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**

Contact details for information submitter:

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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*

1.2 Harmonised classification and labelling proposal

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

	CLP Regulation
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 Aquatic Acute 1; H400 Aquatic Chronic 1 ; H410
Current proposal for consideration	STOT RE 2 ; H373

by RAC	
Initial proposal for consideration dated 2014-06-23	*Repr. 2 ;H361d* *M-ronic = 100* *Re-evaluation of* *Acute Tox. 4 ; H332* *Acute Tox. 4 ; H312* *Acute Tox. 4 ; H302* *Skin Sens. 1B ;H317*
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	STOT RE 2 ;H373 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

2 BACKGROUND TO THE CLH PROPOSAL FOR STOT RE

2.1 Short summary of the scientific justification for the CLH proposal for STOT RE

This proposal supplements the original CLH proposal for Spiroxamine ,submitted in 2014, with an additional proposed classification as STOT RE 2, H373.

Several other endpoints were addressed in the previous submission (commenting phase: 08/07/2014 to 22/08/2014). The present submission addresses solely the need for classification with STOT-RE. All other endpoints are not addressed and are outside the scope of this evaluation.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

In the initial CLH proposal on Spiroxamine, submitted in 2014, a revision of the current Annex VI entry for the substance with regard to the classification of certain hazard classes and the addition of the hazard class of reproductive toxicity (developmental effects) was suggested. Data on repeat dose toxicity studies were not specifically assessed. At the RAC meeting in March 2015, RAC recommended that data on repeat dose toxicity could be valuable to complete the toxicity profile of the substance and should therefore be requested from the dossier submitter.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

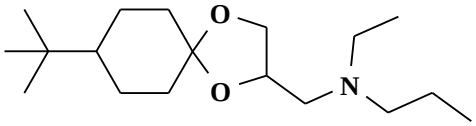
1 IDENTITY OF THE SUBSTANCE

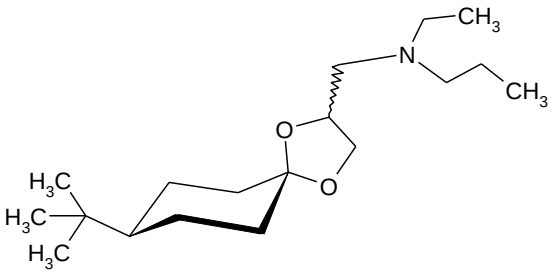
1.1 Name and other identifiers of the substance

Table 3: 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

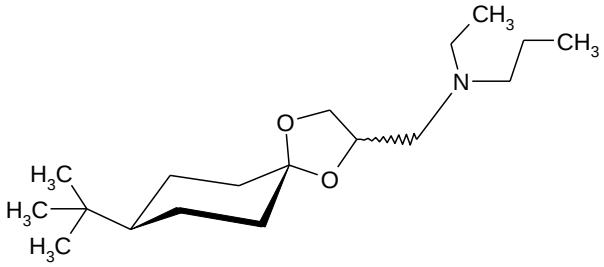
Structural formula:





„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 4: Constituents (non-confidential information)

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

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

Table 5: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
confidential			not relevant for classification

Current Annex VI entry:

Table 6: 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

See the CLH proposal submitted in 2014.

2 HUMAN HEALTH HAZARD ASSESSMENT

2.1 Specific target organ toxicity – repeated exposure

The following information was extracted from the toxicology chapter of the assessment report prepared for the Annex-I-renewal procedure (i.e., Vol. 3, chapter B.6). This chapter was also part of the technical dossier initially submitted by the DS.

2.1.1 Non-human information

2.1.1.1 Short-term toxicity studies

B.6.3 Short-term toxicity (OECD Annex IIA 5.3)

The short-term toxicity data after oral administration of spiroxamine on rats, mice and dogs are summarised in Table B.6.3-1.

Table B.6.3-1: Summary of short-term oral toxicity studies

Study	Dose levels	NOAEL	Targets / Main effects
4 wk, feeding, Wistar rat	0-30-100-300 ppm	30 ppm (3.4 / 3.8 mg/kg bw/d, m/f)	100 ppm (10.8 / 12.2 mg/kg bw/d): liver weight ↑: steatosis of hepatocytes: hyperkeratosis of oesophagal mucosa 300 ppm (33.6 / 35.6 mg/kg bw/d): liver enzyme induction: hyperplasia of bladder epithelium
4 wk, gavage, Wistar rat	0-10-30-90 mg/kg bw/d	LOAEL 10 mg/kg bw/d	10 mg/kg bw/d: clinical symptoms 30 mg/kg bw/d: liver enzyme induction: kidney weight ↑ 90 mg/kg bw/d: liver weight ↑: steatosis of hepatocytes: hyperkeratosis fore-stomach mucosa: hyperplasia of bladder epithelium

Study	Dose levels	NOAEL	Targets / Main effects
13 wk, feeding, Wistar rat	0-25-125-625 ppm	25 ppm (1.9 / 2.7 mg/kg bw/d, m/f)	125 ppm (9.3 / 13.2 mg/kg bw/d, m/f): hyperkeratosis epithelium oesophagus & fore-stomach, hyperplasia/hypertrophy oesophagus, slight liver enzyme induction 625 ppm (54.9 / 75.14 mg/kg bw/d, m/f): body weight ↓; hyperkeratosis epithelium of tongue, hyperplasia of bladder epithelium, liver hyaline droplets
13 wk, feeding, B6C3F1 mice	0-20-80-320-1280 ppm	80 ppm (24.9 / 28.5 mg/kg bw/d, m/f)	320 ppm (88.4 / 126.6 mg/kg bw/d, m/f): epidermal hyperplasia of auricles, hepatocellular hypertrophy & fatty changes 1280 ppm (366.2, 413.7 mg/kg bw/d, m/f): epidermal hyperplasia of tail; liver weight ↑, leucocytes ↑, thrombocytes ↓; hyperplasia bladder epithelium & renal pelvis; kidney weight ↑, water intake ↑, urea ↑
13 wk, gavage, B6C3F1 mice	0-60-180-240 mg/kg bw/d	LOAEL 60 mg/kg bw/d	60 mg/kg bw/d: liver enzyme induction 180 mg/kg bw/d: hepatocellular hypertrophy, ↓ glycogen; hyperkeratosis fore-stomach mucosa; hyperplasia bladder epithelium 240 mg/kg bw/d: Single liver cell necrosis; hyperplasia epidermis of ears & tail
13 wk, feeding, Beagle dog	0-25-750-1500 ppm	25 ppm (0.66 / 078 mg/kg bw/d, m/f)	750 ppm (20.02 / 21.29 mg/kg bw/d, m/f) liver weight ↑, serum albumin ↓, ALP ↑, triglycerides ↓; minimal diffuse hepatocytomegaly
110 d, feeding, Beagle dog	0-150-250-500 ppm	500 ppm (16.2 / 15.1 mg/kg bw/d, m/f)	None
12 mo, feeding; beagle dog	0-25-75-1000-2000 ppm	75 ppm (2.5 mg/kg bw/d)	1000 ppm (28.03 / 25.84 mg/kg bw/d, m/f) cataracts, hepatocytomegaly, albumin ↓; triglycerides ↓; 2000 ppm (56.88 / 52.39 mg/kg bw/d, m/f) Erythrocytes, haemoglobin, haematocrit ↓

In rats, dogs and mice the liver was the main target organ. In mice, signs of liver enzyme induction occurred at doses of 60 mg/kg bw/d. At higher doses, hypertrophy of hepatocytes, degenerative alterations (centrilobular fat deposition) and liver weight increase were seen. Fatty changes of hepatocytes were found also in rats and dogs together with an increase of serum transaminases activity. Investigations using recovery groups revealed that observed liver effects were reversible following cessation of compound administration.
In rats and mice histopathological alterations of the mucosal epithelium of the gastrointestinal and the urogenital tract were found after 4 and 13-week administration. Hyperkeratosis of the epithelium was seen on tongue and in the fore-stomach; the oesophagus showed hyperkeratosis, hyperplasia and hypertrophy. These effects are regarded to be causally related to the strong irritant action of the compound following surface contact. There is no reliable evidence that hyperplasia observed in the urothel of bladder and renal pelvis is likewise related to the irritant potential of spiroxamine. However, the histopathological alterations of the mucosal epithelium were reversible after termination of the exposure.
Ophthalmological findings such as bilateral sub-capsular clouding and cataract changes of the lens were major treatment related effects in dogs following chronic administration of high doses. The liver of dogs treated for 12 months with high doses of spiroxamine exhibited slight signs of hepatocytomegaly. No aggravation of the effects was recorded in comparison with

the findings after subchronic uptake. At 2000 ppm the number of red blood cells was slightly reduced in female dogs only.

Table B.6.3-2: Summary of short-term dermal toxicity studies

Study	Dose levels	NOAEL	Targets / Main effects
3 wk, dermal, NZW rabbit	0-0.5-1-5 mg/kg bw/d	Systemic: 5 mg/kg bw/d Local: < 0.5 mg/kg bw/d	irritation related skin findings (erythema, swelling, hardening, cracking)
3 wk, dermal, NZW rabbit	0-0.05-0.2 mg/kg bw/d	Local: 0.2 mg/kg bw/d	none

No systemic toxicological effects occurred in rabbits following daily dermal application of 5 mg/kg bw/d over a period of 3 weeks. Because of the strong skin irritant action of spiroxamine, 5 mg/kg bw/d (corresponding to a concentration of 0.25 %) was the highest dose which could be tested. The skin of animals treated with a concentration of 0.025 % (0.5 mg/kg bw/d) exhibited erythema; at higher concentrations swelling, hardening and cracking of the skin developed. A concentration of 0.01 % (0.2 mg/kg bw/d) was tolerated without any visible signs of skin damage.

Table B.6.3-3: Summary of short-term inhalation toxicity study

Study	Dose levels	NOAEC [mg/m³ air]	Targets / Main effects
4 wk, inhal., Wistar rat	0-14.3-87.0-518.4 mg/m³ air	14.3	87.0 mg/m³ air irritation-related findings in the respiratory tract; increase of polymorphonuclear granulocytes; haemoglobin ↓; 518.4 mg/m³ air irritation-related findings in respiratory tract and eyes; liver weight ↑, body weight ↓, clinical chemistry (liver related), urinary bladder (hyperplasia)

In a sub-acute inhalation study, irritation-related findings were prominent in the respiratory tract at high concentrations. These changes consisted of metaplasia, hyperplasia and hyperkeratosis of the epithelium of larynx and nasal cavity. In the lungs, the number of macrophages was increased and there was bronchiolo-alveolar proliferation. Similar as after oral administration, the liver was another target organ following sub-acute inhalation.

B.6.3.1 Oral studies

B.6.3.1.1 Rat

Reference: KIIA 5.3 (OECD)

Report: Krötlinger, F., P. Gröning, E. Hartmann (1992) KWG 4168 - Subacute oral toxicity study in rats (feeding study) - Report no.: 21644 (August 31, 1992; report); Bayer AG, Institute for Toxicology, D-42096 Wuppertal, Germany, Dates of exp. work: April 1990 - May 1990.
TOX9552596

Guidelines: OECD TG 407

Deviations: T3, T4 and thyroxine in the blood were measured

GLP: Yes

Acceptability: The study is considered to be acceptable.

Reference: KIIA 5.3 (OECD)
Report: Hartmann, E. (1995) KWG 4168: Subacute oral toxcity study in rats. - 21644A (July 07, 1995; addendum), Amendment - Fatty change in the liver re-evaluation comparison with other toxicity studies with KWG 4168, Bayer AG, Institute for Toxicology, D-42096 Wuppertal
21644 ! 3368 ! T6035521
TOX9552597
Acceptability: The study is considered to be acceptable.

Material and methods:
Test system: 4 Groups of 10 male and 10 female Wistar rats (strain: Bor: WISW [SPF-Cpb], source: Winkelmann, Borchen, Germany) received spiroxamine (batch no.: 17002/90; purity: 94.6 %) in the feed at concentrations of 0-30-100-300 ppm for 28 successive days. In order of increasing doses, mean levels of 3.4, 10.8, 33.6 (males) and 3.8, 12.2, 35.6 (females) mg/kg bw/d were ingested, averaged over a period of four weeks.
Observation period: four weeks. Five male and five female animals from each dose group were selected for haematology, clinical chemistry, urinalysis and histopathology.

Findings:
General observations: The survival rates were unaffected up to 300 ppm. Female rats exhibited slight transient apathy at 300 ppm. The food and water intake did not differ significantly from those in controls throughout the entire study. A transient retardation of the body weight development occurred in males of the 300 ppm group (Table B.6.3-4)

Table B.6.3-4: Body weights (g)

	0 ppm	30 ppm	100 ppm	300 ppm
Males				
Day 0	92	93	92	92
Day 6	126	128	124	118 ++
Day 14	171	172	168	157 ++
Day 21	202	204	200	190 +
Day 28/29	232	236	232	221
Females				
Day 0	84	82	84	84
Day 28/29	155	153	159	153

+ = U-test, 5 % significance level; ++ = U-test, 1 % significance level

Haematology, clinical chemistry, urinalyses: No evidence for a treatment-related effect on the red or white blood cell population, or on the haematopoetic organs up to 300 ppm. The leucocyte counts (LEUCO) were elevated, and the numbers of polymorphs (SEGM) were lower in male rats at 300 ppm.

The clinical chemistry of liver tissue showed elevated levels of the cytochrome P-450 mono-oxygenase system (P 450) in male rats at highest dose level. Furthermore at 300 ppm, the males exhibited lower protein (PROT) and cholesterol (CHOL) levels, and females lower creatinine (CREA) and glucose concentrations. The sodium (Na) levels in both sexes were lower than in the controls (Table B.6.3-5).

Table B.6.3-5: Haematology and clinical chemistry

	0 ppm	30 ppm	100 ppm	300 ppm
Males				
LEUCO [10^9/L]	5.9	7.6§	7.6§§	6.8 +
SEGM [%]	5.2	5.8	6.2	3.5 +
P 450 [nmol/g]	34.4	36.0	35.9	41.6 +
PROT [g/L]	59.0	57.8	58.2	54.8 ++
CHOL [mmol/L]	2.32	2.14	2.22	1.87 ++
Na [mmol/L]	144	144	144	143 +
Females				
P 450 [nmol/g]	33.7	34.6	33.3	39.0
CREA [mcmol/L]	50	61	44	39 +
Glucose [mmol/L]	4.75	4.60	4.55	4.18 ++
Na [mmol/L]	144	145	143	142 +

+ = U-test, 5 % significance level; ++ = U-test, 1 % significance level
§ animal no. 13 = 11.4; §§ animal no. 23 = 9.6

Gross pathology, organ weights, histopathology: At 300 ppm the absolute brain weights were depressed in males, and the relative spleen weights were elevated in females. At 100 ppm and above relative liver weight was increased in males (Table B.6.3-6).

Table B.6.3-6: Organ weights

	0 ppm	30 ppm	100 ppm	300 ppm
Males				
abs. brain weight [mg]	1734	1711	1753	1674 +
rel. liver weight [mg/100g]	4276	4415	4507 ++	4429 +
Females				
rel. spleen weight [mg/100g]	211	229	230	243 +

+ = U-test, 5 % significance level; ++ = U-test, 1 % significance level

Slight to moderate fatty deposits in the hepatocytes were observed in the livers of male and female rats at 100 ppm and above. At 30 ppm the incidence/severity of this finding was not significantly different from the control animals. Hyperkeratosis of the oesophageal mucosa was observed at 100 ppm and above. In addition, at 300 ppm, one female exhibited moderate hyperplasia of the urinary bladder epithelium. Ophthalmic examinations revealed no evidence for treatment-related changes of the eyes.

Table B.6.3-7: Microscopic findings

	0 ppm	30 ppm	100 ppm	300 ppm
Males				
Fatty deposits in hepatocytes (mean grade)*	4/5 (1.0)	4/5 (1.2)	5/5 (2.6)	5/5 (2.6)
Hyperkeratosis of oesophagus	0/5	0/5	5/5	5/5
Females				
Fatty deposits in hepatocytes (mean grade)*	5/5 (1.6)	4/5 (1.2)	4/5 (2.0)	5/5 (2.2)
Hyperkeratosis of oesophagus	0/5	0/5	1/5	5/5
Epithelial hyperplasia of urinary bladder	0/5	0/5	0/5	1/5

* fat deposits in hepatocytes: grade 1 (minimal), grade 2 (slight), grade 3 (moderate)

Conclusion:
The NOEL in males: 30 ppm, equal to 3.4 mg/kg bw /d; females: 30 ppm, equal to 3.8 mg/kg bw /d was based on histopathological findings at 100 ppm (hyperkeratosis of the oesophagus mucosa, increased number of hepatocellular fat deposits) and the elevated relative liver weights in males suggesting slight hepatic enzyme induction. The NOEL in male rats (3.4 mg/kg bw/d) served as basis for calculation of the proposed AOEL for spiroxamine.

Re-evaluation 2009:
The NOAEL 30 ppm (equal to 3.4 / 3.8 mg/kg bw /d, m/f) was based on histopathological findings at 100 ppm (hyperkeratosis of the oesophagus mucosa, slightly increased incidence / severity of hepatocellular fat deposits) and elevated relative liver weights in males.

B.6.3.1.2 Rat

Reference: KIIA 5.3 (OECD)

Report: Krötlinger, F.and E. Hartmann (1992) KWG 4168 – Sub-acute oral toxicity study in rats - Report no.: 21841 (November 11, 1992); Bayer AG, Institute for Toxicology, D-42096 Wuppertal, Germany, Dates of exp. work: February 1991 - March 1991.
TOX9552599

Guidelines: OECD TG 407

Deviations: T3, T4 and thyroxine in the blood were measured; individual data on body weight development (exception terminal sacrifice) and food intake were not submitted

GLP: Yes

Acceptability: The study is considered to be acceptable.

Material and methods:
Test system: 4 Groups of 10 male and 10 female Wistar rats (Bor: WISW [SPF-Cpb], source: Winkelmann, Borchen, Germany) received spiroxamine (batch no.: 17002/90; purity: 93.6 %) at dose-levels of 0-10-30-90 mg/kg bw once a day by gavage for 28 successive days. Observation period: 4 weeks. Five male and five female animals from each dose group were selected for haematology, clinical chemistry, urinalysis and histopathology.

Findings:
General observations: In all dose groups clinical symptoms such as salivation, tremor, digging and preening activities were observed (Table B.6.3-8). At 10 mg/kg bw/d first clinical symptoms (salivation) were observed not before day 6 of the treatment period. At 90 mg/kg bw/d the onset of single clinical findings was day 1 and day 2.

Table B.6.3-8: Clinical findings

	0 mg/kg bw	10 mg/kg bw	30 mg/kg bw	90 mg/kg bw
Males				
Salivation	0/10	2/10	6/10	10/10
Transient tremor	0/10	3/10	10/10	9/10
Dacryosialosis	0/10	1/10	2/10	8/10
Digging activities	0/10	0/10	9/10	10/10
Preening activities	0/10	0/10	2/10	1/10
Females				
Salivation	0/10	3/10	9/10	10/10
Transient tremor	0/10	1/10	9/10	8/10
Dacryosialosis	0/10	0/10	5/10	10/10
Digging activities	0/10	2/10	10/10	10/10
Preening activities	0/10	0/10	0/10	2/10

At 90 mg/kg bw/d body weight gain was slightly retarded and water intake was increased (Table B.6.3-9). The survival rates were unaffected in all dose groups.

Table B.6.3-9: Water intake & body weight

	0 mg/kg bw	10 mg/kg bw	30 mg/kg bw	90 mg/kg bw
Water intake (g/animal/d) – Males				
Day 14	24	25	26	26
Day 29	24	26	27	26
Water intake (g/animal/d) – Females				
Day 14	19	21	22	21
Day 29	20	21	22	23
Body weight (g) – Males				
Day 0	102	101	99	102
Day 14	179	178	176	172
Day 28/29	241	240	243	229
Body weight (g) – Females				
Day 0	93	91	93	93
Day 14	140	141	137	132 +
Day 28/29	166	177	165	158

+ = U-test, 5 % significance level; ++ = U-test, 1 % significance level

Haematology, clinical chemistry, urinalyses: The results of clinical chemistry indicated an induction of hepatic enzymes: increased N-demethylase activities (N-DEM) at 90 mg/kg bw/d (males) and cytochrome P-450 at 30 mg/kg bw/d (males) and at 90 mg/kg bw/d (males/females). At 10 mg/ kg bw/d and above values of triglyceride (TRIGL) in females were decreased, but not clearly dose related. At 30 mg/kg bw/d and above (males) values of

ASAT were increased but without dose relation. At 90 mg/kg bw/d increased values of ALAT (males) and ALP (females) were measured and decreased values of creatinine (males) and protein (females). The albumin levels were depressed in both sexes at 90 mg/kg bw/d (Table B.6.3-10).

Table B.6.3-10: Clinical chemistry

	0 mg/kg bw	10 mg/kg bw	30 mg/kg bw	90 mg/kg bw
Males				
N-DEM [mU/g]	125.1	151.4	159.9	189.9 ++
P 450 [nmol/g]	41.8	43.8	49.4 ++	57.7 ++
ASAT [U/L]	33.6	39.5	44.4 ++	42.8 +
ALAT [U/L]	33.6	33.8	38.7	48.9 ++
ALBUMIN [g/L]	32.2	31.7	31.2	30.3 +
CREA [mcmol/L]	52	52	49	43 +
Females				
N-DEM [mU/g]	61.8	51.4	57.0	72.1
P 450 [nmol/g]	36.8	35.7	38.8	48.4 ++
ALP [U/L]	238	242	265	297 +
TRIGL [mmol/L]	1.19	0.80 ++	0.85	0.55 +
ALBUMIN [g/L]	35.2	35.1	35.7	32.3 ++
PROT [g/L]	65.9	65.0	66.2	60.8 ++

+ = U-test, 5 % significance level; ++ = U-test, 1 % significance level

Gross pathology, organ weights, histopathology: At 90 mg/kg bw/d relative liver weights were increased in both sexes (Table B.6.3-11), spleen, kidneys and testes in males and adrenals in females.

