invertebrate and marine diatom toxicity results (96-h $EC_{50}$ of 0.85 mg/L and 96-h $E_rC_{50}$ of 0.96 mg/L, respectively) for the acute CLP and the DSD classification, while a long-term invertebrate toxicity result (28-d NOEC of 0.1 mg/L in a spiked water-sediment test with *Chironomus riparius*) together with the fact that the substance is not rapidly (or readily) biodegradable supports chronic classification under the CLP Regulation.

### Comments received during public consultation

Three MSCAs commented during the public consultation, two indicating their support for the proposed environmental classification. One MSCA queried the use of oyster growth data for acute classification purposes, and also suggested that a species sensitivity distribution might be appropriate to take account of the range of algal data available, particularly as the 95% confidence intervals for the most sensitive result straddle the 1 mg/L threshold. A second Member State, while noting that toxicity data from a sediment-dwelling species had been used for chronic classification, did not object to this.

### Assessment and comparison with the classification criteria

***Degradability***

Spirotetramat hydrolyses under standard conditions, with calculated half-lives at 25 °C of 32.5, 8.6 and 0.32 days at pH 4, 7 and 9, respectively (longer half-lives may be expected at lower temperatures). One major product was formed in all tests (Spirotetramat-enol), which can be considered to be stable to hydrolysis. Aqueous photolysis is rapid, with extensive photo-rearrangement after 7 days' irradiation and an estimated half-life of about 9 – 12 days under natural summer sunlight conditions at pH 5. Maximum amounts of four products formed were 42.9, 22.9, 11.5 and 19.3% of applied radioactivity (AR), respectively. Whilst photolysis is not relevant for classification purposes, it might be a factor in the interpretation of aquatic toxicity tests.

Spirotetramat failed a test for ready biodegradation, achieving at most 1% mineralisation in 28 days. Simulation tests in two aerobic water-sediment systems using radio-labelled substance indicated fast primary degradation, with no Spirotetramat detectable in the water or sediment phases in either system after 7 days. The first order degradation $DT_{50}$ value for the whole system was 1 day in both cases, but a maximum of only 24% mineralisation occurred over 120 days in one system. Major metabolites were Spirotetramat-enol (reaching a maximum in the total system of 92 – 99.0% of AR after 7 – 14 days, decreasing thereafter) and Spirotetramat-ketohydroxy (with an increasing trend towards the end of the studies, reaching a maximum in the total system 50.8% of AR at study termination). Other identified metabolites (Spirotetramat-MA-amide, Spirotetramat-oxo-enol isomer and Spirotetramat-di-hydroxy) did not exceed 9.8% of AR in the total system individually. Non-extractable residues accounted for a maximum of 40.7% of AR by day 91. Aerobic degradation in soils followed a similar pattern, with rapid primary degradation, formation of non-extractable residues and limited mineralisation (up to 19.4% of AR after 50 days in EU soils, but only 15.3% of AR after 360 days in a US soil).

Despite hydrolysis half-lives above 16 days at some relevant pH values, lack of ready biodegradation and limited mineralization in water-sediment and soil simulation tests, the substance does undergo rapid primary degradation in aquatic systems. The conclusion about rapid degradability therefore depends on whether any of the metabolites are classifiable.

The most sensitive ecotoxicity values for one of the major metabolites, Spirotetramat-enol, are a 48-h $LC_{50}$ of 74.9 mg/L for *Chironomus riparius* (static, water only exposure, nominal

concentrations) and a 7-d $E_rC_{50}$ of 19.3 mg/L and a 7-d $NOEC_{yield}$ of 0.95 mg/L for *Lemna gibba* (both based on nominal concentrations, since test concentrations were well maintained). Whilst the acute *Lemna* value would trigger chronic classification using the surrogate approach (this metabolite has a degradation $DT_{50}$ of 37.9 – 59 days in aerobic water/sediment systems), the 7-d $NOE_rC$ is 3.05 mg/L, which is above the classification threshold. Since growth rate is the preferred measure for classification purposes, this means that the *Lemna* data do not lead to classification for this metabolite. Nevertheless, the absence of a chronic toxicity result for *Chironomus riparius* means that the surrogate approach is also appropriate for invertebrates, and Spirotetramat-enol is therefore classifiable as H412 (harmful to aquatic life with long lasting effects). No information was presented in the CLH report for other metabolites.

