Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

Spiroxamine (ISO); 8-tert-butyl-1,4-dioxaspiro[4.5]decan-2-ylmethyl (ethyl)(propyl)amine

EC number: N.A.
CAS number: 118134-30-8

CLH-O-0000001412-86-76/F

Adopted

11 September 2015

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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 harmonised classification and labelling (CLH) of:

Chemical name: **Spiroxamine (ISO); 8-tert-butyl-1,4-dioxaspiro[4.5]decan-2-ylmethyl(ethyl)(propyl)amine**

EC Number: **N.A.**

CAS Number: **118134-30-8**

The proposal was submitted by **Germany** and received by RAC on **2 July 2014.**

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

PROCESS FOR ADOPTION OF THE OPINION

The Dossier Submitter 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 **08 July 2014**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **22 August 2014.**

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Bogusław Barański**

Co-Rapporteur, appointed by RAC: **Stephen Dungey**

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 **11 September 2015** 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 statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	612-150-00-X	Spiroxamine (ISO); 8-tert-butyl-1,4-dioxaspiro[4.5]decan-2-ylmethyl(ethyl)(propyl)amine	-	118134-30-8	Acute Tox. 4* Acute Tox. 4* Acute Tox. 4* Skin Irrit. 2 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H332 H312 H302 H315 H317 H400 H410	GHS07 GHS09 Wng	H332 H312 H302 H315 H317 H410	-	-	
Dossier submitters proposal	612-150-00-X	Spiroxamine (ISO); 8-tert-butyl-1,4-dioxaspiro[4.5]decan-2-ylmethyl(ethyl)(propyl)amine	-	118134-30-8	Repr. 2 Acute Tox. 4 Acute Tox. 4 Acute Tox. 4 Skin Sens. 1B Aquatic Acute 1 Aquatic Chronic 1	H361d H332 H312 H302 H317 H400 H410	GHS08 GHS07 GHS09 Wng	H361d H332 H312 H302 H317 H410	-	M=100 M=100	
RAC opinion	612-150-00-X	Spiroxamine (ISO); 8-tert-butyl-1,4-dioxaspiro[4.5]decan-2-ylmethyl(ethyl)(propyl)amine	-	118134-30-8	Repr. 2 Acute Tox. 4 Acute Tox. 4 Acute Tox. 4 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H361d H332 H312 H302 H317 H400 H410	GHS08 GHS07 GHS09 Wng	H361d H332 H312 H302 H317 H410	-	M=100 M=100	
Resulting Annex VI entry if agreed by COM*	612-150-00-X	Spiroxamine (ISO); 8-tert-butyl-1,4-dioxaspiro[4.5]decan-2-ylmethyl(ethyl)(propyl)amine	-	118134-30-8	Repr. 2 Acute Tox. 4 Acute Tox. 4 Acute Tox. 4 STOT RE 2 Skin Irrit. 2 Skin Sens. 1 Aquatic Acute 1 Aquatic Chronic 1	H361d H332 H312 H302 H373 (eye) H315 H317 H400 H410	GHS08 GHS07 GHS09 Wng	H361d H332 H312 H302 H373 (eye) H315 H317 H410	-	M=100 M=100	

*NOTE: This RAC opinion refers to one of two CLH proposals which were discussed and adopted by RAC in parallel - see RAC opinion on additional proposal for classification for STOT-RE. The classifications displayed in the last row of the table above reflect therefore the CLH resulting from both adopted opinions.

GROUNDS FOR ADOPTION OF THE OPINION

HUMAN HEALTH HAZARD ASSESSMENT

RAC evaluation of acute toxicity

Summary of the Dossier submitter's proposal

Spiroxamine has been re-evaluated under the former Plant Protection Product Directive 91/414/EC, and during this process it was noted that the current harmonised classification should be amended.

The current entry for spiroxamine in Annex VI of CLP includes Acute Tox. 4* (minimum classifications) for all routes of exposure, i.e. inhalation, dermal and oral. The dossier submitter (DS) has proposed to reevaluate the acute toxicity studies to remove the minimum classification designation.

Oral

Spiroxamine was tested for acute oral toxicity in rats and mice. The highest doses tested were 710 mg/kg bw and 630 mg/kg bw in rats and mice, respectively. The LD_{50} values calculated were 595 mg/kg bw and 460 mg/kg bw in rats and mice, respectively. The DS proposed to remove the minimum classification and to classify spiroxamine as Acute Tox. 4, H302.

Inhalation

Spiroxamine was tested for acute toxicity in rats via inhalation (dynamic spraying head nose only). The highest concentration tested was 3880 mg/m^3. The LC_{50} value calculated was 1982 mg/m^3, i.e. 1.982 mg/L. The DS proposed to remove the minimum classification and classify spiroxamine as Acute Tox. 4, H332.