Table B.6.3-11: Relative organ weights (mg/100 g)

	0 mg/kg bw	10 mg/kg bw	30 mg/kg bw	90 mg/kg bw
Males				
Liver	3900	4086	3972	4322 ++
Spleen	210	233	219	253 ++
Kidneys	658	684	696 +	743 ++
Testes	1217	1248	1187	1357 ++
Females				
Pituitary	7	5	6	5 +
Adrenals	29	27	30	32 +
Liver	4170	4256	4302	4435 +

+ = U-test, 5 % significance level ++ = U-test, 1 % significance level

At 90 mg/kg bw/d histopathological examination revealed slight degenerative effects (hepatocellular steatosis in periportal lobular zones), hyperplasia of the urinary bladder epithelium in females and hyperkeratosis of the cornifying, multilayer squamous epithelium of the fore-stomach mucosa in both sexes (Table B.6.3-12).

Table B.6.3-12: Microscopic findings

	0 mg/kg bw	10 mg/kg bw	30 mg/kg bw	90 mg/kg bw
Males				
Hyperkeratosis of stomach	0/5	0/5	0/5	3/5
Fatty deposits in hepatocytes (mean grade)*	1/5 (2.0)	0/5	0/5	2/5 (1.0)
Females				
Hyperkeratosis of stomach	0/5	0/5	0/5	2/5
Urothelial hyperplasia	0/5	0/5	0/5	2/5
Fatty deposits in hepatocytes (mean grade)*	2/5 (1.0)	3/5 (1.0)	1/5 (1.0)	4/5 (1.0)

* fat deposits in hepatocytes: grade 1 (minimal), grade 2 (slight), grade 3 (moderate)

The ophthalmic examinations afforded no evidence for damage to the eyes up to 30 mg/kg bw/d. However, visible lenticular fibres in animals receiving 90 mg/kg bw/d (1/10 m, 4/10 f) are regarded to be treatment related.

Conclusion:
There was no clear NOEL in this study, but a NOAEL was established at the lowest dose level of 10 mg/kg bw/d. The clinical symptoms observed in all dose groups were assumed to be caused by the irritant action of spiroxamine on gastrointestinal mucosa after gavage application, as histopathology showed local changes in the gastric mucosa of these animals. With regard to systemic effects, the NOEL of 10 mg/kg bw/d was based on liver enzyme induction at 30 mg/kg bw/d.

Re-evaluation in 2009
In this study an NOAEL could not be established due to clinical symptoms which occurred even at the lowest dose level in male and female rats. Relevant histopathological findings were restricted to the highest dose group. Furthermore, there is no reliable evidence that the observed clinical symptoms may be related to irritant properties of the test substance. The LOAEL was the lowest dose tested of 10 mg/kg bw/d.

B.6.3.1.3 Rat

Reference: KIIA 5.3 (OECD)

Report: Eiben, R. and E. Hartmann (1992) KWG 4168 – Subchronic toxicity study in Wistar rats (13-week administration in the diet with four-week recovery period) - Report no.: 21627 (August 18, 1992); Bayer AG, Institute for Toxicology, D-42096 Wuppertal, Germany, Dates of exp. work: October 1990 - February 1991.
TOX9552602

Guidelines: OECD TG 408.

Deviations: None

GLP: Yes

Acceptability: The study is considered to be acceptable.

Material and methods:
Test method: Groups of 10 male and 10 female Wistar rats (Bor: WISW [SPF-Cpb], source: Winkelmann, Borchen, Germany) received spiroxamine (batch no.: 17002/90; purity: 93.6 %) in the feed at concentrations of 0-25-125-625 ppm over a period of 13 weeks. Additional recovery groups made up of ten rats of each sex were treated at levels of 0 or 625 ppm over a period of 13 weeks, and then observed for four weeks. Mean consumption of spiroxamine in mg/kg bw/d was 1.9, 9.3 and 54.9 for males and 2.7, 13.2 and 75.14 for females in ascending order of dose.

Findings:
General observations: No animal died during the study. At 625 ppm (recovery group included), several animals exhibited depressed general condition (2/20 females) and un-groomed coat (4/20 females, 1/20 males). These findings were reversible.
The retarded body weight gain observed at high dose level (Table B.6.3-13) was not fully reversible within a post observation period of four weeks (Table B.6.3-14). Food intake was not affected up to 625 ppm, water intake was slightly reduced at 625 ppm.

Table B.6.3-13: Body weight development (main groups)

week	0 ppm		25 ppm		125 ppm		625 ppm	
	males	females	males	females	males	females	males	females
0	131	117	133	118	131	117	130++	117
1	185	134	187	137	184	138	165++	128
2	221	147	225	147	218	149	190++	137+
3	249	160	253	157	243	160	215++	144++
4	272	169	275	166	267	167	237++	149++
5	287	175	294	171	281	175	251++	152++
6	305	181	309	178	297	180	269+	157++
7	320	185	323	181	310	186	282+	160++
8	333	190	338	187	321	190	297+	165++
9	339	192	346	190	331	195	308+	168++
10	352	199	350	206	342	201	320+	172++
11	361	200	363	199	347	203	328+	173++
12	368	205	369	202	356	205	339	175++
13	355	198	362	196	347	200	331	167++

+ = U-test, 5 % significance level ++ = U-test, 1 % significance level

Table B.6.3-14: Body weight development (recovery groups)

week	0 ppm		625 ppm	
	males	females	males	females
0	121	118	131	114
1	181	136	166++	127+
2	219	148	191++	136++
3	247	158	213++	144++
4	271	166	233++	150++
5	290	175	249++	154++
6	308	181	265++	159++
7	320	186	278++	164++
8	335	192	290++	167++
9	342	196	298++	169++
10	354	204	307++	181++
11	362	205	315++	176++
12	370	208	322++	178++
13	373	210	329++	180++
14	379	213	340+	185++
15	385	215	346+	188++
16	390	218	353+	190++
17	387	215	353+	188++

+ = U-test, 5 % significance level ++ = U-test, 1 % significance level

Haematology, clinical chemistry, urinalyses: No adverse effects on red and white blood cell populations were detected at levels up to 625 ppm. Evidence for impaired blood coagulation (transiently lower thrombocyte counts (THRO) and elevated Hepato-Quick readings (HQUICK) could be seen in the high dose group, but no longer existed following the recovery period (Table B.6.3-15).
The determination of the cytochrome P-450 levels (P 450) in the liver samples from rats treated over a period of 13 weeks showed a statistical significant increase at 125 ppm and above in males. Effects on liver were observed in high dose group animals: liver enzyme activities in the serum (aspartate- and alanine-aminotransferase, alkaline phosphatase) were elevated in both sexes. At high dose blood cholesterol (CHOL) levels were statistically significant decreased in both sexes. No evidence for disturbances in kidney function or damage to kidneys was found at levels up to 625 ppm.

Table B.6.3-15: Haematology, clinical chemistry at week 5, 13 and 17 (recovery)

	0 ppm			25 ppm		125 ppm		625 ppm		
Week	**5**	**13**	**17 rec.**	**5**	**13**	**5**	**13**	**5**	**13**	**17 rec.**
Males										
THRO [10^9/L]	1057	1073	946	1042	1046	1050	1093	1007	1056	1072
HQUICK [sec]	28.0	28.1	28.5	27.4	27.8	28.6	28.4	29.9+	29.2	28.5
P 450 [nmol/g]		36.3			39.9		42.8+		49.9++	
ASAT [U/L]	35.8	36.9	32.2	37.1	36.1	37.2	38.8	40.1+	41.4+	35.0
ALAT [U/L]	43.6	44.3	42.0	43.9	50.1	46.4	50.0	55.7++	57.5++	43.5
SAP [U/L]	480	230	185	465	243	439	241	479	270+	203 +
CHOL [mmol/L]	2.28	2.46	2.42	2.29	2.53	2.32	2.50	1.68++	2.00+	1.95+

	0 ppm			25 ppm		125 ppm		625 ppm		
Week	**5**	**13**	**17 rec.**	**5**	**13**	**5**	**13**	**5**	**13**	**17 rec.**
Females										
THRO [10^9/L]	1109	1092	990	1126	1076	1132	1015	1022+	1038	1059
HQUICK [sec]	26.0	25.4	25.5	25.3	27.2++	26.1	26.5	29.0++	28.6++	26.6
ASAT [U/L]	39.0	35.0	40.3	37.8	35.9	37.5	35.9	43.0	41.4+	38.8
ALAT [U/L]	42.9	42.0	40.4	41.5	44.5	41.0	46.8	52.3	54.1+	38.8
SAP [U/L]	282	161	152	294	177	281	180	249	185+	167
CHOL [mmol/L]	2.44	2.14	2.19	2.35	2.13	2.20	2.04	1.60++	1.51++	1.87++

Rec. = recovery groups; + = U-test, 5 %; ++ = U-test, 1 %

Gross pathology, organ weights, histopathology (Table B.6.3-16): At highest dose level slight degenerative liver changes (hyaline droplets) were observed in three of ten males. After 4 weeks of recovery, these effects were no longer manifest or were observed to a lesser degree.
The urinary bladder epithelia of several 625 ppm animals exhibited hyperplastic changes, which were reversible.
Hyperkeratosis in the superficial epithelium was determined in both sexes at 125 ppm and above (oesophagus, fore-stomach) and at 625 ppm (tongue), and was also accompanied by hyperplastic changes and hypertrophy in the oesophagus of the affected animals. Hyperkeratosis, which also occurred in a few control rats, could no longer be observed - or was only seen at a considerably lower incidence at the end of the recovery period.

Table B.6.3-16: Incidence of histopathological findings

	0 ppm		25 ppm		125 ppm		625 ppm	
	males	females	males	females	males	females	males	females
Bladder urothel: hyperplasia (multifocal)	0/10	0/10	0/10	0/10	0/10	0/10	3/10	4/10
Tongue: hyperkeratosis	0/10	0/10	0/10	0/10	0/10	0/10	7/10	10/10
Oesophagus: hyperkeratosis	1/10	0/10	0/10	0/10	9/10	5/10	10/10	10/10
Hyperplasia / hypertrophy	1/10	0/10	0/10	0/10	9/10	5/10	10/10	10/10
Forestomach: hyperkeratosis	0/10	0/10	0/10	0/10	1/10	0/10	3/10	8/10
Liver: hyaline droplets	0/10	0/10	0/10	0/10	0/10	0/10	3/10	0/10

The ophthalmic examinations and histopathology revealed no evidence for toxic effects at 625 ppm.

Conclusion:
The NOEL of 25 ppm in males and females rats (equal to 1.9 mg/kg bw/d (males) and 2.7 mg/kg bw/d (females)) was based on histopathological findings in the oesophagus and forestomach (hyperkeratosis of the epithelium at 125 ppm).

Re-evaluation in 2009
The NOAEL of 25 ppm in males and females rats (equal to 1.9 and 2.7 mg/kg bw/d, m/f) was based on histopathological findings in the oesophagus and fore-stomach (hyperkeratosis of the epithelium at 125 ppm).

B.6.3.1.4 Mouse

Reference:	KIIA 5.3 (OECD)
Report:	Eiben, R. and E. Hartmann (1992), KWG 4168 – Subchronic range-finding testing for a two-year study in B6C3F1 mice (administration in the diet over a period of about 13 weeks) - Report no.: 21022 (January 15, 1992); Bayer AG, Institute for Toxicology, D-42096 Wuppertal, Germany, Dates of exp. work: May 1990 - August 1990 TOX9552603
Guidelines:	OECD TG 408
Deviations:	None
GLP:	Yes
Acceptability:	The study is considered to be acceptable.

Material and methods:
Test system: Groups of 10 male and 10 female B6C3F1/Bor mice (source: Winkelmann, Borchen, Germany) received spiroxamine (batch no.: 17002/90; purity: 94.6 %) in the feed at concentrations of 0-20-80-320-1280 ppm over a period of 13 weeks. Mean consumption of spiroxamine per kg bw per day was 6.2, 24.9, 88.4 and 366.2 mg for males and 7.3, 28.5, 126.3 and 413.7 mg for females in ascending order of dose.

Findings:
General observations: At 1280 ppm depressed general condition and emaciation, hair loss and un-groomed fur were observed in isolated male mice. In this dose group mice exhibited desiccated or crusted areas of skin at the auricles and/or tail and the histology examination revealed marked epidermal hyperplasia. Minimal epidermal hyperplasia of the auricles was also observed in several 320 ppm males. Two males and one female died with causal relationship to the treatment at 1280 ppm. Therefore, a slightly elevated rate of mortality was noted in both sexes at the high dose.
The food and water intakes underwent no significant effect at levels up to 320 ppm. At the high dose, females consumed less food, and males drank more water than the control animals (Table B.6.3-17).

Table B.6.3-17: Food intake and water intake (g/kg bw/d)

	0 ppm		**20 ppm**		**80 ppm**		**320 ppm**		**1280 ppm**	
	males	females	males	females	males	females	males	females	males	females
Food intake	283.7	378.4	308.3	366.4	311.8	356.5	276.4	394.7	286.1	323.2
Water intake	281.7	331.9	298.3	344.7	316.2	366.1	297.7	367.8	385.0	362.5

The body weight development was not altered to a toxicologically relevant extent at 20 ppm in males, or at levels up to 320 ppm in females. Marginal effects on the weight development were noted in males at 80 and 320 ppm. At the high dose, males and females initially lost weight. As the study progressed, growth in the males was retarded, but was unaffected in females at 1280 ppm (Table B.6.3-18).

Table B.6.3-18: Mean body weight

week	0 ppm		20 ppm		80 ppm		320 ppm		1280 ppm	
	males	females	males	females	males	females	males	females	males	females
0	24.9	23.1	24.8	22.9	24.4	22.5	24.5	22.5	25.0	22.7
1	27.1	24.5	26.6	24.5	26.0+	23.9	26.3	23.9	24.5++	22.0++
2	27.8	26.2	27.7	25.2	27.1	25.4	27.0	25.2+	25.5++	25.1+
3	28.3	26.3	28.6	26.0	27.0+	26.1	27.4	25.7	26.1++	24.9+
4	28.0	25.4	27.2	25.5	26.9+	25.9	26.9+	25.2	25.6++	25.5
5	28.8	26.9	28.1	26.0	27.6+	26.5	27.4+	25.8+	27.2+	26.1
6	29.0	26.5	28.7	27.2	27.7++	26.9	28.1	26.7	26.9++	26.4
7	29.1	26.2	28.0+	26.4	27.9++	26.5	27.8+	25.9	27.3++	26.9
8	29.3	26.4	28.0+	26.5	27.8+	26.7	27.9+	26.1	27.3++	26.8
9	29.4	26.4	28.1+	26.7	28.1++	26.9	28.1++	26.3	26.3++	27.0
10	30.0	27.1	28.7+	27.3	28.5++	27.8	28.5+	26.8	27.9++	27.3
11	30.4	28.0	28.9	28.1	28.5+	28.0	28.9+	27.0	28.8	27.1
12	29.1	27.3	28.1	27.7	27.9	27.8	28.1	26.6	27.7	26.9

+ = U-test, 5 %; ++ = U-test, 1 %

Haematology, clinical chemistry, urinalysis: The haematology tests performed at the end of the study revealed no evidence for treatment related effects on the red blood cell population at levels up to 1280 ppm.
At high dose level, leucocyte counts (LEUCO) in both sexes were slightly elevated, but the differential blood count remained unaffected. Furthermore, significantly fewer thrombocytes (THRO) were counted.
Results for urea and cholesterol (CHOL) were within the physiological range up to 320 ppm, but were elevated (urea) or depressed (cholesterol) to a statistically significant extent at the high dose in both sexes (Table B.6.3-19).

Table B.6.3-19: Haematology and clinical chemistry

	0 ppm	20 ppm	80 ppm	320 ppm	1280 ppm
Males					
LEUCO [10^9/L]	6.7	5.4	5.8	6.7	8.3
THRO [10^9/L]	1238	1194	1259	1230	1057 ++
UREA [mmol/L]	14.68	15.45	14.89	14.50	20.48 ++
CHOL [mmol/L]	2.90	2.78	2.73	2.67	1.75 ++
Females					
LEUCO [10^9/L]	3.0	3.5	3.8	3.6	5.2 ++
THRO [10^9/L]	1054	1045	1039	1099	950 +
UREA [mmol/L]	8.89	10.01	10.11 ++	9.68	13.70 ++
CHOL [mmol/L]	2.37	2.26	2.29	2.35	1.32 ++

+ = U-test, 5 % significance level; ++ = U-test, 1 % significance level

Gross pathology, organ weights, histopathology: Increased centrilobular fatty change of the hepatic lobules in females at 320 ppm and above, as well as elevated liver weights and hepatocellular hypertrophy (both at 1280 ppm) are interpreted as evidence for a change in the metabolic function in the liver (Table B.6.3-20, Table B.6.3-21).
The epithelium of the urinary bladder and renal pelvis exhibited hyperplastic changes in the 1280 ppm group. Effects on the kidneys such as elevated relative kidney weights, increased water intakes and elevated urea levels were found in high dose group animals.

Table B.6.3-20: Organ weights, absolute (mg) and relative (mg/100 g)

	0 ppm		20 ppm		80 ppm		320 ppm		1280 ppm	
	male	female	male	female	male	female	male	female	males	female
Liver, absolute	1440	1465	1364	1410	1338+	1389	1375	1380	1646+	1595
relative	4931	5368	4838	5112	4767	4982+	4912	5205	5961+	5927+
Kidney, absolute	501	433	490	435	499	458	484	421	526	441
relative	1714	1591	1737	1580	1775	1640	1729	1586	1908++	1639

+ = U-test, 5 % significance level; ++ = U-test, 1 % significance level

Table B.6.3-21: Incidence of treatment related histopathological findings

	0 ppm		20 ppm		80 ppm		320 ppm		1280 ppm	
	m	f	m	f	m	f	m	f	m	f
Skin										
-epidermal hyperplasia auricle	0/10	0/10	0/10	0/10	0/10	0/10	6/10	0/10	9/10	10/10
-epidermal hyperplasia tail	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	4/10	9/10
Kidney, epithelial hyperplasia	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	4/10	7/10
Urinary bladder, hyperplasia	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	9/10	9/10
Liver, cellular hypertrophy	0/10	0/10	0/10	0/10	0/10	0/10	1/10	7/10	0/10	3/10
- fatty change	3/10	7/10	8/10	9/10	3/10	6/10	4/10	9/10	8/10	9/10
grade 1	3/10	7/10	8/10	9/10	3/10	6/10	3/10	6/10	8/10	5/10
grade 2	0/10	0/10	0/10	0/10	0/10	0/10	1/10	3/10	0/10	3/10
grade 3	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10

Conclusion:
The NOEL for male mice of 20 ppm (equal to 6.2 mg/kg bw/d) was based on marginally reduced body weight development at 80 ppm. The NOEL for female mice of 80 ppm (equal to 28.5 mg/kg bw/d) was based on morphological findings in the liver at 320 ppm (increased centrilobular fatty change of hepatic lobules).

Re-evaluation in 2009
The NOAEL in this study is 80 ppm (equal to 24.9 and 28.5 mg/kg bw/d m/f) based on observed epidermal hyperplasia (auricles) and morphological findings in the liver at 320 ppm. The marginal reduced body weight in male mice at 80 ppm (< 10 %) is considered to be of no toxicological relevance.

B.6.3.1.5 Mouse

Reference: KIIA 5.3 (OECD)

Report: Eiben, R. and E. Hartmann, U. Schmidt (1992), KWG 4168 – Subchronic toxicological study in B6C3F1 mice to examine effects on the skin, kidneys, liver and urinary bladder (thirteen-week administration by gavage and eight-week recovery period) - Report no.: 21330 (April 29, 1992); Bayer AG, Institute for Toxicology, D-42096 Wuppertal, Germany, (Dates of exp. work: January 1991 - June 1991).
TOX9552626

Guidelines: Sim. OECD TG 408

Deviations: Only 5 animals/sex/dose; not all organs were examined

GLP: Yes

Acceptability: The study is considered to be supplementary.

Material and methods:
Test system: Groups of 5 male and 5 female B6C3F1/Bor mice (source: Winkelmann, Borchen, Germany) received spiroxamine (batch no.: 17002/90; purity: 93.6 %) at dose levels of 0-60-180-240 mg/kg bw/d by gavage over a period of 13 weeks. Five additional animals of each sex were included in the 0, 180 and 240 mg/kg bw/d groups, and following the 13-week treatment period were left untreated for observation over an eight week recovery period.

Findings:
No treatment related clinical signs were observed at doses up to 180 mg/kg bw/d. High dose mice exhibited extension spasms shortly after treatment. The body weight development, mortality, and food and water intake underwent no significant effect over the examined range of doses.
Haematology, clinical chemistry, urinalysis: Cholesterol levels were dose related decreased, but even at highest dose level not statistically verified due to the small number of animals. No significant deviations in this parameter were apparent at the end of the recovery period. Induction of microsomal mono-oxygenases in the liver (7-Ethoxycoumarin deethylase (EOD); 7-Ethoxyresorufin deethylase (ERD); Aldrin epoxidase (ALD); Epoxide hydrolase (EH); Glutathione-S-transferase (GSH-T); UDP-Glucuronyl transferase (GLU-T) was noted in all treatment groups. (Table B.6.3-22).

