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: -
CAS Number: 118134-30-8

CLH-O-0000001412-86-72/F

Adopted

11 September 2015

11 September 2015

CLH-O-0000001412-86-72/F

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

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

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

The proposal was submitted by **Germany** and received by the RAC on **28 April 2015.**

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

PROCESS FOR ADOPTION OF THE OPINION

Germany has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **05/05/2015**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **04 June 2015**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Boguslaw Baranski**

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 harmonized classification and labelling was adopted on **11 September 2015** by **consensus**.

Existing Annex VI entry (CLP, Table 3.1)

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) No 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	H302 H312 H332 H315 H317 H400 H410	GHS07 GHS09 Wng	H302 H312 H332 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	STOT RE 2	H373	GHS08	H373	-	-	-
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	STOT RE 2	H373	GHS08	H373	-	-	-
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	Acute Tox. 4 Acute Tox. 4 Acute Tox. 4 Skin Irrit. 2 Skin Sens. 1 STOT RE 2 Repr. 2 Aquatic Acute 1 Aquatic Chronic 1	H332 H312 H302 H315 H317 H373 (eye) H361d H400 H410	GHS07 GHS08 GHS09 Wng	H332 H312 H302 H315 H317 H373 (eye) H361d 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 proposals for removal of minimum classification for Acute Tox. (all routes of exposure), Skin Sens. 1B, Repr. 2 and M-factors (acute and chronic). 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 general comments

During the evaluation of toxicity to reproduction as proposed by the Dossier Submitter (DS) Germany, RAC requested to have a summary of the repeat dose effects to aid in their interpretation of the reproduction data. Germany responded by submitting a further CLH proposal supporting STOT RE 2. This opinion therefore concerns the evaluation by RAC of this second submission.

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

Summary of the Dossier submitter's proposal

In the CLH report submitted to ECHA on 28 April 2015 the Dossier Submitter (DS) proposed to classify spiroxamine as STOT RE 2; H373 (May cause damage to organs through prolonged or repeated exposure) without specifying affected organs.

According to the DS, classification is justified by the following findings:

- Markedly increased mortality observed in oral developmental toxicity studies in rats at dose levels compatible with STOT RE 2.
- Ocular findings observed in dogs at dose levels compatible with STOT RE 2.

Comments received during public consultation

Two MSCAs and one industrial organization supported the STOT RE classification proposal of the DS.

Assessment and comparison with the classification criteria

The following adverse effects were observed in the repeated dose toxicity studies included in the CLH report dated on 24 April 2015 for the assessment of classification for STOT RE.

Oral studies

Oral range-finding studies on rats

High mortality was noted in the preliminary developmental toxicity studies in rats. In these studies, 21/25 (84%) or 5/5 (100%) dams were dead after exposure to spiroxamine by gavage during pregnancy at doses of 150 or 250 mg/kg bw/d, respectively. Since the deaths occurred on GD 10 – 17, the effect cannot be explained solely by acute toxicity, but could be due to a greater sensitivity of pregnant animals to repeated dosing with spiroxamine.

28-day studies

1. Hyperkeratosis of the oesophagus mucosa, slightly increased incidence/severity of hepatocellular fat deposits and elevated relative liver weights were observed in male rats receiving spiroxamine in the feed at 10.8 and 33.6 mg/kg bw/d and in female rats at 35.6 mg/kg bw/d over 28 days. The NOEL was equivalent in males and females: 3.4 mg/kg bw/d and 3.8 mg/kg bw/d, respectively (Krötlinger *et al.*, 1992).

2. Hyperkeratosis of the stomach mucosa, increased frequency of hepatocellular fat deposits and elevated relative liver weights were observed in male and female rats receiving spiroxamine at dose-levels of 90 mg/kg bw/d by gavage for 28 successive days. The lowest dose of 10 mg/kg bw/d was taken as the LOAEL (Krötlinger and Hartmann, 1992).

90-day studies

1. Hyperkeratosis of the tongue, oesophagus and forestomach mucosa were seen in male and female rats receiving spiroxamine in the feed at concentrations of 625 ppm (54.9 and 75.14 mg/kg bw/d for males and females, respectively) over a period of 13 weeks. In the groups receiving spiroxamine in the feed at concentrations of 125 ppm (9.3 and 13.2 mg/kg bw/d for males and females, respectively) hyperkeratosis of the oesophagus was found in 90% of males and 50% of females. The NOAEL of 25 ppm in rats (equivalent to 1.9 and 2.7 mg/kg bw/d in males and females, respectively) was based on histopathological findings in the oesophagus and fore-stomach (hyperkeratosis of the epithelium at 125 ppm) (Eiben and Hartmann, 1992).

