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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CONTENTS

PART A.

PART B.

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

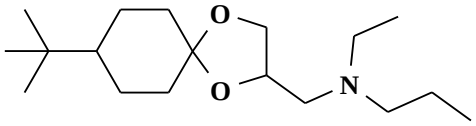
1 IDENTITY OF THE SUBSTANCE

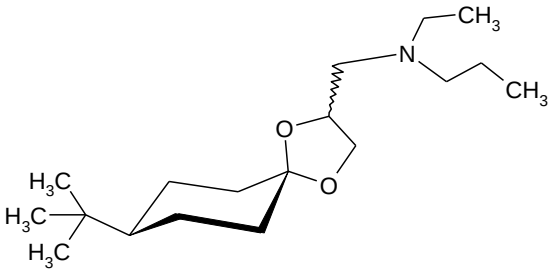
1.1 Name and other identifiers of the substance

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

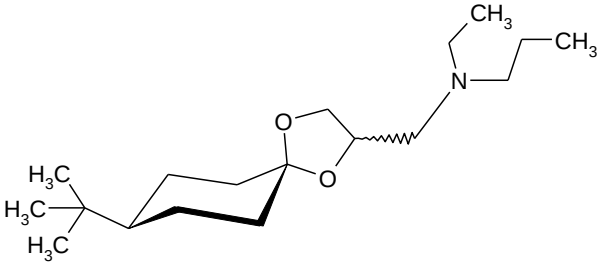
Structural formula:





„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

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.

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 7). 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

	Dose Group			
Observations/study week	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 7; 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 7.

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:

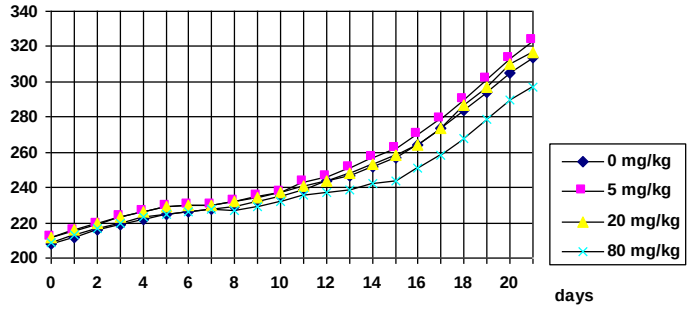
Test system: In a dermal developmental toxicity study, Wistar rats (HanIbm:WIST [SPF], source: Biological Research Laboratories Ltd., Füllinsdorf, Switzerland) were exposed to spiroxamine (batch no. 17002/90; purity: 94.4 - 95.3 %) under occlusive conditions for 6 h/day from day 6 - 15 post coitum at 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).

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**	
Degradation Spiroxamine	< **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.		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.
Bioaccumulation Spiroxamine	Log K_{ow} is < 4 Spiroxamine diastereomer A log K_{ow} = 2.79 Spiroxamine diastereomer B log K_{ow} = .292 at 20 °C	Log K_{ow} is < 3 Spiroxamine diastereomer A log K_{ow} = 2.79 Spiroxamine diastereomer B log K_{ow} = 2.92 at 20 °C	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**.
Acute aquatic toxicity Spiroxamine	**0.001 < L(E)C_{50} ≤ 0.01 mg/L** *Skeletonema costatum* E_rC_{50} = 0.0063 mg a.s./L		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.
Chronic aquatic toxicity Spiroxamine	**For non rapidly degradable substances: 0.0001 < NOEC ≤ 0.001** *Skeletonema costatum* NOEC = 0.00063 mg a.s./L		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)
SUMMARY	**H400, M-factor $_{acute}$ = 100** **H410, M-factor $_{chronic}$ = 100**	**R50-53** SCL are based on ErC50(96h) of *S. costatum* Classification: Concentration [in %] N, R50-53: Cn ≥ 0.25 N, R51/53: 0.025 ≤ Cn < 0.25 R52/53: 0.0025 ≤ Cn < 0.025	**PROPOSED CLASSIFICATION**

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) 21716 ! M-006309-01-1 GLP: Y, published: N 1797568 / TOX9552595	N	BAY
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	Y	BAY

[2] Only notifier listed

			1797957 / ASB2008-2231		
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 administration 23662 ! M-006707-02-1 GLP: Y, published: N 1797612 / TOX9552623	N	BAY

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

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)

RCC PROJECT 281507
KWG 4168 TECHNICAL

357

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.

56

RCC PROJECT 281507
KWG 4168 TECHNICAL

358

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.

57

RCC PROJECT 281507
KWG 4168 TECHNICAL

359

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.

58

RCC PROJECT 281507
KWG 4168 TECHNICAL

360

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 iliac bone positioned at 7th lumbar vertebra	1	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