Table B.6.3-22: Clinical chemistry

	0 mg/kg bw	60 mg/kg bw	180 mg/kg bw	240 mg/kg bw
Males				
CHOL [mmol/L]				
- main group	3.61	3.29	3.25	2.53
- recovery group	3.28	--	3.05	2.96
EOD [nmol/g/min]				
- main group	13.0	19.4	28.9	30.5
- recovery group	15.2	--	--	16.6
EOR [nmol/g/min]				
- main group	0.81	1.59	1.82	1.35
- recovery group	2.05	--	--	1.53
ALD [nmol/g/min]				
- main group	32.8	109.7	156.4	115.8
- recovery group	42.0	--	--	41.8
EH [nmol/g/min]	515	536	624	551
GSH-T [µmol/g/min]	295.8	267.6	332.0	323.9
GLU-T [nmol/g/min]	54	63	74	74
Females				
CHOL [mmol/L]				
- main group	2.71	2.66	2.49	2.13
- recovery group	2.36	--	2.48	2.44
EOD [nmol/g/min]				
- main group	23.5	18.8	38.7	31.8
- recovery group	21.4	--	--	23.6
EOR [nmol/g/min]				
- main group	1.27	1.17	2.84	2.15
- recovery group	1.67	--	--	1.39
ALD [nmol/g/min]				
- main group	74.2	82.9	239.7	216.8
- recovery group	41.2	--	--	46.6
EH [nmol/g/min]	343	269	274	345
GSH-T [µmol/g/min]	137.5	128.9	151.1	150.0
GLU-T [nmol/g/min]	88	99	79	68

Gross pathology, histopathology: Morphological evidence for liver stress (hepatocellular hypertrophy and reduced glycogen levels) was found at 180 mg/kg bw/d and above. In addition, single cell necrosis also occurred at 240 mg/kg bw/d. Urinary tract epithelial hyperplasia was detected in the bladders of the 180 and 240 mg/kg bw/d dose group mice. Mice in the high dose group exhibited hyperplastic changes in the epidermis of the auricles and tails (males only) (Table B.6.3-23).
The described effects on the liver, urinary bladder and epidermis were found to be reversible.

Table B.6.3-23: Incidence of treatment related histopathological findings

	0 ppm		60 ppm		180 ppm		240 ppm	
	male	female	male	female	male	female	male	female
Skin -epidermal hyperplasia ears	0/5	0/5	0/5	0/5	0/5	0/5	3/5	1/5
-epidermal hyperplasia tail	0/5	0/5	0/5	0/5	0/5	0/5	2/5	0/5
Stomach, hyperceratosis	0/5	0/5	0/5	0/5	1/5	0/5	3/5	3/5
Urinary bladder, simple hyperplasia	0/5	0/5	0/5	0/5	1/5	0/5	4/5	1/5
Liver -hepatocellular hypertrophy	0/5	0/5	0/5	0/5	1/5	0/5	3/5	0/5
-single cell necrosis	0/5	0/5	0/5	0/5	0/5	0/5	1/5	1/5
-glycogen reduced	1/5	1/5	0/5	0/5	4/5	2/5	5/5	3/5

Conclusion:
As evidence of liver enzyme induction was seen in all treatment groups, the no observed effect level was < 60 mg/kg bw/d. This dose can be regarded as a low observed effect level. The described effects on the liver, urinary bladder and epidermis were approximately equivalent to those which had been seen following treatment with spiroxamine in the diet.

Re-evaluation in 2009
No NOAEL was determined due to increased liver enzyme activity at 60 mg/kg bw/d and above. The observed hyperplastic changes in the epidermis (auricle, tail) occurred independently by the route of treatment.

B.6.3.1.6 Dog

Reference: KIIA 5.3 (OECD)

Report: Jones, R.D. and L.E. Elcock (1994): KWG 4168 - 13-week subchronic feeding study in beagle dogs - Report no.: 7442 (December 07, 1994); Bayer Corporation, Agriculture Division, South Metcalf, Stilwell, Kansas, USA, Dates of exp. work: November 1991 - February 1992
TOX9552604

Guidelines: OECD TG 409

Deviations: None

GLP: Yes

Acceptability: The study is considered to be acceptable.

Material and methods:
Test system: Groups of 4 male and 4 female pure-bred beagle dogs (source: White Eagle Laboratories, Doyleston, Pennsylvania, USA) received spiroxamine (batch no.: 17002/90; purity: 93.5 %-94.9 %) in the feed at concentrations of 0-25-750-1500 ppm over a period of 13 weeks. The average consumption of spiroxamine in the male dose groups were 0.66, 20.02, 42.76 mg/kg bw/d; the females consumed 0.78, 21.29, 43.69 mg/kg bw/d.

Findings:
General observations: There was no difference in body weight and feed consumption between treated and control groups. Only incidental clinical signs were observed, none of which were considered treatment related. There were no treatment related ophthalmological findings.
Haematology, clinical chemistry, urinalysis: At 1500 ppm decreased albumin levels (males; females even at 750 ppm), increased alkaline phosphatase levels (females) and decreased triglyceride (TRIGL) levels (females) were observed (Table B.6.3-24).

Table B.6.3-24: Clinical chemistry (day 90)

	0 ppm	25 ppm	750 ppm	1500 ppm
Males				
ALBUMIN [g/dL]	3.3	3.2	3.2	2.9 *
ALP [u/L]	78	70	91	112
TRIGL [mg/dL]	65	60	61	57
Females				
ALBUMIN [g/dL]	3.3	3.2	2.9 *	2.8 *
ALP [u/L]	64	70	89	168 *
TRIGL [mg/dL]	59	61	52	46 *

Anova + Students t-tests (two-sided): * $p \leq 5$ %

At 1500 ppm albumin was decreased significantly at the terminal bleeding interval on day 90 in males, and in females on day 35, 63 and 90. In females at 750 ppm decreased albumin level were noted at various time points during the study, even on pre-treament day 0 (statistically significant) and did not increase in magnitude over the course of the study. Furthermore, these females exhibited statistically significant decrease in the total protein level on day 0, 35, 63 and 90.
Gross pathology, organ weights, histopathology: Statistical significant increases in the relative liver weight were evident at 750 ppm and above in males and at 1500 ppm in females (Table B.6.3-25).

Table B.6.3-25: Liver weight

	0 ppm		25 ppm		750 ppm		1500 ppm	
	males	female	male	female	male	female	male	female
Weight, absolute (g)	375	298	404	282	477	299	458	350
Weight, relative (g/kg)	2.8	3.0	3.2	2.9	3.7*	3.2	3.6*	3.8*

Anova + Students t-tests (two-sided): * $p \leq 5$ %

Microscopic observation revealed minimal diffuse hepatocytomegaly in males at 750 ppm (2/4) and statistically significant at 1500 ppm (4/4 males, 4/4 females).

Conclusion:
The NOEL in males of 25 ppm (equivalent to 0.7 mg/kg bw/d) was based on effects on the liver at 750 ppm (increased relative weight, minimal hepatocytomegaly in males). The findings at 1500 ppm (hepatocytomegaly, increased alkaline phosphatase levels, decreased albumin levels and decreased triglyceride levels) are regarded to be indicative for an influence of spiroxamine on the liver function. The decreased albumin level noted in the 750 ppm females was considered to be spurious, for it was decreased on pre-treatment day 0 and did not increase in magnitude over the course of the study.

Re-evaluation in 2009
NOAEL of 25 ppm (equivalent to 0.66 and 0.78 mg/kg bw/d, m/f) was confirmed.

B.6.3.1.7 Dog

Old study, submitted after the peer review examination and not cited in the monograph (date of submission: 04.07.1997) but cited in the EU review report 7584/VI/97-Rev.7 from 12.5.1999.

Reference:	KIIA 5.3 (OECD)
Report:	Jones, R. D.; Hastings, T. F.: Technical grade KWG 4168: A subchronic toxicity study in the beagle dog – Report no.:BC8105 (April 19, 1997) Bayer Corporation Agriculture Division Toxicology, South Metcalf, Stilwell, Kansas Dates of exp. work: November 1995 – March 1996 TOX9750962
Guidelines:	OECD TG 409
Deviations:	None
GLP:	Yes
Acceptability:	The study is considered to be acceptable.

Material and methods:
Test system: Spiroxamine (lot/batch no.: 17002/90, purity: 95.5 – 96.2 %, vehicle: 1 % corn oil) was administered in the diet to Beagle dogs (source: White Eagle Laboratories, Doylestown, Pennsylvania, USA) (4 animals/sex/dose) for approx. 110 days at concentrations of 0-150-250-500 ppm. The average consumption of spiroxamine active substance was 4.84, 9.16, 16.19 mg/kg bw/d in males and 5.45, 8.92, 15.05 mg/kg bw/d in females.

Findings:
No mortality occurred in the study. There were no relevant clinical signs or observations that were attributed to subchronic spiroxamine intake. Weekly body weight measurements showed no relevant treatment related changes in either sex. There were no relevant changes in weekly food consumption. Ophthalmological investigations revealed no test compound related findings. No dose related changes were found with regard to the ECG or BP parameters measured in this study. There were no clinical neurology findings related to subchronic spiroxamine administration. Measurements of body temperature also revealed no treatment related findings.
Haematology: With regard to haematological parameters no significant variations from concurrent control values were observed.
Clinical chemistry: The only noteworthy value was male alkaline phosphatase (ALP), which was significantly higher in both the 250 and 500 ppm male groups at terminal sampling compared to the control. This is a statistical aberration generated by the chance grouping of animals with higher background ALP in the higher dose groups. For example, the pre-treatment values for the 250 and 500 ppm male groups were considerable higher than the control mean. The wide mean difference between the control and the treated groups was

maintained throughout the study, with similar or identical relative changes with time. In females the pre-treatment variation between groups was not as pronounced and no significant differences of biological relevance were observed (Table B.6.3-26).

Table B.6.3-26: Mean serum alkaline phosphatase in U/L (SD)

	0 ppm		150 ppm		250 ppm		500 ppm	
treatment	**males**	**females**	**males**	**females**	**males**	**females**	**males**	**females**
-13 days	170 (58)	199 (37)	227 (39)	201 (34)	249 (55)	225 (55)	227 (48)	211 (33)
-7 days	161 (52)	183 (30)	216 (36)	193 (44)	229 (54)	206 (42)	204 (44)	193 (33)
1 month	131 (40)	153 (16)	170 (17)	153 (29)	199 (54)	185 (39)	193 (44)	172 (35)
2 month	119 (36)	143 (21)	145 (20)	138 (17)	184 (42)	180 (34)	195 (53)	154 (32)
3 month	84 (29)	108 (13)	103 (17)	98 (9)	140 (42)*	133 (41)	165 (49)*	117 (27)

* p<=5 %

Urinalysis parameters showed no treatment-related significant variations from concurrent control values.
Organ weights: There were no significant differences in male absolute/relative organ weights or in female relative organ weights. Absolute lung weights in females were dose related decreased, significantly at 250 and 500 ppm. There were no significant differences in relative lung weights. In the absence of any clinical, gross or microscopic changes in the lungs in this study and in two previous studies with substantially higher dose rate, and in the absence of corresponding relative lung weight change, this effect was not considered to be of toxicological relevance.
Gross pathology and histopathology revealed no significant findings.

Table B.6.3-27: Mean lung weight in females

	0 ppm	150 ppm	250 ppm	500 ppm
absolute in g (SD)	90.9 (9.7)	83.6 (9.5)	77.3 (3.9)*	69.5 (2.5)*
relative in % of body weight (SD)	0.99 (0.20)	0.83 (0.17)	0.88 (0.11)	0.78 (0.14)

* p<=5 %

Conclusion:
The NOAEL in this study is 500 ppm (16.2 and 15.1 mg/kg bw/d, m/f), the highest dose tested.

B.6.3.1.8 Dog

Reference: KIIA 5.3 (OECD)

Report: Jones, R. D., L. E. Elock: Technical grade KWG 4168: a chronic toxicity feeding study in the beagle dog - Report no.: 7461 (January 16,1995). Miles Inc., Agriculture Division, 17745 South Metcalf, Stilwell, Kansas, USA, Dates of exp. work: January 1993 - January 1994.
TOX9552605

Guidelines: OECD TG 452

Deviations: None

GLP: Yes

Acceptability: The study is considered to be acceptable.

Material and methods:
Test system: Spiroxamine (batch no.: 17002/90; purity: 93.5 - 95.6 %) was administered in the diet to Beagle dogs (source: White Eagle Laboratories, Doylestown, Pennsylvania, USA) (4 animals/sex/dose) at concentrations of 0, 25, 75, 1000 or 2000 ppm for 52 weeks. The average consumption of spiroxamine active substance was 0.66, 2.47, 28.03 and 56.88 mg/kg bw/d in males and 0.76, 2.48, 25.84, 52.39 mg/kg bw/d in females.

Findings:
General observations: There were no significant changes in feed consumption or body weights that were related to compound administration. No clinical symptoms that can be attributed to compound administration occurred. No adverse effects were detected by clinical neurology examination or by computerised electrocardiography analysis. About 9 months after the start of the study, changes of the eyes (cataract, opacity) were observed in one male and three female dogs of the 2000 ppm dose group; no effects were seen at lower dose levels. At termination also two males of the 1000 ppm dose group had evidence of bilateral sub-capsular clouding and cataracts, and one female had bilateral lens opacities (cataract). At the final examination of the 2000 ppm dose group dogs, one male had developed a bilateral cataract, and three females had bilateral lens clouding and cataracts. Microscopic evidence of ocular cataract formation was found in the high dose group.
Haematology, clinical chemistry, urinalysis: The only haematological changes considered to be compound related consisted of a decrease in the erythrocyte count (RBC), haemoglobin (HGB) and haematocrit levels (HCT) in 2000 ppm females, occurring on day 273 and/or day 359 (Table B.6.3-28).

Table B.6.3-28: Haematology

		0 ppm	25 ppm	75 ppm	1000 ppm	2000 ppm
Females						
RBC	day 0	6.88	6.50	6.85	6.63	6.95
[10*6/mm³]	day 91	7.65	7.58	7.70	7.75	7.25
	day 182	7.87	7.70	7.33	6.85	7.00
	day 273	7.55	7.60	7.33	7.28	6.75
	day 359	7.63	7.60	7.75	6.90	6.45*
HGB [g/dL]	day 0	15.9	15.1	16.1	15.3	16.3
	day 91	18.1	17.9	18.4	18.3	17.1
	day 182	18.5	18.4	17.7	16.4	16.6
	day 273	17.8	17.8	17.3	17.1	15.6*
	day 359	18.0	18.0	18.5	16.3	15.2*
HCT [%]	day 0	44.8	42.7	45.4	43.4	46.1
	day 91	50.1	49.6	51.1	50.7	47.6
	day 182	50.2	49.6	47.8	44.3	45.3
	day 273	49.9	50.1	48.1	47.9	44.2*
	day 359	51.1	50.6	52.4	46.5	43.2*

* Statistics: Anova + Students t-tests (two-sided): $p \leq 5\ \%$

In dose groups receiving 1000 and 2000 ppm decreased serum albumin levels (males/females), decreased triglyceride levels (females) and increased alanine aminotransferase levels (males) were measured and considered treatment related (Table B.6.3-29): Urinalysis parameters were not affected by compound administration.

Table B.6.3-29: Clinical chemistry

		0 ppm	25 ppm	75 ppm	1000 ppm	2000 ppm
Males						
ALBUMIN [g/dL]	day 0	3.2	3.0	3.1	3.1	3.2
	day 91	3.4	3.3	3.5	3.1*	3.0*
	day 182	3.4	3.4	3.4	3.1*	3.0*
	day 273	3.3	3.3	3.4	2.9*	2.9*
	day 359	3.3	3.5	3.4	3.1	3.1
ASAT [U/L]	day 0	34	28	34	27	30
	day 91	30	31	46	29	40
	day 182	27	35	31	36	34
	day 273	27	32	29	31	32
	day 359	27	31	32	34	38
Females						
ALBUMIN [g/dL]	day 0	3.2	3.2	3.2	3.2	3.1
	day 91	3.5	3.6	3.6	3.3	3.0*
	day 182	3.5	3.5	3.4	3.1*	2.9*
	day 273	3.3	3.3	3.4	3.0*	2.9*
	day 359	3.4	3.3	3.5	3.1*	2.8*
TRIGL [mg/dL]	day 0	63	83	68	69	77
	day 91	66	69	62	49	45
	day 182	78	81	72	53*	52*
	day 273	42	53	42	31	22*
	day 359	31	45*	38	33	24

* Statistics: Anova + Students t-tests (two-sided): $p \leq 5$ %

Gross pathology, organ weights, histopathology: there were no effects on any absolute or relative organ weights in the dose groups tested. Histopathologically, there was evidence of a minimal diffuse hepatocytomegaly occurring in the 1000 and 2000 ppm males and females, characterised by a granular, ground glass cytoplasmic appearance and occasional enlargement of hepatocytes (Table B.6.3-30).

Table B.6.3-30: Incidence of histopathological findings in the liver

	0 ppm	25 ppm	75 ppm	1000 ppm	2000 ppm
Liver (no. examined)	4	4	4	4	4
hepatocytomegaly m	0	0	0	4+(1.0)+	4+(1.0)+
hepatocytomegaly f	0	0	0	1 (1.0)	4+(1.0)+

+ significantly different from control ($p \leq 0.05$); () = Average severity of animals with lesion: 1 (minimal) to 5 (severe)

Conclusion:
The NOAEL was determined at 75 ppm (equivalent to 2.47 and 2.48 mg/kg bw/d, m/f) based on findings at 1000 ppm concerning the liver (altered clinical chemistry parameters, minimal diffuse hepatocytomegaly) and the eyes (sub-capsular clouding, cataractic changes). Haematological parameters suggested a mild anaemia according to decreases in erythrocyte counts, haemoglobin and haematocrit levels of the 2000 ppm females.

Re-evaluation in 2009
In this study the NOAEL is 75 ppm (equal to 2.5 mg/kg bw/d) based on findings concerning eyes and liver.

B.6.3.2 Dermal studies

B.6.3.2.1 Rabbit

Reference: KIIA 5.3 (OECD)
Report: Vohr, H.-W., M. Rinke: KWG 4168 – Sub-acute dermal study on the rabbit - Report no.: 23710 (February 06, 1995); Bayer AG, Institute for Toxicology, D-42096 Wuppertal, Germany, Dates of exp. work: January 1994 - March 1994.
TOX9552600
Guidelines: OECD TG 410

Deviations: None

GLP: Yes

Acceptability: The study is considered to be acceptable.

Material and methods:
Test system: Local and systemic effects of spiroxamine (batch no.: 17002/90; purity: 95.5 %) were examined in a subacute dermal toxicity study on New Zealand rabbits (HC:NZW, source: Interfauna UK Limited, Huntingdon, Great Britain). The test substance was formulated with Cremophor EL (2 % v/v) in sterile physiological saline solution. The animals were treated with the test compound in doses of 0, 0.5, 1 and 5 mg/kg bw/d (6h/d) for 3 weeks (corresponding concentrations were: 0, 0.025, 0.05 and 0.25 %). Because of strong irritant reactions, higher concentrations (and thus higher dose levels) than 0.25 % (5 mg/kg bw/d) could not be tested in rabbits. Five males and 5 females were used per group. A satellite group (5 mg/kg bw/d) and a further control group were observed over a 14-day post-treatment period.

Findings:
The appearance, behaviour, feed consumption and body weights of the dose animals corresponded to that of the control animals. There were no mortalities. Skin erythema occurred in nearly all animals in the dose groups (Table B.6.3-31).

Table B.6.3-31: Mean degree of skin erythema

Dose	0 mg/kg/bw/d		0.5 mg/kg/bw/d		1 mg/kg/bw/d		5 mg/kg/bw/d	
	males	females	males	females	males	females	males	females
Day 1	0	0	0	0	0	0	0	0.1
Day 10	0	0	0.4	0.2	0.6	1.0	3.7	3.5
Day 20	0	0	0.4	0.2	1.0	0.6	3.7	3.9
Day 21	0	0	0.4	0.2	0.8	0.8	3.3	3.9

No redness = 0; very slight erythema = 1; moderate to slight erythema = 2; definite erythema = 3; severe erythema (deep red) = 4

Other findings such as scales, swelling, hardening and cracking occurred among all animals in the highest dose group, in two females at 1 mg/kg bw/d and in one male at 0.5 mg/kg bw/d (Table B. 6.3-32).

Table B. 6.3-32 Incidence of local skin findings

Local skin: findings *	0 mg/kg bw/d (n = 10)		0.5 mg/kg bw/ (n = 5)		1 mg/kg bw/d (n = 5)		5 mg/kg bw/d (n = 10)	
	males	females	males	females	males	females	males	females
Scaly in places	0	0	1	0	0	2	10	10
Scaly	0	0	0	0	0	1	6	6
Slightly swollen	0	0	0	0	0	0	10	9
Swollen in places	0	0	0	0	0	0	0	1
Swollen	0	0	0	0	0	0	10	8
Cracked in places	0	0	0	0	0	0	9	8
Cracked	0	0	0	0	0	1	3	2
Hardened in places	0	0	0	0	0	0	2	4
Hardened	0	0	0	0	0	0	3	5

* where a finding occurs more than once during the course of the study (also each summarised finding where an incidence greater than N = 5 is possible), it is only shown once per animal in the incidence table.

Skin fold thickness was significantly increased among both sexes at the highest dose and to a lower extent at 1 mg/kg bw/d (Table B.6.3-33).

Table B.6.3-33: Mean skin fold thickness (mm)

Dose	0 mg/kg/bw/d		0.5 mg/kg/bw/d		1 mg/kg/bw/d		5 mg/kg/bw/d	
	males	**females**	**males**	**females**	**males**	**females**	**males**	**females**
Day 0	2.74	2.21	3.14	2.16	2.78	2.12	3.07	2.37
Day 6	2.74	2.31	3.16	2.32	2.94	2.24	3.17	2.51
Day 13	2.93	2.26	3.04	2.48	3.18	2.44	3.52	2.90
Day 20	3.17	2.34	3.38	2.78	3.62	3.00	5.38	4.50

No treatment-related haematological or clinical chemistry effects occurred. No treatment-related changes to the examined organs were observed in terms of gross pathological, gravimetric or histopathological findings.
Following histopathological changes in the skin among all treated animals were observed: diffuse epidermal hyperplasia, focal epidermal hyperplasia, hyperkeratosis, inflammation reaction. These effects were mainly reversible at the end of the post-treatment period.