2. Depressed general condition and emaciation, hair loss and un-groomed fur were observed in isolated male mice given spiroxamine in the diet at 1280 ppm (366.2 mg/kg bw/d). The levels of urea and cholesterol were within the physiological range up to 320 ppm, but were elevated (urea) or decreased (cholesterol) to a statistically significant extent at the high dose (1280 ppm) in both sexes. In this dose group mice exhibited desiccated or crusted areas of skin at the auricles and/or tail and the histology examination revealed marked epidermal hyperplasia. Minimal epidermal hyperplasia of the auricles was also observed in several 320 ppm males. Two males and one female died with causal relationship to the treatment at 1280 ppm (366.2 and 413.7 mg/kg bw/d for males and females, respectively). Therefore, a slightly elevated rate of mortality was noted in both sexes at the high dose. Hyperplasia of the urinary bladder epithelium was found in 9/10 mice, both males and females, receiving spiroxamine in the feed at concentrations of 1280 ppm over a period of 13 weeks.

The NOAEL of 80 ppm mice (24.9 and 28.5 mg/kg bw/d for males and females, respectively) was based on epidermal hyperplasia (auricles) and liver hypertrophy being observed at 320 ppm (88.4 and 126.3 mg/kg bw/d for males and females, respectively) (Eiben and Hartmann, 1992).

3. Hepatocellular hypertrophy and reduced glycogen levels were found in male and female B6C3F1 mice receiving spiroxamine at dose levels of 180 and 240 mg/kg bw/d (the highest dose tested) by gavage over a period of 13 weeks. In addition, single cell necrosis occurred at 240 mg/kg bw/d. Urinary tract epithelial hyperplasia was detected in the bladders of the 180 and 240 mg/kg bw/d dose group mice. Mice in the high dose group exhibited hyperplastic changes in the epidermis of the auricles and tails (males only). No NOAEL was determined due to increased liver enzyme activity at 60 mg/kg bw/d and above (Eiben *et al.*, 1992).

6. Hepatocytomegaly, increased alkaline phosphatase levels, decreased albumin levels and decreased triglyceride levels were found in 4 male and 4 female pure-bred beagle dogs receiving spiroxamine in the feed at concentrations of 1500 ppm over a period of 13 weeks (males 42.8 mg/kg bw/d and females 43.7 mg/kg bw/d). The other groups were fed a diet containing 0, 25, 750 or 1500 ppm of spiroxamine (equivalent to 0.66, 20.02 or 42.76 mg/kg bw/d, respectively, in males and 0.78, 21.29 or 43.69 mg/kg bw/d, respectively, in females). The NOEL in males of 25 ppm (0.7 mg/kg bw/d) was based on effects on the liver at 750 ppm (increased relative weight, minimal hepatocytomegaly in males) (Jones and Elcock, 1994).

4. A NOAEL of 500 ppm (16.2 and 15.1 mg/kg bw/d, respectively, for males and females), the highest dose tested, was established in the repeated dose oral toxicity study in which spiroxamine was administered in the diet to Beagle dogs for approximately 110 days at concentrations of 0, 150, 250 or 500 ppm (Jones and Hastings, 1997).

5. Changes in the eyes (cataract, opacity) were observed in one male and three female dogs fed the diet containing 2000 ppm spiroxamine (56.9 and 52.4 mg/kg bw/d in males and females, respectively) about 9 months after the start of the studyNo effects were seen at lower dose levels. In this study spiroxamine was administered in the diet to Beagle dogs (4 animals/sex/dose) at 0, 25, 75, 1000 or 2000 ppm for 52 weeks. At termination also two males of the 1000 ppm dose group had evidence of bilateral sub-capsular clouding and cataracts (28.03 mg/kg bw/d), and one

female had bilateral lens opacities (cataract) at 25.84 mg/kg bw/d. At the final examination of the 2000 ppm dose group dogs, one male had developed a bilateral cataract, and three females had bilateral lens clouding and cataracts. Microscopic evidence of ocular cataract formation was found in the high dose group.

The NOAEL was established at 75 ppm (2.47 and 2.48 mg/kg bw/d, respectively, for males and females), based on findings at 1000 ppm in the liver (altered clinical chemistry parameters, minimal diffuse hepatocytomegaly) and the eyes (sub-capsular clouding, cataractic changes) (Jones and Elock, 1995).