Dermal

Spiroxamine was tested for acute dermal toxicity in rats. The highest dose tested was 2500 mg/kg bw, and the LD_{50} values calculated were 1068 mg/kg bw in females and > 1600 mg/kg bw in males. The DS proposed to remove the minimum classification and classify spiroxamine as Acute Tox. 4, H312.

Comments received during public consultation

Three MSCAs supported the proposed classification and removal of the minimum classification.

Assessment and comparison with the classification criteria

Oral

The oral LD_{50} values calculated for male and female rats were 595 mg/kg bw and 500 mg/kg bw, respectively (Krötlinger, 1991a) and for male and female mice they were 460 mg/kg bw and 561 mg/kg bw, respectively (Krötlinger, 1991b). These values are within the limits of 300 < ATE ≤ 2000 mg/kg bw and therefore spiroxamine meets the criteria in the CLP Regulation for classification as Acute Tox Cat. 4, H302. **RAC agreed to remove the minimum classification as proposed by the DS**.

Inhalation

The inhalation LC_{50} in female rats was reported to be 1.982 mg/L (Pauluhn, 1990), thus it is within the limits of 1.0 < ATE ≤ 5.0 mg/kg for dusts and mists and spiroxamine meets the criteria in the CLP Regulation classification as Acute Tox Cat. 4, H302. It is noted that clinical signs of toxicity were observed in all male and female rats exposed at 1.140 mg/L spiroxamine.

Therefore, as proposed by the DS, RAC agreed to remove the minimum classification and classify spiroxamine as **Acute Tox. 4, H332.**

Dermal

The dermal LD_{50} in female rats was reported as 1068 mg/kg bw (Krötlinger, 1991c) which is within the limits of 1000 < ATE ≤ 2000 mg/kg bw. Therefore spiroxamine meets the criteria in the CLP Regulation for classification as Acute Tox Cat. 4, H312. RAC agreed to remove the minimum classification as proposed by the DS**.**

RAC evaluation of skin sensitisation

Summary of the Dossier submitter's proposal

Spiroxamine has a current entry in Annex VI to the CLP Regulation which includes Skin Sens. 1. The DS proposed to modify the classification to sub-category 1B to take into account the latest changes in the criteria for skin sensitisers (Regulation (EU) No 286/2011; 2nd ATP to CLP).

Skin sensitisation was tested in a Magnusson and Kligman Guinea Pig Maximisation Test (GPMT) (Dreist and Kolb, 1992) and in a Buehler patch test (Krötlinger and Kolb, 1992), both performed according to OECD TG 406 and claimed to be GLP compliant. In 2001, the substance was also tested in human volunteers using the Intensified Shelanski Repeated Insult Patch Test (RIPT) at concentrations up to 0.2%.

In the GPMT study (Dreist and Kolb, 1992), after an intradermal induction with spiroxamine at 5%, 14/20 guinea pigs (70%) were positive after the first challenge with a 1% solution, and 5/20 animals (25%) were positive with a 0.5% solution.

In the Buehler test (Krötlinger and Kolb, 1992), the induction concentrations were 50%, 25% and 12%. No differences with respect to the incidence and intensity of skin reaction were observed after the first challenge with both concentrations (12% and 6%). After the second challenge with the 3% concentration 9/12 treated animals and 2/12 control animals showed skin reactions.

In the human RIPT study, nominal doses of 0.02%, 0.066% and 0.20% were tested on volunteers. After the challenge exposure, no skin reactions were observed, therefore it was concluded that Spiroxamine did not reveal any skin irritation or sensitising properties up to the concentration of 0.20%. Due to the low concentration tested in this study (up to 0.20%), the human study was considered by the DS as supplementary only and the proposed classification as Skin Sens. 1B was based on the results of the animal studies.

Comments received during public consultation

One MSCA disagreed with the proposed change because of insufficient data for sub-categorisation. One MSCA agreed with the proposed change, i.e. Skin Sens. 1B.

Assessment and comparison with the classification criteria

Skin sensitising properties have been evaluated in a GPMT (Dreist and Kolb, 1992) and in a Buehler test (Krötlinger and Kolb, 1992). Both studies were consistent with OECD TG 406.

In the GPMT, the intradermal induction was performed with 5% spiroxamine. After the first challenge with 0.5% and 1% solutions, 5/20 animals (25%) and 14/20 guinea pigs (70%) were positive for sensitisation, respectively. No skin reactions were found after the 2nd challenge with 0.1% or 0.05% solutions of spiroxamine. The concentrations of spiroxamine for the first challenge (1% and 0.5%) were chosen based on the highest non-irritating concentrations in a range finding test using 4 guinea pigs. It is noted that spiroxamine is already classified as Skin Irrit. 2.

