Committee for Risk Assessment

RAC

Annex 1

Background document

to the 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

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'.
Only the RAC text reflects the view of RAC.

Adopted

11 September 2015

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: Spiroxamine

EC Number: **n.a.**

CAS Number: **118134-30-8 (unstated stereochemistry)**

Index Number: **612-150-00-X**

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Version number: 2 **Date: 2014-06-23**

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PART A.

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	***Spiroxamine***
EC number:	***n.a.***
CAS number:	***118134-30-8***
Annex VI Index number:	***612-150-00-X***
Degree of purity:	***≥ 940 g/kg*** *(diastereomers A and B combined)* *490 – 560 g/kg diastereomer A* *440 – 510 g/kg diastereomer B*
Impurities:	***No impurity is considered relevant for the classification of the substance Spiroxamine***

1.2 Harmonised classification and labelling proposal

Table 2 : The current Annex VI entry and the proposed harmonised classification

	CLP Regulation	**Directive 67/548/EEC (Dangerous Substances Directive; DSD)**
Current entry in Annex VI, CLP Regulation	Acute Tox. 4*/ H332 Acute Tox. 4*/ H312 Acute Tox. 4*/ H302 Skin Irrit. 2 / H315 Skin Sens. 1 / H317	Xn; R20/21/22 Xi; R38 R43 N; R50-53

	Aquatic Acute 1 / H400 Aquatic Chronic 1 / H410	
Current proposal for consideration by RAC	Repr. 2 / H361d M-acute = 100 M-chronic = 100 *Reevaluation of* *Acute Tox. 4 / H332* *Acute Tox. 4 / H312* *Acute Tox. 4 / H302* *Skin Sens. 1B / H317*	Repr. Cat. 3; R63 SCL: N; R50-53: Cn≥ 0.25% N; R51-53: 0.025≤ Cn < 0.25 % R52-53: 0.0025 ≤ Cn < 0.025 %
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Repr. 2 / H361d Acute Tox. 4 / H332 Acute Tox. 4 / H312 Acute Tox. 4 / H302 Skin Irrit. 2 / H315 Skin Sens. 1B / H317 Aquatic Acute 1 / H400 Aquatic Chronic 1 / H410 M-acute = 100 M-chronic = 100	Repr. Cat. 3; R63 Xn; R20/21/22 Xi; R38 R43 N; R50-53 SCL: N; R50-53: Cn≥ 0.25% N; R51-53: 0.025≤ Cn < 0.25 % R52-53: 0.0025 ≤ Cn < 0.025 %

1.3 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	none		none	conclusive but not sufficient for classification
2.2.	Flammable gases	none		none	not applicable
2.3.	Flammable aerosols	none		none	not applicable
2.4.	Oxidising gases	none		none	not applicable
2.5.	Gases under pressure	none		none	not applicable
2.6.	Flammable liquids	none		none	conclusive but not sufficient for classification
2.7.	Flammable solids	none		none	not applicable
2.8.	Self-reactive substances and mixtures	none		none	Data lacking
2.9.	Pyrophoric liquids	none		none	Data lacking
2.10.	Pyrophoric solids	none		none	not applicable
2.11.	Self-heating substances and mixtures	none		none	conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	none		none	conclusive but not sufficient for classification
2.13.	Oxidising liquids	none		none	conclusive but not sufficient for classification
2.14.	Oxidising solids	none		none	not applicable
2.15.	Organic peroxides	none		none	not applicable
2.16.	Substance and mixtures corrosive to metals	none		none	Data lacking
3.1.	Acute toxicity - oral	Acute Tox. 4; H302		Acute Tox. 4*; H302	
	Acute toxicity - dermal	Acute Tox. 4; H312		Acute Tox. 4*; H312	
	Acute toxicity - inhalation	Acute Tox. 4; H332		Acute Tox. 4*; H332	
3.2.	Skin corrosion / irritation	Skin Irrit. 2; H315		Skin Irrit 2; H315	
3.3.	Serious eye damage / eye irritation	none		none	Data lacking
3.4.	Respiratory sensitisation	none		none	Data lacking
3.4.	Skin sensitisation	Skin Sens. 1B; H317		Skin Sens. 1; H317	
3.5.	Germ cell mutagenicity	none		none	Data lacking
3.6.	Carcinogenicity	none		none	Data lacking
3.7.	Reproductive toxicity	Repr. 2.; H361d		none	
3.8.	Specific target organ toxicity – single exposure	none		none	Data lacking
3.9.	Specific target organ toxicity – repeated exposure	none		none	Data lacking
3.10.	Aspiration hazard	none		none	Data lacking
4.1.	Hazardous to the aquatic environment	Aquatic Acute 1; H400 Aquatic Chronic 1; H410	M-acute = 100 M-chronic = 100	Aquatic Acute 1; H400 Aquatic Chronic 1; H410	
5.1.	Hazardous to the ozone layer	none		none	Data lacking

[1)] Including specific concentration limits (SCLs) and M-factors

[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling:

Pictograms:	GHS07, GHS08, GHS09
Signal word:	Warning
Hazard statements:	H361d, H332, H312, H302, H315, H317, H410
Precautionary statements:	(P102), P260, P273, P281, P302 + P352, P308 + P313, P362, P391, P405, P501

Proposed notes assigned to an entry:

Table 4: Proposed classification according to DSD

Hazardous property	Proposed classification	Proposed SCLs	Current classification [1)]	Reason for no classification [2)]
Explosiveness	none		none	conclusive but not sufficient for classification
Oxidising properties	none		none	conclusive but not sufficient for classification
Flammability	none		none	conclusive but not sufficient for classification
Thermal stability	none		none	conclusive but not sufficient for classification
Acute toxicity	R20/21/22		R20/21/22	
Acute toxicity – irreversible damage after single exposure	none		none	Data lacking
Repeated dose toxicity	none		none	Data lacking
Irritation / Corrosion	R38		R38	
Sensitisation	R43		R43	
Carcinogenicity	none		none	Data lacking
Mutagenicity – Genetic toxicity	none		none	Data lacking
Toxicity to reproduction – fertility	none		none	Data lacking
Toxicity to reproduction – development	R63		none	
Toxicity to reproduction – breastfed babies. Effects on or via lactation	none		none	Data lacking
Environment	N; R50/53	N; R50-53: Cn≥ 0.25% N; R51-53: 0.025≤ Cn < 0.25 % R52-53: 0.0025 ≤ Cn < 0.025 %	N; R50/53	

[1)] Including SCLs
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling:

Indication of danger:	Xn; N
R-phrases:	R: 20/21/22-38-43-50/53-63
S-phrases:	S: (2-)23-36/37-46-60-61

2 BACKGROUND TO THE CLH PROPOSAL

2.1 Short summary of the scientific justification for the CLH proposal

Currently, Spiroxamine has a legal classification (regulation (EC) 1272/2008) for following toxicological endpoints: acute effects (H302*-H312*-H332*, R20/21/22), skin irritation (H315, R38) and skin sensitisation (H317, R43). For aquatic ecotoxicological endpoints, a legal classification exists as very toxic to aquatic life with long lasting effects for acute (Aquatic acute 1, H400) and chronic (Aquatic Chronic 1, H410) endpoints, but there are no M-factors or specific concentration limits fixed.

During the renewal procedure of Spiroxamine under directive 91/414/EC, it was noted that this current legal classification should be amended to include a classification for developmental toxicity (H361d, R63) based on increased incidences of malformations (palatoschisis) in rats. The existing classification for other toxicological hazards than developmental toxicity was considered appropriate. During the preparation of this CLH-dossier, the existing minimum classification (which was based on the conversion rules) for acute toxicity was double-checked and classification with acute toxicity 4 was considered appropriate in all three cases. Considering the changed criteria for skin sensitizers (regulation (EU) No 286/2011), sub-category 1B is proposed. Furthermore, the addition of suitable M-factors for the environmental classification is proposed.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Spiroxamine is an active substance in the meaning of Regulation (EC) No. 1107/2009 (replaces Directive 91/414/EEC). Following article 36(2) of Regulation (EC) 1272/2008 such substances should normally be subject to harmonised classification.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

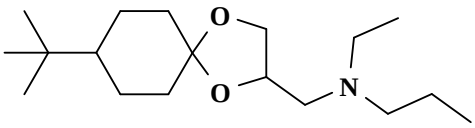
1 IDENTITY OF THE SUBSTANCE

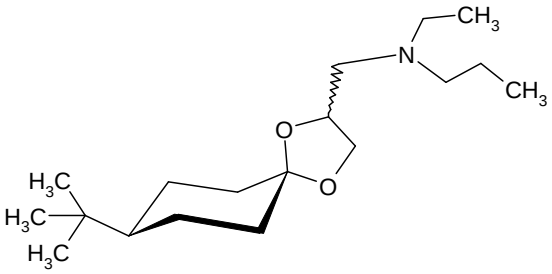
1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	n.a.
EC name:	n.a.
CAS number (EC inventory):	-
CAS number:	118134-30-8
CAS name:	1,4-Dioxaspiro[4.5]decane-2-methanamine, 8-(1,1-dimethylethyl)-N-ethyl-N-propyl-
IUPAC name:	8-*tert*-butyl-1,4-dioxaspiro[4.5]decan-2-ylmethyl(ethyl)(propyl)amine (ISO) *N*-{[8-(1,1-dimethylethyl)-1,4-dioxaspiro[4.5]dec-2-yl]methyl}-*N*-ethylpropan-1-amine
CLP Annex VI Index number:	612-150-00-X
Molecular formula:	$C_{18}H_{35}NO_2$
Molecular weight range:	297.5 g/mol

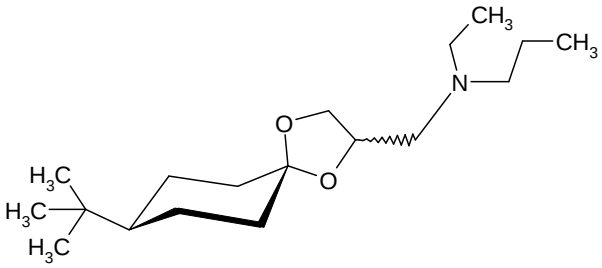
Structural formula:





„cis“ = diastereomer A

equatorial/axial (ea) configuration



“trans” = diastereomer B

equatorial/equatorial (ee) configuration

1.2 Composition of the substance

The confidential information can be found in the “Confidential Annex” or the technical dossier.

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
spiroxamine	Min. ≥ 94.0 %		

Current Annex VI entry: 612-150-00-X

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
confidential			not relevant for classification

Current Annex VI entry:

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
confidential				

Current Annex VI entry:

1.2.1 Composition of test material

For significant impurities see confidential annex.

Physico-chemical properties

Table 9: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	liquid	Draft Assessment Report (DAR) Monograph EFSA List of Endpoints	
Melting/freezing point	< - 170 °C		
Boiling point	no boiling until decompositions starts at 120 °C		
Relative density	0.930 g/mL at 20 °C		
Vapour pressure	diastereomer A: 7.1×10^{-3} Pa at 25 °C diastereomer B: 1.0×10^{-2} Pa at 25 °C		extrapolated
Surface tension	47 mN/m at 200 mg/L, pH 7 and 20 °C		
Water solubility	mixture of A and B: > 200 g/L at pH 3 diastereomer A: 470 mg/L at pH 7 14 mg/L at pH 9 diastereomer B: 340 mg/L at pH 7 10 mg/L at pH 9, all at 20 °C		
Partition coefficient n-octanol/water	diastereomer A: 2.79 diastereomer B: 2.92 at 20 °C		
Flash point	147 °C		97.2 %
Flammability	Does not liberate gasses in hazardous amounts in case of contact with water or moist air.		
Explosive properties	not explosive in the sense of EC A14		97.2 %
Self-ignition temperature	ignition point 255 °C		
Oxidising properties	no oxidising properties in the sense of EC A21		97.0 %
Granulometry	not applicable		
Stability in organic solvents and identity of relevant degradation products	no data available		
Dissociation constant	pKa = 6.9		
Viscosity	no data available		

2 MANUFACTURE AND USES

2.1 Manufacture

Confidential information.

2.2 Identified uses

Spiroxamine is a fungicide to be used in agriculture and viticulture under field conditions only.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 10: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
EEC A14 Explosive properties	not explosive	none	DAR
EEC A15 Self-ignition temperature	255 °C	none	DAR
EEC A09 Flash point	147 °C	none	DAR
EEC A21 Oxidising properties	none	none	DAR
EEC A12 Flammability	Does not liberate gasses in hazardous amounts in case of contact with water or moist air.	none	DAR

3.1 *Physico-Chemical Hazards*

No classification and labelling based on physico-chemical properties of Spiroxamine.

3.1.1 Summary and discussion of physico-chemical hazards

3.1.2 Comparison with criteria

3.1.3 Conclusions on classification and labelling

Spiroxamine has no properties with respect to flammability, explosive and oxidising properties. No change of the existing classification is proposed.

4 HUMAN HEALTH HAZARD ASSESSMENT

Currently, Spiroxamine has a legal classification (regulation (EC) 1272/2008) for following toxicological endpoints: acute effects (H302*-H312*-H332*, R20/21/22), skin irritation (H315, R38) and skin sensitisation (H317, R43). During the renewal procedure of Spiroxamine under directive 91/414/EC, it was noted that this current legal classification should be amended to include a classification for developmental toxicity (H361d, R63). The existing classification for other hazards than developmental toxicity was considered appropriate.

During the preparation of this CLH-dossier, the existing minimum classification (which was based on the conversion rules) for acute toxicity was double-checked and classification with acute toxicity 4 was considered appropriate in all three cases. Considering the changed criteria for skin sensitizers (regulation (EU) No 286/2011), sub-category 1B is proposed.

Hence only acute toxicity and developmental toxicity endpoints are addressed in this dossier. For information on other endpoints please refer to Vol. 3, chapters B.4 and B.6 of the draft re-assessment report (DRAR), which is available under http://dar.efsa.europa.eu/dar-web/provision and which is attached to the IUCLID file.

Information on the impurity profile of the batches used in toxicological studies is included in the confidential annex to this dossier.

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

This endpoint is not addressed by this proposal.

4.2 Acute toxicity

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Reference:	KIIA 5.2 (OECD)
Report:	Krötlinger, F. (1991a), KWG 4168 - Study for acute oral toxicity in rats - Report no.: 20416 (July 05, 1991 a); Bayer AG, Institute for Toxicology, Wuppertal, Germany, Dates of exp. work: October 1990 - November 1990. TOX9552588
Guidelines:	OECD TG 401
Deviations:	No analytic confirmation of homogeneity and stability was provided at the beginning of the study
GLP:	Yes
Acceptability:	The study is considered to be acceptable.

Material and methods:

Test system: Spiroxamine (batch no.: 17002/90, purity: 93.6 % in Cremophor EL/demineralised water (2 % v/v)) was administered once per os in dosages of 10-100-500-710 mg/kg bw to fasted male and in dosages of 10-100-500-560-600-710 mg/kg bw to fasted female Wistar rats, 5 animals/dose (strain: Bor: WISW [SPF-Cpb]; source: Winkelmann, Borchen, Germany). All animals which died during the study were necropsied as soon as possible. Survivors were sacrificed on day 14 after treatment. Recording period: 0-14 days.

Findings:

Clinical signs: A dose of 10 mg/kg bw was tolerated by both sexes without any clinical signs (Table 11). Following a dose of 100 mg/kg bw both sexes showed signs of apathy and increased salivation. In higher doses both sexes exhibited piloerection, laboured or faster breathing, reduced motility, staggering gait, lying on side, spasms and outstretched extremities. Isolated signs of soft faeces, spastic gait, foaming at the muzzle, splayed rear extremities, and periodic rolling over were also observed.

The signs were mainly moderate in degree, occurred in some cases directly after administration and lasted in both sexes until the second day of the study.

Mortality data are summarised in Table 11.

Table 11: Results of acute oral toxicity testing in rats (number of dead animals, number of animals with clinical signs)

Dose	Males	Females	Clinical signs	Time of death
10 mg/kg bw	0/5	0/5	0/10	-
100 mg/kg bw	0/5	0/5	10/10	-
500 mg/kg bw	2/5	2/5	10/10	3h15’ - 6h45’
560 mg/kg bw	-	5/5	5/5	1h15’ - 4h00’
600 mg/kg bw	-	5/5	5/5	1h15’ - 6h30’
710 mg/kg bw	3/5	5/5	10/10	1h30’ - 5h00’

Gross necropsy: Animals which died during the post-treatment observation period: lung distended; spleen pale. The region of the small intestine following the stomach was reddened in isolated cases in females.

Animals sacrificed at the end of the post-treatment observation period revealed no evidence of substance-related gross organ lesions.

The oral LD_{50} was calculated to be 595 mg/kg bw in males and 500 – 560 mg/kg bw in females.

Conclusion:

In this study the oral LD_{50} was calculated to be 595 mg/kg bw in male and approx. 500 mg/kg bw in female rats.

Further information:

Following further data on acute oral toxicity can be extracted from the acute neurotoxicity study in rats: When calculating a linear regression with the mortality data (Table 12) between 300 and 400 mg/kg bw, a dose of 375 mg/kg bw is reached for a mortality rate of 50 %.

Table 12: Mortality data in rangefinding part of acute neurotoxicity study (5 animals were dosed per group)

Dose level (mg/kg bw)	Dead males	Dead females
0	0	0
10	0	0
20	0	0
100	0	0
200	0	0
300	1	1
400	3	3

Reference: KIIA 5.2 (OECD)

Report: Krötlinger, F. (1991b) KWG 4168 - Study for acute oral toxicity in mice - Report no.: 20418 (July 05, 1991 b); Bayer AG, Institute for Toxicology, D-42096 Wuppertal, Germany, Dates of exp. work: November 1990 - December 1990.

TOX9552592

Guidelines: OECD TG 401

Deviations: No analytic confirmation of homogeneity and stability was provided at the beginning of the study

GLP: Yes.

Acceptability: The study is considered to be acceptable.

Material and methods:

Test system: Spiroxamine (batch no.: 17002/90, purity: 93.6 % in Cremophor EL/demineralised water (2 % v/v)) was administered once per os in dosages of 100-355-425-500 mg/kg bw to fasted male and in dosages of 100-500-630 mg/kg bw to fasted female mice (strain: Bor: NMRI [SPF-Han]; source: Winkelmann, Borchen, Germany), 5 animals/dose.

All animals which died during the study were necropsied. Survivors were sacrificed on day 14 after treatment. Recording period: 0-14 days.

Findings:

Clinical signs: Following a dose of 355 mg/kg bw in the males and 500 mg/kg in the females following signs were observed: apathy, piloerection, laboured breathing, reduced motility, staggering or creeping, vocalisation, spasms, periodic twitching, periodic rolling over, outstretched extremities and lying on side. In isolated cases animals were in a supine position, or exhibited clonic and tonic spasms (Table 13).

Clinical signs, mainly moderate, occurred in some cases shortly after administration and were observed in males on day 1 and in females until day 2.

Table 13: Results of acute oral toxicity testing in mice (number of dead animals, number of animals with clinical signs)

Dose	Males	Females	Clinical signs	Time of death
100 mg/kg bw	0/5	0/5	0/10	-
355 mg/kg bw	0/5	-	5/5	-
425 mg/kg bw	1/5	-	4/5	1h30’
500 mg/kg bw	4/5	1/5	7/10	38’ - 3h00’
630 mg/kg bw	-	4/5	5/5	49’ - 1h00’

Gross necropsy: Animals which died during the post-treatment observation period: lung slightly distended, mottled; liver pale, lobulation (males only); glandular stomach reddened (females only).

Animals sacrificed at the end of the post-treatment observation period revealed no evidence of substance related gross organ lesions.

Conclusion:

In this study the oral LD_{50} was calculated to be 460 mg/kg bw in male and 561 mg/kg bw in female mice.

4.2.1.2 Acute toxicity: inhalation

Reference: KIIA 5.2 (OECD)

Report: Pauluhn, J.: (1990), KWG 4168 - Study for acute inhalation toxicity in the rat - Report no.: 19806 (December 12, 1990); Bayer AG, Institute for Toxicology, Wuppertal, Germany, Dates of exp. work: May 1990 - June 1990.

TOX9552590

Guidelines: OECD TG 403

Deviations: None

GLP: Yes

Acceptability: The study is considered to be acceptable.

Material and methods:

Test system: Groups of 5 male and 5 female Wistar rats (strain: Bor: WISW [SPF-Cpb]); source Winkelmann, Borchen, Germany; received spiroxamine (batch no.: 17002/90, purity 94.6 %, undiluted) via inhalation (dynamic spraying, head nose only) in analytical concentrations of 869-1140-1982-2284-3880 mg/m³ air for 4 h. All animals which died during the study were necropsied. Survivors were sacrificed on day 14 after treatment. Recording period: 0-14 days.