Dermal studies

1. Diffuse epidermal hyperplasia, focal epidermal hyperplasia, hyperkeratosis and inflammatory reactions in the skin were observed in all treated animals in a subacute dermal toxicity study on New Zealand White (NZW) rabbits. The spiroxamine was formulated with Cremophor EL (2% v/v) in sterile physiological saline solution. The animals were treated with the test compound at doses of 0, 0.5, 1 and 5 mg/kg bw/d for (6h/d) for 3 weeks (corresponding concentrations were: 0, 0.025, 0.05 and 0.25%). Due to strong irritant reactions, higher concentrations (and thus higher dose levels) than 0.25% (5 mg/kg bw/d) could not be tested in rabbits. Five males and 5 females were used per group. A satellite group (5 mg/kg bw/d) and a further control group were observed over a 14 day post-treatment period. The dermal effects were mostly reversible at the end of the post-treatment period. In this study, the systemic NOAEL was 5 mg/kg bw/d, the highest dose tested. Local effects were observed even at the lowest dose level of 0.5 mg/kg bw/d (Vohr and Rinke, 1995).

2. No local skin findings were observed among the animals in the sub-acute dermal toxicity study on NZW rabbits. Spiroxamine was formulated with Cremophor EL (2% v/v) in sterile physiological saline solution. The animals were treated with the test compound in doses of 0, 0.05 and 0.2 mg/kg bw/d (corresponding concentrations were 0, 0.025 and 0.01%) for 6h/d for 3 weeks. Five males and 5 females were used per group. Under the conditions of this sub-acute dermal toxicity study, the NOAEL for local effects was 0.2 mg/kg bw/d, the highest dose tested (Vohr, 1995).

Inhalation studies

Squamous epithelial metaplasia in the nasal cavity, epithelial hyperplasia and hyperkeratosis in the larynx zone, an elevated rate of bronchiolo-alveolar proliferation with an increase in alveolar macrophages in the lungs, and hyperkeratosis in the oesophagus were observed in rats exposed for 4 weeks to spiroxamine at a concentration of 518.4 mg/m³. The eyes exhibited corneal hyperplasia, and the eyelids hyperplasia accompanied by hyperkeratosis. The most severe local dermal lesions (hyperkeratosis, epithelial hyperplasia, extended inflammatory infiltration, scab formation) were found in the muzzle zone. Hyperkeratosis and epithelial hyperplasia were also seen in the mammary zone and on the tail. The male animals exhibited atrophic thymus changes. The urinary bladder urothelium exhibited hyperplastic lesions in the animals in the 518.4 mg/m³ group. In this study, male and female Wistar rats were exposed to spiroxamine at mean analytical aerosol concentrations of 14.3, 87.0 or 518.4 mg/m³ under dynamic conditions for 6 h/d and 5 d/wk (head-nose-only) over a period of four weeks (mass median aerodynamic diameter = 1.2 µm; Geometric Standard Deviation = 1.5, mass fraction of particles with aerodynamic diameter ≤ 3 µm was ≥ 98%).
The NOAEC in this study was 14.3 mg/m³, equivalent to 3.9 mg/kg bw/d (Pauluhn, 1992).

Long-term oral toxicity studies

In long-term oral toxicity studies, rats were fed a diet for 2 years containing spiroxamine at 490 ppm (33.8 and 43.0 mg/kg bw/d in males and females, respectively) (Eiben, 1994). Mice were fed a diet for 6 months containing spiroxamine at 2.5 ppm, and then for the next 18 months the concentration of spiroxamine in the diet was increased to 480 ppm (59.3 and 102.6 mg/kg bw/d in males and females, respectively) (Eiben and Hartman, 1995). Histopathological alterations of the epithelium of the gastrointestinal tract were noted in both rats and mice. These effects (hyperkeratosis and acanthosis of the epithelium of the tongue, oesophagus and fore-stomach) might be interpreted as an adaptive process following a continuous irritant stimulus by spiroxamine. Furthermore, skin alterations at auricles and tips of the tail were observed in mice.

In rats, no adverse effects were noted at 70 ppm (4.22/5.67 mg/kg bw/d, in males/females) or below (Eiben, 1994).
In mice, in the 160 ppm dose group (36.7/59.5 mg/kg bw/d in males/females), lower body weights were noted in males. One female had cysts in the ovaries at the interim sacrifice. Acanthosis and hyperkeratosis in the oesophagus, pinnae and tail were detected. The NOAEL was 20 ppm (4.5/7.8 mg/kg bw/d, in males/females) (Eiben and Hartman, 1995).