On this basis, Spirotetramat does not meet the criteria for being rapidly degradable (nor readily biodegradable) in the environment.

***Bioaccumulation***

The log n-octanol-water partition coefficient ($K_{ow}$) of Spirotetramat is 2.5 at pH 4 – 9. It is therefore not considered to be a bioaccumulative substance for classification purposes.

***Ecotoxicity***

The lowest reliable ecotoxicity results were as follows (the key studies are highlighted in bold):

| **Trophic level** | **Species** | **Short-term result** | **Long-term result** |
|---|---|---|---|
| Fish | *Cyprinodon variegatus* | 96-h $LC_{50}$ = 1.96 mg/L* | - |
| | *Pimephales promelas* | - | 33-d NOEC = 0.534 mg/L |
| Aquatic invertebrates | *Daphnia magna* | 48-h $EC_{50}$ > 42.7 mg/L | 21-d NOEC = 2.0 mg/L |
| | *Americamysis bahia* | 96-h $EC_{50}$ = 5.5 mg/L | **-** |
| | *Chironomus riparius* | 48-h $EC_{50}$ = 1.30 mg/L | **-** |
| | *Crassostrea virginica* | **96-h $EC_{50}$ (shell deposition) = 0.85 mg/L** | **-** |
| Aquatic algae and plants | *Pseudokirchneriella subcapitata* | 72-h $E_rC_{50}$ = 8.15 mg/L | 72-h $NOE_rC$ = 1.46 mg/L |
| | *Navicula pelliculosa* | 96-h $E_rC_{50}$ = 15.0 mg/L | 96-h $NOE_rC$ = 1.00 mg/L |
| | *Anabaena flos-aquae* | 96-h $E_rC_{50}$ > 15. 1 mg/L | 96-h $NOE_rC$ = 5.1 mg/L |
| | *Skeletonema costatum* | 96-h $E_rC_{50}$ = 0.96 mg/L | **96-h $NOE_rC$ = 0.12 mg/L** |
| | *Lemna gibba* | 7-d $E_rC_{50}$ = 6.21 mg/L | 7-d $NOE_rC$ = 1.54 mg/L |

Note: *This is a marine species, but three freshwater fish species had acute $LC_{50}$ values within a factor of two of this result, so there appears to be good consistency in acute sensitivity amongst fish species.

All values except the long-term *Daphnia* result were based on mean measured concentrations. The purity profile of the key studies complies with the specified composition in Section 1 of the CLH report. Although a long-term result is not available for the most sensitive fish species, and there are no acute data for the only species for which long-term data are available, the very

similar acute sensitivity for four species suggests that this is not a significant issue in terms of using the chronic data directly rather than the surrogate approach.

*Daphnia* are less acutely sensitive to Spirotetramat than other aquatic invertebrates (including another crustacean, an insect and a mollusc) by an order of magnitude. As the substance is an insecticide, it is important that an insect is present in the data set. An acute result is available for *Chironomus riparius* from a test using water-only exposure (this test is considered valid, although there were some deviations from standard test guidelines). The DS chose to include a 28-day study on *Ch. riparius* using spiked water but with sediment present, and used this value to derive the chronic classification under the CLP Regulation. The results were based on nominal concentrations, but this was a static test, and test substance concentrations in the overlying water were below the limit of quantitation (0.00636 mg/L) in two of three treatment groups after 7 days, and in all exposures after 28 days (most likely due to rapid hydrolysis, as the pH was in the range 8.3 – 8.7). It is therefore unclear what caused the observed toxicity, and the test system might not have achieved equilibrium. In addition, it cannot be ruled out that the test organisms were exposed to the substance or metabolites adsorbed to the sediment surface. Therefore although the DS considers the study to be valid, the RAC did not consider it to be relevant for classification purposes. This means that no suitable chronic toxicity result was available for the three most sensitive invertebrate species, so the surrogate approach needed to be considered for the invertebrate trophic group.