Findings:

Clinical signs: Piloerection, un-groomed fur, reduced motility, tremors, laboured breathing, stridor, prostration, tonical spasms with rolling over movements, staggering gait

Body weights: A transient effect on the body weights was noted during the post-treatment observation period from 1140 mg/m³ onwards.

Reflex testing: A reduction in the myotactile response was observed in the females at 1982 mg/m³ performed after exposure or on day 1 of the post-treatment observation period. Deaths occurred at 1982 mg/m^3 air in female rats and at 2284 mg/m^3 air in male rats (Table 14). The inhalative LC_{50} was calculated to be 1982 mg/m^3 air in females and 2772 mg/m^3 air in males.

Table 14: Results of acute inhalation toxicity in rats after 4h (number of dead animals, number of animals with clinical signs)

Dose	Males	Females	Clinical signs	Time of death
Air control	0/5	0/5	0/10	-
869	0/5	0/5	0/10	-
1140	0/5	0/5	10/10	-
1982	0/5	2/5	8/10	< 4h
2284	1/5	5/5	4/10	< 4h
3880	5/5	5/5	0/10	< 4h

Gross pathology: Animals which died intercurrently: lungs distended, liver like appearance (hepatisation) and oedema; hydrothorax; spleen and kidneys pale; liver with lobulation and pale; mucosa of the gastrointestinal tract reddened, yellow slimy contents in lumen, renal pelvis reddened.

Animals sacrificed at the end of the observation period: No evidence of concentration related changes in the lungs or other organs.

Conclusion:

The inhalative LD_{50} measured in rats was 1982 mg/m³ air (1.982 mg/L air). The test compound was a liquid.

4.2.1.3 Acute toxicity: dermal

Reference:	KIIA 5.2 (OECD)
Report:	Krötlinger, F. (1991c), KWG 4168 - Study for acute dermal toxicity in rats - Report no.: 20417 (July 05, 1991 c); Bayer AG, Institute for Toxicology, Wuppertal, Germany, Dates of exp. work: October 1990 - December 1990 TOX9552589
Guidelines:	OECD TG 402
Deviations:	No analytical confirmation of the homogeneity and stability was provided.
GLP:	Yes
Acceptability:	The study is considered to be acceptable.

Material and methods:

Test system: Spiroxamine (batch no.: 17002/90, purity: 93.6 % in cellulose powder) was administered via dermal application to five male (100-1000-1600-1800-2000 [10 males]-2500 mg/kg bw) and five female (100-1000-1120-1250-1600 mg/kg bw) Wistar rats (strain: Bor: WISW [SPF-Cpb]; source: Winkelmann, Borchen, Germany).

All animals which died during the study were necropsied. Survivors were sacrificed on day 14 after treatment. Recording period: 0-14 days.

Findings:

Clinical signs: Following a dose of 1000 mg/kg bw both sexes showed signs of apathy, piloerection, reduced motility, staggering gait and laboured breathing. In isolated cases there were also signs of spasms, outstretched extremities, lying on side, spastic gait, periodic shaking, periodic grooming, soft faeces, no faeces or diarrhoea, emaciation, increased salivation, bloody muzzles, encrusted labial committure and loss of hair on muzzle. The signs were mainly moderate, occurred in some cases from 1 hour 30 minutes after application, and lasted in some males until day 13 and in females until day 6. Dead females were observed at 1120 mg/kg bw and above (Table 15). Contrary to female rats observed deaths in males were not dose related.

Table 15: Results of acute dermal toxicity testing in rats (number of dead animals, number of animals with clinical signs)

Dose	Males	Females	Clinical signs	Time of death
100 mg/kg bw	0/5	0/5	0/10	-
1000 mg/kg bw	0/5	0/5	10/10	-
1120 mg/kg bw	-	3/5	5/5	3d – 4d
1250 mg/kg bw	-	4/5	5/5	3d – 4d
1600 mg/kg bw	2/5	5/5	10/10	3d – 5d
1800 mg/kg bw	1/5	-	5/5	4d
2000 mg/kg bw	1/10	-	10/10	4d
2500 mg/kg bw	0/5	-	5/5	-

Local findings: The treatment sites exhibited grossly visible changes in both sexes: redness, scabbing incrustation, in isolated cases wrinkles and thickening at the skin. The skin changes were visible from day 2 of the study until the end of the post-treatment observation period.

Gross necropsy: Animals which died during the post-treatment observation period: lungs distended; liver mottled; kidneys mottled; mesenteric vessels severely injected; stomach engorged, or engorged with food and shavings; proventriculus reddened; glandular stomach reddened.

Animals sacrificed at the end of the post-treatment observation period: One female has shown increased adipose tissue and a deformed spleen. Spleen, stomach and left uterus horn fused to adipose tissue. Left kidney surrounded by excessive adipose tissue.

No evidence of test article-related gross organ lesions in the males.

Conclusion:

In this study the dermal LD_{50} was calculated to be 1068 mg/kg bw in female and > 1600 mg/kg bw in male rats.

4.2.1.4 Acute toxicity: other routes

Reference: KIIA 5.2 (OECD)

Report: Krötlinger, F. (1991d) KWG 4168 - Study for acute intra-peritoneal toxicity in rats - Report no.: 20419 (July 05, 1991d); Bayer AG, Institute for Toxicology, Wuppertal, Germany, Dates of exp. work: October 1990 - November 1990.

TOX9552591

Guidelines: OECD TG 401.

Deviations: None

GLP: Yes

Acceptability: The study is considered to be acceptable.

Material and methods:

Test system: Spiroxamine (batch no.: 17002/90, purity: 93.6 % in Cremophor EL/demineralised water (2 % v/v) was administered intra-peritoneally in doses of 10-100-112-125 mg/kg bw to 20 male and 10-100-125-140-180 mg/kg bw to 25 female Wistar rats (strain: Bor: WISW [SPF-Cpb]); source: Winkelmann, Borchen, Germany. All animals which died during the study were necropsied. Survivors were sacrificed on day 14 after treatment. Recording period: 21 days.

Findings:

The observed clinical signs (apathy, motility and respiratory disorders, piloerection, staggering, spastic of creeping gait, lying on side or prostration, spasms, periodic twitching and outstretched extremities, foam at the muzzle, e.g.) occurred in some cases directly post administration and lasted in some males up to the end of the study. A dose of 10 mg/kg bw was tolerated without signs in male and female rats.

Table 16: Acute intraperitoneal toxicity in rats (number of dead animals, number of animals with clinical signs)

Dose	Males	Females	Clinical signs	Time of death
10 mg/kg bw	0/5	0/5	0/5	-
100 mg/kg bw	0/5	0/5	10/10	-
112 mg/kg bw	2/5	-	5/5	34’ – 45’
125 mg/kg bw	4/5	1/5	10/10	36’ – 7d
140 mg/kg bw	-	0/5	5/5	-
180 mg/kg bw	-	4/5	5/5	22’ – 1d

Conclusion:

The LD_{50} after intraperitoneal administration was calculated to be 114 mg/kg bw in male rats and 150 mg/kg bw in females.

4.2.2 Human information

No information submitted by the notifier.

4.2.3 Summary and discussion of acute toxicity

Results of the acute toxicity studies are summarised in the table below.

4.2.4 Comparison with criteria

Classification	Finding	Criteria according to in Annex VI to directive 67/548/EEC	Criteria according to Annex I to regulation (EC) no. 1272/2008
R20 and H332	Rat LD_{50} inhalation (4 h): 1982 mg/m^3 air, f; 2772 mg/m^3 air, m The test material was a liquid Pauluhn (1990)	Section 3.2.3.: Inhalation, rat (aerosols or particulates): 1 < LC_{50} < 5 mg/L/4hr ⇨ Harmful by inhalation	Section 3.1.: Inhalation (dust/mist): 1 < ATE ≤ 5.0 mg/L ⇨ Acute toxicity category 4
R21 and H312	Rat LD_{50} dermal: 1068 mg/kg bw, f >1600 mg/kg bw, m Krötlinger (1991c)	Section 3.2.3.: Dermal: 400 < LD_{50} < 2000 mg/kg ⇨ Harmful in contact with skin	Section 3.1.: Dermal: 1000 < ATE ≤ 2000 mg/kg bw ⇨ Acute toxicity category 4
R22 and H302	Rat LD_{50} oral: ~500 mg/kg bw, f 595 mg/kg bw, m Krötlinger (1991a) Mouse LD_{50} oral: 561 mg/kg bw, f 460 mg/kg bw, m Krötlinger (1991b)	Section 3.2.3.: Oral: 200 < LD_{50} < 2000 mg/kg ⇨ Harmful if swallowed	Section 3.1.: Oral: 300< ATE ≤ 2000 mg/kg bw ⇨ Acute toxicity category 4

4.2.5 Conclusions on classification and labelling

Following classification is proposed based on the results of the available acute toxicity studies and

- considering the DSD-classification criteria:
  Xn, R20/R21/R22
- considering the CLP-classification criteria:
  Acute Tox. 4, H302-H312-H332

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.

4.3 Specific target organ toxicity – single exposure (STOT SE)

This endpoint is not addressed by this proposal.

4.4 Irritation

4.4.1 Skin irritation

This endpoint is not addressed by this proposal. Spiroxamine is listed in Annex VI of CLP regulation as skin irritant cat. 2 (H315) and as irritant (R38)

4.4.2 Eye irritation

This endpoint is not addressed by this proposal.

4.4.3 Respiratory tract irritation

This endpoint is not addressed by this proposal.

4.5 Corrosivity

This endpoint is not addressed by this proposal.

4.6 Sensitisation

4.6.1 Skin sensititsation

4.6.1.1 Non-human information

Reference:	KIIA 5.2 (OECD)
Report:	Dreist, M. and J. Kolb.(1992), KWG 4168 - Studies on skin sensitising effect in guinea pigs (Maximisation Test according to Magnusson and Kligman) - Report no.: 21687 (September 22, 1992); Bayer AG, Institute for Toxicology, Wuppertal, Germany, Dates of exp. work: June 1992 - July 1992.
	TOX9552594
Guidelines:	OECD TG 406

Deviations: None

GLP: Yes

Acceptability: The study is considered to be acceptable.

Material and methods:

Test system: 40 male guinea pigs (one test article group consisting of 20 animals, two control groups consisting of 10 animals; strain: BOR:DHPW, source: Winkelmann, Borchen, Germany) were treated with spiroxamine (batch no.: 17002/90; purity: 95.6 %) in 0,9 % NaCl solution/ Cremophor El (2 % v/v) in following concentrations: intradermal induction: 5 %; topical induction: 6 %; 1st topical challenge: 1 % and 0.5 %; 2nd topical challenge: 0.1 % and 0.05 %.

Findings:

Range finding for intracutaneous induction: One guinea pig was injected intradermally with 0.1 ml of the test article at concentrations of 0 %, 1 %, 2.5 % and 5 %.

After 24 and 48 hours injection sites were assessed: 0 % no reaction, 1 % - 5 % grey region with red margin.

Range finding for topical induction: 4 concentrations were tested twice on 4 guinea pigs, respectively. The results of the treatment for 24 hours under occlusive conditions with 4 dressings soaked in 0.5 mL of the test article formulation are shown in Table 17.

Table 17: Number of animals exhibiting skin reddening in the range finding test for topical induction (48 and 72 hours after application) (4 animals tested)

	6 %		12 %		25 %		50 %	
Hours	48	72	48	72	48	72	48	72
1st test	4	4	4	4	4	4	4	4
	0.5 %		1 %		3 %		6 %	
Hours	48	72	48	72	48	72	48	72
2nd test	0	0	0	0	0	0	4	4

1st and 2nd challenge: The treatment was tolerated by all animals without any signs. Body weight gain amongst the treatment group animals corresponded to that of the control groups.

After the 1st challenge, 14 out of 20 test group animals responded to the 1 % test article formulation while none of 9 control animals showed skin reactions; 5 animals showed a positive response to the 0.5 % formulation. No skin reactions were found after the 2nd challenge (Table 18). Body weight gain amongst the treatment group animals corresponded to that of the control groups.

Table 18: Number of animals exhibiting skin reactions in the maximisation test (48 and 72 hours after initiation of challenge)

	Test substance group (20 animals)				**1st and 2nd control group (9 and 10 animals, resp.)**			
	Test patch		**Control patch**		**Test patch**		**Control patch**	
Hours	48	72	48	72	48	72	48	72
1st - 1 %	11	10*	0	0	0	0	2	2

1st - 0.5 %	4	2#	1	0	0	0	1	1
2nd - 0.1 %	0	0	0	0	0	0	0	0
2nd - 0.05 %	1	0	0	0	0	0	0	0

* 3 animals exhibited skin redness; # 1 animal exhibited skin redness

Conclusion:

Under conditions of this Maximisation Test with spiroxamine, following effects were observed: After the 1st challenge, 14 out of 20 test group animals responded to the 1 % test article formulation while none of 9 control animals showed skin reactions; 5 animals showed a positive response to the 0.5 % formulation. No skin reactions were found after the 2nd challenge with 0.1 or 0.05 % test article formulation.

Reference: KIIA 5.2 (OECD)

Report: Krötlinger, F. and J. Kolb.(1992), KWG 4168 - Studies for skin sensitising effect in guinea pigs (Buehler Patch Test) - Report no.: 21716 (October 05, 1992); Bayer AG, Institute for Toxicology, Wuppertal, Germany, Dates of exp. work: April 1992 - May 1992.

TOX9552595

Guidelines: OECD TG 406

Deviations: None

GLP: Yes

Acceptability: The study is considered to be acceptable.

Material and methods:

Test system: 36 male guinea pigs (12 per group, 2 control groups, 1 test substance group; strain: BOR:DHPW, source: Winkelmann, Borchen, Germany) were treated with spiroxamine (batch no.: 17002/90; purity: 94.1 %) in 0,9 % NaCl solution / Cremophor El (2 % v/v).

Concentrations: 1st induction: 50 %; 2nd induction: 25 %; 3rd induction: 12 %; 1st challenge: 12 % and 6 %; 2nd challenge: 3 % and 1 %.

Findings:

Treatment was tolerated by all animals without any signs. No mortalities occurred. Body weight gain amongst treatment groups was comparable to control groups. After 1st challenge of both concentrations no difference with regard to incidence and intensity of skin reactions was seen between treatment groups and control animals. After the 2nd challenge a difference was seen at 3 %: Nine of twelve test group animals and two of twelve control animals showed skin reactions. No dermal reactions occurred in treated or control animals following challenge with a non-irritant concentration of 1 % (Table 19).

Table 19: Number of animals exhibiting skin reactions in the Buehler patch test (24, 48 and 72 hours after initiation of challenge)

	Test substance group (12 animals)						**1st and 2nd control group (12 animals each)**					
	Test patch			**Control patch**			**Test patch**			**Control patch**		
Hours	24	48	72	24	48	72	24	48	72	24	48	72
1st - 12 %	10	8	9	0	0	0	12	7	4	0	0	0
1st - 6 %	7	3	4	0	0	0	7	2	4	0	0	0
2nd - 3 %	9	9	3	0	0	0	2	2	0	0	0	0
2nd -1 %	0	0	0	0	0	0	0	0	0	0	0	0

Conclusion:

Under conditions of the Buehler Test with spiroxamine following findings were observed: After 1st challenge of both concentrations (12 and 6 %) no difference with regard to incidence and intensity of skin reactions was seen between treatment groups and control animals. After the 2nd challenge a difference was seen at 3 %: Nine of twelve test group animals and two of twelve control animals showed skin reactions. No dermal reactions occurred in treated or control animals following challenge with a non-irritant concentration of 1 %.

4.6.1.2 Human information

Reference: KIIA 5.2 (OECD)

Report: Shelanski, M. V.; 2001-02-20, amended 2001-08-15 A patch test procedure to facilitate the expression and detection of the irritating and sensitising propensities of KWG 4168, Report no 107791, Dates of work: 1998-02-23 to 1998-07-30

ASB2008-2231

Guidelines: Not applicable

Deviations: Not applicable

GLP: No, but GCP

Acceptability: The study is considered to be supplementary.

Material and Methods:

Spiroxamine, Lot/Batch no: 17002/90, Purity: 5.6 %, Vehicle: Cremophor®EL/physiological saline 0.20 %; physiological saline, Test system: humans, 19 – 84 years old males and 20 – 84 years old females.

Study design: An intensified version of the Shelanski and Shelanski Repeated Insult Patch Test (RIPT) was conducted under double blind conditions. Group size: 45 males and 166 females. The study was conducted in two stages, i.e., on two panels. The effects of nominal doses of 0.02 %, 0.066 %, and 0.20 % solutions of spiroxamine (as solution in 0.2 % Cremophor® EL in physiological saline) were studied on the subjects in both stages. Volumes of 0.15 mL of each solution were used to load the patching devices. This corresponds to doses of 7.5 µg/cm², 25.0 µg/cm² and 75.0 µg/cm² spiroxamine, available on the 2 cm x 2 cm contact area. Dose selection was based on preliminary investigations. These investigations revealed that 0.20 % was the highest spiroxamine concentration which was tolerated without any gross skin changes after repeated dermal application for up to 4 days. Higher concentrations of 0.30 % up to 1.02 % induced gross skin changes. Application route: dermal, occlusive patching (lateral aspects of the upper arms), Application volume: 0.15 mL/patch, Exposure: initial (induction) phase was 3 weeks, repeated daily application for 4 days/week, intermediate phase: rest period allowing normalisation of the skin following any adverse effects. It also affords an opportunity for the patching of subjects who may not have completed the patch application phase, challenge (elicitation) phase: 4 consecutive days. Procedure Flow Chart is shown in Table 20.

Table 20: Procedure Flow Chart

	Monday	**Tuesday**	**Wednesday**	**Thursday**	**Friday**
Activation/Induction					
Week 1	B/A	R/E/A	R/E/A	R/E/A	R/E
Week 2	E/A	R/E/A	R/E/A	R/E/A	R/E
Week 3	E/A	R/E/A	R/E/A	R/E/A	R/E
Week 4	E/H	(E)H	(E)H	(E)H	(E)
Challenge Phase					
Week 5	B/A	R/E/A	R/E/A	R/E/A	R/E
Week 6	E/D				

B baseline examination, R patch removed under supervision, D subject discharged, A patch applied, E site examined and grade recorded

H hiatus (rest period) or application to make up for any missed during induction phase

Findings:

Initial Phase: There were no gross changes of the skin perceptible at the application sites after repeated dermal application of solutions containing 0.02, 0.066 and 0.20 % spiroxamine.

Challenge Phase: The absence of perceptible gross changes of the skin during the challenge phase indicated that non-irritating solutions of 0.02, 0.066 and 0.20 % spiroxamine have no skin sensitising properties in humans.

Follow-up Phase: No skin findings at the application sites were reported from any of the human volunteers during the 2 weeks of the follow-up phase.

Conclusions:

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.

4.6.1.3 Summary and discussion of skin sensitisation

Results of the sensitization studies are summarised in the table and section below.

4.6.1.4 Comparison with criteria

Toxicological result	DSD criteria	CLP criteria
Guinea pig (M&K): Intradermal induction concentration: 5 % 14 / 20 animals positive with 1 % solution 5 / 20 animals positive with 0.5 % solution Dreist & Kolb (1992)	Adjuvant type test method: ≥ 30 % of the animals positive	**Guinea pig maximisation test** Category 1A: ≥ 30 % responding at ≤ 0.1 % intradermal induction dose or ≥ 60 % responding at > 0.1 % to ≤ 1 % intradermal induction dose Categrory 1B: ≥ 30 % to < 60 % responding at > 0.1 % to ≤ 1 % intradermal induction dose or ≥ 30 % responding at > 1 % intradermal induction dose

Guinea pig (Buehler): 50-12 % dermal induction concentration 9/12 animals positive with 3 % solution (control: 2/12) Krötlinger & Kolb (1992)	Other test method: ≥ 15 % of the animals positive ⇨ R43	**Buehler assay** Category 1A: ≥ 15 % responding at ≤ 0.2 % topical induction dose or ≥ 60 % responding at > 0.2 % to ≤ 20 % topical induction dose Categrory 1B: ≥ 15 % to < 60 % responding at > 0.2 % to ≤ 20 % topical induction dose or ≥ 15 % responding at > 20 % topical induction dose

Both studies fulfil the DSD-criteria to be classified as a skin sensitiser.

Based on the results with the M&K test, a classification into sub-category 1B according to the CLP-criteria is necessary (intra dermal induction concentration > 1 %).

In the Buehler test 9/12 (75 %) treated animals and 2/12 (~17 %) control animals reacted upon challenge (excess rate of sensitised animals: ~58 %). Considering the dermal induction concentration (50 to 12 %) and the rate of animals with skin reaction upon challenge, sub-category 1B is proposed from this study, too.