Taking into account the concentration in the intradermal induction (5%), the extent of response in the challenge test with 1% spiroxamine (70%) corresponds to the criteria given in table 3.4.3 in Annex I of the CLP Regulation for classifying a substance in subcategory Skin Sens. 1B (≥ 30% of animals responding at > 1% intradermal induction dose).
In order to be classified in subcategory Skin Sens. 1A, spiroxamine would have to be sensitising after intradermal induction at a concentration of 1% in at least 60% of guinea pigs. This intradermal induction concentration has not been tested, however a response of 60% of animals after intradermal induction with 1% solution does not seem improbable. This concentration is only 5 times lower than tested and spiroxamine is skin irritating which may facilitate induction of sensitisation.

The Buehler test (Krötlinger and Kolb, 1992) was conducted with successive decreasing topical inductions with concentrations of 50%, 25% and 12% of spiroxamine. After the first challenge with 12% spiroxamine, 10/12 Guinea pigs (83%) of the test group and 12/12 animals of the control group (100%) had positive skin reactions. No differences with regard to incidence and intensity of skin reactions were seen between treatment groups and control animals, which demonstrates that the substance has skin irritating properties also at a concentration of 12%. After the 2nd challenge with spiroxamine at a concentration of 3 %, 9/12 test group animals (75%) and 2/12 control animals (16.7%) showed skin reactions (the net difference in incidence between treated and control animals was 58.3%). However, no dermal reactions occurred in treated or control animals following a challenge with a non-irritant concentration of 1%. The results of the test indicate that spiroxamine has low skin sensitising potential.

The concentrations in the topical induction (50%, 25% and 12%) and the level of response in the 2nd challenge test with 3% spiroxamine (75%) fulfilled the criteria for Skin Sens. subcategory 1B (≥ 15% of animals responding at > 20% topical induction dose) as ~~given~~ provided in table 3.4.3, Annex I, in the CLP Regulation.

In order to be classified as subcategory 1A, spiroxamine would have to be sensitising in the Buehler test after a topical induction dose of up to 20% in at least 60% of the animals, which does not seem improbable.

The existing animal data indicate that the sensitising potential of spiroxamine is rather low. However, it has not been demonstrated that it does not fulfil criteria for Skin Sens. subcategory 1A. Furthermore it is not possible to exclude that in adequately conducted assays it would meet these cirteria.

Under the conditions of the Intensified Shelanski Repeated Insult Patch Test (RIPT) solutions of up to 0.20% spiroxamine did not reveal any skin irritating or sensitising properties in human volunteers (Shelanski, 2001).

Therefore, RAC is of the opinion that sub-categorisation is not appropriate in this case, and spiroxamine warrants classification as **Skin Sens. 1 with the hazard statement H317: "May cause an allergic skin reaction"**.

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

Effects on sexual function and fertility

This endpoint is not addressed by the DS proposal. Nevertheless, the Dossier Submitter included the summaries of two 2-generation studies in rats (Pickel, 1993; Milius and Stuart, 2008), both performed according OECD TG 416 and under GLP in theCLH report at the request of the ECHA Secretariat.

Effects on development of the offspring

The developmental toxicity of spiroxamine has been assessed based on the results of OECD TG 414 compliant oral studies in rats (Becker and Biedermann, 1992) and in rabbits (Holzum, 1995) and on the results of an OECD 414 compliant dermal study in rats (Becker and Biedermann, 1993). The results of three range-finding preliminary developmental toxicity studies were also briefly summarised.

In the oral developmental toxicity study in rats (Becker and Biedermann, 1992), palatoschisis was observed at a dose of 100 mg/kg bw/day in 3 pups from 3/24 litters,. At this dose level other developmental toxic effects, such as delayed ossification and reduced foetal body weight were also reported. At the same dose level, slight maternal toxic effects (reduced feed intake and marginally decreased body weight) occurred.

As noted in the CLH report, the incidences of palatoschisis were outside the respective historical control range of the performing laboratory, as submitted by the notifier. The historical control data indicated that whenever palatoschisis was detected, only one litter per study was affected except for one study in 1995 (2 foetuses out of 2 litters). Additionally, palatoschisis was detected only in a few of the performed studies.

The following summary of historical control data was submitted (Henninger, 2009):

Table: Incidences of palatoschisis in vehicle controls of developmental rat studies in WIST HALBbm: WIST (SPF) rats conducted at RCC between 1988 and 1995.