Conclusion:
A NOAEL of > 5 mg/kg bw/d was determined since no systemic effects were observed up to and including the highest dose level. Local effects first occured among both sexes at the lowest dose group of 0.5 mg/kg bw/d.

Re-evaluation in 2009
In this study the systemic NOAEL was 5 mg/kg bw/d, the highest dose tested. Local effects were observed even at the lowest dose level of 0.5 mg/kg bw/d. Contrary to the previous assessment, skin fold thickness was already increased at 1 mg/kg bw/d in both female and male rabbits, however to a lower extent.

B.6.3.2.2 Rabbit

Reference: KIIA 5.3 (OECD)
Report: Vohr, H.-W: KWG 4168 – Sub-acute dermal study on the rabbit - Report no.: 23727 (February 10, 1995); Bayer AG, Institute for

	Toxicology, D-42096 Wuppertal, Germany, Dates of exp. work: July 1994. TOX9552601
Guidelines:	OECD TG 410
Deviations:	Only limited parameter were tested (local skin tolerability, general observations, body weight), two dose groups
GLP:	Yes
Acceptability:	The study is considered to be supplementary

Material and methods:
Test system: The local and systemic tolerability of KWG 4168 (batch no.: 17002/90; purity: 95.1 %) was examined in a sub-acute dermal toxicity study on New Zealand rabbits (HC:NZW, source: Interfauna UK Limited, Huntingdon, Great Britain). The test substance was formulated with Cremophor EL (2 % v/v) in sterile physiological saline solution. The animals were treated with the test compound in doses of 0, 0.05 and 0.2 mg/kg bw/d (corresponding to concentrations of 0, 0025 and 0.01 %) 6h/d for 3 weeks. Five males and 5 females were used per group.

Findings:
There were no treatment related findings in all dose groups. No local skin findings were observed among the animals. The skin fold thickness of the treated animals corresponded to that of the controls.

Conclusion:
A no observed effect level for local effects to the skin was 0.2 mg/kg bw/d for both sexes.

Re-evaluation in 2009
Under the conditions of this sub-acute dermal toxicity study the NOAEL for local effects was 0.2 mg/kg bw/d, the highest dose tested.

B.6.3.3 Inhalation study

B.6.3.3.1 Rat

Reference:	KIIA 5.3 (OECD)
Report:	Pauluhn, J. (1992) KWG 4168 Aerosol - Study for sub-acute inhalation toxicity in the rat according to OECD Guideline no. 412 - Report no.: 21785 (October 21, 1992) Bayer AG, Institute for Toxicology, Wuppertal, Germany, Dates of exp. work: January 1991 - February 1991. TOX9552598
Guidelines:	OECD TG 412
Deviations:	None

GLP: Yes

Acceptability: The study is considered to be acceptable.

Material and methods:
Test system: Groups of 10 male and 10 female Wistar rats (Bor: WISW [SPF-CpbJ, source: Winkelmann, Borchen, Germany) were exposed to spiroxamine (batch no.: 17002/90; purity: 95.3 %) at mean analytical aerosol concentrations of 14.3, 87.0 or 518.4 mg/m³ air under dynamic conditions for 6 h/d and 5 d/wk (head-nose-only) over a period of four weeks (MMAD = 1.2 µm; GSD = 1.5, mass fraction of particles with aerodynamic diameter ≤ 3 µm was ≥ 98 %) Rats exposed to conditioned air or to an aerosol of the vehicle (blend of polyethylene glycol 400 and ethanol) were used as control animals.

Findings:
Physical parameters - test atmosphere: The results show that exposure conditions which met the standards for stability and exhibited the necessary degree of reproducibility existed throughout the exposure period.

Table B.6.3-34: Physical parameters - test atmosphere

Nominal concentration (mg PE/m³ air)	0 [air]	0[veh.]	14.3	87.0	518.4
Aerosol concentrations (mg/m³ air)	0	0	14.3	87.0	1204.3

[air] = air control group; [veh.] = vehicle control group

General observations: Levels of 14.3 and 87.0 mg/m³ air were tolerated without clinical symptoms or mortality. There were no effects observed in reflex tests, rectal temperature or on body weight.
Rats exposed to 518.4 mg/m³ air exhibited un-groomed fur and decreased motility during exposure weeks zero and one. Clinical symptoms were observed at the beginning of exposure week two (among others: staggering gait, decreased motility, narrowed palpebral fissure, hypersalivation, un-groomed fur and piloerection, reddened conjunctivae, reddened and bloody rhinal zone, transient breathing sounds, abnormal digging and preening activities and an upright tail). One female rat died on day 25 of the study. Predominantly at the end of the study local dermal reactions were observed, particularly at the less densely haired contact sites. The ophthalmic examinations afforded evidence for test substance-induced corneal damage in this group. No evidence was seen for a change in the reflex pattern. The rectal temperature was marginally depressed in the rats exposed to 518.4 mg/m³ air (Table B.6.3-35).

Table B.6.3-35: Rectal temperatures (°C)

mg/m³	**0 [air]**	**0 [veh.]**	**14.3**	**87.0**	**518.4**
males					
Day 0	37.6	37.2	37.2	37.4	36.3 +
Day 7	37.9	38.1	37.5	37.8	37.1
Day 21	37.9	37.4	37.6	37.6	36.7 +

females					
Day 0	37.0	36.3	37.2	37.4	36.1
Day 7	38.0	37.5	38.1	37.9	36.8 +
Day 21	38.4	38.0	37.9	37.9	36.3

Toxicologically significant decreased body weight was observed in males at 518.4 mg/m³ air (Table B.6.3-36).

Table B.6.3-36: Mean body weights (g)

	air		vehicle		14.3 mg/m3		87.0 mg/m3		518.4 mg/m3	
	males	females	males	females	males	females	males	females	males	females
Week 0	200	176	198	182*	201	180	196	179	200	179
Week 1	203	172	200	177	204	180*	197	178**	200	180**
Week2	212	167	207	170	215	172	204	169	197*	168
Week 3	225	167	219	170	227	174*	216	172	195**	165
Week 4	235	168	228	174	239	176	224	174	190**	165

+ = U-test, 5 % significance level: ++ = U-test, 1 % significance level

Haematology: At highest dose level an increase in blood coagulation time (H-Quick), depressed thrombocyte (THRO) and elevated leucocyte counts (LEU) were observed. The differential blood count revealed relative increase in polymorphonuclear granulocyte fraction (SEGM) and relative decrease in the lymphocyte fraction (LYM) at levels of 87.0 mg/m³ air and above. These effects are regarded as causally related to the inflammatory changes which occurred in the skin areas. Marginal decreases in the haemoglobin level (HGB) and haematocrit (HCT) were determined in females at 87.0 mg/m³ air and above. With respect to changes in the haematology, a level of 14.3 mg/ m³ air was tolerated without effect (Table B.6.3-37).

Table B.6.3-37: Haematological parameters

mg/m³	0 [air]	0 [veh.]	14.3	87.0	518.4
Males					
HQUICK [sec]	33.1	33.8	32.9	34.5	37.1 ++
LEU [10E9/L]	6.4	6.0	6.0	5.1	8.1
THRO [10E9/L]	918	986	896	983	831
SEGM [%]	8.3	9.6	10.1	13.0 ++	28.8 ++
LYM [%]	87.5	85.1	86.8	84.0 +	67.6 ++
HGB [g/L.]	146	154 +	150	144	138
HCT [l/L]	0.479	0.497	0.485	0.468	0.448 +
Females					
HQUICK [sec]	29.7	30.3	30.6	28.9	33.7 ++
LEU [10E9/L]	3.8	4.6	5.6	4.1	6.5 +
THRO [10E9/L]	951	1052	1027	908	777 ++
SEGM [%]	8.4	9.5	9.9	12.2	27.4 ++
LYM [%]	88.0	88.0	86.8	85.4	69.9 ++
HGB [g/L.]	140	139	145	131 +	127 +
HCT [l/L]	0.446	0.443	0.471	0.425	0.415

+ = U-test, 5 % significance level: ++ = U-test, 1 % significance level

Clinical chemistry: Particularly at 518.4 mg/m³ air elevated serum ALAT and ASAT activities, depressed plasma cholinesterase activity (CHE, females only), reduced total protein

(PROT) and albumin levels, and an increase in globulin fraction (GLOB) and relative re¬duction in the albumin fraction in protein electrophoresis were measured. The cholesterol level (CHOL) was dose related reduced at 87.0 mg/m³ air and above. Evidence for a significant change in the N-demethylase/O-demethylase (N-DEM/O-DEM) or cytochrome P-450 activities (P 450) was only found in the 518.4 mg/m3 air group (N-DEM/P-450 depress¬ed in males, not affected to a toxicologically significant extent in females; O-DEM slightly elevated in males and females) (Table B.6.3-38).

Table B.6.3-38: Clinical chemistry

mg/m³	0 [air]	0 [veh.]	14.3	87.0	518.4
Males					
ASAT [U/L]	51.6	57.4	54.1	56.2	75.4 ++
ALAT [U/L]	43.9	47.0	46.0	45.4	84.4 ++
ALBUMIN [g/L]	31.7	32.4	31.7	31.0	27.6 ++
ALBUMIN [%]	59.9	60.6	61.2	59.8	52.9 ++
a1-GLOB [%]	16.7	16.7	16.3	16.9	14.1 ++
a2-GLOB [%]	6.5	6.3	6.2	6.4	7.4 ++
b-GLOB	14.8	14.3	14.0	14.6	20.8 ++
g-GLOB	2.1	2.2	2.3	2.3	4.8 ++
O-DEM [mU/g]	11.0	11.7	12.0	14.0 +	13.5
N-DEM [mU/g]	121.5	130.9	119.0	121.5	94.8 ++
P450 [nmol/g]	40.5	39.9	41.4	42.1	31.2 ++
Females					
ASAT (GOT) [U/L]	62.9	57.2	56.4	52.9	88.4 ++
ALAT (GPT) [U/L]	39.6	43.2	41.6	45.3	86.1 ++
ALBUMIN [g/L]	31.8	33.3	31.8	32.5	25.7 ++
PROT [g/L]	61.0	66.1 ++	60.1	58.9	56.1 +
CHE [kU/L]	1.66	1.78	1.71	1.47	0.78 ++
ALBUMIN [%]	61.9	60.2 +	61.7	62.2	51.8 ++
a1-GLOB [%]	14.5	14.6	14.2	14.4	13.8
a2-GLOB [%]	5.5	6.2 +	5.8	5.4	7.6 ++
b-GLOB	14.6	14.9	15.2	15.1	21.9 ++
g-GLOB	3.5	4.1	3.0	2.9 ++	4.9 ++
O-DEM [mU/g]	10.2	9.7	11.1	11.3	13.4 ++
N-DEM [mU/g]	78.7	67.4	63.5	69.0	88.5
P450 [nmol/g]	32.3	32.1	34.8	35.3	31.7

+ = U-test, 5 % significance level; ++ = U-test, 1 % significance level

Urinalysis: At 518.4 mg/m³ air urinalysis revealed increased levels of proteins (PROT), bilirubin (BILI), urobilinogen (UBG), ketone bodies (KETO), ammonium-magnesium (triple) phosphate and corpuscular components in the group animals. A concentration related increase in ammonium-magnesium phosphate level was present in female rats at levels of 87.0 mg/m³ air and above (Table B.6.3-39).

Table B.6.3-39: Urinalysis

mg/m³	0 [air]	0 [veh.]	14.3	87.0	518.4
Males					
PROT (GRADE 3)	1/10	0/10	0/10	0/10	6/10 +
BILI (GRADE 1)	2/10	0/10	0/10	0/10	9/10 ++
UBG (GRADE 1)	2/10	0/10	0/10	0/10	9/10 ++
KETO (GRADE 1)	6/10	7/10	3/10	1/10	10/10 +

Females					
PROT (GRADE 3)	0/10	0/10	0/10	0/10	6/10 ++
BILI (GRADE 1)	0/10	0/10	0/10	0/10	8/10 ++
UBG (GRADE 1)	0/10	0/10	0/10	0/10	7/10 ++
KETO (GRADE 1)	1/10	0/10	0/10	0/10	6/10 +

+ = U-test, 5 % significance level; ++ = U-test, 1 % significance level

Organ weights: At 518.4 mg/m³ air absolute organ weights were statistically significant affected: reduced thymus weight in males and females; reduction in heart and spleen weights in males; increased liver and kidney weights in females. Relative organ weights were also affected at this dose level (Table B.6.3-40).

Table B.6.3-40: Absolute (mg) and [relative (mg/100 g bw)] organ weights

	0 [air]		**0 [veh.]**		**14.3**		**87.0**		**518.4**	
	males	females	males	females	males	females	males	females	males	females
Thymus	308 (130)	204 (119)	290 (125)	194 (110)	338 (142)	204 (114)	268 (119)	206 (119)	94** (48**)	73** (44**)
Heart	824 (350)	660 (386)	826 (361)	671 (382)	858 (361)	668 (377)	788 (351)	662 (382)	723** (380)	655 (399)
Spleen	460 (196)	315 (184)	398 (173)	293 (168)	464 (195)	337 (189)	422 (188)	301 (173)	301** (157*)	262 (159)
Liver	8433 (3579)	5399 (3155)	8072 (3508)	5625 (3206)	8274 (3462)	5740 (3232)	7573 (3370)	5593 (3225)	7767 (4087**)	6341* (3850**)
Kidneys	1503 (639)	1085 (634)	1363 (594)	1109 (633)	1437 (604)	1133 (638)	1385 (616)	1131 (652)	1397 (736*)	1184 (720**)
Adrenals	48 (20)	53 (31)	45 (20)	54 (31)	47 (20)	60** (34)	43 (19)	55 (32)	51 (27)	65** (40**)
Testes	2764 (1175)	-	2691 (1173)	-	2875 (1215)	-	2718 (1211)	-	2702 (1419**)	-

* = U-test, 5 % significance level; ** = U-test, 1 % significance level

Pathology: The histopathology of the 518.4 mg/m³ air group showed squamous epithelial metaplasia in the nasal cavity, as well as epithelial hyperplasia and hyperkeratosis in the larynx zone. An elevated rate of bronchiolo-alveolar proliferation with an increase in alveolar macrophages was noted in lungs, and hyperkeratosis in the oesophagus. The eyes exhibited corneal hyperplasia, and the eyelids hyperplasia accompanied by hyperkeratosis. The severest local dermal lesions (hyperkeratosis, epithelial hyperplasia, extended inflammatory infiltration, scab) were found in the muzzle zone. Hyperkeratosis and epithelial hyperplasia were also seen in the mamma zone and on the tail. The male animals exhibited atrophic thymus changes. The urinary bladder urothelium exhibited hyperplastic lesions in the 518.4 mg/m³ air group animals.

Table B.6.3-41: Incidence of histopathological findings

mg/m³ **Sex**	**0 [air]** **m / f**	**0 [veh.]** **m / f**	**14.3** **m / f**	**87.0** **m / f**	**518.4** **m / f**
EYES AND EYELIDS (no. of animals examined)	10 / 10	10 / 10	10 / 10	10 / 10	10 / 10
corneal hyperplasia	0 / 0	0 / 0	0 / 0	0 / 1	4 / 4
eyelid hyperplasia	0 / 0	0 / 0	0 / 0	0 / 0	9*/ 9*
eyelid hyperkeratosis	0 / 0	0 / 0	0 / 0	0 / 0	10*/10*
NASAL / PARANASAL CAVITIES (no. of animals examined)	10 / 10	10 / 10	10 / 10	10 / 10	10 / 10
squamous-cell hyperplasia	1 / 4	1 / 5	2 / 2	3 / 4	8*/ 3
goblet-cell hyperplasia	0 / 4	3 / 5	4 / 4	2 / 5	2 / 5
hyperaemia	6 / 8	7 / 7	3 / 5	8 / 7	5 / 4
LARYNX					

mg/m³ Sex	0 [air] m / f	0 [veh.] m / f	14.3 m / f	87.0 m / f	518.4 m / f
(no. of animals examined)	10 / 10	10 / 10	10 / 10	10 / 10	10 / 10
hyperplasia	0 / 0	0 / 0	0 / 1	3 / 2	7*/ 8*
hyperkeratosis	0 / 0	0 / 0	0 / 1	2 / 2	7*/ 7*
round-cell infiltration	0 / 1	0 / 0	0 / 2	0 / 1	6+/ 5
LUNGS (no. of animals examined)	10 / 10	10 / 10	10 / 10	10 / 10	10 / 10
hyperaemia	4 / 5	7 / 8	6 / 7	8 / 5	8 / 9
bronch./alveol. prolif.	0 / 0	1 / 0	0 / 0	0 / 0	7*/ 2
thickening of septa	1 / 0	1 / 0	1 / 1	0 / 3	8* / 1
OESOPHAGUS (no. of animals examined)	10 / 10	10 / 10	10 / 10	10 / 10	10 / 10
hyperkeratosis	0 / 0	0 / 0	0 / 0	0 / 0	2 / 8*
LIVER (no. of animals examined)	10 / 10	10 / 10	10 / 10	10 / 10	10 / 10
hyperaemia	2 / 4	3 / 6	3 / 2	2 / 5	8+/ 7
vacuolation hepatocytes	0 / 6	5+ / 1	2 / 4	5+/ 1	9*/ 5
MESENTERIAL LYMPH NODES (no. of animals examined)	10 / 10	10 / 10	10 / 10	10 / 10	10 / 10
sinus catarrh	3 / 6	3 / 2	9+ / 5	7 / 4	10*/ 4
BLADDER (no. of animals examined)	10 / 10	10 / 10	10 / 10	10 / 10	10 / 10
hyperplasia	0 / 0	0 / 0	0 / 0	0 / 0	4 / 5+

+ = U-test, 5 % significance level; ++ = U-test, 1 % significance level

Conclusion:

A concentration of 14.3 mg KWG 4168 /m³ air was the no observed effect concentration. Assuming a minute volume of one litre per kilogram rat, this concentration is approximately equivalent to a nominal exposure dose of about 5.1 mg/kg bw and exposure day. It was based on haematological findings at 87.0 mg/m³ air (main effect: increase in the polymorphonuclear granulocyte fraction which is considered to be causally related to the irritation induced epithelium alterations).

A subchronic inhalation study is not necessary, because the 28-day study showed low toxicity based mainly on local effects. Furthermore the vapour pressure is < 10^{-2} Pa.

Re-evaluation in 2009

In this study the NOAEC was 14.3 mg KWG 4168 /m³ air, equivalent to 3.9 mg/kg bw/d. (according to the AOEL Guidance document: 45 L/kg bw/h x 14.3 mg/m³/1000 x 6h) due to haematological effects at 87.0 mg/m³ air (equivalent to 23.49 mg/kg bw/d).

2.1.1.2 Long-term toxicity and carcinogenicity studies

B.6.5 Long-term toxicity and carcinogenicity (OECD Annex IIA 5.5)

In addition to the available long-term studies in rats and mice a second long-term study in mice was submitted in 2008.

In long-term toxicity studies in rats and mice, histopathological alterations of the epithelium of the gastrointestinal tract were noted. These effects (hyperkeratosis and acanthosis of the epithelium of tongue, oesophagus and fore-stomach,) might be interpreted as an adaptive process following the continuous irritant stimulus by spiroxamine. Furthermore, skin alterations at auricles and tips of the tail were observed in mice. Additionally, clear systemic signs of toxicity (such as low body weights, findings in uterus, ovaries or liver) occurred. Hence the NOAEL do not change, whether some findings are interpreted as local effects.
No evidence of an oncogenic potential of spiroxamine was found in the long-term feeding studies in rats and mice.

In the two available long-term studies in mice, a dose level of 160 ppm was tested in both, however with different result: In the first study this was the LOAEL, whereas it was the NOAEL in the other. This can not be explained with the achieved compound intake, because they are comparable. As a conservative approach, the dose level of 20 ppm (equal to 4.5/7.8 mg/kg bw/d) is considered the overall NOAEL for mice.
The NOAEL for systemic effects in rats was 70 ppm (equal to 4.22/5.7 mg/kg bw/d).

Table B.6.5-1: Summary of long-term toxicity and carcinogenicity studies

Study	Dose levels	NOAEL	Targets / Main effects
24 mo, feed; Wistar rats 50 M+50 F	0-10-70-490 ppm (equal to 0/0-0.66/0.77-4.22/5.67-32.81/43.04 mg/kg bw/d for M/F)	70 ppm (equal to 4.22/5.7 mg/kg bw/d)	490 ppm: acanthosis & hyperkeratosis of oesophagus mucosa; hyperplasia of urinary bladder urothel; bw ↓; mortality ↑; uterus (masses, distention)
26 mo, feed B6C3F1 mice 50 M+50 F OECD 451	0-2.5/480*-20-160 ppm (equal to 0/0-59.3/102.6-4.5/7.8-36.7/59.3 mg/kg bw/d for M/F)	20 ppm (equal to 4.5/7.8 mg/kg bw/d)	2.5/480 ppm: acanthosis & hyperkeratosis of tongue, oesophagus & tail; acanthosis of auricles; cysts in ovaries, bw ↓ 160 ppm: acanthosis & hyperkeratosis of oesophagus & tail; acanthosis of auricles; cysts in ovaries (1 animal), bw ↓
104 wk, feed; B6C3F1 mice 50 M+50 F 10 M+10 F (interim) OECD 451	0-160-600 ppm (equal to 0/0-41.0/64.4-149.8/248.1 mg/kg bw/d for M/F)	160 ppm (equal to 41.0/64.4 mg/kg bw/d)	600 ppm: acanthosis & hyperkeratosis of tongue, oesophagus, fore-stomach, pinna, tail; bw ↓; liver (histological changes) No carcinogenic potential

* = increased to 480 ppm from week 32 to termination based on the results of the supplemental study

B.6.5.1 Rat

Reference: KIIA 5.5 (OECD)

Report:	Eiben, R. (1994) KWG 4168 - Investigations of chronic toxicity and carcinogenicity in Wistar rats (administration in diet over 2 years) - Report no.: 23580 (December 28, 1994) Bayer AG, Institute for Toxicology, Germany TOX9552606
Guidelines:	OECD TG 453 (1981)
Deviations:	Only 10 animals/dose group/sex used for haematological tests and these tests were not conducted 3 mo after study initiation. In wk 102, pH of spontaneous urine was determined.
GLP:	Yes
Acceptability:	The study is considered to be acceptable.