Under the conditions of the human study, no skin sensitising properties were observed. However, the tested dose levels were relatively low; therefore, more weight is put on the results of the animal studies.

4.6.1.5 Conclusions on classification and labelling

Considering the study results and the classification criteria, a classification of spiroxamine as a skin sensitiser with R43 or H317 (skin sensitisation 1B) is proposed according to DSD and CLP criteria, respectively.

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".

4.6.2 Respiratory sensitisation

This endpoint is not addressed by this proposal.

4.7 Repeated dose toxicity

This endpoint is not addressed by this proposal.

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

This endpoint is not addressed by this proposal.

4.9 Germ cell mutagenicity (Mutagenicity)

This endpoint is not addressed by this proposal.

4.10 Carcinogenicity

This endpoint is not addressed by this proposal.

4.11 Toxicity for reproduction

4.11.1 Effects on fertility

This endpoint is not addressed by this proposal. Nevertheless, based on a request by ECHA/RAC Secretariat, the reproductive toxicity studies are included in this dossier.

4.11.1.1 Non-human information

Study 1

Reference: KIIA 5.6 (OECD)

Report: Pickel, M. (1993): KWG 4168 - Two generation study on rats - Report no.: 23115 (June 17, 1994; Dates of exp. work: 03 – 12/91), Bayer AG, Institute for Toxicology, Wuppertal, Germany

TOX9552619

Guidelines: OECD TG 416

Deviations: None

GLP: Yes

Acceptability: The study is considered to be acceptable.

Material and methods:

In a two-generation study on Wistar rats [Bor: WISW (SPF-Cpb), source: Winkelmann, Borchen, Germany] spiroxamine [batch no.: 17002/90; purity: 94.3 - 95.3 %] was examined for possible effects on reproduction. The compound was administered with the feed to 30 male and 30 female rats each at the following dose levels: 0, 20, 80, and 300 ppm, respectively.

Formulas used for the calculation of indices:

$$\text{Insemination index} = \frac{\text{number of inseminated females}}{\text{number of mated females}} \times 100$$

$$\text{Fertility index} = \frac{\text{number of pregnant females}}{\text{number of inseminated females}} \times 100$$

$$\text{Gestation index} = \frac{\text{number of females with live pups}}{\text{number of pregnant females}} \times 100$$

$$\text{Viability index Day 0} = \frac{\text{number of live pups at birth}}{\text{number of pups born}} \times 100$$

$$\text{Viability index Day 4} = \frac{\text{number of live pups 4 days after birth (before reduction)}}{\text{number of live pups at birth}} \times 100$$

$$\text{Viability index Day 21} = \frac{\text{number of live pups 3 weeks after birth}}{\text{number of live pups 4 days after birth (after reduction)}} \times 100$$

Findings:

The actual test compound uptake during the pre-mating periods is given in Table 21

Table 21: Uptake of spiroxamine [mg/kg bw/d]

	20 ppm	**80 ppm**	**300 ppm**
F0 - males	2.13	9.19	35.88
F0 - females	2.38	10.59	41.85
F1 - males	2.87	12.33	53.65
F1 - females	3.02	13.15	55.81

General observations of parental animals: Regarding appearance, behaviour and mortality, no test substance related findings were observed in male or female F0 animals up to 300 ppm. F1 animals at 300 ppm exhibited increased incidence of piloerection, bloody noses, polyuria and muzzles to which feed adhered. At the beginning of the study, one male animal died both in the control and 80 ppm group, and two F1 males and four F1 females in the 300 ppm group died or were sacrificed when moribund. Based on these findings, a treatment-related increase in mortality rate can be assumed for male and female F1 animals at 300 ppm although no additional animals in this group died during the further course of the study.

During the entire study period, body weight gain in F0 and F1 animals up to 80 ppm was comparable to corresponding control animals (data reproduced in section 0). 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 (parental) and in female F1 animals at 300 ppm during the entire treatment period.

Starting at 80 ppm, feed consumption in F0 parental animals was temporarily increased (Table 22). Furthermore, feed consumption was reduced in females (parental) at 80 and 300 ppm during lactation period and in F1 females at 300 ppm temporarily also during pre-mating period.

Table 22: Feed intake (F0, g/kg bw/d)

	0 ppm	**20 ppm**	**80 ppm**	**300 ppm**
Males (day 70)	105.0	106.7	114.9	119.6
Females (day 70)	126.3	118.9	132.4	139.5
Females (PND 4)	137.4	147.9	102.9**	112.7**

Haematology, clinical chemistry, urine analysis of parental animals: At 300 ppm following clinical chemistry parameters in plasma and blood of parental females were altered: increased activities of ASAT and creatine kinase (CK) in F0 animals and increased urea values in F1 animals. The values for cholesterol (CHOL) were reduced in F0 and F1 animals and protein (PROT) and triglycerides

(TRIGL) were lowered only in F1 females. The thrombocyte count (THRO) for female parental animals at 300 ppm was reduced (Table 23, Table 24).

Table 23: Haematology, clinical chemistry (F0)

	0 ppm	**20 ppm**	**80 ppm**	**300 ppm**
F0 - males				
LYM [%]	82.8	88.6	87.3	91.0**
SEGM [%]	13.9	9.0	8.6	6.3*
F0 - females				
HCT [l/L]	0.470	0.469	0.455	0.450*
MCHC [g/L] ERY	325	328	327	331*
THRO [10E9/L]	920	933	891	750**
HQUICK [sec]	27.0	27.1	27.4	30.5*
ASAT [U/L]	54.1	54.6	53.5	80.7**
ALAT [U/L]	114.4	100.4	108.0	163.3
CK [U/L]	94	106	110	177**
CHOL [mmol/L]	2.39	2.52	2.29	1.79**

U-test: * significant $p < 0.05$; ** significant $p < 0.01$

Table 24: Haematology, clinical chemistry (F1)

	0 ppm	**20 ppm**	**80 ppm**	**300 ppm**
F1 - males				
TRIGL [mmol/L]	1.46	1.33	0.97*	0.82**
UREA [mmol/L]	7.74	8.12	8.16	8.56*
F1 – females				
LEUCO [10E9Ll]	8.9	8.2	7.7	7.4*
THRO [10E9/L]	1118	1092	1082	974*
HQUICK [sec]	28.9	29.8	29.5	31.8
MONO [%]	2.6	3.0	2.3	4.0*
ASAT [U/L]	52.3	56.7	55.6	75.2**
ALAT [U/L]	114.8	111.8	114.6	148.6
LDH [U/L]	86	103	81	67*
PROT [g/L]	56.4	54.9	56.9	53.3*
CHOL [mmol/L]	2.10	2.11	1.91	1.60**
TRIGL [mmol/L]	0.73	0.62	0.61	0.36**
UREA [mmol/L]	10.15	11.25	11.32	12.94**

U-test: * significant $p < 0.05$; ** significant $p < 0.01$

Gross pathology, organ weights, histopathology of parental animals: At histopathological examination of F0 and F1 paternal animals, hyperkeratosis in the oesophagus was detected at 80 ppm (8 females) and 300 ppm (29 females, 27 males). Organ weights: in both sexes decreased liver and kidney weight (F0 and F1) at 300 ppm were observed and additionally reduced liver weights in F0 males at 80 ppm; in females reduced thymus and ovary weights starting at 80 ppm (F1) and at 300 ppm (F0); increased adrenal (F0 and F1) and spleen weights (F1) at 300 ppm; in males reduced testes weights (F1) at 300 ppm (Table 25, Table 26).

Table 25: Absolute organ weights (F0)

	0 ppm	**20 ppm**	**80 ppm**	**300 ppm**
F0 - males				
Liver (mg)	13018	12775	12168*	11838**
Kidneys (mg)	2284	2314	2230	2166*
Thymus (mg)	342	365	326	336
Adrenals (mg)	50	49	48	47
Testes (mg)	3416	3351	3284	3348
F0 - females				
Liver (mg)	10729	10949	10092	9492*
Kidneys (mg)	1543	1602	1587	1449*
Thymus (mg)	209	204	193	139**
Adrenals (mg)	68	72	73	82**
Ovaries (mg)	129	130	134	110**

* significant $p < 0.05$; ** significant $p < 0.01$

Table 26: Absolute organ weights (F1)

	0 ppm	**20 ppm**	**80 ppm**	**300 ppm**
F1 - males				
Liver (mg)	12690	12992	12508	11485**
Kidneys (mg)	2197	2282	2223	2021*
Spleen (mg)	584	585	590	580
Thymus (mg)	337	327	333	326
Adrenals (mg)	44	48	48*	43
Testes (mg)	3361	3309	3394	3163*
F1 - females				
Liver (mg)	9175	9326	9497	7938**
Kidneys (mg)	1543	1560	1578	1373**
Spleen (mg)	462	449	467	423*
Thymus (mg)	279	248	196**	190**
Adrenals (mg)	67	71	71	73*
Testes (mg)	147	141	136*	113**

U-test: * significant $p < 0.05$; ** significant $p < 0.01$

Reproduction parameters: Up to 300 ppm 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. Between day 4 and 21 p.n. high mortality of F2 pups was observed. Despite the fact, that the viability index (d 21) for all groups (F2) was below the range of historical control data, the high mortality was not regarded as treatment related, since highest mortality occurred in the control group (Table 27, Table 28 and Table 29). An infection as a possible cause for the high mortality was stated in the study report, but the infection itself was not established.

During lactation, the number of F1 and F2 pups in this group that exhibited laboured breathing or cyanosis was also increased, and F1 pups with cold external surface areas and thin appearance were observed. At 300 ppm, the number of thin F1 pups and pups with piloerection, bloody noses and polyuria was increased in the 4th week after birth. Sporadically, animals in this group had muzzles to which feed adhered and bloody discharge from the eyes. At 300 ppm body weight of F1 pups was decreased in week 3 and 4 after birth (Table 30).

Table 27: Reproduction Data F0

	0 ppm	**20 ppm**	**80 ppm**	**300 ppm**
Insemination Index (%)	100	100	100	100

Fertility Index (%)	83.3	86.2	76.7	90.0
Gestation Index (%)	96.0	96.0	100	100
Gestation Period (d)	22.4	22.3	22.4	22.1
Mated Females (n)	30	30	30	30
Viability Index day 4 (%)	97.0	96.9	99.6	96.7
Viability Index day 21(%)	94.2	93.2	97.7	97.5

Table 28: Reproduction Data F1

	0 ppm	**20 ppm**	**80 ppm**	**300 ppm**
Insemination Index (%)	100	100	100	100
Fertility Index (%)	90.0	96.7	93.1	86.7
Gestation Index (%)	100	100	100	100
Gestation Period (d)	21.9	22.3	22.2	22.2
Mated Females (n)	30	30	30	30
Viability Index day 4 (%)	89.0	85.6	87.6	89.2
Viability Index day 21(%)	35.3	45.1	73.1***	65.6***

*** p>0.001

Table 29: Pup Data

Dose ppm	**Number**		**Live birth index**	**Males [%]**	**Females [%]**	**Litter size**
	total	dead				
	F1-generation					
0	268	0	100	50.0	50.0	11.2
20	288	2	99.4	52.8	47.2	11.9
80	260	4	97.8	52.0	48.0	11.1
300	278	4	98.4	48.2	51.8	10.1*
	F2-generation					
0	302	3	99.0	51.5	48.5	11.1
20	327	1	99.7	48.5	51.5	11.2
80	308	2	99.4	50.7	49.3	11.3
300	265	6	97.7	52.9	47.1	10.0*

U-test: * significant $p < 0.05$

Table 30: Mean body weights (g) of pups (F1- & F2-generation)

	males				**females**			
F1	**0 ppm**	**20 ppm**	**80 ppm**	**300 ppm**	**0 ppm**	**20 ppm**	**80 ppm**	**300 ppm**
Day 0	5.87	6.03	6.16	5.98	5.55	5.77	5.84	5.67
Day 4 pre-culling	8.67	8.73	9.00	8.57	8.07	8.44	8.65	8.09
Day 4 culling	8.64	8.80	8.97	8.54	8.04	8.43	8.68	8.11
Day 7	13.10	13.26	13.34	12.19	12.57	13.15	12.84	11.48
Day 14	25.00	25.15	24.28	21.20**	23.81	25.60	23.26	19.79**
Day 21	37.91	39.35	36.58	31.30**	36.63	39.41	35.44	29.32**
Day 28	60.15	61.07	57.18	42.15**	54.85	58.64	53.15	38.38**
F2								
Day 0	5.50	5.89	5.83**	5.68	5.27	5.52	5.52	5.38
Day 4 pre-culling	7.25	7.66	8.18**	7.72	7.01	7.07	7.79	7.20
Day 4 culling	7.21	7.63	8.15**	7.73	7.00	7.07	7.75	7.21
Day 7	10.13	10.45	12.46**	10.44	10.19	9.96	11.48	10.15
Day 14	24.11	23.75	24.22	18.90**	24.32	23.47	23.11	18.27**
Day 28	40.06	38.73	35.77**	27.59**	38.16	36.48	34.11	26.92**

U-test: * significant $p < 0.01$
U-test: ** significant $p < 0.05$

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

For information only:

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. For reproductive and offspring toxicity an NOAEL of 80 ppm (9.19 mg/kg bw/d) was based on reduced litter size, reduced body weight development and clinical signs in pups at 300 ppm.

Effects at other dose levels are summarised above.

Study 2

Reference: KIIA 5.6 (OECD)

Report: Milius, A.D.; Stuart, B.P.(2008): KWG 4168 - Two generation reproductive toxicity study in the Wistar rat - Report no.: 201823 (July, 2007, Dates of exp. Work: 03 – 07/07), Bayer AG, Institute for Toxicology, Wuppertal, Germany

ASB2008-2232

Guidelines: OECD TG 416

Deviations: Two different summary tables regarding relative organ weights in F1-21-day pups resulted in inconsistent values predominantly for the 80 ppm dose group. For micropathology some tissues of different organs of F0, F1 and F2 adults or pups were missing.

GLP: Yes

Acceptability: The study is considered to be acceptable.

Material and methods:

Test system: In a two-generation study on Wistar rats [(Wistar Han Crl:WI (HAN)], source: Charles River Laboratories, Raleigh, NC, USA), spiroxamine (batch no.: EDTH004650); purity: 95.1 %) was examined for possible effects on reproduction. The compound was administered with the feed to 30 male and 30 female rats each at the following dose levels: 0, 20, 80, and 300 ppm, respectively. Stability was guaranteed for study duration. Vehicle: Acetone mixed in rat feed.

Findings:

The mean daily intake of spiroxamine (mg/kg bw/d) throughout this two-generation is summarised in Table 31. The concentration of the test substance in the feed for females was adjusted down by 50 % during lactation period (d 0-21) to avoid the large increase in dosage (mg/kg bw/d) that is otherwise associated with increased feed consumption during lactation.

Table 31: Mean daily intake of spiroxamine (mg/kg bw/d)

	20 ppm	**80 ppm**	**300 ppm**
F0 – males pre-mating	1.4	5.5	21.0
F1 – males pre-mating	1.5	5.7	23.3
F0 – females pre-mating	1.7	6.7	24.5
F1 – females pre-mating	1.8	6.9	26.7
F0 – females gestation	1.6	6.1	21.2
F1 – females gestation	1.6	6.3	25.9
F0 - females lactation	1.7	6.5	22.2
F1 - females lactation	1.8	6.7	27.7

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

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

Body weight (Table 32, Table 33 and Table 34):

P-generation adults: 300 ppm females exhibited declines in body weight gain during the pre-mating period as well as declines in absolute body weight and body weight gain during gestation. During lactation, slight declines in body weight were observed with significance on day 14. Females also exhibited subtle declines in terminal body weights.

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

F1-generation adults: During the pre-mating period at 300 ppm males and females exhibited declines in body weight with females also showing declines in body weight gain. Females continued with declines in body weight throughout gestation and lactation. Significant declines in terminal body weight were noted in both genders.

F2-Offspring: There were no effects on birth weight considered to be directly attributed 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 historical control values for this laboratory in this strain of rat and the decline in birth weight observed is considered to be secondary to a higher percentage of animals in this dose group delivering on d 21 when compared to the majority of controls delivering on d 22. At 300 ppm pup absolute body weight was decreased during lactation period and overall body weight gain was less compared to control pups (9.1 %).

Food consumption (Table 32, Table 33 and Table 34):

Parental animals, pre-mating: Incidental declines in food consumption were observed for the P-generation females at 300 ppm during the first three weeks of pre-mating. Food consumption was comparable to controls by week 4. There were no further effects on food consumption in male and female parental animals of either generation at any dietary level that were considered to be attributed to the test substance.

Gestation: No treatment related effects on food consumption were noted in P- and F1-generation females at any dietary level tested.

Lactation: There were no test substance-related effects on food consumption observed in P- and F1-generation females at any dietary level tested.

Table 32: Mean (S.E.) body weight (bw) and food consumption – Pre-mating/mating

Observations/study week	**Dose Group**			
	0 ppm	20 ppm	80 ppm	300 ppm
F0 Generation Males				
Mean bw (g) Week 15 (S.E.)	463.2 (7.14)	455.8 (8.48)	456.8 (6.39)	451.5 (8.30)
Mean weight gain (g) Weeks 1-15	195.6	190.6	183.8	181.3
Mean food (g/animal/day) Weeks 1-10	23.8	24.3	24.0	24.2
Mean food (g/kg/day) Weeks 1-10	66.6	68.8	66.8	68.3

F0 Generation Females - Pre-mating				
Mean bw (g) Week 10 (S.E.)	252.3 (2.67)	251.8 (3.88)	250.4 (2.88)	246.2 (3.09)
Mean weight gain (g) Weeks 1-10	70.3	70.7	65.6	60.3
Mean food(g/animal/day) Weeks 1-10	17.8	18.5	17.9	17.3
Mean food (g/kg/day) Weeks 1-10	81.1	84.0	81.7	79.8
F_1 Generation Males				
Mean bw (g) Week 14 (S.E.)	454.9 (6.66)	451.9 (9.16)	448.2 (7.46)	418.0** (5.62)
Mean weight gain (g) Weeks 1-14	188.7	181.1	173.6	174.8
Mean food (g/animal/day) Weeks 1-10	23.6	23.8	24.2	23.1
Mean food (g/kg/day) Weeks 1-10	66.0	67.1	66.6	70.6
F_1 Generation Females - Pre-mating				
Mean bw (g) - Week 10 (S.E.)	240.4 (3.35)	245.0 (3.99)	245.3 (3.30)	221.0** (2.44)
Mean weight gain (g) Weeks 1-10	63.7	63.1	64.0	56.6
Mean food (g/animal/day) Weeks 1-10	16.8	17.7	17.4	16.1
Mean food (g/kg/day) Weeks 1-10	78.7	81.4	80.7	82.2

**: Statistically different from control, $p \leq 0.01$

Table 33: Mean (S.E.) body weight (bw) and food consumption – Gestation

	Dose Group			
Observations/study week	0 ppm	20 ppm	80 ppm	300 ppm
F0 Generation females				
Mean bw (g) day 0 (S.E.)	248.6 (3.43)	249.8 (3.38)	246.9 (3.09)	241.9 (3.55)
Mean bw (g) day 6 (S.E.)	266.9 (2.93)	267.2 (3.22)	262.1 (3.15)	254.3* (4.25)
Mean bw (g) day 13 (S.E.)	286.6 (2.97)	287.9 (3.81)	282.2 (3.41)	275.2* (3.47)
Mean bw (g) day 20 (S.E.)	348.4 (3.74)	348.6 (5.2)	339.5 (4.53)	331.5* (4.50)
Mean weight gain (g) day 0-20 (S.E.)	99.8 (2.52)	98.8 (3.21)	92.6 (2.62)	89.6* (2.40)
Mean food (g/animal/day) day 0-20	18.8	19.4	19.1	17.9
Mean food (g/kg/day) day 0-20	70.5	72.2	72.4	69.9
F1 Generation Females				
Mean body weight (g) day 0 (S.E.)	237.4 (3.43)	243.1 (3.94)	242.0 (3.53)	222.3**(2.68)
Mean body weight (g) day 6 (S.E.)	250.6 (3.20)	258.6 (4.09)	256.7 (3.40)	236.1**(2.81)
Mean body weight (g) day 13 (S.E.)	270.8 (3.57)	279.7 (4.16)	277.4 (3.83)	253.1**(3.63)
Mean body weight (g) day 20 (S.E.)	326.3 (4.76)	342.2 (5.16)	335.6 (5.45)	316.3 (4.27)
Mean weight gain (g) day 0-20 (S.E.)	88.9 (2.66)	99.1 (2.50)	93.4 (2.7)1	94.0 (2.65)
Mean food (g/animal/day) day 0-20	17.7	18.9	19.4	19.2
Mean food (g/kg/day) day 0-20	69.0	72.6	75.0	81.2**

*: Statistically different from control, $p \leq 0.05$; **: Statistically different from control, $p \leq 0.01$

Table 34: Mean (S.E.) body weight (bw) and food consumption - Lactation

	Dose Group			
Observations/study week	0 ppm	20 ppm	80 ppm	300 ppm
F0 Generation Females - Lactation				
Mean bw (g) day 0 (S.E.)	274.1 (2.84)	274.3 (4.43)	269.1 (4.23)	262.0 (3.54)
Mean bw (g) day 4(S.E.)	284.0 (3.16)	282.5 (4.51)	277.1 (4.17)	271.4 (3.92)
Mean bw (g) day 7 (S.E.)	292.5 (2.79)	288.5 (4.60)	279.9 (5.40)	278.4 (3.99)
Mean bw (g) day 14 (S.E.)	307.1 (2.82)	302.3 (5.23)	299.5 (3.81)	283.1** (4.53)
Mean bw (g) day 21 (S.E.)	290.0 (3.06)	288.7 (5.33)	286.2 (3.99)	277.0 (4.09)
Mean food (g/animal/day) day 0-21	47.3	44.3	43.5	41.5

Mean food (g/kg/day) day 0-21	162.6	153.3	153.5	150.7
F1 Generation Females - Lactation				
Mean body weight (g) day 0 (S.E.)	256.7 (3.88)	265.1 (4.23)	263.0 (3.61)	240.3*(4.28)
Mean body weight (g) day 4 (S.E.)	268.0 (4.25)	279.2 (4.71)	270.8 (3.56)	251.9*(3.39)
Mean body weight (g) day 7 (S.E.)	273.8 (3.97)	281.9 (4.76)	275.6 (3.31)	260.5*(3.62)
Mean body weight (g) day 14 (S.E.)	2.87.7 (3.96)	298.9 (5.29)	290.2 (3.39)	2.73.9* (3.94)
Mean bw (g) day 21 (S.E.)	283.9 (4.58)	283.8 (4.21)	280.5 (3.92)	264.7** (3.32)
Mean food (g/animal/day) day 0-21	47.7	48.0	46.7	46.2
Mean food (g/kg/day) day 0-21	174.3	170.0	168.7	179.6

*: Statistically different from control, $p \leq 0.05$; **: Statistically different from control, $p \leq 0.01$

Clinical chemistry: There were no adverse test substance-related clinical chemistry, haematology or coagulation profile findings at any dietary dose level tested. At 300 ppm a tendency for a subtle increase in APTT values (Activated partial thromboplastin time) relative to concurrent controls was noted particularly in males (F0) and females (both generations). However, values for APTT were both dose dependent and statistically significant increased only in F1-females (Table 35).