Malformation		Incidences of palatoschisis	
Year	No. of studies	No. of studies	No. of affected foetuses and litters in the study
1988	7	0	0
1989	12	0	0
1990	7	3	1 foetus in one litter 4 foetuses in one litter 2 foetuses in one litter
1991	6	1	1 foetus in one litter
1992	4	0	0
1993	2	1	2 foetuses in one litter
1994	1	0	0
1995	1	1	2 foetuses in two litters

In the range-finding experiments summarised in Table 42 of the background document, palatoschisis was observed at 100 and 150 mg/kg bw/day. However at 150 mg/kg bw/d, a high mortality rate in dams was observed (84%); therefore the results of this latter experiment did not contribute to the classification proposal. At 100 mg/kg bw/d, the following maternal findings were observed: clinical symptoms, lower feed intake, lower body weight gain and lower body weight at termination.

Considering the criteria in 3.7.2.4, Annex I, CLP Regulation and 4.2.3, Annex VI, Directive 67/548/EC, the following was proposed by the DS:

- No data from humans were available, hence a classification with H360 (category 1A) is not possible.
- Although palatoschisis is a malformation, it was observed in only one species (rat), and at low incidences (3 foetuses in 3 litters). This finding was also reported at the same dose in a range-finding study (background document Table 42, experiment R6072), indicating reproducibility of the finding. Slight signs of maternal toxicity (lower feed intake and body weight) were observed in dams at the dose levels at which the malformation was observed both in the main study and the range-finding study. According to the DS, these observations (one species, one study, low incidences, maternal toxicity) reduce the concern for developmental hazard; hence, classification as Repr. 1B, H360 seems not to be appropriate. In summary, classification as Repr. 2, H361 for developmental effects was proposed.

Comments received during public consultation

Three MSCAs agreed with the DS on the proposed classification as Repr. 2, H361 for developmental effects. One MSCA suggested to correlate individual data for offspring and their mothers in the main study in rats, and indicated that even when a causal relationship is established, the effects in offspring can still be relevant for classification for developmental toxicity, depending on the severity of the effects and therefore a classification Cat. 2 can be warranted.

Assessment and comparison with the classification criteria

Fertility and sexual function

1. In a two-generation reproductive toxicity study (Pickel, 1993) performed according to OECD TG 416 and compliant with GLP, groups of 30 male and 30 female rats were fed a diet containing 0, 20, 80 and 300 ppm of spiroxamine resulting in the uptake of spiroxamine in a range of 2.13 – 3.02, 9.19 – 13.15 and 35.88 – 55.81 mg/kg bw /day respectively. No signs of toxicity were observed in any group in F0 animals, while in F1 male and female animals treated with 300 ppm an increase in incidence of piloerection, bloody noses, polyuria and mortality was observed. During the entire study period, body weight gain in F0 and F1 animals up to 80 ppm was comparable to corresponding control animals. Significantly decreased body weight gain was observed at 300 ppm in F0 males starting week 1. The body weights of F0 females at 300 ppm were reduced between day 4 and day 21 *post partum*. Body weight gain was reduced in male and female F1 (parental) animals at 300 ppm during the entire treatment period, demonstrating mild parental toxicity at that dose level.

On histopathological examination of F0 and F1 parental animals, hyperkeratosis in the oesophagus was detected at 80 ppm (8 females) and 300 ppm (29 females, 27 males).

In both sexes, decreased liver and kidney weights (F0 and F1) at 300 ppm were observed and additionally, reduced liver weights in F0 males at 80 ppm.

The insemination index, insemination performance, oestrus frequency and cycle classification (F1), fertility index, gestation index, gestation period, sex ratio and birth weight did not exhibit any treatment-related effects. The litter size at birth was slightly reduced at 300 ppm in F0 and

F1 generations. Between postnatal day (PND) 4 and 21, a high mortality of F2 pups was observed. Despite the fact that the viability index (on PND 21) for all groups (F2) was below the range of historical control data, the high mortality was not regarded as treatment related, since the highest mortality occurred in the control group.

Examination of the pups up to 300 ppm revealed no relevant gross-pathological or histopathological findings. There were no treatment-related external malformations.

The NOAEL of 20 ppm (2.13 mg/kg bw/d) for parental toxicity was based on hyperkeratosis of the oesophagus epithelium and reduced feed consumption at 80 ppm.

It cannot be excluded that the slightly reduced litter size at birth could be related to maternal toxicity and that the reduced pup body weight from PND 14 and clinical signs in pups during the lactation period at 300 ppm could be related to a direct exposure of the pups to spiroxamine via consumption of feed containing spiroxamine. The study does not provide evidence of effects of spiroxamine on fertility and sexual function or developmental toxicity.

2. In a two-generation reproductive toxicity study (Milius and Stuart, 2008) performed according to OECD TG 416 with some deviations and compliant with GLP, groups of 30 male and 30 female rats were fed a diet containing 0, 20, 80 and 300 ppm of spiroxamine resulting in the uptake of spiroxamine in a range of 1.4 – 1.8, 5.5 – 6.9 and 21.0 -27.7 mg/kg bw/day.