Dates of exp. work: April 1991 - May 1993.

Material and methods:
Test system: Spiroxamine (batch no. 17002/90; purity: 94.3 - 95.6 %) was administered to 50 male and 50 female Wistar rats (Bor: WISW [SPF Cpb], source: Winkelmann, Borchen, Germany) per dose group at diet concentrations of 0, 10, 70 or 490 ppm up to 24 months. Further 10 animals per dose and sex were treated for 12 months and then necropsied for interim analysis. Mean consumption of spiroxamine per kg bw per day was 0.61, 4.22 and 32.81 mg for males and 0.77, 5.67 and 43.04 mg for females in ascending order of dose.

Findings:
Feed analysis indicated that the individual batches prepared reached the intended concentration within a range of 81.4-110 %, 85.4-109 % or 86.3-106 % for 10, 70 or 490 ppm dietary concentrations, respectively. Within 14 d of storage, feed concentration reached 80 % (2.5 ppm) or 87 % (3000 ppm) of the initial concentration [results in this report were taken from the first mouse carcinogenicity study or the 13-wk, rat study, respectively].

No treatment-related clinical signs were observed at doses up to 490 ppm. There were no toxicologically relevant changes in feed intake at doses up to 490 ppm or in water consumption at doses up to 70 ppm (Table B.6.5-2). Rats in the high dose group drank 10-12 % less, which was observed consistently throughout the study period and reached statistical significance in most instances. There was no effect on body weight gain at doses of up to 70 ppm. At the end of the treatment period, body weights in animals treated with 490 ppm were reduced by 7-11 % in both sexes. Body weight at this dose level was reduced during the whole treatment period.

Table B.6.5-2: Selected mean data on body weight and feed, test compound and water intake

	Males				Females			
Dose level (ppm)	**0**	**10**	**70**	**490**	**0**	**10**	**70**	**490**
Body weight (g)								
Wk 0	116	116	117	119	109	109	107	108
Wk 1	154	157	156	144**	128	130	125*	122**
Wk 4	249	250	249	224**	167	169	167	161*
Wk 13	359	363	357	337**	215	218	211	200**
Wk 29	405	415	410	387**	239	241	235	220**
Wk 53	443	448	438	415**	265	264	261	240**
Wk 79	444	446	441	419**	281	275	273	255**
Wk 103	455	454	453	424**	301	285	290	268**
Feed intake								
g/d	20.5	20.8	20.5	20.9	17.4	16.9	17.4	17.8
g/kg bw/d	60.3	61.0	60.3	67.0	79.4	70.3	81.1	87.8
Test compound intake								
mg/kg bw/d	0	0.61	4.22	32.81	0	0.77	5.67	43.04
Water intake								
g/d	26.5	26.2	26.3	23.9	24.1	24.3	23.7	21.3
g/kg bw/d	78.2	77.1	77.6	75.5	107.4	108.7	107.3	103.4

Mortality was not increased significantly in males of all groups. In 490 ppm females, there was a statistically increased incidence of deaths in the late phase of the study (Table B.6.5-3); the increase noted in the 70 ppm group was not statistically significant. Several females in 70 ppm dose group were killed in the lasts 3 weeks due to humane reasons (e.g. large subcutaneous masses).

Table B.6.5-3: Cumulative mortality

Dose [ppm] **Sex**	**0** **M / F**	**10** **M / F**	**70** **M / F**	**490** **M / F**
Wk 26 (n=60)	0/1 (0 %/2 %)	0/0 (0 %/0 %)	0/0 (0 %/0 %)	0/1 (0 %/2 %)
Wk 52 (n=60)	1/2 (2 %/3 %)	1/2 (2 %/3 %)	0/1 (0 %/2 %)	0/3 (0 %/5 %)
Wk 78 (n=50)	2/6 (4 %/12 %)	3/8 (6 %/16 %)	1/3 (2 %/6 %)	0/7 (0 %/14 %)
Wk 101 (n=50)	7/15 (14 %/30 %)	9/14 (18 %/28 %)	7/16 (14 %/32 %)	7/21 (14 %/42 %)
Wk 104 (n=50)	8/15 (16 %/30 %)	11/16 (22 %/32 %)	8/23 (16 %/46 %)	8/24 (16 %/48 %)

There was no evidence of treatment-related haematological changes. No toxicological relevant changes of clinical chemistry or urinalysis parameters were detected.
Ophthalmological evaluation revealed an increased incidence of lens turbidities in high dose animals after 2 yr of treatment. However the increase was small and within the normal range as indicated by historical control data.

Table B.6.5-4: Findings in ophthalmological evaluation

	Males				Females			
Dose level (ppm)	**0**	**10**	**70**	**490**	**0**	**10**	**70**	**490**
Number of eyes	86	78	85	86	72	68	62	58
Turbidity in cornea	26	15	21	17	3	2	1	0
Turbidity in lens	34	32	36	41	14	21	19	21

Gross pathological examination in animals of interim kill did not indicate any treatment-related effects. Especially in prematurely killed females of the high dose group (scheduled for final necropsy), more uterine masses (1, 1, 1, 6 for 0, 10, 70, 490 ppm, respectively) and distention (2, 5, 4, 8) were observed. In females, decreased incidences of thickening of skin at mammary gland were noted in high dose group (6, 5, 7, 3; all animals scheduled for final necropsy).
Absolute and relative organ weights did not indicate any treatment-related effects.
Acinar hyperplasia at mammary area was noted in animals that died prematurely; however incidences in all animals combined did not indicate an effect, therefore, this finding is considered to be no treatment-related effect. Hyperkeratosis and acanthosis were observed in the oesophagus of animals receiving 490 ppm (interim and final necropsy). In this dose group, an increased number of females exhibited hyperplasia in the urinary bladder.

Table B.6.5-5: Incidence of histopathological findings

	Males				Females			
Dose (ppm)	**0**	**10**	**70**	**490**	**0**	**10**	**70**	**490**
INTERIM NECROPSY§								
OESOPHAGUS (no. examined)	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*
- Acanthosis	0C	0	0	10c	0C	0	0	9c
- Hyperkeratosis	0C	0	0	10c	0C	0	0	9c
FINAL NECROPSY								
OESOPHAGUS (no. examined)	*50*	*49*	*50*	*50*	*50*	*50*	*50*	*49*
- Acanthosis	0C	0	0	40c	0C	0	0	39c
- Hyperkeratosis	0C	0	0	40c	0C	0	0	39c
- Acute inflammation	0	1	0	0	0	0	0	0
URINARY BLADDER (no. examined)	*50*	*48*	*50*	*50*	*50*	*50*	*50*	*48*
- Transitional cell hyperplasia	0	2	1	0	0A	1	0	4
MAMMARY AREA (no. examined) [premature dead/killed animals]	*8*	*11*	*8*	*10*	*16*	*16*	*23*	*23*
- Acinar hyperplasia	0	0	0	0	4	6	11	10
MAMMARY AREA (no. examined) [premature death animals]	*50*	*49*	*50*	*50*	*50*	*50*	*50*	*49*
- Acinar hyperplasia	0	0	0	0	13	23	19	19

A = trend $p < 0.05$ b = $p < 0.01$ significant different from control
C = trend $p < 0.001$ c = $p < 0.001$ significant different from control
§. Incidences were taken from the tables in the pathology report (the numbers are not in agreement with the data in the results section of the study report)

The incidence, time of occurrence and nature of tumours provided no indication of any relationship with treatment (Table B.6.5-6). [In mammary gland, a decreased incidence of carcinoma was seen in females of high dose group, however the reason is unknown.]

Table B.6.5-6: Incidence of animals with tumours

INTERCURRENT DEATHS								
Sex	Males				Females			
Dose (ppm)	0	10	70	490	0	10	70	490
No. of animal examined	8	12	8	10	16	16	23	24
Animals with tumours	5	6	6	7	14	9	20	16
Animals with benign tumours only	4	4	0	2	6	4	7	11
Animals with malignant tumours only	1	2	4	3	5	3	4	3
Animals with benign/malignant tumours	0	0	2	2	3	2	9	2
FINAL NECROPSY								
Sex	Males				Females			
Dose (ppm)	0	10	70	490	0	10	70	490
No. of animals examined	42	38	42	40	34	34	27	26
Animals with tumours	20	20	23	18	19	24	11	16
Animals with benign tumours only	12	14	17	13	12	17	6	12
Animals with malignant tumours only	4	5	4	5	2	2	1	0
Animals with benign/malignant tumours	1	1	2	0	5	5	4	4

Conclusion:
The NOEL was determined at 70 ppm, equal to 4.22 mg/kg bw/d in males and 5.67 mg/kg bw/d in females. It was based on histopathological findings at 490 ppm (hyperkeratosis and acanthosis of oesophagus epithelium, hyperplasia of urinary bladder epithelium).

Re-evaluation in 2009:
In 490 ppm dose group (equal to 32.8 or 43.0 mg/kg bw/d in males or females, respectively), lower body weights and increased mortalities (during the final part of the study) were observed. In prematurely killed animals, higher incidences of uterine masses and distentions were noted. Hyperkeratosis and acanthosis were observed in the oesophagus. An increased number of females exhibited hyperplasia in the urinary bladder.
No effects were noted in 10 ppm (equal to 0.61/0.77 mg/kg bw/d) and 70 ppm dose group (equal to 4.22/5.67 mg/kg bw/d), which was the NOAEL.

In this study no carcinogenic potential was detected.

B.6.5.2 Mouse

Reference: KIIA 5.5 (OECD)
Report: Eiben, R. and E. Hartmann (1995) KWG 4168 - Summary report of an oncogenicity study and a supplementary six months chronic toxicity study in B6C3F1 mice - Report no.: 23975 (May 05, 1995) Bayer AG, Institute for Toxicology, Wuppertal, Germany. TOX9552613
Guidelines: OECD TG 451

Deviations:	In addition to guideline-required parameters, haematology and clinical chemistry were performed after 12, 18 (differential blood count only) and 26 mo as required by OECD TG 453. Additional groups of 10 animals/sex/dose group were sacrificed after 1 yr of treatment (evaluated parameters: gross-necropsy, organ weights, histopathology). pH of spontaneous urine was determined in 10 animals/group/sex at study termination.
GLP:	Yes (oncogenicity study) No (supplementary study)
Acceptability:	The study is considered to be acceptable.

Dates of exp. work: main study: January 1991- March 1993; supplementary study: January 1991-July 1991

Material and methods:
Test system: Groups of 50 male and 50 female mice (B6C3F1/BOM, source: Bomholtgaard Experimental Animal Breeders, Denmark) were administered spiroxamine (batch no. 17002/90; purity: 94.3 - 95.6 %) at diet concentrations of 0, 20, 160 or 480 ppm. The study was started at dose levels of 0, 2.5, 20 and 160 ppm. But as in an additional study started simultaneously (see below) no signs of toxicity were found after 6 months in animals receiving 160 ppm, the low dose group concentration (2.5 ppm) was increased to 480 ppm beginning at week 32. The study then lasted for another 18 months. The duration of the animal experimental part was 26 month. Ten additional animals per group, which were scheduled for interim necropsy after one year, were administered the test substance at identical concentrations.
The treated mice ingested (in order of increasing doses: 20-160-2.5/480 ppm) the following mean levels of spiroxamine: 4.5, 36.7 and 59.3 mg/kg bw/d (males); 7.8, 59.5 and 102.6 mg/kg bw/d (females).

Findings:
Supplementary six months chronic toxicity study:
In an additional study started simultaneously with the main oncogenicity study, groups of seven male and seven female B6C3F1 mice were administered spiroxamine at levels of 0 or 160 ppm in their diet over a period of six months. The purpose of this study was to examine whether the 160 ppm level, which corresponded to the high dose in the oncogenicity study, leads to adverse effects on the skin, kidneys and urinary bladder.
No clinical signs were observed. One female of 160 ppm dose group died in week 13. The body weight development of the treated mice was not affected to a toxicologically significant extent. No macroscopic or microscopic evidence was found for any effects on kidneys, urinary bladder, skin or other examined organs in the treated mice. A concentration of 160 ppm administered for 6 months was thus tolerated by mice without any adverse effects.

Oncogenicity study:
General observations: There was no increase in mortality at levels up to 480 ppm. Treated and untreated mice could not be differentiated on the basis of their appearance, behaviour or general condition at levels up to 160 ppm. Mice with increased test compound-concentration (2.5 to 480 ppm in wk 32) showed desiccation of skin on auricles from wk 41 to wk 51.
The food and water intakes were not affected to a toxicologically significant extent at levels up to 480 ppm. The body weight development at 20 ppm in males, and at levels up to 160

ppm in females, was comparable to that of the control group. Statistically significant (up to 6 %) decreases in the body weights were determined at the high dose in each case (Table B.6.5-7). Results of haematological and clinical chemistry evaluations were unremarkable. Determination of urine pH revealed no treatment-related differences in males. In females, the urine in 480 ppm dose group was slightly more alkaline: 7.96 vs. 7.55 (range: 7.63-8.84 vs. 7.0-8.14).

Table B.6.5-7: Mortality, body weight and achieved test compound intake

Dose [ppm] **Sex**	**0** **M / F**	**20** **M / F**	**160** **M / F**	**480** **M / F**
Mortality (111 wk) [%]	20/22	6/38	6/24	14/34
Body weight (0 wk) [g]	23/19	23/19	22/19	23/19
Body weight (55 wk) [g]	37/28	37/28	35**/28	34**/27*
Body weight (111 wk) [g]	33/29	33/29	33/29	31**/28
Test compound intake [mg/kg bw/d]	0/0	4.5/7.8	36.7/59.5	59.3/102.6

* = U-test, 5 % significance level; ** = U-test, 1 % significance level

Gross pathology in animals killed after one year of treatment did not indicate treatment-related effects. At terminal sacrifice, in stomach of high dose animals (2 M, 3 F) areas of change were observed. Cystic uterus was observed in 15 of 50 animals in intermediate dose group. This finding was also observed in incidences of 2, 5 or 1 in control group, low dose or high dose groups, respectively.
No evidence for organ damage could be determined at levels up to 480 ppm on the basis of the organ weight determinations performed in animals of interim and terminal sacrifice.
Desiccation of the auricular epidermis was observed shortly after the 2.5 ppm dose was increased to 480 ppm. Acanthosis of this section of skin could be determined as a histological correlative in these animals. Comparable epithelial changes were also observed at the oesophagus and the tip of the tail at 160 ppm and above, as well as on the tongue at 480 ppm (Table B.6.5-8). Ovaries in intermediate and high dose animals showed bursal cysts. In addition acantholysis (both sexes) and chronic or acute inflammation (females only) of the oesophagus and tongue were evident in the high dose groups.

Table B.6.5-8: Incidence of pathological findings

INTERIM NECROPSY								
Sex	**Males**				**Females**			
Dose (ppm)	**0**	**20**	**160**	**480**	**0**	**20**	**160**	**480**
OESOPHAGUS (no. examined)	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*
- Acanthosis	0C	0	5a	6b	0C	0	6a	9c
- Hyperkeratosis	0B	0	2	5b	0C	0	0	9c
PINNAE (no. examined)	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*
- Extensive acanthosis bilat.	0	0	0	0	0B	0	0	4
- Extensive acanthosis unilat.	0	0	0	0	0	0	0	1
OVARIES (no. examined)					*10*	*10*	*10*	*10*
- Bursal cyst					0A	0	1	4
FINAL NECROPSY								
Sex	**Males**				**Females**			
Dose (ppm)	**0**	**20**	**160**	**480**	**0**	**20**	**160**	**480**
OESOPHAGUS (no. examined)	*50*	*50*	*50*	*50*	*48*	*50*	*49*	*47*
- Acanthosis	0C	0	1	36c	0C	0	1	22c
- Hyperkeratosis	1C	0	7	45c	0C	0	5	41c
- Acantholysis	0	0	0	2	0B	0	0	6a
- Chronic inflammation	0	0	0	0	0	0	0	1
TONGUE (no. examined)	*50*	*50*	*50*	*50*	*49*	*50*	*50*	*47*
- Hyperkeratosis	0A	0	0	4	0C	0	0	7b
- Acantholysis	0	0	0	2	0	0	0	2
- Acute inflammation	0	0	0	0	0	0	0	1
PINNAE (no. examined)	*50*	*50*	*50*	*50*	*50*	*50*	*50*	*50*
- Extensive acanthosis bilat.	0C	2	1	46c	0C	0	1	32c
- External surface: peripheral acanthosis bilat.	1	1	3	0	0A	0	1	4
TAIL (no. examined)	*49*	*49*	*49*	*50*	*47*	*49*	*49*	*46*
- Hyperkeratosis	0B	0	0	6a	0	0	0	1
- Extensive acanthosis	1C	2	12b	31c	0A	0	3	4

A = trend $p < 0.05$ a = $p < 0.05$ significant different from control
B = trend $p < 0.01$ b = $p < 0.01$ significant different from control
C = trend $p < 0.001$ c = $p < 0.001$ significant different from control

An increased incidence of malignant lymphomas in females of the high dose group (intercurrent deaths) was observed (Table B.6.5-9). A similar increase was not observed in females of terminal sacrifice of either sex or in decedent males. Hence it was considered by the pathologist to be a chance occurrence. Historical control data showed a range of 2-17 lymphoma / control group. Additionally, the combined incidences (intercurrent deaths + terminal kill) showed no dose response.
The slight increase of harderian gland adenoma was within the range of historical control data (1-7 adenoma/control group).
Therefore, both findings were considered to be no indication of a carcinogenic effect.

Table B.6.5-9: Incidence of selected neoplastic findings

Sex	Males				Females			
Dose (ppm)	**0**	**20**	**160**	**480**	**0**	**20**	**160**	**480**
H'poietic tumours (no. examined)$	10	4	3	7	10	19	12	15
- Malignant Lymphoma	2	1	0	1	1B	3	3	8a
H'poietic tumours (no. examined)§	40	46	47	43	39	31	38	33
- Malignant Lymphoma	0	1	2	2	3	7	2	3
Harderian gland (no. examined)$	10	4	3	7	10	19	12	14
- Benign adenoma	2	0	0	0	0	1	0	1
Harderian gland (no. examined)§	40	45	47	42	39	31	38	33
- Benign adenoma	1	1	0	1	0A	1	3	3

$: animals that died during treatment period; §: animals killed at terminal sacrifice

No carcinogenic potential of spiroxamine can be inferred from the type, incidence and time to occurrence of benign or malignant tumours or from the rate of animals with tumours (Table B.6.5-10).

Table B.6.5-10: Incidence of animals with tumours

INTERCURRENT DEATHS								
Sex	Males				Females			
Dose (ppm)	0	20	160	480	0	20	160	480
No. of animal examined	10	4	3	7	11	19	12	17
Animals with tumours	7	2	3	5	8	16	9	13
Animals with benign tumours only	1	0	2	2	1	3	0	2
Animals with malignant tumours only	4	2	1	3	7	12	9	10
Animals with benign/malignant tumours	2	0	0	0	0	1	0	1
FINAL NECROPSY								
Sex	Males				Females			
Dose (ppm)	0	20	160	480	0	20	160	480
No. of animals examined	40	46	47	43	39	31	38	33
Animals with tumours	9	16	16	16	14	15	21	16
Animals with benign tumours only	6	7	8	6	7	5	10	7
Animals with malignant tumours only	2	9	7	8	4	8	7	5
Animals with benign/malignant tumours	1	0	1	2	3	2	4	4

Conclusion:
The NOEL in this long-term study was determined at 20 ppm, equal to 4.5 mg/kg bw/d in male mice and equal to 7.8 mg/kg bw/d in females. It was based on reduced body weight development and epithelial changes at the oesophagus and the tip of the tail at 160 ppm.

Re-evaluation in 2009:
In 480 ppm dose group (equal to 59.3/102.6 mg/kg bw/d), desiccated auricular skin, acanthosis, hyperkeratosis, acanthalysis and inflammation at tongue, oesophagus, tail, and auricular skin were noted. Additionally, cysts in ovaries and lower body weights were noted.
In 160 ppm dose group (equal to 36.7/59.5 mg/kg bw/d), lower body weights were noted in males. One female showed cyst in ovaries in the interim sacrifice. Acanthosis and hyperkeratosis in oesophagus, pinnae and tail were detected.
No effects were noted in 20 ppm dose group (equal to 4.5/7.8 mg/kg bw/d), which was the NOAEL.
No carcinogenic potential was detected in this study.

B.6.5.3 Mouse

The following study was submitted for the re-evaluation of spiroxamine in 2009. In the first carcinogenicity study in mice (*cf.*, above), MTD was not reached. Probably due to this reason, a second study with a higher top dose level was initiated.