Table 35: Activated partial thromboplasttin time (APTT) in seconds

	Dose Group			
Males	0 ppm	20 ppm	80 ppm	300 ppm
F0-generation (S.E.)	15.0 (1.7)	16.3* (1.3)	16.8* (1.5)	16.6* (1.5)
F1-generation (S.E.)	16.3 (2.7)	16.2 (1.7)	16.4 (1.9)	16.2 (1.7)
Females				
F0-generation (S.E.) non-pregnant	16.8 (0.0)	18.8 (4.1)	16.9 (2.5)	15.9 (0.6)
F0-generation (S.E.) pregnant	18.1 (2.5)	18.0 (1.4)	18.8 (2.1)	19.4 (2.9)
F1-generation (S.E.) non-pregnant	17.8 (0.0)	-	21.6 (0.0)	-
F1-generation (S.E.) pregnant	17.9 (3.3)	18.2 (2.0)	19.1*(2.5)	20.3 (4.6)*

*: Statistically different from control, $p \leq 0.05$;

Organ weights: Absolute organ weights were not affected by treatment with spiroxamine in either F0-males or -females. The statistical increase in the relative brain weight of the females at 300 ppm was considered to be attributed to the decline in terminal body weight observed in these animals. No effects on organ weights were recorded in the F1-generation.

F1- and F2-pups: Two summary tables regarding relative organ weights in F1-21-day pups have been submitted (p. 736 and p. 738 of the study report). However, these tables resulted in inconsistent values for the 80 ppm dose group (brain, thymus, spleen) and for all treatment groups concerning the relative uterus weights.

In the 300 ppm dose group, organ weight changes were observed on brain (relative, increased in F1-female pups), spleen (absolute, decreased in F2-males & females). Further changes in organ weights were not statistically significant at 300 ppm. Organ weight changes were not evident at any other dietary level tested.

At gross necropsy no test substance related findings were observed in this study.

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 and two F1-males hyperkeratosis of the epididymides. Despite some missing tissues of animals of different organs and different generations no other test substance related findings were observed in this

study. Histopathological examinations in F1- and F2-pups also revealed no test substance related findings.

Reproductive performance: A slight increase in cycle length (with concomitant decrease in cycle number) was observed for F1-females of the 300 ppm dose group when compared to the concurrent controls. However, mean values in F1-females at 300 ppm of 4.4 days compared to 4.0 days in the controls are well within provided historical control values (F1 mean oestrous cycle length 4.1 – 5.1 days) and no effect was observed on "days to insemination" or fertility. Furthermore, the mean value of the concurrent controls with 4.0 days is just below the lower limit of historical control means for F1-generation females. F0-generation females did not show any increase in oestrous cycle length in any dose.

No test substance related effects were observed on any sperm parameter evaluated at any dietary level tested for either generation.

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 dietary level.

At 300 ppm F1-females exhibited an increased incidence of slightly shorter gestation lengths. The median gestation length in days was statistically significant, although it was the identical value of 22.0 days in all dose groups including the control. The mean value of 21.6 days at 300 ppm (control: 22.1 days) is within historical control values for this laboratory (range of 21.6 – 22.3 days). The F0-generation was not affected. Therefore, this finding is considered to be no effect of spiroxamine treatment.

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 declines observed in both genders at this dose level (Table 36). In the second generation, anogenital distance for F2-pups was measured on lactation day 0, but was not affected by treatment at any dose level tested.

Table 36: Developmental landmarks in F1 pups (age, day)

		0 ppm	**20 ppm**	**80 ppm**	**300 ppm**
Preputial separation	Mean ± SE	42.0±0.32	42.3±0.61	42.8±0.38	44.6±0.58**
	N	28	25	26	26
	% pups reaching criteria	100	100	100	100
Vaginal opening	Mean ± SE	34.3±0.51	34.6±5.6	35.2±0.58	38.4±0.55**
	N	28	27	27	25
	% pups reaching criteria	100	100	97	100

In this second 2-generation study none of the pronounced clinical symptoms observed in the first study in 300 ppm pups (F1, F2) and in F1-adults occurred (e.g. piloerection, laboured breathing, cold external surface, cyanosis, bloody noses, polyuria, increased mortality in F1 adults and their pups). Furthermore, no increased mortality was observed in both the F1- and the F2-generation pups. The missing treatment relationship of mortality in the first study together with the fact that clinical symptoms and increased mortality could not be reproduced in the second study indicate that these severe findings of the first 2-generation study rather might have been caused by an infection

of the animals than by a systemic effect of spiroxamine. However, in the second 2-generation study the doses expressed as mean daily intake in mg/kg bw/d during treatment period were noticeable lower (obviously due to higher food consumption of the smaller rats in the first study).

For information only:

In this two-generation study the parental systemic NOAEL is 80 ppm (5.5 mg/kg bw/d) based on declined body weight, increased incidence of hyperkeratosis of the oesophagus and increased APTT values. The reproductive NOAEL is 300 ppm (21.0 mg/kg bw/d) based on absence of test substance related findings. The offspring NOAEL is 80 ppm (5.5 mg/kg bw/d) based on reduced pup weight and weight gain, delayed balanopreputial separation and vaginal patency.

Effects at other dose levels are summarised above.

4.11.1.2 Human information

No data submitted by the notifier.

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

Oral study in rats

Reference:	KIIA 5.6 (OECD)
Report:	Becker, H. and K. Biedermann (1992): Embryotoxicity study (including teratogenicity) with KWG 4168 technical in the rat - Report no.: R 5574 (May 22, 1992), Research and Consulting company Ltd. and RCC Umweltchemie, Itingen, Switzerland, Dates of exp. work: December 1990 - January 1991. TOX9552620
Guidelines:	OECD TG 414
Deviations:	None
GLP:	Yes
Acceptability:	The study is considered to be acceptable.

Material and methods:

Test system: Spiroxamine (batch no.: 17002/90; purity: 93.6 %) was tested for developmental toxicity in pregnant Wistar rats (HanIbm: WIST [SPF], source: Biological Research Laboratories Ltd., Füllinsdorf, Switzerland). The test compound was administered orally by gavage once daily from days 6 to 15 post coitum at dose levels of 0, 10, 30 or 100 mg/kg bw/d. Each group consisted

of 25 mated female rats. Control animals were dosed with the vehicle alone (water with 0.5 % Cremophor EL). The rats were sacrificed on day 21 post coitum and the foetuses were removed.

Findings:

Observations in dams: No clinical signs or symptoms were observed and no deaths were observed which were considered to be related to the test substance. At 100 mg/kg bw/d only slight signs of maternal toxicity occurred: decreased food consumption (Table 37) and body weight (Table 39). Body weight gain was statistically significant decreased only after correction for uterus weight (Table 38). At terminal necropsy, one dam at 100 mg/kg bw/d had a perforating gastric ulcer. No abnormal macroscopic changes were noted at 0, 10 or 30 mg/kg bw/d.

Notifier's comment: 'The fact that also in the one 100 mg/kg female which suffered a perforating gastric ulcer no symptoms were noted could possibly indicate that in this study, which was conducted at RCC 1990 over Christmas, the intensity of clinical observations might have been reduced' (Henninger, K., 2009, ASB2009-2108). This allegation was not confirmed by the performing laboratory. Based on the available data and information in the study report, dossier submitter cannot comment on the validity and soundness of this argument.

Table 37: Food intake (g/animal/d) of dams post coitum

	0 mg/kg	**10 mg/kg**	**30 mg/kg**	**100 mg/kg**
Days 0-6	22.1	22.6	21.4	21.5
Days 6-11	22.8	23.0	22.0	19.9 **
Days 11-16	24.7	24.9	24.1	18.3 **
Days 16-21	24.7	24.6	23.9	24.1

Dunnet-Test based on pooled variance significant at 5 % (*) or 1 % (**) level

Table 38: Calculation of corrected body weight gain of dams (day 6 – 21 p.c.)

	0 mg/kg	**10 mg/kg**	**30 mg/kg**	**100 mg/kg**
Number of dams	25	25	24	24
Body weight gain (g)	96.5	95.5	92.6	81
Uterus weight (g)	76.7	75.2	74.3	72.5
Corrected body weight gain (g)	19.8	20.3	18.3	8.5**

significant at 1 % (**) level

Table 39: Mean body weights in g (S.E.) of dams (day 5 – 21 p.c.)

	0 mg/kg	**10 mg/kg**	**30 mg/kg**	**100 mg/kg**
Day 5	224 (10.0)	225 (11.4)	220 (10.1)	225 (11.0)
Day 6	227 (10.6)	227 (12.0)	222 (10.3)	227 (10.2)
Day 7	230 (11.0)	229 (11.7)	223 (9.8)*	228 (9.8)
Day 8	233 (11.3)	231 (11.9)	225 (10.5)*	230 (9.9)
Day 9	236 (11.2)	234 (12.1)	228 (10.3)*	231 (10.1)
Day 10	241 (10.9)	239 (12.0)	234 (10.4)	235 (10.2)
Day 11	246 (11.8)	245 (12.4)	239 (10.6)	240 (10.1)
Day 12	250 (11.6)	249 (12.6)	242 (10.5)*	242 (10.3)*
Day 13	253 (12.0)	254 (12.0)	247 (11.1)	245 (11.4)*
Day 14	258 (12.2)	258 (12.8)	251 (10.8)	245 (12.6)**
Day 15	262 (12.8)	263 (12.6)	257 (10.2)	247 (13.9)**
Day 16	271 (12.8)	271 (13.8)	263 (11.1)	251 (14.5)**
Day 17	279 (13.2)	280 (14.7)	272 (12.7)	263 (16.6)**
Day 18	292 (15.5)	291 (15.9)	284 (14.2)	275 (17.2)**
Day 19	303 (15.8)	301 (16.5)	293 (15.3)	285 (18.9)**
Day 20	314 (17.7)	315 (17.2)	307 (16.2)	297 (22.3)**
Day 21	323 (16.0)	322 (17.8)	315 (16.3)	308 (23.1)*

significant at 5 % (*) or 1 % (**) level

The evaluation of the reproduction data did not indicate any test article related effects. All differences were within the normal range of variation (Table 40).

Table 40: Reproduction data (total/dose group and mean/dam)

	0 mg/kg	**10 mg/kg**	**30 mg/kg**	**100 mg/kg**
Number of dams	25	25	24	24
Corpora lutea	349	341	327	330
mean (+)	14.0	13.6	13.6	13.8
Pre-implantation loss	34	45	19	36
% of corp. lutea (#)	9.7	13.2	5.8#	10.9
mean (+)	1.4	1.8	0.8	1.5
number of dams affected	16	18	14	11
Implantation sites	315	296	308	294
% of corp. lutea (#)	90.3	86.8	94.2#	89.1
mean (+)	12.6	11.8	12.8	12.3
Post-implantation loss	29	11	40	29
% of impl. sites (#)	9.2	3.7##	13.0	9.9
mean (+)	1.2	0.4	1.7	1.2
Embryonic deaths: total	28	11	40	29
% of impl. sites (#)	8.9	3.7##	13.0	9.9
mean (+)	1.1	0.4	1.7	1.2
Embryonic resorptions	27	11	40	27
% of impl. sites (#)	8.6	3.7##	13.0#	9.2
mean (+)	1.1	0.4	1.7	1.1
Foetal resorptions	1	0	0	2
% of impl. sites (#)	0.3			0.7
mean (+)	0.0			0.1

(#) Fisher's Exact Test significant at level 5 % (#) or 1 % (##), (+) = Steel Test significant at level 5 %

The sex ratio of foetuses was not affected by treatment. The body weights of the foetuses were statistically significantly reduced at 100 mg/kg bw/d on individual basis as well as on litter basis. The reason for the statistical significance (on individual basis only) only at 10 mg/kg bw/d and not at 30 mg/kg bw/d was a result of calculation which used the exact raw data values and not the presented rounded-off results.

At 100 mg/kg bw/d in three foetuses out of three litter palatoschisis[1] were detected and were outside of control data (concurrent and historical range).

The notifier submitted following summary of historical control data (Henninger, 2009, ASB2009-2008):

[1] RAC's rapporteur commented during accordance check: „palatoschisis may comprise both cleft palate and cleft lip so a more exact description of the malformations detected would be very useful. Was it cleft palate or both cleft palate and cleft lip?" However no further details on the observation „palatoschisis" is included in the study report. According to www.devtox.org, „palatoschisis, uranoschisis" is defined as „fissure of the palate".

Incidences of palatoschisis and caudal malposition of the left hindleg in vehicle controls of developmental rat studies in WIST HanIbm: WIST (SPF) rats conducted at RCC between 1988 and 1995.

Malformation		Incidences of palatoschisis [no. of studies, affected fetuses]
Year	Studies	
1988	7	0
1989	12	0
1990	7	3 (1 fetus in one litter, 4 fetuses in 1 litter and 2 fetuses in 1 litter)
1991	6	1 (1 fetus in 1 litter)
1992	4	0
1993	2	1 (2 fetuses in 1 litter)
1994	1	0
1995	1	2 (2 fetuses from 2 litters with palatoschisis + multiple malformations)

In summary, the provided historical control data (1988 to 1995) showed that findings of palatoschisis occurred very rarely in the rat strain used by RCC. Except for one study in 1995 at most one litter per study was affected.

Furthermore, in a previously conducted range finding study with spiroxamine (R6072, please see below) palatoschisis was already observed at the same dose level: three out of 46 foetuses had palatoschisis. These foetuses descended from two out of four litters. Both females showed clinical signs in the second half of treatment period (Table 43). However, food consumption and body weight gain were only slightly affected (Table 42).

During visceral examination of the foetuses by Wilson technique, no further abnormal findings were noted which were considered to be substance related.

The abnormal findings noted at skeletal examination were mostly wavy ribs and dumbbell shaped thoracic vertebrae (Table 41).

Table 41: Results of developmental toxicity in rats: Foetal data (total/dose group and mean/dam)

	0 mg/kg	**10 mg/kg**	**30 mg/kg**	**100 mg/kg**
Number of litters examined	25	25	24	24
Total foetuses	287	285	268	265
% of impl. Sites (#)	91.1	96.3##	87.0	90.1
mean (+)	11.5	11.4	11.2	11.0
Live foetuses	286	285	268	265
% of impl. Sites	90.8	96.3	87.0	90.1
mean (+)	11.4	11.4	11.2	11.0
External examination				
-palatoschisis (n/litters)				3/3
- caudal malposition of left hindleg				1/1
External examination	1	0	0	0
% of abnormal dead foetuses	0.3			
mean	0.2			
Skeletal examination (n/litters)				
- wavy ribs (n/litters)	2/2	11/4	7/5	6/4
- dumbbell shaped thoracic vertebrae	1/1	0	0	2/2
- bipartite sternebrae	0	1/1	1/1	0
- abnormally ossified sternebrae	1/1	0	0	1/1
Weights of live foetuses n =	286	285	268	265
mean (*)	4.8	4.7**	4.7	4.6**
Weights of male foetuses n =	135	158	138	128
mean (*)	4.9	4.8**	4.8	4.7**
Weights of female foetuses n =	151	127	130	137
mean (*)	4.6	4.5	4.6	4.5**

(#) Fisher's Exact Test significant at level 5 % (#) / 1 % (##); (*) Dunett-Test based on pooled variance significant at level 5 % (*) / 1 % (**); (+) Steel Test significant at level 5 %

Furthermore at 100 mg/kg bw/d skeletal examination resulted in significant increased incidences of incomplete ossification (cranium, sternebrae) or non-ossification (phalanges).

Conclusion:

At 30 mg/kg bw/d no adverse maternal effects were reported. At the next higher dose level of 100 mg/kg bw/d, reduced feed intake (13-26 %) and marginal reduced body weight were noted. Body weight gain was statistically significant decreased only after correction for uterus weight. One dam at 100 mg/kg bw/d had a perforating gastric ulcer. No further signs of maternal toxicity were reported. However, it is assumed that clinical signs occurred at 100 mg/kg bw/d: in a previously conducted range finding study (for details see below) and even in the acute oral toxicity study in rats (*c.f.*, section 4.2.1.1) clinical signs were observed at the same dose level.

Beside delayed ossification and reduced body weight clearly signs of developmental toxicity were detected at 100 mg/kg bw/d: In three foetuses out of three litters palatoschisis occurred. Furthermore, in a previously conducted range-finding study palatoschisis was also observed at the same dose level.

Oral range-finding studies in rats

Reference:	KII 5.6 (OECD)
Report:	Becker, H. and K. Biedermann (1995): Combined report of embryotoxicity screening study (incl. teratogenicity) and supplementary study to the embryotoxicity screening study (incl. teratogenicity) with KWG 4168 technical in the rat, RCC Projects 263068 and 281507 - Report no.: R6355, M-006780-01-1, Dates of exp. work: January 1990 - March 1990, ASB2009-2096 Becker, H. (1995): Range-finding studies with KWG 4168 technical in the rat, RCC Projects 268075, 272610 and 277931 - Report no.: R6343, M-008093-02-1, Dates of exp. work: April 1990 - August 1990, ASB2009-2106 Becker, H. (1993): Dose range-finding embryotoxicity study (incl. teratogenicity) with KWG 4168 technical in the rat, RCC Project 286648, Report no.: R6072, M-007009-01-1, Dates of exp. work: October 1990 - November 1990, ASB2009-2026 All studies conducted by Research and Consulting Company Ltd. and RCC Umweltchemie, Itingen, Switzerland
Guidelines:	Not appropriate
Deviations:	Not applicable
GLP:	No
Acceptability:	The studies are considered to be supplementary

Results of preliminary developmental toxicity studies with spiroxamine are summarised in Table 42.