In the second long-term oral toxicity study (Eiben and Sander, 1997) mice were fed for 24 months a diet containing spiroxamine at concentrations of 0, 160, 600 ppm (0, 41.0, 149.8 mg/kg bw/d and 0, 64.6, 248.1 mg/kg bw/d, in males and females respectively). In the 600 ppm dose group, decreased body weights in males and females were detected. During histological examination hyperkeratosis and/or acanthosis on tongue, oesophagus, forestomach, pinnae and tail of females were detected. Additionally, hepatocellular liver changes were detected predominantly in females that died during the treatment period. The NOAEL was established at 160 ppm (41.0 or 64.6 mg/kg bw/d for males or females, respectively) in this study.

Comparison with the classification criteria

Substances are classified in category 2 for target organ toxicity (repeated exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations.

Guidance dose/concentration values applicable to the different study durations (based on Haber's rule) are provided below:

Oral, rat:

8-day: 105 < C ≤ 1050 mg/kg bw/d corresponding to exposure during GD 10 - 17
28-day: 30 < C ≤ 300 mg/kg bw/d
90-day: 10 < C ≤ 100 mg/kg bw/d
1-yr: 2.5 < C ≤ 25 mg/kg bw/d
2-yr: 1.25 < C ≤ 12.5 mg/kg bw/d

Dermal, rat or rabbit:

28-day: 60 < C ≤ 600 mg/kg bw/d
90-day: 20 < C ≤ 200 mg/kg bw/d

Inhalation (dust/mist/fume), rat:

28-day: 0.06 < C ≤ 0.6 mg/L/6 h/d or 60 < C ≤ 600 mg/m^3/6 h/d
90-day: 0.02 < C ≤ 0.2 mg/L/6 h/d or 20 < C ≤ 200 mg/ m^3/6 h/d

Classification of spiroxamine as STOT RE 2; H373 (May cause damage to organs through prolonged or repeated exposure) is justified by the following findings observed at or below guidance dose/concentration values for STOT RE 2:

- High mortality of 84% and 100% was seen at doses of 150 and 250 mg/kg bw/d, respectively, in the dose-range finding developmental toxicity studies in rats after oral exposure during GD 10 - 17. These are within the limits of the guidance values of 105 < C ≤ 1050 mg/kg bw/d based on exposure during 8 days and of 30 < C ≤ 300 mg/kg bw for the 28 day repeated toxicity study.
- Changes in the eyes (cataract, opacity) were observed in one male and three female dogs fed the diet containing spiroxamine at 2000 ppm about 9 months after the start of the study (56.9 and 52.4 mg/kg bw/d in males and females, respectively). After one year of exposure, bilateral sub-capsular clouding and cataracts were observed in two males of the 1000 ppm dose group (28.0 mg/kg bw/d), and bilateral lens opacities (cataract) in one female at 25.8 mg/kg bw/d, thus slightly above the guidance value of ≤ 25 mg/kg bw/d for that duration of exposure. According to the CLP Regulation, Annex I, 3.9.2.9.8, "The guidance values and ranges […] are intended only for guidance purposes, i.e. to be used as

part of the weight of evidence approach, and to assist with decisions about classification. They are not intended as strict demarcation values."

- Marked or severe hyperkeratosis of the oesophagus was observed in the 13 week dietary study in rats (Eiben and Hartmann, 1992) in 4/10 males and 6/10 females at doses of 32.8 and 43.0 mg/kg bw/d and of the forestomach in 1/10 males and 2/10 females at the same doses. These were below the guidance value of ≤ 100 mg/kg bw/d. These marked or severe findings were noted after subchronic oral exposure in rats at doses > 10 and ≤ 100 mg/kg bw/d, leading to a classification as STOT-RE 2; H373.

The hyperkeratosis and acanthosis of the epithelium of tongue, oesophagus and fore-stomach observed in long-term feeding studies in rats and mice occurred at concentrations above the guidance value for STOT RE 2: 1.25 < C ≤ 12.5 mg/kg bw/d.

Taking the above data into account and noting that the adverse effects were occurred within the guidance values for STOT RE 2 or only slightly above these values, and giving more weight to systemic effects than to local adaptive effects, RAC is of the opinion that spiroxamine warrants **classification as STOT RE 2**; H373 (May cause damage to eyes through prolonged or repeated exposure).

ANNEXES:

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

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