Mortality: There were no mortalities during the course of the study at any dose tested in either generation of parental animals.

Clinical signs: No test substance related clinical observations were noted in parental animals or in the offspring during this study in either generation at any dose tested.

Body weight:

– P-generation adults: The body weight gain of females at 300 ppm was reduced during the pre-mating period and during gestation. During lactation, a slight decline in body weight as compared to controls, were observed on PND 14.

- F1-Offspring: Pup body weights at birth were comparable to controls in all treated groups. Pups at 300 ppm exhibited non-statistically significantly lower absolute body weights by day 21 (6.9% less than control) with overall body weight gain (lactation day 14-21) lower in males by 10.4% and females by 11.6% relative to control.

- F1-generation adults: During the pre-mating period at 300 ppm, males and females exhibited slightly lower body weight gains relative to controls . Females had a slightly lower body weight gain relative to controls during gestation and lactation. Significant reductions in terminal body weight were noted in both sexes as compared to controls.

- F2-Offspring: There were no effects on birth weight considered to be directly attributable to the test substance. The mean birth weight in the 300 ppm dose group was lower than in the concurrent controls (5.8 vs. 6.2 g). However, the value is well within the laboratory's historical control values in this strain of rats and the lower birth weight observed is considered to be secondary to a higher percentage of animals in this dose group delivering on day 21 when compared

to the majority of controls delivering on day 22. At 300 ppm pup absolute body weight was decreased during the lactation period as compared to controls and overall body weight gain was lower with respect to control pups (9.1%).

Histopathology: At 300 ppm, 17/30 males and 25/30 females of the F0-generation and 22/30 males and 27/30 females of the F1-generation exhibited hyperkeratosis of the oesophagus.

Overall, reproductive performance was not affected for any parameter (mating, fertility or gestation indices, days to insemination or the median number of implants) in either generation at any dose level. No test substance related effects were observed on any sperm parameters evaluated at any dose level tested for either generation.

Pup viability and clinical signs: There were no test substance related effects on the viability of the pups or any clinical observations observed in either generation at any dietary level tested.

Sexual maturation (F1): Slight delays in balanopreputial separation and vaginal patency observed at 300 ppm are considered to be secondary to body weight reductions observed in both sexes at this dose level.

In this two-generation study, the parental systemic NOAEL was 80 ppm (5.5 mg/kg bw/d) based on reduced body weight, increased incidence of hyperkeratosis of the oesophagus and increased activated partial thromboplastin time values observed at 300 ppm. The reproductive NOAEL was 300 ppm (21.0 mg/kg bw/d) based on the absence of test substance related findings.

The study did not provide evidence of effects of spiroxamine on fertility and sexual function and it did not indicate a potential for developmental toxicity.

Developmental toxicity

Developmental toxicity of spiroxamine has been assessed in three studies: An oral study in rats (Becker and Biedermann, 1992), an oral study in rabbits (Holzum, 1995) and a dermal study in rats (Becker and Biedermann 1993).

Nine range-finding studies were performed before the main study in 1990. In two range-finding studies (R6072 (1993) and R6355 (1995)) palatoschisis was detected. In study R6072 this malformation was observed in three foetuses (out of 46, i.e. 6.5%) in two litters out of 4 pregnant female rats dosed with 100 mg/kg bw/day of spiroxamine during gestation. The female rats with these foetuses exhibited a number of symptoms during one or more days 10-17 post coitum (post coitum) (ruffled fur, lateral recumbency (one female), dyspnea, sedation and/or hunched posture) while no such symptoms were reported in the other 2 pregnant females. In the second preliminary study (R6355, 1995), 3 foetuses (out of 18, i.e. 16%) in 2 litters out of 4 surviving dams had palatoschisis, after dosing dams during pregnancy with 150 mg/kg bw/day. In this study, 21/25 pregnant female rats (84%) died showing marked maternal toxicity. No palatoschisis was observed in the other range-finding developmental toxicity studies in which pregnant female rats were receiving spiroxamine during pregnancy at 10, 25, 75, 100 or 150 mg/kg bw/day (where 3/5 dams showed clinical symptoms, but no mortality).

1. In the main developmental toxicity study in rats (Becker and Biedermann, 1992) performed according OECD TG 414 and with GLP, groups of 25 pregnant female Wistar rats were treated with spiroxamine by gavage at 0, 10, 30 or 100 mg/kg bw/d on day 6 – 15 *post coitum*.

Observations in dams: At 100 mg/kg bw/d only slight signs of maternal toxicity occurred: decreased food consumption (13 - 26%) on GD 6-16 and significantly lower body weight from GD 12 to GD 21 (4.6% - 7.4%%) as compared to controls. Body weight gain on GD 21 was 57.1% lower in comparison with controls at 100 mg/kg bw/d and the decreases were

statistically significant only after correction for uterus weight. The uterus weight was not statistically significantly different from the control group. At terminal necropsy, one dam at 100 mg/kg bw/d had a perforating gastric ulcer. No other clinical signs or symptoms and no deaths were observed which were considered to be related to the test substance.