Table 42: Results of preliminary developmental toxicity studies in rats

	Maternal toxicity	Reproductive toxicity	Foetal findings	Histopathol. dams
R6355, batch E 343928 10 mg/kg bw/d	None (0/25)	None	None	3/25 slight erosion of gastric mucosa
R6355, batch E 343928 25 mg/kg bw/d	None (0/25)	None	None	1/25 slight erosion of gastric mucosa
R6343, batch E 343928 75 mg/kg bw/d	None (5/5)	None	Omphalocele (1/61) hydrocephalus (1/61)	Not investigated
R6072, batch 17002/90 75 mg/kg bw/d	None	None	None	Not investigated
R6343, batch E 343928 100 mg/kg bw/d	None (5/5)	None	None	Not investigated
R6072, batch 17002/90 100 mg/kg bw/d	Clinical symptoms* (2/5) feed slightly & bw gain marginal ↓	None (4/5 pregnant females)	Palatoschisis (3/46 foetuses in 2 litters)*	Not investigated
R6343, batch E 343928 150 mg/kg bw/d	Clinical symptoms (3/5) feed intake ↓	implantation loss, reduced foetuses	Bw ↓	Not investigated
R6355, batch 17002/90 150 mg/kg bw/d	Mortality (21/25), clinical symptoms (25/25)§	3/25 pregnant and surviving females: implantation loss, reduced foetuses	Palatoschisis (3/18 foetuses in 2 litters); omphalocele (1/18)§	Not investigated
R6343, batch E 343928 250 mg/kg bw/d	Mortality (5/5)	All females died until day 13 p.c.	-	Not investigated

* Clinical symptoms given in Table 43; litters by dams no. 13 and 14 were affected;
§ Clinical symptoms including individual data are presented in section 0; only litters/pups of dams surviving until terminal sacrifice were evaluated for foetal findings, litters by dams no. 38 (1 pup) and 48 (2 pups) were affected by palatoschisis and one pup in dam no. 41 was affected by omphalocele

Table 43: Dose range-finding study in rats (R6072): Clinical symptoms observed at 100 mg/kg bw/d

Female No.	10 p.c.	11 p.c.	12 p.c.	13 p.c.	14 p.c.	15 p.c.	16 p.c.	17 p.c.
13	**A, B, C**		**C**	**A, D, E**	**A, D, E**	**A, D, E**	**A, D**	**A**
14	**C**				**A, D, E**	**A, D, E**	**A, D**	**A**

A = ruffled fur, B = lateral recumbency, C = dyspnea, D = sedation, E = hunched posture

Oral study in rabbits

Reference: KIIA 5.6 (OECD)

Report: Holzum, B.: KWG 4168 - Studies for embryotoxic effects in rabbits following oral administration - Report no.: 23662 (January 20, 1995), Bayer AG, Institute for Toxicology, D-42096 Wuppertal, Germany; Dates of exp. work: main study: January 1991 - October 1992; supplementary study: July 1991 - June 1992.

TOX9552623

Guidelines: OECD TG 414

Deviations: None

GLP: Yes

Acceptability: The study is considered to be acceptable.

Material and methods:

Test system: Groups of 15 female Himalayan rabbits (CHBB:HM, source: Thomae Breeders, Biberach a. d. Riss, Germany) each received spiroxamine (batch no. 17002/90; purity: 94.3 %-95.3 %) at daily doses of 0, 5, 20 or 80 mg/kg bw/d (first study) and of 0 or 80 mg/kg bw/d (supplementary study, purity: 94.3 %) by gavage from day 6 to 18 post coitum. The 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.

Findings:

Observations in dams: 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 (first study only). In addition, animals exhibited impaired body weight gain and reduced food intakes at this dose (Table 44). One dam at 20 mg/kg bw/d died on day 16 p.c. probably due to misapplication.

Table 44: Main study: Mean food intake and body weight development

	0 mg/kg	**5 mg/kg**	**20 mg/kg**	**80 mg/kg**
Mean food intakes [g/animal/d]				
p.c. day 0- 6	67.2	74.7	75.5	78.2*
p.c. day 6-10	65.9	60.6	66.8	50.2*
p.c. day 10-14	56.0	56.8	60.8	43.0*
p.c. day 14-19	65.5	60.7	65.3	38.8**
p.c. day 19-24	74.7	78.7	76.5	76.2
p.c. day 24-29	83.7	84.8	86.7	80.4
p.c. day 0-29	69.3	70.3	72.6	62.7
Body weight gain [g]				
p.c. day 6-18 (mean)	52.1	8.2	37.9	-58.3**
p.c. day 0-29 (mean)	206.3	219.6	200.9	104.4
p.c. day 0-29 (corrected)	-162.2	-125.6	-135.3	-239.7

* statistically significant deviation to control ($p < 0.05$)
** statistically significant deviation to control ($p < 0.01$)

Animals of the supplementary study excreted few or soft faeces and one dam at 80 mg/kg died on day 16 post coitum. Due to autolytic changes, gross pathological examination was not possible. As shown in Table 45 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.

Table 45: Supplementary study: Mean food intake and body weight development

	0 mg/kg	80 mg/kg
Mean food intakes [g/animal/d]		
p.c. day 0- 6	76.0	84.1
p.c. day 6-10	66.1	58.3
p.c. day 10-14	57.1	55.0
p.c. day 14-19	58.3	52.2
p.c. day 19-24	71.0	79.4
p.c. day 24-29	80.8	88.5 *
p.c. day 0-29	68.9	70.9
Body weight gains [g]		
p.c. day 6-18 (mean)	40.6	-2.6
p.c. day 0-29 (mean)	209.1	212.3
p.c. day 0-29 (corrected)	-150.3	-122.9

* statistically significant deviation to control ($p < 0.05$)

The rate of gestation, resorption rate, numbers and sexes of foetuses (Table 46, Table 47) were comparable up to 80 mg/kg bw/d.

Table 46: Main study: Reproduction data

	0 mg/kg	5 mg/kg	20 mg/kg	80 mg/kg
Fertilised animals	14+	15	14+	14+
Animals with implantations	14	15	14	14
Corpora lutea	8.9	8.3	8.7	8.4
Implantations	7.6	7.0	7.0	7.2
Animals with viable foetuses	14	14	14	14
Placental weight [g]	4.24	4.51	4.43	4.25
Number of foetuses per dam	6.8	6.2	6.2	6.4
Resorptions per dam	0.8	1.4	0.8	0.9
Males:females	1:0.95	1:0.50	1:0.99	1:0.98
Weight of live foetuses (litter based) in g				
- total	37.99	40.16	38.55	38.05
- males	38.61	40.38	38.83	37.64
- females	37.61	39.67	37.83	38.47
Weight of live foetuses (individual) in g,				
- total	37.37	38.86*	37.82	37.56
- males	37.65	38.94	38.46	36.84
- females	37.06	38.70	37.11	38.32

\+ animal no. 822, which died, and animal nos. 848 and 866, which exhibited uterine anomalies, were not included in the calculation

Table 47: Supplementary study: Reproduction data

	0 mg/kg	80 mg/kg
Fertilised animals	15	13+
Animals with implantations	15	13
Corpora lutea	8.0	8.9
Implantations	7.3	7.5
Animals with viable foetuses	15	13
Number of dams with		
- implantations	15	13
- viable fetuses	15	13
Placental weight [g]	4.35	4.04
Number of foetuses per dam	6.6	6.5
Resorptions per dam	0.7	1.0

Males:females	1:1.11	1:0.66
Weight of live foetuses (litter based) in g - total - males - females	 39.58 40.03 39.27	 37.09 37.65 35.91
Weight of live foetuses (individual) in g, n foetuses - total - males - females	99 38.55 39.48 37.67	85 36.43* 36.86* 35.79

* $p < 0.05$ %

The degree of ossification and the rate of variations in the foetal skeletal system, as well as the external appearance of the placentas underwent no treatment related effect up to 80 mg/kg bw/d. A dose of 80 mg/kg bw/d induced developmental toxicity including a slight increase in the rate of foetuses exhibiting malformations (Table 48, Table 49). Except for hydrocephalus internus with caudal displacement of the ears in one foetus at 80 mg/kg bw/d all observed malformations were covered by historical control data (1982-1996). (However, cases of hydrocephalus internus *without* displacement of the ears occurred occasionally in this strain of rabbits.) Malformations observed in control animals were above the historical range.

The supplementary study revealed a slight depression of the foetal weight which correlates with a slight decrease in placental weights at 80 mg/kg bw/d.

Table 48: Main study: Incidence of malformations

	0 mg/kg	**5 mg/kg**	**20 mg/kg**	**80 mg/kg**
Foetuses per group (n)	95	87	87	89
Foetuses with malformations (n) (%)	8 8.4	1 1.1	3 3.4	9 10.1
Litters per group (n)	14	14	14	14
Litters with malformations (n) (%)	6 42.9	1 7.1	3 21.4	6 42.9
Malformation (no./litter)				
Multiple malformation	2/2*			1/1
Hydrocephalus internus, caudal displacement of ears				1/1
Twelfth thoracic vertebral body missing, 12th thoracic vertebral arch fused with 1st lumbar vertebral arch	1/1			
Missing thoracic vertebra, 12th rib bilateral at first lumbar vertebra, pre-sacral dislocation of pelvis				2/1
Supernumerary lumbar vertebra with 13th rib	1/1			1/1
Enlargement of second proximal phalange of left forelimb			1/1	
Missing proximal, medial and distal phalangeal digits				1/1
Arthrogryposis	5/3	1/1	2/2	3/3
Chicken breast (conjoined sternebrae)	1/1*			2/2

* chicken breast was detected in one foetus with multiple malformations

Table 49: Supplementary study: Incidence of malformations

	0 mg/kg	**80 mg/kg**
Foetuses per group (n)	99	85
Foetuses with malformations per group (n) (%)	4 4.0	6 7.1
Litters per group (n)	15	13
Litters with malformations per group (n) (%)	4 26.7	4 30.8
Arthrogryposis		2/2
Missing thoracic vertebra, 12th rib bilateral at 1st lumbar vertebra, pre-sacral dislocation of pelvis	1/1	
Missing thoracic vertebra, 12th rib right at first lumbar vertebra, 12th rib left missing		1/1
Slight curvature in spinal column due to absence of 10th thoracic vertebral body and left 10th thoracic vertebral arch; floating 10th rib left		1/1
Iliac bone positioned at seventh lumbar vertebra	1/1	
Anomaly of coccygeal vertebra	2/2	2/2

A summary of historical control data on spontaneous malformations was included in the study report. Additional historical (control) data of studies performed during 1989 to 1996 were provided for re-evaluation in 2009. Both are reproduced in section 0.

Conclusion:

No maternal effects were reported at a dose level of 20 mg/kg bw/d. At the next higher dose level of 80 mg/kg bw/d, clinical findings, reduced body weight gain and feed consumption were reported. One dam died and exhibited encrustation at the labial angles and marginal body weight loss (7 %). Gross pathological examination was impossible in this female due to autolytic changes. No toxicological findings were detected at necropsy of the other animals.

In a pilot developmental toxicity study with doses of 50, 75 and 100 mg/kg bw/d one dam (out of three) died at highest dose level and a gastric ulcer was detected at gross pathology (Anon., 2009, ASB2009-2104).

No effects on development were reported at a dose level of 20 mg/kg bw/d. Slightly increased incidences of malformations were reported at 80 mg/kg bw/d. With the exception of one malformation in the first study (hydrocephalus internus with caudal displacement of the ears) all malformations at the 80 mg/kg level correspond to changes previously observed as spontaneous malformations in the strain of rabbits used. It is assumed that the development of an hydrocephalus internus may result in a caudal displacement of the ears. At high dose level foetal body weight was slightly (but significantly) decreased on individual basis, which was observed in the supplementary study only.

Dermal study in rats

Reference: KIIA 5.6 (OECD)

Report: Becker, H. and K. Biedermann (1993): Embryotoxicity study (including teratogenicity) with KWG 4168 technical in the rat

	(dermal application) - Report no.: R 5952 of March 30, 1993 (report); R 5952A of July 16, 1993 (addendum), Research and Consulting company Ltd. and RCC Umweltchemie, Itingen, Switzerland; Dates of exp. work: October 1991 - November 1991.
	TOX9552621
Guidelines:	OECD 414
Deviations:	None
GLP:	Yes
Acceptability:	The study is considered to be acceptable.

Material and methods:

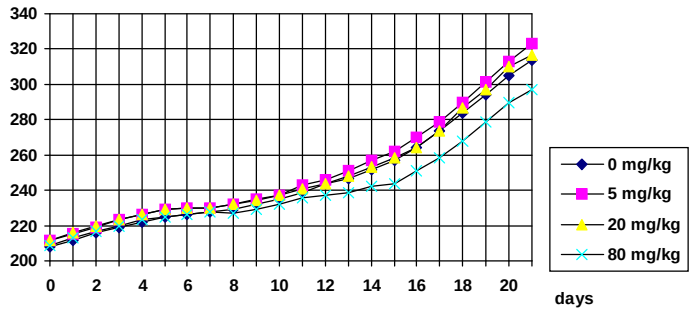
Test system: In a dermal developmental toxicity study, Wistar rats (HanIbm:WIST [SPF], source: Biological Research Laboratories Ltd., Füllinsdorf, Switzerland) were exposed to spiroxamine (batch no. 17002/90; purity: 94.4 - 95.3 %) under occlusive conditions for 6 h/day from day 6 - 15 post coitum at dose levels of 0, 5, 20 or 80 mg/kg bw. Each group consisted of 25 mated female rats. 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.

Findings:

Observations in dams: No deaths occurred and no test article-related systemic signs and/or symptoms were observed. Dermal application caused dose related skin reactions (slight erythema and scaling) from 5 mg/kg upwards.

Body weight was decreased at 80 mg/kg bw/d (Figure 1) and corrected body weight gain (corrected for uterus weight) was slightly decreased at 20 mg/kg (10.0 g vs. 19.3 g in control group) and above (-2.6 g). Due to lowest uterus weights in control animals, corrected body weight at 20 mg/kg bw/d was considered to be not adverse. The mean food consumption was not affected in any dose group. During terminal necropsy, no macroscopic changes were noted in any female of any group.

Figure 1: Results of a dermal developmental toxicity study in rats: Mean body weights [g] of dams post coitum



No indication for substance related effects was noted on reproductive parameters at any dose level. The incidental differences evident were within the normal range of variations for rats of this strain and age.

Table 50: Reproduction data (total/dose group and mean/dam)

	0 mg/kg	**5 mg/kg**	**20 mg/kg**	**80 mg/kg**
Number of dams	25	25	25	24
Corpora lutea	326	341	347	331
mean (+)	13.0	13.6	13.9	13.8
Pre-implantation loss	33	36	29	36
% of corp. lutea (#)	10.1	10.6	8.4	10.9
mean (+)	1.3	1.4	1.2	1.5
Implantation sites	293	305	318	295
% of corp. Lutea (#)	89.9	89.4	91.6	89.1
mean (+)	11.7	12.2	12.7	12.3
Post-implantation loss	22	11	13	21
% of corp. lutea (#)	7.5	3.6#	4.1#	7.1
mean (+)	0.9	0.4	0.5	0.9
Embryo / foetal deaths:total	22	11	13	20
% of impl. sites (#)	7.5	3.6#	4.1#	6.8
mean (+)	0.9	0.4	0.5	0.8
Embryo resorptions	22	11	13	20
Total foetuses	271	294	305	275
% of impl. sites (#)	92.5	96.4#	95.9#	93.2
mean (+)	10.8	11.8	12.2	11.5
Live foetuses	271	294	305	274
% of impl. sites	92.5	96.4	95.9	92.9
mean (+)	10.8	11.8	12.2	11.4
External examination	1	3	1	1
% of abnormal live foetuses	0.4	1.0	0.3	0.4
mean	0.0	0.1	0.0	0.0
External examination	0	0	0	0
% of abnormal dead foetuses				
mean				
Weights of live foetuses n =	271	294	305	274
mean (*)	4.6	4.7**	4.7	4.7**
Weights of male foetuses n =	121	155	144	150
mean (*)	4.7	4.8	4.8	4.9*
Weights of female foetuses n =	150	139	161	124
mean (*)	4.5	4.6*	4.6	4.6

= Fisher's Exact Test significant at level 5 % (#) / 1 % (##; * = Dunett-Test based on pooled variance significant at level 5 % (*) / 1 % (**)

+ = Steel Test significant at level 5 %

External and visceral examination of the foetuses revealed no indication of substance related effects. Mean body weight of foetuses was not affected. The skeletal examination of foetuses showed a slight toxic effect: at highest dose level 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.

Conclusion:

The NOAEL for local skin effects was < 5 mg/kg bw/d based on skin irritation (slight erythema and scaling) at 5 mg/kg bw/d and above.

The NOAEL for systemic maternal toxicity was 20 mg/kg bw/d based on reduced body weight at 80 mg/kg bw/d.

The NOAEL for developmental toxicity was 20 mg/kg bw/d based on slight signs of toxicity (increased incidence of wavy ribs) at 80 mg/kg bw/d.

4.11.2.2 Human information

No data submitted by the notifier.

4.11.3 Other relevant information

ECHA/RAC secretariat considered it helpful, if reduced litter size seen in the reproduction toxicity study 1 was mentioned. DS notes, that the reduction was by approx. 1 foetus per litter only.

4.11.4 Summary and discussion of reproductive toxicity

The endpoint “effects on fertility” is not addressed by this proposal.

The developmental toxicity of spiroxamine was studied in developmental toxicity studies in rats and rabbits (Table 51).

Table 51: Summary of developmental toxicity studies

Study	Dose levels	NOAEL parental	NOAEL reproduction	NOAEL Offspring
developmental, gavage, rat	0-10-30-100 mg/kg bw/d	30 mg/kg bw/d		30 mg/kg bw/d
developmental, gavage, rabbit	0-5-20-80 mg/kg bw/d	20 mg/kg bw/d		20 mg/kg bw/d
developmental, dermal, rat	0-5-20-80 mg/kg bw/d	20 mg/kg bw/d (systemic) < 5 mg/kg bw/d (local)		20 mg/kg bw/d

* compound uptake considering the food consumption during the pre-mating period

In an oral developmental toxicity study in rats, in 3 pups out of 3 litter palatoschisis was observed at a dose of 100 mg/kg bw/d together with other developmental effects such as delayed ossification and reduced body weight. These effects were observed at slight maternal toxic effects (reduced feed

intake and marginal decreased body weight). Additionally, palatoschisis was observed in range-findings experiments at similar dose-ranges.

In the oral developmental toxicity study in rabbits maternal effects such as clinical findings, reduced body weight and feed consumption were observed at 80 mg/kg bw/d. Developmental toxicity was reported for groups receiving 80 mg/kg bw/d: slightly increased incidence in spontaneous skeletal malformation.

In a dermal developmental toxicity study in rats, treatment related effects on intrauterine development were limited to slight toxicity at high dose level of 80 mg/kg bw/d (increased number of foetuses with wavy ribs) and were observed at maternal toxic dose level. Local skin reactions in dams occurred in all treatment groups. No evidence for teratogenicity was seen after dermal application.

4.11.5 Comparison with criteria

The endpoint “effects on fertility” is not addressed by this proposal.

In an oral developmental toxicity study in rats, in 3 pups out of 3 litter palatoschisis was observed at a dose of 100 mg/kg bw/d together with other developmental effects such as delayed ossification and reduced body weight. These effects were observed at slight maternal toxic effects (reduced feed intake and marginal decreased body weight). The incidences of palotoschisis were outside of the respective historical control range of the performing laboratory as submitted by the notifier: 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 few of the performed studies.

In range-finding experiments (Table 42), palatoschisis was observed at a dose level of 100 mg/kg bw/d (experiment R6072) and 150 mg/kg bw/d (experiment R6355). However at a dose level of 150 mg/kg bw/d, high mortality rate in dams was observed, therefore, the results of this latter experiment do not contribute to the classification proposal. At 100 mg/kg bw/d, following maternal findings were observed: clinical symptoms, slightly lower feed intake and marginally lower body weight gain.

Considering the criteria in Annex I, section 3.7.2.4 to regulation (EC) No 1272/2008 (as amended) and Annex VI, section 4.2.3 to directive 67/548/EC (as amended) following case is proposed:

- No data from humans is available, hence a classification with H360 (category 1A) respectively R61 (category 1) is not possible.
- Palatoschisis is a malformation, however it was observed in one species (rat), only, and in low incidences (3 foetuses in 3 litters). This finding was also reported in a range-finding study (Table 42, experiment R6072), indicating reproducibility. 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. In our understanding, these observations (one species, low incidences, maternal toxicity) reduce the concern for developmental hazard; hence, classification with H360 (category 1B)/R61 (category 2) seems to be not appropriate. In summary, classification with H361 (category 2) respectively R63 (category 3) is proposed.

4.11.6 Conclusions on classification and labelling

According to EU Directive 67/548/EEC classification of spiroxamine with R63 is proposed by the dossier submitter based on observed malformations in the oral developmental study in rats. And according to regulation (EC) No. 1272/2008 classification of spiroxamine with H361d (reproductive toxicity 2) is proposed.

Remark: the notifier did not agree with this conclusion and stated that no classification for developmental effects was needed (low incidences, high maternal toxicity).

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.

Comparison with the 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.