Reference:	KIIA 5.5 (OECD)
Report:	Eiben, R.; Sander, E. KWG 4168: Oncogenicity study in B6C3F1 mice (administration in diet over 2 years) - Supplementary Report numbers: 26780, MO-99-011327, T3055616 Report date: 1997-10-30 Bayer AG, Institute of Toxicology, Wuppertal, Germany ASB2007-5080
Guidelines:	OECD TG 451 (1981)
Deviations:	In addition to guideline-required parameters, haematology and clinical chemistry were performed after 51/52, 78 (differential blood count only) and 104/105 wks as required by OECD TG 453. Additional groups of 10 animals/sex/dose group were sacrificed after 1 yr of treatment (evaluated parameters: gross-necropsy, organ weights).
GLP:	Open
Acceptability:	The study is considered to be acceptable.

Material and Methods:

Test material:	KWG 4168
Development no.:	30-0097368
Description:	brown liquid
Lot/Batch no:	17002/90
Purity:	95 %
Stability of test compound:	guaranteed for study duration; expiry date: 1996-07-22
Vehicle:	1 % peanut oil (DAB 10) in mouse feed

Test animals:

Species:	mouse
Strain:	B6C3F1/Bom, SPF-bred
Age:	5 – 6 weeks
Weight at dosing:	males: 20 g – 24 g
females:	15 g – 20 g
Source:	Bomholtgård Breeding and Research Center Ltd., Ry, Denmark
Acclimatisation period:	one week
Diet:	Altromin® 1321 meal (Altromin GmbH, Lage, Germany), *ad libitum*
Water:	tap water, *ad libitum*
Housing:	individually, conventionally in Type II Makrolon® cages

bedding: low-dust soft-wood shavings (Ssniff GmbH, Soest, Germany)

Study design and methods
Animal assignment and treatment:
Dose: 0-160-600 ppm (dose selection based on previous studies) equal to:
males: 0-41.0-149.8 mg/kg bw/day
females: 0-64.6-248.1 mg/kg bw/day
Duration: terminal sacrifice: 24 months, interim sacrifice after 12 months
Application route: oral via feed
Group size: main groups: 50/sex/dose group
interim groups: 10/sex/dose group
Observations: mortality, clinical signs, body weight, feed intake, water intake, haematology, clinical chemistry, gross necropsy, organ weight, histopathology

In-life phase of the study: June 8, 1994 - June 26, 1996

Findings:
Feed was analysed for the amount of test substance. Feed containing a nominal concentration of 160 ppm was in a range between 100 and 85.3 % of the intended concentration. Feed containing a nominal concentration of 600 ppm was in a range between 103.8 and 83.3 % of the intended concentration.

Survival rates of male and female mice treated with spiroxamine were similar to those in the control groups throughout the study period.
No abnormalities were found in any of the animals treated with spiroxamine up to doses of 600 ppm when animals were checked for clinical signs or at detailed clinical examination; no evidence of treatment-related effects could be deduced from the frequency, localisation and time of appearance of palpable masses.
There was no toxicologically relevant effect on body weights of 160 ppm males and females. In 600 ppm high dose males mean body weight was up to 9.1 % lower than in controls during the whole in-life phase. In high dose females sporadically a slight body weight reduction was evident reaching statistical significance in isolated weeks (7.0 % in week 70 to 74).

Table B.6.5-11: Mean body weight and achieved dose level

	Males			Females		
Dose level (ppm)	**0**	**160**	**600**	**0**	**160**	**600**
Mean body weight (g)						
Wk 0	22	22	22	18	18	18*
Wk 4	24	23	23**	20	20	20
Wk 13	27	27	26**	23	23*	22**
Wk 26	31	31	29**	25	25	26
Wk 52	35	35	33**	28	28	27
Wk 78	35	36	33*	29	28	28
Wk 104	34	34	32*	29	28	28
Test compound intake (mg/kg bw/d)						
	0	41.0	149.8	0	64.6	248.1

The feed intake in all dose groups was comparable to that of untreated mice. Furthermore, there was no toxicologically relevant change in water intake.

Haematological investigations did not reveal any toxicologically relevant changes after doses up to 600 ppm spiroxamine. There was no effect on clinico-chemical parameters in this study. A few mean values marked as significant are of no relevance, since a dose dependence is missing or all individual values are within the 2s range of historical controls (e.g. protein and creatinine concentrations in females in week 52 or 105, respectively).

Table B.6.5-12: Clinical chemistry

	Males			Females		
Dose level (ppm)	**0**	**160**	**600**	**0**	**160**	**600**
Wk 52						
Glucose (mmol/L)	6.54	5.83*	5.68**	5.65	6.08	5.84
CHOL (mmol/L)	3.67	3.67	3.59	2.59	2.65	2.55
CREA (µmol/L)	28	24**	26	32	25**	25**
Urea (mmol/L)	10.59	11.13	10.46	10.09	9.75	10.70
BILI-t (µmol/L)	1.5	1.6	1.6	1.8	1.9	1.9
PROT (g/L)	60.8	57.7**	58.0**	62.4	59.5*	58.7**
ALB (g/L)	30.5	29.8	30.2	32.6	32.0	31.8
Wk 105						
Glucose (mmol/L)	7.21	7.23	7.33	6.28	6.22	6.01
CHOL (mmol/L)	3.63	3.84	3.46	2.38	2.23	2.53
CREA (µmol/L)	29	26*	26	28	26	23**
Urea (mmol/L)	11.75	10.96	11.99	10.78	10.69	10.38
BILI-t (µmol/L)	1.1	1.4*	1.6*	1.9	1.9	1.9
PROT (g/L)	60.5	59.7	59.8	59.0	59.9	58.4
ALB (g/L)	29.0	30.2	29.0	30.5	29.8	30.5

No treatment related macroscopical findings were observed in mice scheduled for interim sacrifice after a treatment period of 12 months. Gross-pathological findings recorded at necropsies performed during the study or at terminal sacrifice revealed no evidence of treatment related effects.

At interim necropsy absolute and relative organ weights recorded in the treatment groups did not differ remarkably from those of the control animals. At 600 ppm the slightly lower relative spleen weights in females and the slightly higher relative kidney and brain weights in males were observed. They might be attributed to the differences in body weight.
At terminal necropsy organ weights of brain, liver, spleen, kidneys and testes revealed no toxicologically relevant differences between spiroxamine treated animals and controls. Some deviations gained statistical significance (reduced absolute kidney weights in 600 ppm males, enhanced relative brain weights in 600 ppm females and elevated relative testes weights in 600 ppm males). They might be considered to be the consequence of differences in body weight.

Table B.6.5-13: Organ weight data at interim sacrifice

Absolute organ weights							
Dose **ppm**	**week**	**Body-weight** **g**	**Brain** **mg**	**Liver** **mg**	**Spleen** **mg**	**Kidneys** **mg**	**Testes** **mg**
Male							
0	52	37	488	1632	85	657	225

Absolute organ weights							
160	52	37	483	1598	90	666	211
600	52	33	493	1565	85	665	224
Female							
0	52	27	516	1339	111	394	
160	52	28	517	1367	112	404	
600	52	28	499	1371	102	392	
Relative organ weights							
Dose **ppm**	**week**	**Body-weight** **g**	**Brain** **mg/100 g**	**Liver** **mg/100 g**	**Spleen** **mg/100 g**	**Kidneys** **mg/100 g**	**Testes** **mg/100 g**
Male							
0	52	37	1346	4454	235	1801	621
160	52	37	1300	4278	241	1788	566
600	52	33	**1491***	4709	256	**1997***	675
Female							
0	52	27	1889	4885	405	1439	
160	52	28	1871	4893	401	1448	
600	52	28	1796	4914	**366***	1408	

Table B.6.5-14: Organ weight data at terminal sacrifice

Absolute organ weights							
Dose **ppm**	**week**	**Body-weight** **g**	**Brain** **mg**	**Liver** **mg**	**Spleen** **mg**	**Kidneys** **mg**	**Testes** **mg**
Male							
0	105-107	35	496	1741	113	737	201
160	105-107	35	489	1632	115	736	208
600	105-107	33	498	1697	105	**692***	204
Female							
0	105-107	31	504	1569	215	466	
160	105-107	29	506	1460	252	443	
600	105-107	**28***	510	1536	228	445	
Relative organ weights							
Dose **ppm**	**week**	**Body-weight** **g**	**Brain** **mg/100 g**	**Liver** **mg/100 g**	**Spleen** **mg/100 g**	**Kidneys** **mg/100 g**	**Testes** **mg/100 g**
Male							
0	105-107	35	1421	4970	326	2103	574
160	105-107	35	1411	4661	331	2105	599
600	105-107	33	1502	5137	315	2073	**613***
Female							
0	105-107	31	1683	5218	706	1545	
160	105-107	29	1755	5018	861	1526	
600	105-107	**28***	**1809***	5423	808	1571	

Histopathological examination of tissues for animals killed in interim sacrificed was not performed, since it is no guideline requirement.

In 600 ppm females the administration of spiroxamine led to an increased incidence of findings in surface epithelia. These included hyperkeratosis of the tongue, oesophagus and forestomach mucosa and hyperkeratosis and acanthosis in the epidermis of pinnas and tail. No increased incidences of these effects were observed in 160 ppm females or in males.

Hepatocellular cytoplasmic changes (reduced glycogen storage, distinct cytoplasmic basophilia) was observed in 4/5 animals necropsied before term.
Some statistically significant incidences of age dependent lesions at 600 ppm found to be above or below the corresponding control value are regarded by the study pathologist to be incidental considering their relatively wide variability.

Table B.6.5-15: Selected non-neoplastic lesions (all animals)

	Males			Females		
Dose level (ppm)	**0**	**160**	**600**	**0**	**160**	**600**
Oesophagus - Hyperkeratosis	0	0	1	2	1	10**
Tongue - Hyperkeratosis	0	0	0	0	0	5*
Pinnas - Hyperkeratosis	0	0	0	0	0	2
Pinnas - Acanthosis	0	0	1	0	2	8**
Tail (tip) - Hyperkeratosis	0	0	0	2	0	5
Tail (tip) - Acanthosis	0	1	1	2	3	11**
Forestomach - Hyperkeratosis	0	-§	0	0	-§	3*
Lacrimal glands - round cell inftr.	24	-§	30	37	-§	40
Heart - fibrosis	0	0	3*	3	0	4
Kidney - mineralisation	29	-§	39*	4	-§	7
Kidney - hydronephrosis	0	-§	0	0	-§	3*
Brain - thalamic area mineralisation	40	-§	33	31	-§	25
Adrenals - cortical hyperplasia	34	40	44**	8	3	4
Adrenals - focal cortic. degen. (unilateral)	0	0	0	0	2	4*
Spleen - lymphoid hyperplasia	2	-§	7*	7	-§	9
Liver - hepatocellular cytoplasmic changes	2	-§	3	1	-§	5*
Pituitary gland - cysts	4	-§	2	0	-§	3*
Salivary glands - round cell infiltr. (sublingual)	6	-§	2	2	-§	10*

* p<0.05; **p<0.01
§, tissue of animals in this group not examined

In treated animals cortical or subcapsular adenomas as well as medullary (females only) tumours were noted in the adrenal glands with higher incidences than at 0 ppm (Table B.6.5-16). These results are not considered to reflect an oncogenic effect of the compound for the following reasons:

- A statistical significance is lacking in any case.
- With regard to cortical adenoma there is a dose dependent increase in males with 10 % - 14 % - 20 % after 0 -160 – 600 ppm and an increased incidence of 4 % in high dose females. The incidence of 10 % in untreated control males is already markedly higher than the incidences in historical controls of 0-4 % in male B6C3F1 mice obtained from 5 other oncogenicity studies conducted at Bayer between 1991 to 1996 (including the previous oncogenicity study with spiroxamine and 4 other studies). This shows the high spontaneous variability of this finding particularly in aging male mice which is also reflected by historical control data from other sources: Historical controls from the RITA database reveal a range of 0-16 % for cortical adenoma in males and 0-2.1 % in females. The NTP as the largest source of neoplasm incidence data in B6C3F1 mice indicate a wide range of incidences between 0-22 % in males and 0-6 % in females, thus covering the incidences of 20 % in high dose males and 4 % in high dose females observed in the present study. Additionally, it has to be kept

in mind that there was no increase in cortical adenoma in the first oncogenicity study with spiroxamine in mice (incidence 2-0-1-3 in males [n=50] and 0-1-1-1 in females [n=50] after 0-20-160-480 ppm).

- With regard to subcapsular adenoma of the adrenal glands no increased incidence of subcapsular adenoma A (developing from spindle cells) was observed in treated males, but the incidences of subcapsular adenoma B (developing from polygonal cells) were increased in treated males up to 6 % in the high dose group. Overall incidences for subcapsular cell adenoma in males with 2 % - 4 % - 6 % after 0 – 160 – 600 ppm are well within the historical control range of 0-20 % from the NTP data base. While there was no subcapsular adenoma B in females, subcapsular adenoma A was found in 2/50 high dose females (4 %). Since this value just exceeds the range of historical control values in female B6F3C1 mice of 0 – 2 % by only one case, no effect on subcapsular adenoma A was observed in males in the present study and no case of subcapsular cell adenoma at all occurred in the previous oncogenicity study with spiroxamine in B6C3F1 mice, this is not assessed as a treatment related effect.
- Benign medullary tumours (syn. benign pheochromocytoma) in high dose females appeared with the same incidence of 4 % as in the control females of the previous oncogenicity study with spiroxamine (Eiben & Hartmann, 1995, TOX9552613).

In conclusion, the observed increased incidences of benign tumours of the adrenal glands in spiroxamine treated animals are not considered to represent an oncogenic effect, since the increased incidences were not statistically significant, were generally covered by historical control data and/or were confined to only one sex. Furthermore, no increased incidences of these adrenal tumours occurred in the previous oncogenicity study with spiroxamine in B6C3F1 mice.

Incidences calculated for the other organs were inconspicuous at 600 ppm when compared to those of controls. There was no increase in the total tumour frequency or the time of onset of tumours.

Table B.6.5-16: Incidence of neoplastic lesions of the adrenals and respective historical control data

	Males			**Females**		
Dose level (ppm)	**0**	**160**	**600**	**0**	**160**	**600**
No. of adrenal glands examined	49	50	49	49	50	50
Cortical adenoma	5 (10 %)	7 (14 %)	10 (20 %)	0 (0 %)	0 (0 %)	2 (4 %)
Adenoma subcapsular A	1 (2 %)	1 (2 %)	0 (0 %)	0 (0 %)	0 (0 %)	2 (4 %)
Adenoma subcapsular B	0 (0 %)	1 (2 %)	3 (6 %)	0 (0 %)	0 (0 %)	0 (0 %)
Medullary tumor # (benign)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	2 (4 %)
Control data from the previous oncogenicity study with spiroxamine in B6C3F1 mice conducted 1991 (Eiben & Hartmann, 1995, TOX9552613)						
Cortical adenoma	4 %			0 %		
Phaeochromocytoma #, benign	0 %			4.2 %		
Historical control data from 4 oncogenicity studies in B6C3F1 mice conducted at Bayer from 1992 to 1996						
Cortical adenoma	0-4 %			0 %		
Subcapsular cell adenoma	0-4 %			0-2 %		
Medullary tumor # (benign)	0-2 %			0-2 %		
Historical control data from 18 oncogenicity studies in B6C3F1 mice conducted between 1988 and 2001 from the RITA database[1]						

	Males			Females		
Dose level (ppm)	**0**	**160**	**600**	**0**	**160**	**600**
Cortical adenoma	0-16 %			0-2.1 %		
Subcapsular cell adenoma	0-4 %			0 %		
Medullary tumor # (benign)	0-2 %			0-2 %		
NTP oncogenicity data from 27 studies (untreated/vehicle controls) or 21 studies (chamber controls) in B6C3F1 mice according to Haseman et al., 1999[2]						
	untreated (vehicle) control	chamber control		untreated (vehicle) control	chamber control	
Cortical adenoma	0-22 %	0-10 %		0-6 %	0-2 %	
Adrenal capsule: adenoma	0-20 %	0-2 %		0 %	0-2 %	
Phaeochromocytoma #	0-5 %	0-3 %		0-4 %	0-6 %	

#: Phaeochromocytoma = medullary tumor; *: $p < 0.05$; **: $p < 0.01$

[1]: Deschl, U., Kittel, B., Rittinghausen, S., Morawietz, G., Kohler, M., Mohr, U., Keenan, C.: The value of historical control data – scientific advantages for pathologists, industry and agencies. Toxicol Pathol 30, 80-87, 2002 (ASB2009-1778)

[2]: Haseman, J.K., Elwell, M.R., Hailey, J.R., Neoplasm incidences in B6C3F1 mice: NTP historical data. In: Pathology of the mouse. Ed. Maronpot, R.R., Boorman, G.A., Gaul, B.W.. Cache River Press, Vienna, USA: 679-689, 1999 (ASB2009-8154))

Conclusions:

In the 600 ppm dose group (equal to 149.8 or 248.1 mg/kg bw/d for males or females, respectively), decreased body weights in males and females were detected. During histological examination hyperkeratosis and/or acanthosis on tongue, oesophagus, forestomach, pinnas and tail of females were detected. Additionally, hepatocellular liver changes were detected predominantly in females that died during the treatment period. No effects were observed in 160 ppm dose group (equal to 41.0 or 64.6 mg/kg bw/d for males or females, respectively), which was the NOAEL in this study.

No carcinogenic potential was detected in this study.

2.1.1.3 Reproductive and developmental toxicity studies

B.6.6 Reproductive toxicity (OECD Annex IIA 5.6)

The reproductive toxicity of spiroxamine was studied in two 2-generation studies in rats and in developmental toxicity studies in rats and rabbits (Table B.6.6-1).

Table B.6.6-1: Summary of reproduction toxicity studies

Study	Dose levels	NOAEL parental	NOAEL reproduction	NOAEL Offspring
2-generation, feeding, rat	0-20-80-300 ppm	20 ppm (2.13 mg/kg bw/d*)	80 ppm (9.19 mg/kg bw/d)	80 ppm (9.19 mg/kg bw/d)
2-generation, feeding, rat	0-20-80-300 ppm	80 ppm (5.5/6.7 mg/kg bw/d ,m/f)	300 ppm (21.0/24.5 mg/kg bw/d)	80 ppm (5.5 mg/kg bw/d)
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 the first 2-generation study a concentration of 80 ppm in the feed had no negative influence on the reproduction. At 300 ppm, litter size at birth was slightly reduced and some pups exhibited clinical signs such as laboured breathing or cyanosis. Later, F1- and F2-pups with thin appearance, piloerection, bloody noses and polyuria were observed. Increased mortality in F1 adults and their pups was seen. In parent animals, hyperkeratosis of the oesophagus epithelium was seen at 80 ppm and above in females and at 300 ppm in males. At 80 ppm and above, feed consumption was decreased in parent animals. At 300 ppm, body weight gain in parent animals was reduced. The NOAEL for parental toxicity was 20 ppm and 80 ppm for both reproductive and offspring toxicity.
In addition to the originally submitted study on reproduction a second 2-generation study was performed recently on request of US EPA in order to address the uncertainty with regard to postnatal toxicity of spiroxamine arising from the first 2-generation study. In this second study the same dietary concentrations as in the previous study were administered. However, the doses expressed as mean daily intake (mg/kg bw/d) were noticeable lower. At the high dose of 300 ppm parental toxicity in males and females consisted in declined body weights and terminal body weights, subtle increases in activated partial thromboplastin time (APTT) and hyperkeratosis of the oesophagus. There were no test substance related effects on reproduction in this study. Pups of both generations at 300 ppm showed decreased body weight, body weight gain and changes in organ weights. Furthermore, slightly delayed balanopreputial separation and vaginal patency were noted in F1-pups at 300 ppm. The NOAELs in this study were 80 ppm for parental toxicity and offspring effects and 300 ppm for reproductive toxicity.
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 increased mortality could not be reproduced in the second study indicate that these severe findings of the first 2-generation study were rather caused by an infection of the animals than by a systemic effect of spiroxamine. Whether the severe clinical symptoms observed in the first study at 300 ppm were related to a possible infection or to the higher daily dose intake (mg/kg bw/d) cannot be answered.

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).
In the oral developmental toxicity study in rabbits a maternal NOAEL of 20 mg/kg bw/d based on clinical findings, reduced body weight and feed consumption at 80 mg/kg bw/d was established. The NOAEL of 20 mg/kg bw/d for developmental toxicity was determined based on slightly increased spontaneous skeletal malformation at 80 mg/kg bw/d.
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.

According to EU Directive 67/548/EEC classification of spiroxamine with R63 is proposed by RMS 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 cat. 2) is proposed by RMS.

Remark: The proposal for classification / labelling (R63 - Possible risk of harm to the unborn child, Cat. 3 / H361d - Suspected of damaging the unborn child) is not supported by Co-RMS. A detailed comment of the Co-RMS is given in Annex 14 to chapter B.6.

B.6.6.1 Multi-generation study in rats

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.

Findings:
The actual test compound uptake during the pre-mating periods is given in Table B.6.6-2.

Table B.6.6-2: 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. 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 B.6.6-3). 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 B.6.6-3: 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 B.6.6-4, Table B.6.6-5).

Table B.6.6-4: 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 B.6.6-5: 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 [10E9L]	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 B.6.6-6, Table B.6.6-7).

Table B.6.6-6: 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 B.6.6-7: 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 B.6.6-8; Table B.6.6-9 and Table B.6.6-10). An infection as a possible cause for the high mortality was stated in the study report.
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 B.6.6-11).

Table B.6.6-8: 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 B.6.6-9: 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 B.6.6-10: 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 B.6.6-11: 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.

Conclusion:
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 the reproduction toxicity, a NOAEL of 80 ppm (corresponding to 9.19 mg/kg bw/d) was determined on reduced litter size, reduced body weight development and clinical signs in pups of the 300 ppm group.

Re-evaluation in 2009
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.