Observations in offspring: The incidence of total embryonic resorptions and foetal resorptions were not affected by treatment with spiroxamine at any dose. The total number of foetuses, live foetuses, % of abnormal dead foetuses and results of skeletal examination were not different between the experimental groups. However, at 100 mg/kg bw/d skeletal examination resulted in significantly increased incidences of incomplete ossification (cranium, sternebrae) or non-ossification (phalanges) which might be associated with delayed development. The body weights of the male and female foetuses at 100 mg/kg bw/d were statistically significantly reduced, by 4.1% and 2.2%, respectively, in comparison with controls. Thus the changes were comparable to the reductions in body weights of the dams during GD 12-21 as compared to controls (4.6% - 7.4%). At 100 mg/kg bw/d, palatoschisis was detected in three foetuses from three litters.

2. In a developmental toxicity study in rabbits (Holzum, 1995), which was performed according OECD TG 414 and with GLP, groups of 15 pregnant female Himalayan rabbits were treated with spiroxamine by gavage at 0, 5, 20 or 80 mg/kg bw per day on days 6 – 18 *post coitum*. A supplementary study became necessary because of partially equivocal findings in the first study. Control animals were dosed with the vehicle (water with 0.5% Cremophor EL). The dams were sacrificed on day 29 *post coitum* and foetuses were removed.

Observations in dams:

Main study: No significant gross pathological findings were observed at necropsy. Isolated dams at 80 mg/kg bw/d displayed encrustation at the labial angles or anal prolapse. In addition, animals exhibited impaired body weight gain and reduced food intakes at this dose.

Supplementary study: Animals excreted few or soft faeces and one dam at 80 mg/kg bw/d died on day 16 *post coitum*. Food intake of treated animals did not differ significantly from those in the control group. At 80 mg/kg bw/d reduced weight gain during treatment period was observed. Body weight development throughout the entire gestation period and corrected body weight did not differ significantly from the control group.

Observations in offspring:

Numbers of implantation sites, number of resorptions and live featuses per dam were not affected by treatment with spiroxamine at any dose. Weight of live foetuses was not affected in the main study, but in the supplementary study foetal body weight was slightly lower (by 5%) in males than in controls. The degree of ossification and the incidences of variations in the foetal skeletal system, as well as the external appearance of the placentas showed no treatment related effects up to 80 mg/kg bw/d. The total incidence of internal malformations in individual foetuses and in foetuses per litter did not differ between control and treated groups in the main or supplementary study. All observed malformations were within historical control data (1982 - 1996), except for hydrocephalus internus with caudal displacement of the ears in one foetus at 80 mg/kg bw/d.

3. In a developmental dermal toxicity study in rats (Becker and Biedermann, 1993) performed according OECD TG 414 and GLP compliant, groups of 25 mated female Wistar rats were exposed to spiroxamine under occlusive conditions for 6 h/day from day 6 - 15 *post coitum* at 0, 5, 20 or 80 mg/kg bw/d. Control animals were dosed with the vehicle alone (water with 1% Cremophor EL). The rats were sacrificed on day 21 *post coitum* and the foetuses were removed.

Observations in dams:

No deaths occurred and no test substance -related systemic signs and/or symptoms were observed. Dermal application caused dose related skin reactions (slight erythema and scaling) from 5 mg/kg bw/d upwards. Body weight ~~was~~ decreased during gestation at 80 mg/kg bw/d as compared to controls and corrected body weight gain (corrected for uterus weight) was slightly decreased at 20 mg/kg bw/d (10.0 g vs. 19.3 g in the control group) and at 80 mg/kg bw/day (-2.6 g as compared to controls). Since the lowest uterus weights were seen in control animals, the corrected body weight at 20 mg/kg bw/d was not considered to be adverse. The mean food consumption was not affected in any dose groups. During terminal necropsy, no macroscopic changes were noted in any female of any group.

Observations in offspring:

No indication of substance related effects was noted on reproductive parameters at any dose level. External and visceral examination of the foetuses also revealed no indication of substance related effects. The mean body weight of foetuses was not affected. The skeletal examination of foetuses showed a slight toxic effect: at the highest dose the number of foetuses with wavy ribs was increased (11/143) compared to control animals (1/143). The stage of skeletal development in the foetuses of all dose groups was comparable to the control group.

Assessment and comparison with the classification criteria

In the three studies reviewed above, spiroxamine caused no embryonic or foetal mortality. Slightly reduced foetal weight in some studies can be secondary to maternal toxicity, which presented as reduced food consumption as well as reduced corrected maternal body weight gain and body weight.