4.12 Other effects (neurotoxicity, immunotoxicity, specific investigations: other studies)

This endpoint is not addressed by this proposal.

5 ENVIRONMENTAL HAZARD ASSESSMENT

It is not proposed to change the current environmental classification and labelling of spiroxamine. However, according to the 2nd ATP to Regulation (EC) No 1272/2008, M-factors for the environmental categories Aquatic Acute 1 and Aquatic Chronic 1 have to be set. Therefore, the aquatic effect studies that are relevant for the selection of the respective M-factors are presented in the following:

5.1 Degradation

5.1.1 Stability

5.1.1.1 Hydrolytic degradation

Author:	Brumhard, B.
Title:	Hydrolysis of KWG 4168 in sterile aqueous buffer solutions
Date:	1995
Doc ID:	PF4074 (BVL reg no 1797715) M-006003-01-1
Guidelines:	EPA Ref.: 161-1, Hydrolysis Studies (Oct. 1982)
Deviations:	None
Status:	Old study, originally submitted for first Annex I inclusion
GLP:	Yes
Validity:	Acceptable

Author:	Krohn, J.
Title:	Hydrolysis of KWG 4168 (Spiroxamine, proposed) as a function of pH
Date:	1997
Doc ID:	145000922 (BVL reg no 1798093) M-006002-01-1
Guidelines:	OECD - Guideline for the Testing of Chemicals No.: 111 Hydrolysis Studies
Deviations:	None
Status:	New study, not submitted for first Annex I inclusion Justification for including this new study in the Annex I renewal dossier: This study was included to give a consistent overview on the fate and behaviour in the E-Fate section in the OECD dossier.
GLP:	Yes
Validity:	Acceptable

In the hydrolysis study by Brumhard (1995, refer to RAR: IIA7.5/01) conducted at 25 °C using buffer solutions of pH 5, 7 and 9 spiroxamine showed hardly any degradation over the examined testing period of 30 days. At termination of the experiment spiroxamine (KWG 4168) was accounted for 97.3 - 99.5 % of the radioactivity recovered in the solutions. In a supplemental study at pH 9 only very limited degradation of active substance was observed. As a result, small amounts of three metabolites were detected (max. 4 %) which in their behaviour corresponded to the reference compounds N-oxide (M03), despropyl (M02) and desethyl (M01). Considering the hydrolytic stability determined under environmental pH and temperature conditions it is not expected that hydrolytic processes will contribute to the degradation of spiroxamine (KWG 4168) in the environment to any significant extent.

A second hydrolysis study by Krohn (1997, refer to DAR: IIA7.5/02) generally confirmed the results for Spiroxamine outlined above: The half-lives of isomer A of spiroxamine (KWG 4168) at pH 4 was approx. one year at 25 °C and 2 years at 20 °C. The isomer B was slightly unstable at pH 4 with half-lives of 68 days at 25 °C and 120 days at 20 °C, calculated by extrapolation from the rates of hydrolysis measured at 30 °C and 50 °C.

In conclusion it is shown that spiroxamine is hydrolytically stable under environmental relevant conditions.

5.1.1.2 Photochemical degradation in water

Author:	Hellpointner, E.
Title:	Determination of the quantum yield and assessment of the environmental half-life of the direct photodegradation of KWG 4168 in water (buffer pH 7)
Date:	1994
Doc ID:	PF4001 (BVL reg no 1797717) M-006008-01-1
Guidelines:	Phototransformation of Chemicals in Water, Part A: Direct Phototransformation, UBA, Berlin, FRG (Dec. 1992)
Deviations:	None
Status:	Old study, originally submitted for first Annex I inclusion
GLP:	Yes
Validity:	Acceptable

Author:	Brumhard, B
Title:	Photolysis of KWG 4168 in aqueous solution
Date:	1995
Doc ID:	PF4075 (BVL reg no 1797719) M-006004-01-1
Guidelines:	EPA Ref.: 161-2, Photodegradation Studies in Water
Deviations:	None
Status:	Old study, originally submitted for first Annex I inclusion
GLP:	Yes
Validity:	Acceptable

Investigations regarding the UV-Spectrum of spiroxamine (KWG 4168) showed no maximum of absorbance in the range of 200 - 400 nm for both isomers.

Under the experimental conditions used, [cyclohexyl-1-^{14}C]KWG 4168 degraded slowly with an experimental half-life of 50.5 days. The experimental half-life corresponds to a calculated environmental half-life of 236 days under worst case solar conditions.

It can be concluded from this study that photolysis in aqueous solution probably will be only of minor importance for the degradation of spiroxamine (KWG 4168) in the environment.

5.1.2 Biodegradation estimation

Water/sediment study

Author:	Scholz, K.
Title:	Aerobic metabolism of KWG 4168 in an aquatic model ecosystem
Date:	1995
Doc ID:	PF4029 (BVL reg no 1797725) M-006015-01-1
Guidelines:	BBA Ref.: Degradability and Fate of Plant Protection Products in Water sediment System 5-1 (Dec. 1990)
Deviations:	None
Status:	Old study, originally submitted for first Annex I inclusion
GLP:	Yes
Validity:	Acceptable

The degradation of spiroxamine (KWG 4168) in two water/sediment systems in the laboratory was investigated (Scholz, 1995) using [cyclohexyl-1-^{14}C]KWG 4168. The sediments and the water were collected from an artificially dammed pond (Hönninger Weiher, = HW; Germany) and from a pond in an agriculturally used area (Stilwell, = ST, Kansas, USA). The sediments were classified as silt loam and silty clay loam, with organic carbon contents of 4.4 % and 1.6 % and a pH of 6.2 and 7.8 respectively. The concentration of the active substance tested corresponded to the maximum commercial use rate of 750 g as/ha calculated as being directly-applied to a water body of 30 cm depth. The samples were incubated in the dark at 20 ± 2° C for a period of 100 days.

The distribution pattern of the radioactivity in the two water-sediment systems depended somewhat upon the type of system. The radioactive compounds were translocated during the incubation period somewhat quicker into the HW sediment (containing larger amounts of organic substance) than into the ST sediment. Thus, 72 % of the applied radioactivity was localised in the sediment of the HW system and 47 % in the sediment of the ST system after 24 hours. The degradation of the active substance was somewhat slower in the HW system than in the ST system.

As the incubation period progressed, the amount of radioactivity that was not extracted with organic solvents increased continuously until day 14 and day 56, respectively. The decrease of the unextracted part proved that the bound residue (or a part of it) is accessible to degradation and mineralisation. A total of 3-7 % of the applied radioactivity was liberated from the bound residue by reflux (3-6 % parent compound). The majority of the bound radioactivity remained in the sediment matrix.

A rapid disappearance of the active substance occurred in the supernatant water in both microecosystems. Only 1 % of the applied active substance could be detected in the HW supernatant water after 7 days and 2 % after 14 days in the ST water. In the HW sediment 43 % of unchanged parent compound was found using thin-layer chromatography after an incubation period of 100 days, compared to 24 % in the ST sediment. The DT_{50} for the degradation of the active substance in the test systems can be taken from table 37.

Table 52: DT_{50} values of spiroxamine (KWG 4168) in water/sediment systems

Water-Sediment System	**Supernatant Water**	**Water and Sediment**
	DT_{50} Values	**DegT50 Values**
Hönninger Weiher	13 hours	106 days
Stilwell	12 hours	28 days

Spiroxamine (KWG 4168) bound rapidly to the sediment. The compound was degraded in the ST system quicker than in the HW system. Independent of the type of water-sediment system used, the active substance was mineralised (to the point of $^{14}CO_2$). Toward the end of the test, 7 % of the applied radioactivity in the HW system and 17 % in the ST system were detected as $^{14}CO_2$.

Conclusion:

The results of this test show that spiroxamine (KWG 4168) was degraded in aquatic systems to ($DegT_{50}$ in the system 28 days and 106 days). Compound applied to the supernatant water bound rapidly to the sediment. The $DisT_{50}$ values for the supernatant water were 12 and 13 hours, respectively. Six metabolites were detected in the water sediment systems. Of these, metabolites acid (M6) and N-oxide (M3) were the main components. In one system was the mean value of one metabolite >10 % of the applied dose (11 % N-oxide, M3). This metabolite which was assumed to be in equilibrium with the active substance in the water, exceeded the 10 % mark at the first processing date (hour 0) in the ST system. The occurrence of the other metabolites was below 5 % at all sampling times.

No studies on ready biodegradability according to OECD 301 B and on inherent biodegradability were delivered. However, these studies are not deemed to be necessary, since higher tiered studies, namely simulation tests for the relevant environmental compartments ‘water/sediment’ and ‘soil’, are available, thus skipping the readily and inherent biodegradation test. Hence, spiroxamine is considered as not rapidly biodegradable.

In conclusion all available data show that spiroxamine did not actually degrade biotically or abiotically in the aquatic environment by > 70 % in 28 days. The criterion for rapid degradability in the sense of CLP regulation is not met.

5.2 Aquatic Bioaccumulation

5.2.1 Measured bioaccumulation data

Guideline / Test method	**Species**	**Endpoint / Type of test**	**Exposure**		**Results**	**Remarks**	**Reference**
			design	**duration**	**BCF**		
EPA-FIFRA 72-6 OECD 305E	*Lepomis macrochirus*	Bioconcentration whole fish	flow through system	28 d exposure 14 d depuration	87	steady-state approach	Grau, 1995

Spiroxamine rapidly concentrated in the fish and reached a plateau within a few days. The mean steady state BCF was calculated to be 70 to 90 with a single maximum value of 117. When

exposure ceased, the residue was depurated very quickly with a half-life of approximately 13 to 19 hours. The worst – case BCF for the whole fish is 87, based on total radioactivity.

5.3 Aquatic toxicity

This section provides the study results which reveal the most sensitive endpoints for each taxonomic group. Other fish and algae species have been tested. However, for reason of clarity these are not listed here. Tested species are: *Oncorhynchus mykiss*, *Lepomis macrochirus*, *Pimephales promelas*, *Scenedesmus subspicatus* (*Desmodesmus subspicatus*), *Selenastrum capricornutum* (*Pseudokirchneriella subcapitata*), *Anabaena flos-aquae* and *Navicula pelliculosa.*

5.3.1 Fish

5.3.1.1 Short-term toxicity to fish

Guideline / Test method	Species	Endpoint / Type of test	Exposure		Results [mg a.s./L]	Remarks	Reference
			design	duration	LC_{50}		
OECD 203 (rev. 1992)	*Danio rerio*	mortality	static	96 h	2.41	results based on geometric mean measured conc. of spiroxamine	Teigeler, 2008

Acute toxicity to Zebra fish (*Danio rerio*) was investigated according to OECD Guideline 203. Ten Zebra fish (mean body length 2.0 cm) per aquarium were exposed under static conditions for 96 h to nominal concentrations of 0.31, 0.63, 1.25, 2.5, 5.0 and 10 mg a.s./L. Mean measured concentrations were 0.278, 0.589, 1.22, 2.22, 4.27 and 9.27 mg a.s./L. pH values were in a range of 8.1 to 8.6 in the control group and test concentrations up to 5 mg a.s./L from test initiation until 96 h. In the highest test concentration the pH varied between 7.7 and 8.5 in the first 48 h. Afterwards no pH measuring has been conducted due to 100% mortality. The number of surviving fishes and possible sublethal effects were observed after 3, 24, 48, 72 and 96 h. A 96h-LC_{50} of 2.41 mg a.s./L related to mean measured concentration was determined.

5.3.1.2 Long-term toxicity to fish

Guideline / Test method	Species	Endpoint / Type of test	Exposure		Results [mg a.s./L]	Remarks	Reference
			design	duration	NOEC/EC_{10}		
see footnote[1)]	*Danio rerio*	mortality	flow through system	230 d	0.002	results based on nominal conc. of spiroxamine	Teigeler, 2009

[1)] OECD Guideline for Testing of Chemicals, 210 “Fish, Early Life Stage Toxicity Test”, 1992
OECD Guideline for Testing of Chemicals, 215 “Fish, Juvenile Growth Test”, 2000
OECD "Draft Proposal for a new Guideline: Fish Two-generation Test", 2002.
EPA-FIFRA § 72-5/SEP-EPA-540/9-86-137 "Standard Evaluation Procedure: Fish Life-Cycle Toxicity Tests", 1986
Nagel, R. (1998): Der vollständige Life Cycle Test (Complete Life Cycle Test, CLC Test) mit dem Zebrabärbling (Danio rerio, vormals Brachydanio rerio), Entwuf. UBA-Texte 58/98

A fish life cycle study was performed to examine the potential for long term adverse effects of the test item spiroxamine to fish populations. The fungicide is an ergosterol biosynthesis inhibitor, predominantly affecting the activity of 2 target enzymes: Δ^{14}-reductase and Δ^{8} - Δ^{7}-isomerase. Therefore, additional endocrine test parameters were included in the study to assess possible disturbance of the endocrine system of the exposed fish. The study investigated the effects of a continuous exposure to spiroxamine on different life stages of zebra fish (*Danio rerio*) during a full life cycle, including early life stages, juvenile growth, reproduction and early life stages of the filial generation. pH values were in a range of 7.4 to 8.6 (min. and max. values). The overall mean recovery of the test substance over the whole test period ranged between 92 and 101 %. Therefore the nominal values (0.0026, 0.0064, 0.016, 0.040 and 0.100 mg as/L) were used for the evaluation. The overall NOEC for the FFLC test was the EC_{10} for the survival observed in the F1-ELS of 2.0 µg as/L.

5.3.2 Aquatic invertebrates

5.3.2.1 Short-term toxicity to aquatic invertebrates

Guideline / Test method	Species	Endpoint / Type of test	Exposure		Results [mg a.s./L]	Remarks	Reference
			design	duration	EC_{50}		
OECD 202	*Daphnia magna*	Immobilisation	flow through system	48 h	3.0	results based on mean measured conc. of spiroxamine	Heimbach, 1997

The acute toxicity of spiroxamine to *Daphnia magna* was determined according to OECD 202. Juvenile daphnids were exposed under flow-through conditions to a series of seven test concentrations and a control. Nominal concentrations of spiroxamine were 0.44, 0.73, 1.4, 2.4, 4.3, 7.3 and 8.8 mg a.s./L. The pH values were in a range of 7.9 to 8.1. A 48 h-EC_{50} of 3.0 mg a.s./L related to mean measured concentration was determined.

5.3.2.2 Long-term toxicity to aquatic invertebrates

Guideline / Test method	Species	Endpoint / Type of test	Exposure		Results [mg a.s./L]	Remarks	Reference
			design	duration	NOEC		
OECD 202	*Daphnia magna*	Reproduction	flow through system	21 d	0.034	results based on mean measured conc. of spiroxamine	Heimbach, 1998

Effects of spiroxamine on reproduction, mortality, body length and dry weight of *Daphnia magna* were investigated according to OECD 202. Four replicates of five female water fleas each were exposed in a 21-day life cycle study to a series of seven concentrations of ^{14}C-spiroxamine (KWG 4168) and controls under flow-through test conditions. The solutions were renewed every two hours. Nominal test concentrations were 0.0056, 0.010, 0.018, 0.032, 0.056, 0.10 and 0.18 mg a.s./L The stock solutions in the flow-through-system were prepared three times during the test at day 0, 7

and 14. At the beginning and the end of each 7-day exposure period, the measured concentrations in the stock solutions of the test concentrations were determined by Gas Liquid Chromatography and the corresponding radioactivity values were measured. The radioactivity was measured in the test concentrations seven times during the study. Based on the total radioactivity in the test solutions and the measured ^{14}C-spiroxamine (KWG 4168) concentrations in the stock solutions, the actual concentrations in the test solutions were calculated. Based on these results, the mean measured test concentrations for spiroxamine were 0.0065, 0.011, 0.020, 0.034, 0.057, 0.11 and 0.19 mg a.s./L.

No dead offspring or aborted eggs were found in any test levels throughout the study. Also no abnormal behaviour of adult or juvenile organisms was observed. The NOECs have been determined as 0.034 mg as/L (0.032 mg as/L) for number of offspring / parent / reproduction day, 0.11 mg as/L (0.1 mg as/L) for body length of parent animals and dry weight of parent animals both related to mean measured (nominal) concentrations.

In the DAR (2005) and also in the Re-Assessment report (2010) for spiroxamine the mean measured test concentrations of 0.0065, 0.011, 0.020, 0.034, 0.057, 0.11 and 0.19 mg a.s./L and all NOEC/LOEC values inclusive the lowest NOEC of 0.034 mg/as/L were given as nominal by a typing error.

5.3.3 Algae and aquatic plants

Guideline / Test method	Species	Endpoint / Type of test	Exposure		Results [mg a.s./L]	Remarks	Reference
			design	duration			
ASTM, 1990 EPA, 1989	*Skeletonema costatum*	Inhibition of biomass	static	96 h	ErC_{50}: 0.0063 EbC_{50}: 0.0013 NOEC: 0.00063	results based on initial measured conc. of spiroxamine	Bowers, 1998

Effects of spiroxamine (Batch: C-618B; 98.2% purity) on biomass production of *Skeletonema costatum* were investigated according to ASTM and EPA. The marine diatom was exposed to a series of five concentrations of ^{14}C-spiroxamine (KWG 1468) under static test conditions over a period of 96 hours. Three replicates were prepared for each concentration and controls and each was inoculated with *Skeletonema costatum* cells at a nominal density of 10,000 cells/mL. Nominal (initial measured) test concentrations for spiroxamine were 0.63 (0.63), 1.25 (1.29), 2.5 (2.46), 5 (5.35) and 10 (10.36) µg a.s./L. Testing was conducted in an environmental chamber with a mean test temperature of 20.3°C, a photoperiod of 16 hour light and 8 hour dark and a light density of approximately 401 foot candles (4300 lux). The pH measurements ranged from 8.0-9.1 for all test levels during the exposure period. Sterile enriched saltwater media (ASTM 1990), salinity 25 ‰, was used in the test.

Each day, density was determined in all replicates at each concentration using a light microscope and an Improved Neubauer hemocytometer. Actual exposure concentrations were measured on day 0 and day 4 with Liquid scintillation counting and radio-thin layer chromatography (recovery of 97 – 107%). Temperature was recorded daily. The salinity and pH were measured on day 0 and day 4.No undissolved test substance was visually observed in the test vessels throughout the test period. The cell density was determined by direct cell counts. The parameter growth rate was analyzed by comparing the change in cell density from day 0 to day 4. The parameter biomass is based upon the cell density and area under the growth curve. The data were analyzed using the following statistical

tests: Shapiro-Wilks test for normality and Levene`s test for homogeneity of variance; ANOVA followed by Dunnett`s test. Fitting the Logistic Model using non-linear (Weighted) regression analysis was used to estimate the EC_{25} and EC_{50}.

The day 4 growth data was analyzed as cell density, growth rate and cumulative biomass. For each endpoint the data were analyzed using ANOVA followed by the Dunnett`s test to determine the lowest observed effect concentration (LOEC) and the no observed effect concentration (NOEC). The NOEC for both endpoints has been determined to be 0.00063 mg a.s./L and the LOEC 0.00129 mg a.s./L. The 96 h-E_rC_{50} related to growth rate for the marine diatom has been determined as 0.0063 mg as/L. The 96 h-E_bC_{50} related to biomass has been determined as 0.0013 mg a.s./L. All endpoints based on initially measured concentrations.