Another consideration is that the most sensitive invertebrate acute toxicity result was for the marine mollusc *Crassostrea virginica*, with a 96-h $EC_{50}$ for shell deposition of 0.85 mg/L (95% confidence intervals: 0.59 – 1.3 mg/L). The results were based on mean measured concentrations (which were in the range of 59–100% of the nominal). As noted during the public consultation, this end point is related to growth rather than mortality/immobilization (which is the measure used for other invertebrate species). However, the RAC recognised that this test is intended to give information on acute toxicity for this species, and the result is within a factor of 2 – 3 of acute toxicity values for both fish and another invertebrate. It is also supported by data on marine diatoms (but see caveats below), so is considered relevant for the classification of this substance.

As might be expected from the potential for rapid hydrolysis and photolysis, the concentrations in the algal studies were not well maintained. For example, mean measured concentrations were in the range 22–48% of nominal by day 3 for *Pseudokirchneriella subcapitata*, and 0–59% of nominal on day 4 for *Navicula pelliculosa*. Results were therefore expressed as geometric mean concentrations (or time weighted mean measured concentrations in the case of *Lemna*, as concentrations were better maintained by the static renewal regime). The most sensitive aquatic algal/plant species was the marine diatom *Skeletonema costatum*, with a 96-h $E_rC_{50}$ of 0.98 mg/L (95% confidence interval: 0.92 – 1.05 mg/L).

***Classification according to CLP***

Acute aquatic hazard: The lowest reliable short-term aquatic toxicity result was a 96-h $EC_{50}$ of 0.85 mg/L for the mollusc *Crassostrea virginica*. This result was within a factor of 2 – 3 of acute toxicity values for both fish and other invertebrates. It was also supported by acute toxicity data on a marine diatom species which was also slightly below 1 mg/L. Spirotetramat is therefore classifiable as Aquatic Acute 1 (H400), with an M-factor of 1 ($0.1 < L(E)C_{50} < 1$ mg/L).

Chronic aquatic hazard: Spirotetramat is not considered to be rapidly degradable. Reliable and relevant long-term aquatic toxicity data was only available for the fish and aquatic algae/plant trophic levels, with lowest NOEC values of 0.534 and 0.12 mg/L, respectively. These concentrations were below the threshold value of 1 mg/L for non-rapidly degradable substances, leading to classification as Aquatic Chronic 2 (H411). for invertebrates Due to the lack of reliable chronic toxicity data for the most acutely sensitive species, the surrogate approach was used. Based on the lowest acute $L(E)C_{50}$ of 0.85 mg/L combined with the substance's lack of rapid degradability, classification as Aquatic Chronic 1 (H400) was considered appropriate.

In summary, Spirotetramat is therefore classifiable as Aquatic Chronic 1 (H400), with an M-factor of 1.

***Classification according to DSD***
The lack of ready biodegradation and a 96-h $EC_{50}$ of 0.85 mg/L for invertebrates (with a similar value for a marine diatom) meant that Spirotetramat fulfilled the criteria for classification with N; R50-53. The following specific concentration limits are therefore applicable:

| Concentration of Spirotetramat in the mixture, C (w/w) | Classification of the mixture |
|---|---|
| C ≥ 25% | N; R50-53 |
| 2.5% ≤ C < 25% | N; R51-53 |
| 0.25% ≤ C < 2.5% | R52-53 |

In summary, the RAC agrees with the original proposal of the DS, although the base for the lack of rapid degradability and chronic classification is different.

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## ANNEXES:

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

Annex 2 Comments received on the CLH report, response to comments provided by the dossier submitter and rapporteurs' comments (excl. confidential information).