External and visceral examination of the foetuses revealed no indication of substance related effects, except for the occurrence of palatoschisis in 3 pups from 3 litters in pregnant female rats exposed to spiroxamine at 100 mg/kg bw/d. The significance of this increase in frequency of palatoschisis is not easy to assess. Although the rat developmental study reports are from 1992 (main study) and 1995 (range finding studies), the experimental work for the range finding studies was performed in 1990, and for the main developmental study between December 1990 and January 1991. As reported in the CLH report (see Background document), the spontaneous frequency of this malformation in the rats used by the laboratory which carried out all developmental toxicity studies of spiroxamine was variable, with no cases of palatoschisis in rats in years 1988, 1989, 1992 and 1994. In 1990, when the range-finding and main studies of spiroxamine were conducted, up to 7 control foetuses with palatoschisis were observed in 3 litters in 3 studies (i.e. in one litter per study in 3 out of 7 studies), with at most 4/280 affected foetuses (i.e. 1.4%) in one study (from one litter)). In 1995, 2 foetuses in 2 litters was reported in one study in 1995. The observed incidence of this malformation in the main developmental toxicity study (three foetuses with palatoschisis in three litters) (Becker and Biedermann, 1992) was relatively low, and the incidence in foetuses was even below the upper range observed in the historical control group in 1990 when this study was performed (see above). However, the incidence was slightly above historical controls when ~~if~~ the number of affected litters are considered (3 affected litters in the Becker and Biederman study (1992)) while in the historical controls only one litter was affected in each of the three studies in 1990, and 2 litters were affected in one study in 1995. Taking into account the results of two range-finding studies and one main developmental toxicity study, the increase in the palatoschisis should be considered as treatment related, although that increase is low, at least in the main study. This malformation was not observed in rabbits exposed to spiroxamine by gavage or in rats exposed via the skin.

The mechanism of induction of palatoschisis by spiroxamine in rats is not known, therefore it is not possible to exclude that this mechanism is relevant to humans.

Although this malformation is a serious finding, observed in one species, it was reported at a low incidence, in one main study where the incidence was slightly above that in historical

controls at doses causing maternal toxicity. A higher incidence was observed in range finding studies in maternal animals showing some clinical symptoms. Taking into account these considerations, RAC is of the opinion that the evidence on developmental toxicity of spiroxamine fulfill the criteria for category 2 but not for category 1B. RAC concludes that spiroxamine warrants classification as **Repr. 2, H361d (Suspected of damaging the unborn child)** as proposed by the DS.

ENVIRONMENTAL HAZARD ASSESSMENT

RAC evaluation of environmental hazards

Summary of the Dossier submitter's proposal

Spiroxamine is included in Annex VI of the CLP Regulation (Regulation (EU) 1272/2008) with the environmental hazard classifications Aquatic Acute 1 (H400) and Aquatic Chronic 1 (H410), without M-factors. The dossier submitter (DS) proposed an M-factor of 100 for both acute and chronic hazard classes, based on toxicity to the alga *Skeletonema costatum* (96-h E_rC_{50} = 0.0063 mg/L, NOE_rC = 0.00063 mg/L) and lack of rapid degradation in water and water-sediment simulation tests.

Comments received during public consultation

An industrial stakeholder was of the opinion that the evaluation of rapid degradability should be based on higher-tier mesocosm studies (i.e. a mean whole system DT_{50} value of 7.2 days in nine outdoor mesocosm systems (Bruns *et al*., 2008), supported by a total system DT_{50} of ca. 10 days in an additional enclosure study by Heimbach *et al*., 2000). Since the DT_{50} (whole system) was below 16 days in aerobic water-sediment mesocosm systems, they concluded that Spiroxamine undergoes rapid primary degradation in the environment. In their opinion, the two major metabolites in the water-sediment systems (KWG 4168-N-oxide (M03) and KWG 4168-acid (M06)) do not fulfil the criteria for classification as hazardous to the aquatic environment (the E_rC_{50} values for the green alga *Desmodesmus subspicatus*, which is the most sensitive species, are 31.7 mg/L for KWG 4168-N-oxide (M03) and > 3.2 mg/L for KWG 4168-acid (M06)). Consequently this stakeholder considered that Spiroxamine is rapidly degradable in the aquatic environment. In response, the DS stated that the mesocosm studies were not performed according to a standardized protocol or defined laboratory conditions, and are therefore unsuitable for evaluating rapid degradability. RAC's view is given below. They also pointed out some issues regarding the interpretation of the hydrolysis and long-term fish toxicity studies.

Two Member State Competent Authorities agreed with the proposal, but one pointed out that the description of environmental fate properties was incomplete, and asked for all available aquatic toxicity studies to be summarised. The DS did not provide any additional relevant information in its response, and RAC has not sought further data.