5.4 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

Endpoint	Classification Criteria (criteria in bold)		Evidence for Spiroxamine
	CLP (2nd ATP)	**DSD**	

<table>
<tr><td>Degradation Spiroxamine</td><td colspan="2">< 70 % ready or rapid degradation in 28 days
Spiroxamine is hydrolytically stable.
Photodegradation of Spiroxamine was moderate with an experimental half-life of 50.5 days under the test conditions.
No tests on ready biodegradability were available. The degradation half lifes in water/sediment studies are in the range of 28 days and 106 days.</td><td>In conclusion, all available data show that spiroxamine did not actually degrade biotically or abiotically in the aquatic environment by > 70 % in 28 days. The criterion for rapid degradability in the sense of classification and labelling is not met.</td></tr>
<tr><td>Bioaccumulation Spiroxamine</td><td>Log K_{ow} is < 4
Spiroxamine diastereomer A log K_{ow} = 2.79
Spiroxamine diastereomer B log K_{ow} = .292
at 20 °C</td><td>Log K_{ow} is < 3
Spiroxamine diastereomer A log K_{ow} = 2.79
Spiroxamine diastereomer B log K_{ow} = 2.92
at 20 °C</td><td>The measured log K_{ow} is 2.79 for diastereomer A and 2.92 for diastereomer B at 20 °C and is below the two classification criteria of 3 and 4, therefore Spiroxamine is considered to have a low bioaccumulation potential.</td></tr>
<tr><td rowspan="2">Acute aquatic toxicity Spiroxamine</td><td colspan="2">0.001 < L(E)C_{50} ≤ 0.01 mg/L</td><td rowspan="2">Spiroxamine is of high acute toxicity to algae (Skeletonema costatum) with an E_rC_{50} = 0.0063 mg a.s./L and fulfills the criteria for the proposed classification as R50-53 according to Directive 67/548/EEC and the criteria for the proposed classification as H400 according to Regulation EC 1272/2008. An acute M-factor of 100 is applicable based on 0.001 < L(E)C_{50} ≤ 0.01 mg/L.</td></tr>
<tr><td colspan="2">Skeletonema costatum E_rC_{50} = 0.0063 mg a.s./L</td></tr>
<tr><td rowspan="2">Chronic aquatic toxicity Spiroxamine</td><td>For non rapidly degradable substances:
0.0001 < NOEC ≤ 0.001</td><td rowspan="2"></td><td rowspan="2">Spiroxamine is of high chronic toxicity to algae (Skeletonema costatum) with a $NOEC_{growth\ rate}$ = 0.00063 mg a.s./L. Therefore, Spiroxamine fulfills the criteria for the proposed classification as H410 according to Regulation EC1272/2008. A chronic M-factor of 100 is applicable based on 0.0001 < NOEC ≤ 0.001 mg/L (no rapid degradation)</td></tr>
<tr><td>Skeletonema costatum
NOEC = 0.00063 mg a.s./L</td></tr>
<tr><td rowspan="6">SUMMARY</td><td rowspan="6">H400, M-factor $_{acute}$ = 100
H410, M-factor $_{chronic}$ = 100</td><td colspan="2">R50-53</td><td rowspan="6">PROPOSED CLASSIFICATION</td></tr>
<tr><td colspan="2">SCL are based on ErC50(96h) of S. costatum</td></tr>
<tr><td>Classification</td><td>Concentration [in %]</td></tr>
<tr><td>N, R50-53</td><td>Cn ≥ 0.25</td></tr>
<tr><td>N, R51/53</td><td>0.025 ≤ Cn < 0.25</td></tr>
<tr><td>R52/53</td><td>0.0025 ≤ Cn < 0.025</td></tr>
</table>

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.

<u>Degradation</u>

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

Acute aquatic hazard: 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 $0.001 < E_rC_{50} \leq 0.01$ 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 $0.0001 < NOEC \leq 0.001$ 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.

6 REFERENCES

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BVL registration number	Data protection claimed Y/N	Owner 2
KIIA 5.2 (OECD)	Kroetlinger, F.	1991	KWG 4168 - Study for acute oral toxicity in rats 20416 ! M007791-01-1 GLP: Y, published: N 1797552 / TOX9552588	N	BAY
KIIA 5.2 (OECD)	Kroetlinger, F.	1991	KWG 4168 - Study for acute oral toxicity in mice 20418 ! M-007804-01-1 GLP: Y, published: N 1797554 / TOX9552592	N	BAY
KIIA 5.2 (OECD)	Kroetlinger, F.	1991	KWG 4168 - Study for acute dermal toxicity in the rat 20417 ! M-007795-01-1 GLP: Y, published: N 1797556 / TOX9552589	N	BAY
KIIA 5.2 (OECD)	Pauluhn, J.	1990	KWG 4168 - Study for acute inhalation toxicity in the rat 19806 ! M-006477-01-1 GLP: Y, published: N 1797558 / TOX9552590	N	BAY
KIIA 5.2 (OECD)	Kroetlinger, F.	1991	KWG 4168 - Study for acute intraperitoneal toxicity in rats 20419 ! M-007996-01-1 GLP: Y, published: N 1797626 / TOX9552591	N	BAY
KIIA 5.2 (OECD)	Dreist, M.; Kolb, J.	1992	KWG 4168 - Studies on skin sensitising effect in guinea pigs (maximisation test according to Magnusson and Kligman) 21687 ! M-016682-01-1 GLP: Y, published: N 1797566 / TOX9552594	N	BAY
KIIA 5.2 (OECD)	Kroetlinger, F.; Kolb, J.	1992	KWG 4168 - Study for skin-sensitising effects in guinea pigs (Buehler Patch Test)	N	BAY

[2] Only notifier listed

			21716 ! M-006309-01-1 GLP: Y, published: N 1797568 / TOX9552595		
KIIA 5.2 (OECD)	Shelanski, Y. M.	2001	A patch test procedure to facilitate the expression and detection of the irritating and sensitising propensities of KWG 4168 107791 ! M-086474-02-1 GLP: Y, published: N 1797957 / ASB2008-2231	Y	BAY
KIIA 5.6 (OECD)	Becker, H.; Biedermann, K.	1992	Embryotoxicity study (including teratogenicity) with KWG 4168 technical in the rat R5574 ! M-006733-01-1 GLP: Y, published: N 1797610 / TOX9552620	N	BAY
KIIA 5.6 (OECD)	Anon.	2009	Spiroxamine: Data from prenatal development toxicity studies – Historical control reproduction data on Wistar rat Renewal 2009, M-344107-01-1, ASB2009-1678	?	BAY
KIIA 5.6 (OECD)	Becker, H.	1993	Spiroxamine: Dose range-finding embryotoxicity study (including teratogenicity) with KWG 4168 in the rat Renewal 2009, 286648 ! R6072 ! M 0-99-008769, GLP: N, published: N, ASB2009-2026	?	BAY
KIIA 5.6 (OECD)	Becker, H.	1995	Spiroxamine: Range finding studies with KWG 4168 technical in the rat (confidential), R6343 ! M0-99-009584 ! 268075 ! 272610 ! 277931 ! T 7037395, GLP: N, published: N, ASB2009-2106	?	BAY
KIIA 5.6 (OECD)	Becker, H.; Biedermann, K.	1995	Spiroxamine: Combined report of embryotoxicity screening study (including teratogenicity) and supplementary study to the embryotoxicity screening study (including teratogenicity) with KWG 4168 technical in the rat (Part I of II) Renewal 2009, R 6355 ! M0-99-008687 ! T 0034706 ! T 7037395 ! 263068 ! 281507, GLP: N, published: N, ASB2009-2096	?	BAY
KIIA 5.6 (OECD)	Becker, H.; Biedermann, K.	1993	Embryotoxicity study (including teratogenicity) with KWG 4168 technical in the rat (dermal application) R5952 ! M-006820-02-1 GLP: Y, published: N 1797628 / TOX9552621	N	BAY
KIIA 5.6 (OECD)	Holzum, B.	1995	KWG 4168 - Studies for embryotoxic effects in rabbits following oral	N	BAY

			administration 23662 ! M-006707-02-1 GLP: Y, published: N 1797612 / TOX9552623		
KIIA 5.6 (OECD)	Anon.	1995/ 2000	Spiroxamine: Raw Data Pilot development study on rabbits (cited in report 23662: KWG 4168 – Studies for embryotoxic effects in rabbits following oral administration dated 20.01.1995, amended 10.10.2000), Renewal 2009, M-344109-01-1, GLP: ?, published: N, ASB2009-2104	?	BAY
KIIA 5.6 (OECD)	Anon.	1990	Spiroxamine: Historical control data of malformations in control and treated groups of rabbits (Rabbit CHBB:HM) – Data from prenatal development toxicity studies performed during 1989 to 1996, Renewal 2009, M-344103-01-1; ASB2009-2107	?	BAY
KIIA 5.6 KIIA 5.9 (OECD)	Henninger, K.	2009	Spiroxamine. Regulatory toxicology – Response of BCS to requests raised by BfR after submission of the dossier for Annex I Renewal, Renewal 2009, M-344272-01-2, ASB2009-2108	?	BAY
KIIA 7.5	Brumhard, B.	1995	Hydrolysis of KWG 4168 in sterile aqueous buffer solutions	Y	BAY
KIIA 7.5	Krohn, J.	1997	Hydrolysis of KWG 4168 (Spiroxamine, proposed) as a function of pH	Y	BAY
KIIA 7.6	Hellpointner, E.	1994	Determination of the quantum yield and assessment of the environmental half-life of the direct photodegradation of KWG 4168 in water (buffer pH 7)	Y	BAY
KIIA 7.6	Brumhard, B.	1995	Photolysis of KWG 4168 in aqueous solution	Y	BAY
KIIA 7.8.3	Scholz, K.	1995	Aerobic metabolism of KWG 4168 in an aquatic model ecosystem	Y	BAY
KIIA 8.2.1 (OECD)	Teigeler, M.	2008	Acute Toxicity of Spiroxamine to Zebra fish (*Danio rerio*) over 96 hours	Y	BAY
KIIA 8.2.5	Teigeler, M.	2009	Zebra Fish, Life Cycle Test, Flow through Conditions	Y	BAY
KIIA 8.2.6	Grau, R.	1995	KWG 4168: Bioconcentration in Bluegill-Sunfish.	Y	BAY
KIIA 8.3.1	Heimbach, F.	1997	Acute Toxicity of ^{14}C-Spiroxamine (techn.) to Water fleas (*Daphnia magna*) under Flow-Through Test Conditions	Y	BAY
KIIA 8.3.2.1	Heimbach, F.	1998	Influence of ^{14}C-Spiroxamine (technical) on the Reproduction of Water Fleas under Flow-Through Test Conditions.	Y	BAY
KIIA 8.11.1	Bowers, L.M.	1998	Toxicity of ^{14}C-KWG 4168 to the marine diatom *Skeletonema costatum*.	Y	BAY

Additional references
Additional references not included in the CLH report Bruns E, Arnold M, Krenbber R, Brumhard B, Schöning R, Strauß T (2008). Biological effects and fate of spiroxamine EC 500 in outdoor mesocosm ponds simulating actual exposure conditions in agricultural use. Heimbach F, Brock TCM, Deneer JW (2000). Fate of spiroxamine in enclosures of an experimental ditch.

7 ANNEXES

Becker, H. and K. Biedermann (1995): Dose range-finding study in rats (R6355)

Clinical symptoms observed at 150 mg/kg bw/d

A = Ruffled fur; B = Spasms; C = Ataxia; D = Ventro-lateral recumbency;
E = Paddling movements; F = Dyspnea; G = Sedated; H = Rolling movements;
I = Comatose state; J = Diarrhea mucus; K = Hunched posture

Day post coitum	7	8	9	10	11
Number of live females	25	25	25	25	25
Found dead in the morning					
Died during the day					2
SYMPTOMS					
A	5 (20%)	5 (20%)	25 (100%)	25 (100%)	25 (100%)
B		3 (12%)	25 (100%)	25 (100%)	25 (100%)
C	2 (8%)	3 (12%)	25 (100%)	25 (100%)	25 (100%)
D		3 (12%)	25 (100%)	25 (100%)	21 (84%)
E		3 (12%)	25 (100%)	24 (96%)	21 (84%)
F		2 (8%)	24 (96%)	25 (100%)	24 (96%)
G	5 (20%)	5 (20%)	5 (20%)	15 (60%)	25 (100%)
H		3 (12%)	5 (20%)	4 (16%)	2 (8%)
I					1 (4%)
J					
K	2 (8%)	5 (20%)	5 (20%)	5 (20%)	13 (52%)

Day post coitum	12	13	14	15	16
Number of live females	22	17	13	9	5
Found dead in the morning	1	3	1	2	
Died during the day	2	3	2	4	1
SYMPTOMS					
A	22 100%)	17 (100%)	13 (100%)	9 (100%)	4 (80%)
B	20 (90.9%)	13 (76.5%)	10 (76.9%)	7 (77.7%)	3 (60%)
C	21 (95.5%)	16 (94.1%)	13 (100%)	9 (100%)	3 (60%)
D	10 (45.5%)	5 (29.4%)	5 (38.5%)	2 (22.2%)	
E	10 (45.5%)	5 (29.4%)	5 (38.5%)	2 (22.2%)	
F	22 (100%)	16 (94.1%)	13 (100%)	8 (88.9%)	3 (60%)
G	22 (100%)	17 (100%)	13 (100%)	9 (100%)	4 (80%)
H	3 (13.6%)	5 (29.4%)	5 (38.5%)	2 (22.2%)	
I	3 (13.6%)				
J		2 (11.8%)			
K	22 (100%)	16 (94.1%)	13 (100%)	9 (100%)	4 (80%)

Day post coitum	17	18	19	20	21
Number of live females	4	4	4	4	4
Found dead in the morning					
Died during the day					1
SYMPTOMS					
A	3 (75%)	3 (75%)	2 (50%)	2 (50%)	1 (25%)
B	2 (50%)				
C	3 (75%)				
D					
E					
F	3 (75%)				
G	3 (75%)	1 (25%)			
H					
I					
J					
K	3 (75%)	3 (75%)	1 (25%)		

Further details on clinical signs recorded in the dose range-finding study in rats (R6355)

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INDIVIDUAL CLINICAL SIGNS

Group 1 (vehicle control) : No clinical signs were observed

Group 2 (150 mg/kg) :

Female No.	Days post coitum 7	8	9	10	11	12	13	14
26			A,B,C, D,E,F	A,B,C, D,E,F	A,B,C, D,E,F, G	A,B,C, D,E,F, G,K	A,B,C, F,G,K	A,B,C, F,G,K
27			A,B,C, D,E,F	A,B,C, D,E,F	A,B,C, D,E,F, G	A,B,C, D,E,F, G,K	A,B,C, F,G,K	A,B,C, F,G,K
28			A,B,C, D,E,F	A,B,C, D,E,F	A,B,C, D,E,F, G	A,B,C, D,E,F, G,H,I,K	L	
29			A,B,C, D,E,F	A,B,C, D,E,F	A,B,C, D,E,F, G	A,B,C, D,E,F, G,K	A,B,C, F,G,K	L
30			A,B,C, D,E,F	A,B,C, D,E,F	A,B,C, D,E,F, G	A,B,C, D,E,F, G,K	L	
31			A,B,C, D,E,F	A,B,C, D,E,F	A,B,C, D,E,F, G	A,B,C, D,E,F, G,K	A,B,C, F,G,J, K, M	
32			A,B,C, D,E,F	A,B,C, D,E,F	A,B,C, D,E,F, G	A,B,C, D,E,F, G,K	A,B,C, D,E,F, G,H,K	A,B,C, D,E,F, G,H,K,M
33			A,B,C, D,E,F	A,B,C, D,E,F	A,B,C, D,E,F, G	A,B,C, F,G,K	A,G	A,B,C, F,G,K
34			A,B,C, D,E,F	A,B,C, D,E,F	A,B,C, D,E,F, G	A,B,C, D,E,F, G,K	A,B,C, F,G,K	A,B,C, F,G,K
35			A,B,C, D,E,F	A,B,C, D,E,F	A,B,C, D,E,F, G	A,B,C, D,E,F, G,H,I,K	A,B,C, F,G,J, K,M	

A = Ruffled fur; B = Spasms; C = Ataxia; D = Ventro-lateral recumbency;
E = Paddling movements; F = Dyspnea; G = Sedated; H = Rolling movements;
I = Comatose state; J = Diarrhea mucus; K = Hunched posture;
L = Found dead in the morning; M = Died during the day.

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INDIVIDUAL CLINICAL SIGNS

Group 2 (150 mg/kg, cont'd):

Female No.	Days post coitum 7	8	9	10	11	12	13	14
36			A,B,C, D,E,F	A,B,C, D,E,F, G	A,B,C, D,E,F, G,K	A,C,F G,K	A,B,C, F,G,K	A,B,C, F,G,K,M
37			A,B,C, D,E,F	A,B,C, D,E,F, G	A,B,C, D,E,F, G,K	A,B,C, F,G,K	A,B,C, F,G,K, M	
38			A,B,C, D,E,F	A,B,C, D,E,F, G	A,B,C, D,E,F, G,K	A,B,C, F,G,K	A,B,C, D,E,F, G,H,K	A,B,C, D,E,F, G,H,K
39			A,B,C, D,E,F	A,B,C, D,E,F, G	A,B,C, D,E,F, G,K	A,B,C, F,G,K	L	
40			A,B,C, D,E,F	A,B,C, D,E,F, G	A,B,C, D,E,F, G,K	A,B,C, F,G,K, M		
41			A,B,C, D,E,F	A,B,C, D,E,F, G	A,B,C, G,K	A,B,C, F,G,K	A,B,C, D,E,F, G,H,K	A,B,C, D,E,F, G,H,K
42			A,B,C, D,E,F	A,B,C, D,E,F, G	A,B,C, D,E,F, G,K	A,B,C, D,E,F, G,H,I, K,M		
43			A,B,C, D,E,F	A,B,C, D,E,F, G	A,B,C, D,E,F, G,I,M			
44			A,B,C, D,E,F	A,B,C, D,E,F, G	A,B,C, D,E,F, G,K	A,B,C, F,G,K	A,B,C, D,E,F, G,H,K	A,B,C, D,E,F, G,H,K
45			A,B,C, D,E,F	A,B,C, D,E,F, G	A,B,C, D,E,F, G,K	A,B,C, F,G,K	A,B,C, D,E,F, G,H,K	A,B,C, D,E,F, G,H,K

A = Ruffled fur; B = Spasms; C = Ataxia; D = Ventro-lateral recumbency;
E = Paddling movements; F = Dyspnea; G = Sedated; H = Rolling movements;
I = Comatose state; J = Diarrhea mucus; K = Hunched posture;
L = Found dead in the morning; M = Died during the day.

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INDIVIDUAL CLINICAL SIGNS

Group 2 (150 mg/kg, cont'd):

Female No.	Days post coitum 7	8	9	10	11	12	13	14
46	A,C,G, K	A,G,K	A,B,C, D,E,F, G,H,K	A,B,C, D,E,F, G,H,K	A,B,C, D,E,F, G,H,M			
47	A,C,G, K	A,G,K	A,B,C, D,E,F, G,H,K	A,B,C, D,E,F, G,H,K	A,B,C, D,E,F, G,H,K	A,B,C, F,G,K	A,C,F, G,K	A,C,F, G,K
48	A,G	A,B,C, D,E,G, H,K	A,B,C, D,E,F, G,H,K	A,B,C, D,E,F, G,H,K	A,B,C, F,G,K	A,C,F, G,K	A,C,F, G,K	A,C,F, G,K
49	A,G	A,B,C, D,E,F, G,H,K	A,B,C, D,E,G, H,K	A,B,C, D,F,G, K	A,B,C, F,G,K	A,B,F, G,K	A,C,F, G,K	A,C,F, G,K
50	A,G	A,B,C, D,E,F, G,H,K	A,B,C, D,E,F, G,H,K	A,B,C, D,E,F, G,H,K	A,B,C, F,G,K	L		

A = Ruffled fur; B = Spasms; C = Ataxia; D = Ventro-lateral recumbency;
E = Paddling movements; F = Dyspnea; G = Sedated; H = Rolling movements;
I = Comatose state; J = Diarrhea mucus; K = Hunched posture;
L = Found dead in the morning; M = Died during the day.

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INDIVIDUAL CLINICAL SIGNS

Group 2 (150 mg/kg, cont'd):

Female No.	Days post coitum 15	16	17	18	19	20	21	
26	A,B,C, F,G,K, M							
27	A,B,C, F,G,K	A,B,C, F,G,K	A,B,C, F,G,K	A,G,K	A,K	A	A	
33	A,B,C, F,G,K, M							
34	A,B,C, F,G,K	A,G,K, M						
38	A,B,C, G,K	A,B,C, F,G,K	A,B,C, F,G,K	A,K				
41	A,B,C, D,E,F, G,H,K	A,B,C, F,G,K	A,C,F, G,K	A,K	A	A		
44	A,B,C, D,E,F, G,H,K, M							
45	L							
47	A,C,F, G,K,M							
48	A,C,F, G,K							
49	L							

A = Ruffled fur; B = Spasms; C = Ataxia; D = Ventro-lateral recumbency;
E = Paddling movements; F = Dyspnea; G = Sedated; H = Rolling movements;
I = Comatose state; J = Diarrhea mucus; K = Hunched posture;
L = Found dead in the morning; M = Died during the day.