B.6.6.2 Multi-generation study in rats

This study has been submitted for renewal procedure in 2009

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 B.6.6-12. 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 B.6.6-12: 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 (see Table B.6.6-13, Table B.6.6-14, Table B.6.6-15):
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 B.6.6-13, Table B.6.6-14, Table B.6.6-15):
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 B.6.6-13: Mean (S.E.) body weight (bw) and food consumption – Pre-mating/mating

Observations/study week	Dose Group			
	0 ppm	20 ppm	80 ppm	300 ppm
F0 Generation Males				
Mean bw (g) Week 15 (S.E.)	463.2 (7.14)	455.8 (8.48)	456.8 (6.39)	451.5 (8.30)
Mean weight gain (g) Weeks 1-15	195.6	190.6	183.8	181.3
Mean food (g/animal/day) Weeks 1-10	23.8	24.3	24.0	24.2
Mean food (g/kg/day) Weeks 1-10	66.6	68.8	66.8	68.3
F0 Generation Females - Pre-mating				
Mean bw (g) Week 10 (S.E.)	252.3 (2.67)	251.8 (3.88)	250.4 (2.88)	246.2 (3.09)
Mean weight gain (g) Weeks 1-10	70.3	70.7	65.6	60.3
Mean food(g/animal/day) Weeks 1-10	17.8	18.5	17.9	17.3
Mean food (g/kg/day) Weeks 1-10	81.1	84.0	81.7	79.8

F_1 Generation Males				
Mean bw (g) Week 14 (S.E.)	454.9 (6.66)	451.9 (9.16)	448.2 (7.46)	418.0** (5.62)
Mean weight gain (g) Weeks 1-14	188.7	181.1	173.6	174.8
Mean food (g/animal/day) Weeks 1-10	23.6	23.8	24.2	23.1
Mean food (g/kg/day) Weeks 1-10	66.0	67.1	66.6	70.6
F_1 Generation Females - Pre-mating				
Mean bw (g) - Week 10 (S.E.)	240.4 (3.35)	245.0 (3.99)	245.3 (3.30)	221.0** (2.44)
Mean weight gain (g) Weeks 1-10	63.7	63.1	64.0	56.6
Mean food (g/animal/day) Weeks 1-10	16.8	17.7	17.4	16.1
Mean food (g/kg/day) Weeks 1-10	78.7	81.4	80.7	82.2

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

Table B.6.6-14: 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 B.6.6-15: 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 B.6.6-16).

Table B.6.6-16: 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.
Reproductive performance:
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. 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.

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).
Nevertheless, results of the second 2-generation study should be the basis for the overall NOAELs.

Conclusion:
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.

B.6.6.3 Developmental toxicity studies

B.6.6.3.1 Oral study on 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 (Hanlbm: 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 B.6.6-17) and body weight (< 10 %) (Table B.6.6-19). Body weight gain was statistically significant decreased only after correction for uterus weight (Table B.6.6-18). 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).

Table B.6.6-17: 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 B.6.6-18: 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 B.6.6-19: 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 B.6.6-20).

Table B.6.6-20: 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

	0 mg/kg	10 mg/kg	30 mg/kg	100 mg/kg
% 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 were detected and were outside of control data (concurrent and historical range). Historical control data (1987 to 1995) showed that findings of palatoschisis occurred very rarely in the rat strain used by RCC: In 49 studies 12 cases of palatoschisis were detected and except for one study in 1995 (2 foetuses out of 2 litters) at most one litter per study was affected (Remark: additional historical control data of studies performed during 1991 to 1995 were provided for re-evaluation in 2009, Anon. 2009, ASB2009-1678).
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 B.6.6-23). However, food consumption and body weight gain were only slightly affected (Table B.6.6-22).

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 B.6.6-21).

Table B.6.6-21: 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

	0 mg/kg	10 mg/kg	30 mg/kg	100 mg/kg
External examination % of abnormal dead foetuses mean	1 0.3 0.2	0	0	0
Skeletal examination (n/litters) - wavy ribs (n/litters) - dumbbell shaped thoracic vertebrae - bipartite sternebrae - abnormally ossified sternebrae	 2/2 1/1 0 1/1	 11/4 0 1/1 0	 7/5 0 1/1 0	 6/4 2/2 0 1/1
Weights of live foetuses n = mean (*)	286 4.8	285 4.7**	268 4.7	265 4.6**
Weights of male foetuses n = mean (*)	135 4.9	158 4.8**	138 4.8	128 4.7**
Weights of female foetuses n = mean (*)	151 4.6	127 4.5	130 4.6	137 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:
The maternal NOAEL of 30 mg/kg bw/d was based on reduced body weight gain and feed intake at 100 mg/kg bw/d. The NOAEL for intrauterine development of 30 mg/kg bw/d was based on delay of ossification, slightly reduced weights of foetuses and three cases of palatoschisis at 100 mg/kg bw/d. The delay of skeletal ossification was considered to be the consequence of slightly delayed maturation (indicated also by the reduced body weight of foetuses) and not a specific effect on skeletal development of foetuses. The minor statistically significant differences between groups 1 and 2 were considered to be incidental and, therefore, were not included for the calculation of the NOAEL.

Re-evaluation 2009
The maternal NOAEL of 30 mg/kg bw/d was based on reduced feed intake (13-26 %) and marginal reduced body weight (< 10 %) at 100 mg/kg bw/d. 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 and even in the acute oral toxicity study in rats 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 the previously conducted range finding study palatoschisis was already observed at the same dose level.

B.6.6.3.2 Oral range-finding studies on 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 B.6.6-22.

Table B.6.6-22: 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 B.6.6-23

Table B.6.6-23: 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

B.6.6.3.3 Oral study on 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 B.6.6-24). One dam at 20 mg/kg bw/d died on day 16 p.c. probably due to misapplication.

Table B.6.6-24: 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, no gross pathological findings were possible. As shown in Table B.6.6-25 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 B.6.6-25: 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 B.6.6-26, Table B.6.6-27) were comparable up to 80 mg/kg bw/d.

Table B.6.6-26: 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 B.6.6-27: 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] Number of foetuses per dam Resorptions per dam	4.35 6.6 0.7	4.04 6.5 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 B.6.6-28, Table B.6.6-29). 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). (ControlHowever, 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. (Remark: additional historical control data of studies performed during 1989 to 1996 were provided for re-evaluation in 2009, Anon., 2009, ASB2009-2107).
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 B.6.6-28: 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 B.6.6-29: 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

Conclusion:
The maternal NOEL of 20 mg/kg bw/d was based on clinical findings, reduced body weight gain, reduced food intake and lethality at 80 mg/kg bw/d.
The NOEL for intrauterine development of 20 mg/kg bw/d was based on marginal developmental toxicity at 80 mg/kg bw/d (retardation of foetal weight and slightly increased rate of spontaneous malformations). With the exception of one malformation in the first study (hydrocephalus internus with caudal displacement of the ears) the malformations at the 80 mg/kg level correspond to changes previously observed as spontaneous malformations in the strain of rabbits used.

Re-evaluation 2009
The maternal NOAEL of 20 mg/kg bw/d based on clinical findings, reduced body weight gain and feed consumption at 80 mg/kg bw/d. 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. Furthermore, 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).
The NOAEL of 20 mg/kg bw/d for developmental toxicity based on slightly increased malformations 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.

B.6.6.3.4 Dermal study on 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

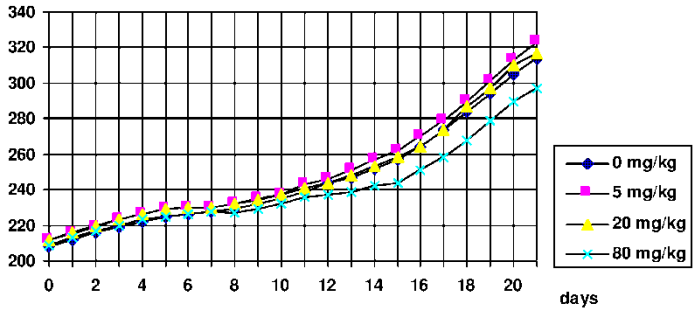
GLP: Yes

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 B.6.6-1) 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 B.6.6-1: Results of a dermal developmental toxicity study in rats: Mean body weights [g] of dams post coitum



No indication for substance related effects were 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 B.6.6-30: 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 mean (+)	326 13.0	341 13.6	347 13.9	331 13.8
Pre-implantation loss % of corp. lutea (#) mean (+)	33 10.1 1.3	36 10.6 1.4	29 8.4 1.2	36 10.9 1.5
Implantation sites % of corp. Lutea (#) mean (+)	293 89.9 11.7	305 89.4 12.2	318 91.6 12.7	295 89.1 12.3
Post-implantation loss % of corp. lutea (#) mean (+)	22 7.5 0.9	11 3.6# 0.4	13 4.1# 0.5	21 7.1 0.9
Embryo / foetal deaths:total % of impl. sites (#) mean (+)	22 7.5 0.9	11 3.6# 0.4	13 4.1# 0.5	20 6.8 0.8
Embryo resorptions	22	11	13	20
Total foetuses % of impl. sites (#) mean (+)	271 92.5 10.8	294 96.4# 11.8	305 95.9# 12.2	275 93.2 11.5
Live foetuses % of impl. sites mean (+)	271 92.5 10.8	294 96.4 11.8	305 95.9 12.2	274 92.9 11.4
External examination % of abnormal live foetuses mean	1 0.4 0.0	3 1.0 0.1	1 0.3 0.0	1 0.4 0.0
External examination % of abnormal dead foetuses mean	0	0	0	0
Weights of live foetuses n = mean (*)	271 4.6	294 4.7**	305 4.7	274 4.7**
Weights of male foetuses n = mean (*)	121 4.7	155 4.8	144 4.8	150 4.9*

	0 mg/kg	5 mg/kg	20 mg/kg	80 mg/kg
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 following NOAELs were determined: NOAEL for systemic maternal toxicity: 5 mg/kg bw/d, based on reduced corrected body weight gain at 20 mg/kg bw/d and above;
Maternal NOAEL for local skin effects: < 5 mg/kg bw/d, based on signs of skin irritation at the application site at 5 mg/kg bw/d and above;
NOAEL for intrauterine development: 20 mg/kg bw/d, based on slight signs of embryotoxicity (increased number of foetuses with "wavy ribs") at 80 mg/kg bw/d;
NOAEL for teratogenicity: 80 mg/kg bw/d (highest dose tested).

Re-evaluation 2009
The NOAEL for local skin effects was < 5 mg/kg bw/d based on skin irritation 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 at 80 mg/kg bw/d.

2.1.1.4 Neurotoxicity studies

B.6.7 Delayed neurotoxicity (OECD Annex IIA 5.7.2)

In an acute neurotoxicity screening study in rats, a dose-related increase in unspecific toxicity but no neurotoxicity was observed using standard toxicological testing, a neurotoxicity-related functional observational battery and automated measurement of movement activity. Minimal effects were found at a dose of 30 mg/kg bw (piloerection, incoordinated gait, laboured breathing in one animal; reduced landing foot splay) and severe toxicity at higher doses of 100 and 220 mg/kg bw. Effects were reversible up to day 4. Histopathological investigations of neural tissues revealed no indication of neurotoxic effects. The NOAEL was 10 mg/kg bw/d.

In a subchronic neurotoxicity screening study in rats doses of 700 and 155 ppm produced clear evidence of unspecific toxicity in both sexes including mortality, reduced body weight development, body-weight-related effects on absolute liver weights, unspecific changes in the functional observational battery (high dose males only), clinical pathology findings (increased activity of alanine-aminotransferase at 700 ppm) and microscopic lesions in non-neural tissues (hyperkeratosis of oesophagus and tongue epithelium). The low dose of 35 ppm (equal to 2.4 or 2.5 mg/kg bw/d in males or females, respectively) was the overall NOAEL for males

and females. Neurobehavioural changes indicative of selective neurotoxicity were not evident at any dose level. There were no pathologic findings in neural or muscle tissues.

As spiroxamine is a fungicide with a completely different molecular structure than the known delayed-neurotoxic substances, a study for delayed neurotoxicity is considered not necessary.

B.6.7.1 Acute neurotoxicity study on rats

Reference:	KIIA 5.7 (OECD)
Report:	Dreist, M. and A. Popp: KWG 4168 - Acute oral neurotoxicity screening study in rats - Report no.: 23503 (November 25, 1994), Bayer AG, Institute for Toxicology, Wuppertal, Germany TOX9552627
Guidelines:	US EPA FIFRA, Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, Guideline Addendum 10, Neurotoxicity, NTIS, 1991, EPA 540/09-91-123, PB 91-154617 (not checked by RMS)
Deviations:	Not applicable
GLP:	Yes
Acceptability:	The study is considered to be acceptable.

Dates of exp. work: September 1993 - October 1993.

Material and methods:
In an acute neurotoxicity screening study, spiroxamine (batch no. 17002/90; purity: 95.8 %) was administered orally by gavage as a single dose to fasted adult male and female Wistar rats (Hsd/Win: WU, source: Harlan Winkelmann GmbH, Borchen, Germany) at doses of 0, 10, 30, 100 and 220 mg/kg bw (12 animals per sex and dose group). The test substance was formulated in aqueous Cremophor EL (2 % v/v), which served also as negative control compound. Administration volume was 10 mL/kg bw.
To detect any possible neurotoxic effects, functional observational battery (FOB), automated measurement of movement activity (figure-eight maze) and specific histopathological investigations of neural tissues were included in the study in addition to standard toxicological testing.

Findings:
General observations: Two females treated with 220 mg/kg bw died on the day of application or on day 7, respectively. Dose-dependent unspecific signs of general toxicity (decreased motility and reactivity, staggering gait, piloerection, laboured breathing, lateral position, increased salivation etc.) were evident after treatment with 100 or 220 mg/kg bw. Clinical signs were generally more pronounced in females than in males. Most of the effects were reversible within one day, but in one high-dose female signs persisted until day 7, when the animal died.
Functional observational battery and motor and locomotor activity: Concerning the results of the FOB, both sexes exhibited treatment related effects on day 0. There was evidence of toxicity (piloerection, incoordinated gait, laboured breathing in one animal) at the dose of 30

mg/kg bw, and numerous effects were recorded at 100 and 220 mg/kg bw (hind limbs dragging, describing exaggerated movements, splayed or hind feet pointing outward; forelimbs dragging, extended or weak; stilted gait (only females) as well as piloerection, laboured breathing, affected responses to external (auditory, tail pinch (only males)) stimuli, incoordinated righting reflex and decreased body temperatures). These effects are attributed to acute unspecific general toxicity. The NOEL with regard to FOB parameters was 10 mg/kg bw. Treatment-related decreases in measures of motor and locomotor activity in the figure-eight maze and the home cage occurred (increased number of animals sitting or lying normally rather than standing normally in open field, animals lying flat in home cage and open field, decreased activity in home cage or arousal in open field, decreased number of rearing events in open field) on day 0 in males and females at dose levels of 100 and 220 mg/kg bw. Complete recovery of effects on motor and locomotor activity was found on the next test occasion (day 7). Landing foot splay was reduced in dose groups 30 to 220 mg/kg bw (only on day of dosing). Forelimb and hindlimb grip strength were reduced in males of 100 and 220 dose group and in females of 220 mg/kg bw (only on day of dosing).
Haematology, clinical chemistry, urine analysis: At the time of FOB and motor activity testing on the day of treatment, clinical pathological investigations were done in satellite group animals. Increased blood levels of aspartate-aminotransferase (males > 100 mg/kg bw), alanine-aminotransferase (males 220 mg/kg bw) and creatine-kinase (males > 100 mg/kg bw; females 220 mg/kg bw) were indicative of liver and muscle damage. The total leukocyte count was decreased in both sexes at > 100 mg/kg bw and differential blood count revealed decreased values for lymphocytes and increased values for polymorphonuclear neutrophils in males and females at 220 mg/kg bw. Clinical pathological parameters were not affected in main groups on day 15-16.
Gross pathology, organ weights, histopathology: There were no treatment related microscopic lesions in skeletal muscle or neural tissues. The only gross lesions in main group animals that are regarded to be treatment-related were a dark discolouration in the liver of one high-dose female which died on the day of treatment and a liver that was reduced in size in another high dose female that died on day 7.

Conclusion and re-evaluation in 2009:
To summarise, this study established a dose-related increase in acute systemic toxicity, with minimal effects at 30 mg/kg bw (piloerection, incoordinated gait, laboured breathing in one animal; reduced landing foot splay) and severe toxicity at higher doses of 100 and 220 mg/kg bw. Histopathological investigations of neural tissues revealed no indication of neurotoxic effects. The NOAEL was 10 mg/kg bw/d.

B.6.7.2 Subchronic neurotoxicity study on rats

Reference:	KIIA 5.7 (OECD)
Report:	Dreist, M. and A. Popp: KWG 4168 - Subchronic neurotoxicity screening study in Wistar rats (thirteen-week administration in the diet) - Report no.: 24089 (June 21, 1995), Bayer AG, Institute for Toxicology, D-42096 Wuppertal, Germany TOX9552631
Guidelines:	US EPA FIFRA, Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, Guideline Addendum 10, Neurotoxicity, NTIS, 1991, EPA 540/09-91-123, PB 91-154617 (not checked by RMS)

Deviations: Not applicable

GLP: Yes

Acceptability: The study is considered to be acceptable.

Dates of exp. work: February 14 - May 27, 1994.

Material and methods:
In a subchronic neurotoxicity screening study twelve young adult Wistar rats (Hsd/Win:Wu, source Harlan Winkelmann, Borchen, Germany) per sex and dose group received spiroxamine (batch no. 17002/90; purity: 95.5 %, in peanut oil 1 % as admixture in the diet at concentrations of 0, 35, 155 and 700 ppm for 13 weeks.
To detect any possible neurotoxic effects, functional observational battery (FOB), automated measurement of movement activity (figure-eight maze) and specific histopathological investigations of neural tissues were included in the study in addition to standard toxicological testing. Neurobehavioural evaluation was performed in all rats; half of the animals were used for neuropathology and the other half for clinical and target organ pathology. Mean consumption of spiroxamine per kg body weight per day was 2.4, 10.6 and 48.5 mg for males and 2.5, 11.1 and 50.6 mg for females in ascending order of dose.

Findings:
Compound concentrations in feed reached a range of 90 to 98 % of the intended concentration.
One male high-dose rat died in week 13. Treatment-related clinical signs were not evident by cage-side observation at any dietary exposure level. In comparison to the control groups, the body weight gain was reduced by 12 % in the high dose group animals at study termination (Figure B.6.7-1 and Figure B.6.7-2). Low body weights in low and intermediate dose groups were considered to be related to the low initial body weights. The water intake was also reduced in 700 ppm dose group.

Figure B.6.7-1: Mean body weights [g] - males

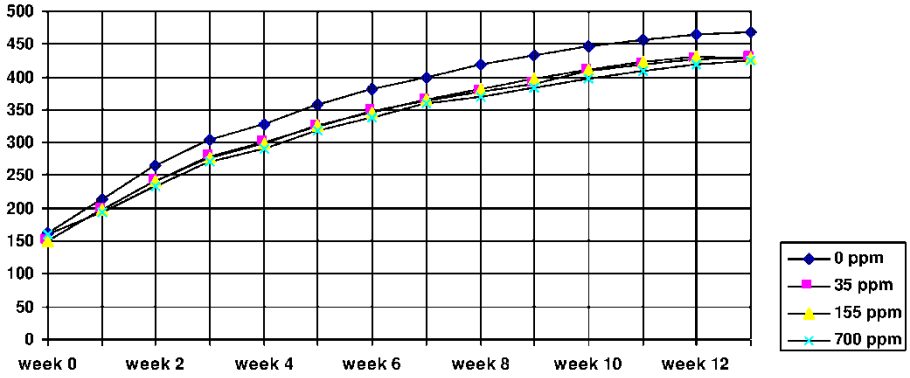
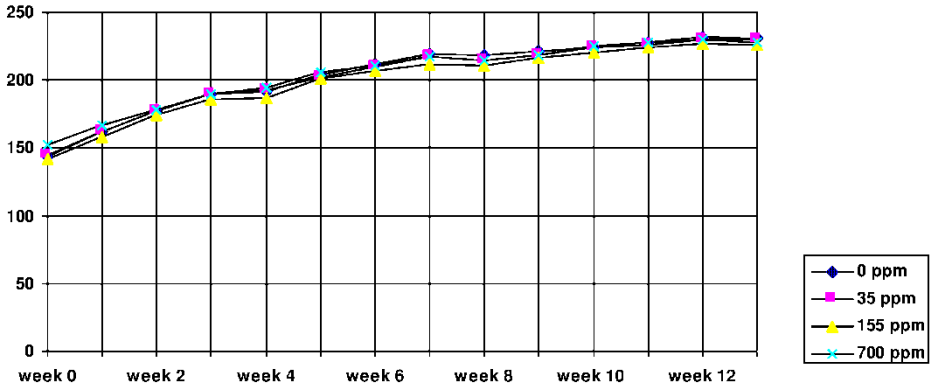


Figure B.6.7-2: Mean body weights [g] - females



Results from the FOB revealed treatment-related effects in high-dose males (reduced activity in home cage, decreased foot splay (related to body weight)) but no effects in females at any exposure level. All changes were attributed to unspecific general toxicity. Motor and locomotor activity was not affected at any dose level. There were no treatment-related ophthalmological findings.

With regard to the results of the clinical pathology investigations, marginally increased activity of alanine-aminotransferase and decreased cholesterol content were found after 4 weeks at 700 ppm (Table B.6.7-1).