Assessment and comparison with the classification criteria

Spiroxamine contains two diastereomers in approximately equal proportions. Studies have been performed on the commercial substance, unless otherwise stated. The substance has a pKa of 6.9, and therefore will be substantially ionised at an environmentally relevant pH.

Degradation

Spiroxamine is hydrolytically stable at 25 ^{0}C at pH 4, 7 and 9, with a maximum of 4.5% degradation occurring at pH 9 after 30 days' incubation. Aqueous photolysis is slow with a

calculated environmental half-life of 236 days under worst case solar conditions. A ready biodegradation test is not available. Simulation tests in two aerobic water-sediment systems using a radio-labelled substance indicated primary degradation and formation of non-extractable residues, with first order degradation DT_{50} values for the whole system of 28 – 106 days, and relatively little mineralisation over 100 days (7 – 17% of applied radioactivity). Higher-tier studies provide a mean whole system DT_{50} value of 7.2 days in nine outdoor mesocosm systems (supported by a total system DT_{50} of ca. 10 days in an additional enclosure study). RAC notes that the CLP Guidance (Annex II, Section II.2.3.2) states that data from mesocosm experiments can in principle be used for assessing the potential for rapid degradation, provided that ultimate degradation can be demonstrated. Since such data have not been supplied during the public consultation, RAC considers that the results of the aquatic simulation tests are more reliable.

Based on the limited hydrolysis, and primary degradation half-lives exceeding 16 days in aquatic simulation studies, RAC agrees with the DS's proposal that Spiroxamine does not meet the criteria for being rapidly degradable in the environment.

Bioaccumulation

The worst case BCF for whole fish is below 100 L/kg, based on total radioactivity (lipid content was not measured). Since the BCF value is significantly below the threshold value of 500 L/kg, lipid normalisation has no effect on the evaluation of bioaccumulation potential. Furthermore, given the presence of adequate chronic toxicity data, the bioaccumulation potential is not relevant to the classification.

Aquatic Toxicity

The lowest reliable ecotoxicity results reported in the CLH dossier were as follows (the key data are highlighted in bold):

Trophic level	**Species**	**Short-term result**	**Long-term result**
Fish	Zebra fish *Danio rerio*	96-h LC_{50} = 2.41 mg/L	230-d EC_{10} = 0.002 mg/L
Aquatic invertebrates	*Daphnia magna*	48-h EC_{50} = 3.0 mg/L	21-d NOEC = 0.034 mg/L
Aquatic algae and plants	*Skeletonema costatum*	**96-h E_rC_{50} = 0.0063 mg/L**	**96-h NOE_rC = 0.00063 mg/L**

The acute fish toxicity and both aquatic invertebrate results were based on mean measured concentrations. Spiroxamine is an ergosterol biosynthesis inhibitor, and so additional endocrine test parameters were included in a fish full life-cycle study to assess possible disruption of the endocrine system of the exposed fish. The most sensitive end point was survival in the F1 early life stage. The results of this study were based on nominal test concentrations only. The algae study results were based on initial measured concentrations. Algae data for a 72-h exposure period are usually preferred if available. There is no information in the CLH dossier to establish whether the algae were in an exponential growth phase over the longer duration of 96 hours. As the study has been accepted for pesticide regulatory purposes, RAC assumes that the 96-h result is reliable in the absence of any other information.

Classification according to CLP

<u>*Acute aquatic hazard*</u>: Reliable acute aquatic toxicity data are available for the three trophic levels fish, aquatic invertebrates and algae. The lowest reliable short-term aquatic toxicity result is a 96-h E_rC_{50} of 0.0063 mg/L for the marine diatom *S. costatum*. This concentration is

below the threshold value of 1 mg/L, so spiroxamine meets the criteria in the CLP Regulation for classification as Aquatic Acute 1; H400. As 0.001 < E_rC_{50} ≤ 0.01 mg/L, the acute M-factor is 100, as proposed by the DS.

Chronic aquatic hazard: Reliable long-term aquatic toxicity data are available for the three trophic levels fish, aquatic invertebrates and algae. The lowest reliable long-term aquatic toxicity result is a 96-h NOE_rC of 0.00063 mg/L for the marine diatom *S. costatum*. Spiroxamine is not rapidly degradable, and as this concentration is below the threshold value of 0.1 mg/L, the substance meets the criteria in the CLP Regulation for classification as Aquatic Chronic 1; H410. As 0.0001 < NOEC ≤ 0.001 mg/L, the chronic M-factor is 100, as proposed by the DS.

In summary, RAC agrees with the proposal of the DS that spiroxamine should be classified as:
Aquatic Acute 1; H400, M=100;
Aquatic Chronic 1; H410, M=100.

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 by RAC (excl. confidential information).