Historical control data for developmental toxicity studies in rabbits performed during 1989 to 1996 at Bayer Institute of Toxicology

Extracted from Anon., 2009, ASB2009-2107

No further details are available to the dossier submitter

Historical Data (1989)

Vehicle

Species: **Rabbit** Strain: **CHBB:HM**

Study	Vehicle
T7032002++	0.5% aqueous cremophor emulsion
T0032834	0.5% aqueous cremophor emulsion

++ dermal application

Malformations in different study groups in an oral developmental toxicity study (T0032834) in rabbits (CHBB:HM) which was performed from May 02, 1989 to January 17, 1990

Malformation	Dose group			
	Control	Low	Medium	High
arthrogryposis[a]			3 (2)	2 (2)
epignathus			1	
number of fetuses per group	84	89	77	70
number of fetuses with malformations	0	0	4	2
malformed fetuses per group (%)	0	0	5.2	2.9
number of litters per group	15	15	12	14
number of litters with malformations	0	0	3	2
malformed litters per group (%)	0	0	25.0	14.3

() number of litters affected

[a] now called malposition of forelimb(s)

Historical Data (1990)

Vehicle

Species: **Rabbit** Strain: **CHBB:HM**

Study	Vehicle
T3033881	0.5% aqueous cremophor emulsion
T9037397	0.5% aqueous cremophor emulsion

Malformations in different study groups in an oral developmental toxicity study (T3033881) in rabbits (CHBB:HM) which was performed from January 08, 1990, to August 22, 1990

Malformation	Dose group			
	Control	Low	Medium	High
arthrogryposis[a]	1		1	3 (3)
blockage of left nostril	1			
vertebral malformation, floating rib				1
small orbital cavity, folded retina				2 (2)
asymmetrical coccygeal vertebra	2 (2)			
number of fetuses per group	80	60	72	72
number of fetuses with malformations	4	0	1	6
malformed fetuses per group (%)	5.0	0	1.4	8.3
number of litters per group	15	12	15	13
number of litters with malformations	4	0	1	4
malformed litters per group (%)	26.7	0	6.7	30.8

() number of litters affected

[a] now called malposition of forelimb(s)

Malformations in different study groups in an oral developmental toxicity study (T9037397) in rabbits (CHBB:HM) which was performed from September 25, 1990, to February 14, 1991

Malformation	Dose group			
	Control	Low	Medium	High
cleft palate				5 (2)
bifurcation of rib		1		
floating rib	1			
vertebrae and ribs changes			1	
arthrogryposis[a]		1	5 (3)	1
tail shortened				1
multiple malformation			2 (2)	
blockage of nostrils	1			
number of fetuses per group	77	78	66	37
number of fetuses with malformations	2	2	7	7*
malformed fetuses per group (%)	2.6	2.5	10.6	18.9
number of litters per group	15	14	13	10
number of litters with malformations	2	2	5	3
malformed litters per group (%)	13.3	14.2	38.4	30.0

() number of litters affected

[a] now called malposition of forelimb(s)

* statistically significant with $p < 0.05$

Historical Data (1991)

Vehicle

Species: **Rabbit** Strain: **CHBB:HM**

Study	Vehicle
T3039597	0.5% aqueous cremophor emulsion
T4040262	0.5% aqueous cremophor emulsion
T4040127	0.5% aqueous tylose suspension

Malformations in different study groups in an intravenous developmental toxicity study (T3039597) in rabbits (CHBB:HM) which was performed from January 02, 1991 to October 06, 1992

Malformation	Dose group			
	Control	Low	Medium	High
multiple malformations	2 (2)			1
arthrogryposis[a]	5 (3)	1	2 (2)	3 (3)
12th thoracic vertebral body missing, 12th thoracic vertebral arch fused with 1st lumbar vertebral arch (left)	1			
missing thoracic vertebra, 12th rib bilateral at 1st lumbar vertebra, presacral dislocation of pelvis				2 (1)
supernumerary lumbar vertebra with 13th rib	1			1
enlargement of second proximal phalange of left forelimb			1	
missing proximal, medial and distal phalangeal digits				1
hydrocephalus internus, caudal displacement of ears				1
chicken breast				2 (2)
number of fetuses per group	95	87	87	89
number of fetuses with malformations	8	1	3	9
malformed fetuses per group (%)	8.4	1.1	3.4	10.1
number of litters per group	14	14	14	14
number of litters with malformations	6	1	3	6
malformed litters per group (%)	42.9	7.1	21.4	42.9

() number of litters affected

[a] now called malposition of forelimb(s)

Malformations in different study groups in an intravenous developmental toxicity study (T4040262) in rabbits (CHBB:HM) which was performed from July 10, 1991 to June 01, 1992

Malformation	Dose group	
	Control	Dose
arthrogryposis[a]		2 (2)
missing thoracic vertebra, 12th rib bilateral at 1st lumbar vertebra, presacral dislocation of pelvis	1	
missing thoracic vertebra, 12th rib right at 1st lumbar vertebra, 12th rib left missing		1
slight curvature in spinal column due to absence of 10th thoracic vertebral body and left 10th thoracic vertebral arch; floating 10th rib left		1
iliac bone positioned at 7th lumbar vertebra	1	
anomaly of coccygeal vertebra (fusion, asymmetry, dislocation)	2 (2)	2 (2)
number of fetuses per group	99	85
number of fetuses with malformations	4	6
malformed fetuses per group (%)	4.0	7.1
number of litters per group	15	13
number of litters with malformations	4	4
malformed litters per group (%)	26.7	30.8

() number of litters affected

[a] now called malposition of forelimb(s)

Malformations in different study groups in an oral developmental toxicity study (T4040127) in rabbits (CHBB:HM) which was performed from September 02, 1991 to January 22, 1993

Malformation	Dose group			
	Control	Low	Medium	High
arthrogryposis[a]	5 (3)	2 (2)	2 (2)	
12th thoracic vertebra missing, 12th rib bilateral at 1st lumbar vertebra	1			
one supernumerary lumbar vertebra, 13th rib bilateral at 1st lumbar vertebra				1
pelvis left shift to caudal		1		
tail vertebral anomaly (supernumerary ossifications center resp. fusion)	1		1	
number of fetuses per group	90	105	97	23
number of fetuses with malformations	7	3	3	1
malformed fetuses per group (%)	7.8	2.9	3.1	4.4
number of litters per group	14	15	14	4
number of litters with malformations	4	3	3	1
malformed litters per group (%)	28.6	20.0	21.4	25.0

() number of litters affected

[a] now called malposition of forelimb(s)

Historical Data (1992)

Vehicle

Species: **Rabbit** Strain: **CHBB:HM**

Study	Vehicle
T4040749	aqua dest.

Malformations in different study groups in an oral developmental toxicity study (T4040749) in rabbits (CHBB:HM) which was performed from January 14, 1992 to July 28, 1992

Malformation	Dose group			
	Control	Low	Medium	High
supernumerary 13th rib, supernumerary lumbar vertebra	1			
iliac bone fused with sacral vertebral arch	2 (1)			
fusion of 11th + 12th rib	1			
fusion of caudal vertebral bodies	1	1	1	
arthrogryposis[a]	1			1
12th rib at 1st lumbar vertebra		1		
only 11 thoracic vertebrae present, 12th rib at 1st lumbar vertebra, presacral dislocation of pelvis				1
number of fetuses per group	80	105	93	79
number of fetuses with malformations	6	2	1	2
malformed fetuses per group (%)	7.50	1.90	1.08	2.53
number of litters per group	13	15	15	13
number of litters with malformations	3	2	1	2
malformed litters per group (%)	23.1	13.3	6.7	15.4

() number of litters affected

[a] now called malposition of forelimb(s)

Historical Data (1993)

Vehicle

Species: **Rabbit** Strain: **CHBB:HM**

Study	Vehicle
T1039496	0.5% aqueous tylose suspension
T5044151	0.5% aqueous tylose suspension
T5044250#	aqua dest.
T8050067	0.5% aqueous tylose suspension

Strain: Mol Russian

Malformations in different study groups in an oral developmental toxicity study (T1039496) in rabbits (CHBB:HM) which was performed from January 14, 1993 to June 23, 1993

Malformation	Dose group			
	Control	Low	Medium	High
arthrogryposis[a]		1	1	
kidney, missing	1			1
ureter, missing	1			
heart, cardiac septum defect			1	
heart, cardiac septum defect + major vessel malformation			1	
hydrocephalus internus				1
12th thoracic vertebra, missing				
12th rib at 1st lumbar vertebra				
- present			1	
- missing (left or right)		2		
- comma shaped	1			
7th lumbar vertebra, missing		1		
coccygeal vertebra				
- fusion		1		
- enlarged		1		
- supernumerary ossification center			1	
number of fetuses per group	115	105	101	86
number of fetuses with malformations	2	6	5	2
malformed fetuses per group (%)	1.74	5.71	4.95	2.33
number of litters per group	15	14	14	11
number of litters with malformations	2	5	3	1
malformed litters per group (%)	13.3	35.7	21.4	9.1

() number of litters affected

[a] now called malposition of forelimb(s)

Malformations in different study groups in an oral developmental toxicity study (T5044151) in rabbits (CHBB:HM) which was performed from March 16, 1993 to December 12, 1993

Malformation	Dose group			
	Control	Low	Medium	High
arthrogryposis[a]		3 (3)	1	1
acrania				1
hernia abdominalis, sternal cleft		1		
missing gallbladder	1			
malformation of heart and major vessels	1		1	
hydrocephalus internus		1		
anomaly of vertebrae	1	1	2 (2)	
12th thoracic vertebra missing, 12 rib at 1st lumbar vertebra, presacral dislocation of pelvis	1		1	
fusion of ribs			1	
presacral dislocation of pelvis	1			
number of fetuses per group	132	120	137	140
number of fetuses with malformations	5	6	5	2
malformed fetuses per group (%)	3.8	5.0	3.7	1.4
number of litters per group	20	19	21	21
number of litters with malformations	4	5	4	2
malformed litters per group (%)	20.0	26.3	19.1	9.5

() number of litters affected

[a] now called malposition of forelimb(s)

Malformations in different study groups in an oral developmental toxicity study (T5044250) in rabbits (females Mol Russian, males CHBB:HM) which was performed from May 17, 1993 to July 23, 1993

Malformation	Dose group			
	Control	Low	Medium	High
arthrogryposis[a]	1	6 (4)	2 (2)	5 (4)
distal and medial phalanx of digits missing	1			
12th thoracic vertebra missing, 12 rib at 1st lumbar vertebra			1	
12th thoracic vertebra missing, 12 rib at 1st lumbar vertebra, presacral dislocation of pelvis	2 (2)	4 (2)	2 (2)	
one supernumerary lumbar vertebra, 13th rib				
- right comma shaped	1			
- bilateral present				1
anomalies of caudal vertebrae (supernumerary ossification center, fusion, asymmetric)	1		1	2 (2)
number of fetuses per group	90	96	93	79
number of fetuses with malformations	6	10	6	7
malformed fetuses per group (%)	6.67	10.42	6.45	8.86
number of litters per group	16	16	16	16
number of litters with malformations	6	4	5	5
malformed litters per group (%)	37.5	25.0	31.3	31.3

() number of litters affected

[a] now called malposition of forelimb(s)

Malformations in different study groups in an oral developmental toxicity study (T8050067) in rabbits (CHBB:HM) which was performed from October 18, 1993 to December 17, 1993

Malformation	Dose group			
	Control	Low	Medium	High
arthrogryposis[a]	1	1	3 (3)	4 (2)
cleft palate	1			
heart malformation				
- septum malformation	1			
+ truncus arteriosus	1			1
vertebral malformation				
- supernumerary lumbar vertebra	1			
- missing thoracic vertebra				1
rib malformation				
- fusion	1			
- fusion at cartilaginous part			1	
- distal end thickened		1		
number of fetuses per group	106	90	91	85
number of fetuses with malformations	5	2	4	5
malformed fetuses per group (%)	4.7	2.2	4.4	5.9
number of litters per group	16	15	14	13
number of litters with malformations	5	2	4	3
malformed litters per group (%)	31.3	13.3	28.6	23.1

() number of litters affected

[a] now called malposition of forelimb(s)

Historical Data (1994)

Vehicle

Species: **Rabbit** Strain: **CHBB:HM**

Study	Vehicle
T6055394	0.5% aqueous tylose suspension
T8055549	0.5% aqueous tylose suspension
T3058028	0.5% aqueous tylose suspension
T6058030	0.5% aqueous tylose suspension

Malformations in different study groups in an oral developmental toxicity study (T6055394) in rabbits (CHBB:HM) which was performed from February 09, 1994 to April 28, 1994

Malformation	Dose group			
	Control	Low	Medium	High
arthrogryposis[a]	2 (2)	1	2 (1)	1
exencephaly (occulta)			1	
heart malformation		1		
malformation of ribs	2 (2)	7 (5)		
malformation of ribs and vertebrae	1			
missing lumbar vertebra			1	
dislocation of pelvis	1			
- with finding of lumbar vertebra	1			1
fused or asymmetrical caudal vertebrae	1	1		1
number of fetuses per group	101	75	88	99
number of fetuses with malformations	8	10	4	3
malformed fetuses per group (%)	7.9	13.3	4.6	3.0
number of litters per group	16	14	15	14
number of litters with malformations	6	6	3	3
malformed litters per group (%)	37.5	42.9	20.0	21.4

() number of litters affected

[a] now called malposition of forelimb(s)

Malformations in different study groups in an oral developmental toxicity study (T8055549) in rabbits (CHBB:HM) which was performed from April 11, 1994 to June 22, 1994

Malformation	Dose group			
	Control	Low	Medium	High
arthrogryposis[a]	1		3 (2)	1
12th thoracic vertebra missing	1			
+ 12th ribs missing				1
supernumerary lumbar vertebra			1	
15th caudal vertebral body asymmetrical position		1		
7th + 8th ribs fused and thickened		1		
dislocation of pelvis	2 (2)	2 (1)		
heart septum defect		1		
gallbladder missing		1		
hydrocephalus internus			1	
multiple malformation				1
number of fetuses per group	81	105	106	96
number of fetuses with malformations	4	6	5	3
malformed fetuses per group (%)	4.9	5.7	4.7	3.1
number of litters per group	16	15	16	16
number of litters with malformations	4	4	4	3
malformed litters per group (%)	25.0	26.7	25.0	18.8

() number of litters affected

[a] now called malposition of forelimb(s)

Malformations in different study groups in an oral developmental toxicity study (T3058028) in rabbits (CHBB:HM) which was performed from August 16, 1994 to February 17, 1995

Malformation	Dose group			
	Control	Low	Medium	High
arthrogryposis[a]		2 (1)	1	1
multiple malformation (including major vessel malformation, missing phalanges)				1
missing phalanx of digit		1		
malformation of ribs	2 (2)	1		1
malformation of ribs and vertebrae			1	
presacral dislocation of pelvis				1
fusion of caudal vertebrae	1		1	1
number of fetuses per group	94	98	91	93
number of fetuses with malformations	3	4	2	5
malformed fetuses per group (%)	3.2	4.1	2.2	5.4
number of litters per group	16	15	15	15
number of litters with malformations	3	4	2	3
malformed litters per group (%)	18.8	26.7	13.3	20.0

() number of litters affected

[a] now called malposition of forelimb(s)

Malformations in different study groups in an oral developmental toxicity study (T6058030) in rabbits (CHBB:HM) which was performed from September 28, 1994 to December 07, 1994

Malformation	Dose group				
	Control	Low	Medium	High I	High II
arthrogryposis[a] alposition of forelimb(s)	1	5 (2)	1	2 (1)	
distal phalanx missing	1				
cleft maxilla		1			
+ cleft palate	1				
skull and maxilla deformation + missing eye		1			
fusion of caudal vertebral bodies					1
rib malformation (fusion, floating, thickened)	2 (2)	1	5 (3)		
heart + major vessel malformation	1			1	2 (2)
gallbladder missing					1
number of fetuses per group	116	122	103	84	115
number of fetuses with malformations	3	7	6	3	4
malformed fetuses per group (%)	2.6	5.7	5.8	3.6	3.5
number of litters per group	16	16	15	15	16
number of litters with malformations	2	4	4	2	3
malformed litters per group (%)	12.5	25.0	26.7	13.3	18.8

() number of litters affected

[a] now called malposition of forelimb(s)

Historical Data (1995)

Vehicle

Species: **Rabbit** Strain: **CHBB:HM**

Study	Vehicle
T0058034	0.5% aqueous tylose suspension
T5059074	0.5% aqueous carboxymethylcellulose (high viscosity)
T0059079++	tap water

++ dermal application

Malformations in different study groups in an oral developmental toxicity study (T0058034) in rabbits (CHBB:HM) which was performed from March 21, 1995 to June 20, 1995

Malformation	Dose group				
	Control	Low	Medium	High I	High II
multiple malformation				1	
arthrogryposis[a]	1	5 (4)	1	2 (2)	2 (2)
malposition of hind limbs		1			
supernumerary lumbar vertebra		1	1		
supernumerary lumbar vertebra and missing phalanges / metacarpal	1				
cardiac septum defect				1	
number of fetuses per group	107	115	96	100	71
number of fetuses with malformations	1	7	2	4	2
malformed fetuses per group (%)	0.9	6.1	2.1	4.0	2.8
number of litters per group	15	15	14	13	11
number of litters with malformations	1	5	2	4	2
malformed litters per group (%)	6.7	33.3	14.3	30.8	18.2

() number of litters affected

[a] now called malposition of forelimb(s)

Malformations in different study groups in an oral developmental toxicity study (T5059074) in rabbits (CHBB:HM) which was performed from June 21, 1995 to September 15, 1995

Malformation	Dose group			
	Control	Low	Medium	High
arthrogryposis[a]	3 (2)	1	3 (3)	5 (2)
small orbital cavity	1			
hydrocephalus internus		1	1	
cardiac septum defect	1	1	3 (3)	2 (1)
missing kidney				1
missing gallbladder	1	1		
fusion of ribs (cartilaginous part)		2 (2)		
supernumerary lumbar vertebra			1	
supernumerary lumbar vertebra with 13 ribs				2 (2)
number of fetuses per group	151	132	172	114
number of fetuses with malformations	6	6	8	10
malformed fetuses per group (%)	4.0	4.6	4.7	8.8
number of litters per group	22	20	24	19
number of litters with malformations	4	5	7	6
malformed litters per group (%)	18.2	25.0	29.2	31.6

() number of litters affected

[a] now called malposition of forelimb(s)

Historical Data (1996)

Vehicle

Species: **Rabbit** Strain: **CHBB:HM**

Study	Vehicle
T4060080	0.5% aqueous tylose suspension
T0060086	0.5% aqueous carboxymethylcellulose (high viscosity)
T1060087	0.5% aqueous carboxymethylcellulose (high viscosity)

Malformations in different study groups in an oral developmental toxicity study (T4060080) in rabbits (CHBB:HM) which was performed from January 23, 1996 to April 23, 1996

Malformation	Dose group			
	Control	Low	Medium	High
combined malformation		1		1
arthrogryposis[a]	2 (1)	1	1	2 (2)
cardiac septum defect				1
missing lumbar vertebra	1	1		2 (2)
fusion of caudal vertebral bodies	1	1		1
fusion of ribs (cartilaginous part)		2 (2)		
number of fetuses per group	126	132	135	127
number of fetuses with malformations	4	4	1	6
malformed fetuses per group (%)	3.2	3.0	0.7	4.7
number of litters per group	17	18	18	18
number of litters with malformations	3	4	1	5
malformed litters per group (%)	17.7	22.2	5.6	27.8

() number of litters affected

[a] now called malposition of forelimb(s)

Malformations in different study groups in an oral developmental toxicity study (T0060086) in rabbits (CHBB:HM) which was performed from May 06, 1996 to August 21, 1996

Malformation	Dose group			
	Control	Low	Medium	High
malposition of forelimb(s)	1	1	2 (2)	11** (8*)
missing lumbar vertebra	3 (1)		3 (2)	1
supernumerary lumbar vertebra with supernumerary pair of ribs			2 (2)	
fusion of caudal vertebral bodies			1	
skull deformed with parts of skull missing				1
encephalomeningocele with parts of skull missing				1
multiple malformation			1	
number of fetuses per group	119	140	136	111
number of fetuses with malformations	4	1	9	14*
malformed fetuses per group (%)	3.4	0.7	6.6	12.6
number of litters per group	19	20	19	17
number of litters with malformations	1	1	6	10**
malformed litters per group (%)	5.3	5.0	31.6	58.8

() number of litters affected

* statistically significant difference to control $p < 0.05$

** statistically significant difference to control $p < 0.01$

Malformations in different study groups in an oral developmental toxicity study (T1060087) in rabbits (CHBB:HM) which was performed from Septemvber 17, 1996 to December 19, 1996

Malformation	Dose group				
	Control	Low	Medium	High I	High II
malposition of forelimb(s)	1		1	7 (3)	3 (2)
missing kidney and ureter			1	1	
supernumerary lumbar vertebra with 13th ribs	1			1	
missing lumbar vertebra		1			
malformation of caudal vertebrae (fusion, supernumerary ossification center)			3 (2)	1	
fusion of ribs	1		1		
number of fetuses per group	126	140	127	115	102
number of fetuses with malformations	3	1	5	10	3
malformed fetuses per group (%)	2.4	0.7	3.9	8.7	2.9
number of litters per group	20	21	21	18	15
number of litters with malformations	3	1	4	6	2
malformed litters per group (%)	15.0	4.8	19.0	33.3	13.3

() number of litters affected