Table B.6.7-1: Clinical chemistry

	0 ppm	35 ppm	155 ppm	700 ppm
Males				
ASAT [U/L]				
Week 4	44.9	42.0	46.4	43.6
Week 14	34.9	39.7	40.1	42.4
ALAT [U/L]				
Week 4	36.5	44.8	45.1	46.4 *
Week 14	33.9	45.5 *	41.2	41.4
CHOL [mmol/L]				
Week 4	2.52	2.28	2.18	1.77 **
Week 14	3.44	2.47	2.63	2.05 **
Females				
ASAT [U/L]				
Week 4	47.4	42.0	44.3	46.8
Week 14	46.3	54.5	46.6	47.4
ALAT [U/L]				
Week 4	32.7	35.8	37.0	42.0
Week 14	44.0	45.9	39.9	44.5
CHOL [mmol/L]				
Week 4	2.35	1.95 *	2.14	1.64 **
Week 14	2.64	2.32	2.77	2.13 *

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

No gross lesions which were related to compound-treatment were detected. Absolute liver weights were marginally decreased in both sexes but without clear dose dependence in males. The relative liver weights were decreased in high-dose females (Table B.6.7-2).

Table B.6.7-2: Organ weights (all animals)

	0 ppm	35 ppm	155 ppm	700 ppm
Absolute organ weights [mg]				
Liver m	17385	14258 *	14782 *	15034
f	8447	8343	7612	7398
Relative organ weights [mg/100g]				
Liver m	3518	3310	3383	3496
f	3533	3383	3271	3122 *

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

In non-neural tissues, following microscopic lesions were observed: hyperkeratosis of epithelium of tongue, oesophagus and stomach in all animals receiving 700 ppm. Hyperkeratosis of oesophagus mucosa was found also in one male and one female animal at 155 ppm. Some high dose animals exhibited very slight focal urothelial hyperplasia in the urinary bladder. There were no treatment-related microscopic lesions in skeletal muscle or neural tissues.

Conclusion and re-evaluation in 2009:
The NOAEL for subchronic neurotoxicity of 700 ppm (equal to 48.5 / 50.6 mg/kg bw/d, m/f) was based on the absence of specific effects at the highest dose tested.

The NOAEL for systemic toxicity of 155 ppm (equal to 10.6/11.1 mg/kg bw/d, m/f) was based on reduced body weight development and clinical chemistry findings at 700 ppm.
The overall NOAEL of 35 ppm (equal to 2.4 / 2.5 mg/kg bw/d, m/f) was based on irritation-induced local effects on epithelium of the oesophagus at 155 ppm.

B.6.7.3 Delayed neurotoxicity studies

As spiroxamine is a fungicide with a completely different molecular structure than the known delayed-neurotoxic substances, a study for delayed neurotoxicity is considered not necessary.

2.1.2 Human information

B.6.9 Medical data and information (OECD Annex IIA 5.9)

Occupational health surveillance did not reveal any health effects. No reports on epidemiological studies were submitted. Clinical cases and poisoning incidences were summarised by the notifier. However, the correlation between Spiroxamine and the observed symptoms is not sure, besides findings of skin and eye irritation from splashes with Spiroxamine containing products.

No specific therapy upon ingestion is available. The usual first aid measures and symptomatic treatment apply. Special care should be taken for possible lesions due to irritation properties along the route of exposure and for liver and eye lesions upon systemic exposure.

B.6.9.1 Report on medical surveillance on manufacturing plant personnel

DAR 1997:
No data are available. At this stage of the development there is no large scale production of spiroxamine.

The following report was submitted for the re-evaluation of Spiroxamine in 2009

Reference:	KIIA 5.9 (OECD)
Report:	Steffens, W.: Occupational medical experiences with Spiroxamine; document number: M-300761-01-1; document date: 2008-04-25 ASB2008-2234
Guidelines:	None
Deviations:	Not applicable
GLP:	Open
Acceptability:	Not applicable

Material and Methods:

Test material:	KWG 4168 (Spiroxamine)
No. of workers exposed:	About 30
Medical examinations:	History, full physical examination with orientating neurological status (reflexes, sensibility, coordination) and skin status
Commenced on:	Since 1997
Examination intervals:	annually
Laboratory examinations:	BSR, full blood count, AST, ALT, γ-GT, glucose, creatinine, cholesterol, urine status
Technical examinations:	Lung function testing, ecg/ergometry (reason.: respiratory protection use), vision-testing, audiometry, sonography (if necessary),

Occupational medical surveillance of workers exposed to Spiroxamine, performed annually on a routine basis, not directly related to exposures, did not reveal any unwanted effects in the workers. The examinations included the above laboratory parameters and clinical and technical examinations.

Findings:
Since 1997 no accidents with Spiroxamine occurred in the workers and no consultations of the site Medical Department due to work or contact with Spiroxamine were required. No sensitisation to Spiroxamine has been observed since 1997.

Conclusions:
No unusual occurrences were observed. According to the report, annual production was 2000 tons/yr; personal protection measures (full mask with filter ABEK-P3, protective gloves for chemicals, chemical-resistant suite, safety glasses, and safety shoes) were used by the workers.

B.6.9.2 Report on clinical cases and poisoning incidents

The notifier submitted following information (Henninger, 2009 ASB2009-2108):

A case of ingestion of a spiroxamine containing product has been reported in 2007 in Moldova. A four-year-old child ingested one gulp of Falcon, a product containing spiroxamine, tebuconazole and triadimenol, and became rapidly unconscious. Gastric lavage and hemodialysis were performed along with symptomatic and supportive treatment. The child fully recovered within one week. The actual involvement of the different active substances and formulation chemicals in the symptoms cannot be assessed. The offer made by BCS of developing and doing biomonitoring was declined by the hospital. At the moment no recommendation of hemodialysis in case of spiroxamine poisoning is possible.

Another case of spiroxamine ingestion was reported in 2006 in Ecuador - a suicidal attempt of a worker with 100 ml of Prosper 500 EC (spiroxamine). Symptoms were not reported. The treatment was done with gastric lavage and activated charcoal. The patient was released from the hospital one day later.

In 2004 in Germany, a farmer was splashed by the concentrate of Input Set (spiroxamine and prothioconazole), and became unconscious 6 hours later. In all likelihood the somnolence was due to the measured blood alcohol level of 2.7 ‰.

One poisoning incident following aerial application was reported in 2003 in Germany. A vintner was sprayed from a helicopter with a mixture of Prosper (spiroxamine 500 EC) and a competitor's fungicide Equation Pro, a product containing cymoxanil and famoxadone, and suffered from headaches, dizziness, nausea and abdominal pain. An expert judgement, issued in the frame of the court case against the helicopter company, related the symptoms to the combination of the active substances (rather cymoxanil than spiroxamine) and the solvents. The treatment was symptomatic and the recovery spontaneous.

In 2007, Bayer CropScience received a short information from the BfR (Bundesinstitut fur Risikobewertung) on an incident that happened during application of Input set (spiroxamine and prothioconazole). This information mentioned the case of an acute accidental occupational exposure on an adult male leading to mild symptoms.

Possible sensitisation cases have been reported. Three days after application of Input Set (spiroxamine and prothioconazole) and a competitor's pesticide an anaphylactic reaction occurred in a woman weeding treated weeds. A correlation is not likely, but cannot be fully ruled out.

In two further cases that happened in Germany, the correlation of spiroxamine to allergic symptoms was unlikely due to the latency time between exposure and appearance of symptoms or to the duration of the symptoms without further exposure.

There were few further cases of short-termed skin or eye irritation from splashes with spiroxamine containing products responding well to symptomatic treatment namely flushing with water.
Interestingly there were also some comparable cases without any irritation.

B.6.9.3 Observation on exposure of the general population and epidemiological studies

No reports relevant for this section are available. The notifier stated, "Up to now there is no known exposure of the general population to KWG 4168. No epidemiological studies have been performed." (Henninger, 2009 ASB2009-2108)

B.6.9.4 Clinical signs and symptoms of poisoning and details of clinical tests

Compound-specific poisoning signs in men after oral ingestion other than irritation are not expected. The analytical demonstration of parent compound or metabolites in blood, urine or gastrointestinal contents is required for an exact diagnosis of poisoning.

B.6.9.5 First aid measures

DAR 1997:
The removal of ingested compound by (preferably) stomach tube or induction of vomiting followed by symptomatic treatment is recommended in cases of oral uptake of spiroxamine. Any contamination of the skin should be washed off immediately with plenty of water.

No specific antidotal therapy is available for the treatment of poisoning.

Re-evaluation in 2009:

The notifier proposed the following first aid measures (Henninger, 2009 ASB2009-2108):

- *remove patient from/terminate exposure*
- *thorough skin decontamination with copious amounts of water*
- *flushing of the eyes for at least 15 minutes with lukewarm water*
- *induction of vomiting generally is no longer advised, and is forbidden in case of ingestion of a formulation with solvents. Induction of vomiting (by ipecac syrup) might be considered if definite medical help is not available within 1 hours, time since ingestion of a dangerous dose (more than a mouthful of undiluted product) is less than 1 hour, and patient is fully conscious*

Additionally, the notifier proposed the following therapeutic regimes (Henninger, 2009 ASB2009-2108):

Gastric lavage is no longer generally advised. It may be considered in case of ingestion of a potentially lethal dose within the first hours after ingestion, and by an experienced physician in hospital under airway protection.
No specific antidotal therapy is available for the treatment of poisoning. If skin irritation persists after washing, a cortisone creme may be applied, if eye irritation persists after flushing soothing eye drops can be used. Gastrointestinal irritation after ingestion may respond to antacids.

Comment by RMS: The medical basis for these poroposals by the notifier has not been assessed in this evaluation. It is recommended that the information should not be used as a basis for treatment advice in the event of poisoning incident. Specialist advice should be sought from an appropriate source such as a national or regional poisons unit (or similar organisations).

B.6.9.6 Expected effects and duration of poisoning as a function of the type, level and duration of exposure or ingestion

The effects in men following oral uptake of toxic doses of spiroxamine are not known. Irritation-induced effects on the gastrointestinal mucosa can be expected from the studies in experimental animal. Liver and eye were target organs in experimental animals.

2.1.3 Other relevant information

No other relevant information was submitted by the applicant in the PPP procedure. Information and data on spiroxamine's properties after acute exposure is summarised in section 2.2.

2.1.4 Summary and discussion of repeated dose toxicity studies

B.6.10.1.3 Short-term toxicity

The short-term toxicity data after oral administration of spiroxamine on rats, mice and dogs are summarised in Table B.6.10-2.

Table B.6.10-2: Summary of short-term oral toxicity studies

Study	Dose levels	NOAEL	Targets / Main effects
4 wk, feeding, Wistar rat	0-30-100-300 ppm	30 ppm (3.4 / 3.8 mg/kg bw/d, m/f)	100 ppm (10.8 / 12.2 mg/kg bw/d): liver weight ↑; steatosis of hepatocytes; hyperkeratosis of oesophagal mucosa 300 ppm (33.6 / 35.6 mg/kg bw/d): liver enzyme induction; hyperplasia of bladder epithelium
4 wk, gavage, Wistar rat	0-10-30-90 mg/kg bw/d	LOAEL 10 mg/kg bw/d	10 mg/kg bw/d: clinical symptoms 30 mg/kg bw/d: liver enzyme induction; kidney weight ↑ 90 mg/kg bw/d: liver weight ↑; steatosis of hepatocytes; hyperkeratosis fore-stomach mucosa; hyperplasia of bladder epithelium
13 wk, feeding, Wistar rat	0-25-125-625 ppm	25 ppm (1.9 / 2.7 mg/kg bw/d, m/f)	125 ppm (9.3 / 13.2 mg/kg bw/d, m/f): hyperkeratosis epithelium oesophagus & fore-stomach, hyperplasia/hypertrophy oesophagus, slight liver enzyme induction 625 ppm (54.9 / 75.14 mg/kg bw/d, m/f): body weight ↓; hyperkeratosis epithelium of tongue, hyperplasia of bladder epithelium, liver hyaline droplets
13 wk, feeding, B6C3F1 mice	0-20-80-320-1280 ppm	80 ppm (24.9 / 28.5 mg/kg bw/d, m/f)	320 ppm (88.4 / 126.6 mg/kg bw/d, m/f): epidermal hyperplasia of auricles, hepatocellular hypertrophy & fatty changes 1280 ppm (366.2, 413.7 mg/kg bw/d, m/f): epidermal hyperplasia of tail; liver weight ↑, leukocytes ↑, thrombocytes ↓; hyperplasia bladder epithelium & renal pelvis; kidney weight ↑, water intake ↑, urea ↑
13 wk, gavage, B6C3F1 mice	0-60-180-240 mg/kg bw/d	LOAEL 60 mg/kg bw/d	60 mg/kg bw/d: liver enzyme induction 180 mg/kg bw/d: hepatocellular hypertrophy, ↓ glycogen; hyperkeratosis fore-stomach mucosa; hyperplasia bladder epithelium 240 mg/kg bw/d: Single liver cell necrosis; hyperplasia epidermis of ears & tail
13 wk, feeding, Beagle dog	0-25-750-1500 ppm	25 ppm (0.66 / 078 mg/kg bw/d, m/f)	750 ppm (20.02 / 21.29 mg/kg bw/d, m/f) liver weight ↑, serum albumin ↓, ALP ↑, triglycerides ↓; minimal diffuse hepatocytomegaly
110 d, feeding, Beagle dog	0-150-250-500 ppm	500 ppm (16.2 / 15.1 mg/kg bw/d, m/f)	None
12 mo, feeding; beagle dog	0-25-75-1000-2000 ppm	75 ppm (2.5 mg/kg bw/d)	1000 ppm (28.03 / 25.84 mg/kg bw/d, m/f) cataracts, hepatocytomegaly, albumin ↓; triglycerides ↓; 2000 ppm (56.88 / 52.39 mg/kg bw/d, m/f) Erythrocytes, haemoglobin, haematocrit ↓

In rats, dogs and mice the liver was the main target organ. In mice, signs of liver enzyme induction occurred at doses of 60 mg/kg bw/d. At higher doses, hypertrophy of hepatocytes, degenerative alterations (centrilobular fat deposition) and liver weight increase were seen.

Fatty changes of hepatocytes were found also in rats and dogs together with an increase of serum transaminases activity. Investigations using recovery groups revealed that observed liver effects were reversible following cessation of compound administration.
In rats and mice histopathological alterations of the mucosal epithelium of the gastrointestinal and the urogenital tract were found after 4 and 13-week administration. Hyperkeratosis of the epithelium was seen on tongue and in the fore-stomach; the oesophagus showed hyperkeratosis, hyperplasia and hypertrophy. These effects are regarded to be causally related to the strong irritant action of the compound following surface contact. There is no reliable evidence that hyperplasia observed in the urothel of bladder and renal pelvis is likewise related to the irritant potential of spiroxamine. However, the histopathological alterations of the mucosal epithelium were reversible after termination of the exposure.
Ophthalmological findings such as bilateral sub-capsular clouding and cataract changes of the lens were major treatment related effects in dogs following chronic administration of high doses. The liver of dogs treated for 12 months with high doses of spiroxamine exhibited slight signs of hepatocytomegaly. No aggravation of the effects was recorded in comparison with the findings after subchronic uptake. At 2000 ppm the number of red blood cells was slightly reduced in female dogs only.

Table B.6.10-3: Summary of short-term dermal toxicity studies

Study	Dose levels	NOAEL	Targets / Main effects
3 wk, dermal, NZW rabbit	0-0.5-1-5 mg/kg bw/d	Systemic: 5 mg/kg bw/d Local: < 0.5 mg/kg bw/d	irritation related skin findings (erythema, swelling, hardening, cracking)
3 wk, dermal, NZW rabbit	0-0.05-0.2 mg/kg bw/d	Local: 0.2 mg/kg bw/d	none

No systemic toxicological effects occurred in rabbits following daily dermal application of 5 mg/kg bw/d over a period of 3 weeks. Because of the strong skin irritant action of spiroxamine, 5 mg/kg bw/d (corresponding to a concentration of 0.25 %) was the highest dose which could be tested. The skin of animals treated with a concentration of 0.025 % (0.5 mg/kg bw/d) exhibited erythema; at higher concentrations swelling, hardening and cracking of the skin developed. A concentration of 0.01 % (0.2 mg/kg bw/d) was tolerated without any visible signs of skin damage.

Table B.6.10-4: Summary of short-term inhalation toxicity study

Study	Dose levels	NOAEC [mg/m³ air]	Targets / Main effects
4 wk, inhal., Wistar rat	0-14.3-87.0-518.4 mg/m³ air	14.3	87.0 mg/m³ air: irritation-related findings in the respiratory tract; increase of polymorphonuclear granulocytes; haemoglobin ↓ 518.4 mg/m³ air: irritation-related findings in respiratory tract and eyes; liver weight ↑, body weight ↓, clinical chemistry (liver related), urinary bladder (hyperplasia)

In a sub-acute inhalation study, irritation-related findings were prominent in the respiratory tract at high concentrations. These changes consisted of metaplasia, hyperplasia and hyperkeratosis of the epithelium of larynx and nasal cavity. In the lungs, the number of

macrophages was increased and there was bronchiolo-alveolar proliferation. Similar as after oral administration, the liver was another target organ following sub-acute inhalation.

[...]

B.6.10.1.5 Long-term toxicity and carcinogenicity

In addition to the available long-term studies in rats and mice a second long-term study in mice was submitted in 2008.

In long-term toxicity studies in rats and mice, histopathological alterations of the epithelium of the gastrointestinal tract were noted. These effects (hyperkeratosis and acanthosis of the epithelium of tongue, oesophagus and fore-stomach,) might be interpreted as an adaptive process following the continuous irritant stimulus by spiroxamine. Furthermore, skin alterations at auricles and tips of the tail were observed in mice. Additionally, clear systemic signs of toxicity (such as low body weights, findings in uterus, ovaries or liver) occurred. Hence the NOAEL do not change, whether some findings are interpreted as local effects.
No evidence of an oncogenic potential of spiroxamine was found in the long-term feeding studies in rats and mice.

In the two available long-term studies in mice, a dose level of 160 ppm was tested in both, however with different result: In the first study this was the LOAEL, whereas it was the NOAEL in the other. This can not be explained with the achieved compound intake, because they are comparable. As a conservative approach, the dose level of 20 ppm (equal to 4.5/7.8 mg/kg bw/d) is considered the overall NOAEL for mice.
The NOAEL for systemic effects in rats was 70 ppm (equal to 4.22/5.7 mg/kg bw/d).

Table B.6.10-7: Summary of long-term toxicity and carcinogenicity studies

Study	Dose levels	NOAEL	Targets / Main effects
24 mo, feed; Wistar rats 50 M+50 F	0-10-70-490 ppm (equal to 0/0-0.66/0.77-4.22/5.67-32.81/43.04 mg/kg bw/d for M/F)	70 ppm (equal to 4.22/5.7 mg/kg bw/d)	490 ppm: acanthosis & hyperkeratosis of oesophagus mucosa; hyperplasia of urinary bladder urothel; bw ↓; mortality ↑; uterus (masses, distention)
26 mo, feed B6C3F1 mice 50 M+50 F OECD 451	0-2.5/480*-20-160 ppm (equal to 0/0-59.3/102.6-4.5/7.8-36.7/59.3 mg/kg bw/d for M/F)	20 ppm (equal to 4.5/7.8 mg/kg bw/d)	2.5/480 ppm: acanthosis & hyperkeratosis of tongue, oesophagus & tail; acanthosis of auricles; cysts in ovaries, bw ↓ 160 ppm: acanthosis & hyperkeratosis of oesophagus & tail; acanthosis of auricles; cysts in ovaries (1 animal), bw ↓
104 wk, feed; B6C3F1 mice 50 M+50 F 10 M+10 F (interim) OECD 451	0-160-600 ppm (equal to 0/0-41.0/64.4-149.8/248.1 mg/kg bw/d for M/F)	160 ppm (equal to 41.0/64.4 mg/kg bw/d)	600 ppm: acanthosis & hyperkeratosis of tongue, oesophagus, forestomach, pinna, tail; bw ↓; liver (histological changes) No carcinogenic potential

* = increased to 480 ppm from week 32 to termination based on the results of the supplemental study

B.6.10.1.6 Reproductive toxicity

The reproductive toxicity of spiroxamine was studied in two 2-generation studies in rats and in developmental toxicity studies in rats and rabbits (Table B.6.10-8).

Table B.6.10-8: Summary of reproduction toxicity studies

Study	Dose levels	NOAEL parental	NOAEL reproduction	NOAEL Offspring
2-generation, feeding, rat	0-20-80-300 ppm	20 ppm (2.13 mg/kg bw/d*)	80 ppm (9.19 mg/kg bw/d)	80 ppm (9.19 mg/kg bw/d)
2-generation, feeding, rat	0-20-80-300 ppm	80 ppm (5.5/6.7 mg/kg bw/d ,m/f)	300 ppm (21.0/24.5 mg/kg bw/d)	80 ppm (5.5 mg/kg bw/d)
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 the first 2-generation study a concentration of 80 ppm in the feed had no negative influence on the reproduction. At 300 ppm, litter size at birth was slightly reduced and some pups exhibited clinical signs such as laboured breathing or cyanosis. Later, F1- and F2-pups with thin appearance, piloerection, bloody noses and polyuria were observed. Increased mortality in F1 adults and their pups was seen. In parent animals, hyperkeratosis of the oesophagus epithelium was seen at 80 ppm and above in females and at 300 ppm in males. At 80 ppm and

above, feed consumption was decreased in parent animals. At 300 ppm, body weight gain in parent animals was reduced. The NOAEL for parental toxicity was 20 ppm and 80 ppm for both reproductive and offspring toxicity.
In addition to the originally submitted study on reproduction a second 2-generation study was performed recently on request of US EPA in order to address the uncertainty with regard to postnatal toxicity of spiroxamine arising from the first 2-generation study. In this second study the same dietary concentrations as in the previous study were administered. However, the doses expressed as mean daily intake (mg/kg bw/d) were noticeable lower. At the high dose of 300 ppm parental toxicity in males and females consisted in declined body weights and terminal body weights, subtle increases in activated partial thromboplastin time (APTT) and hyperkeratosis of the oesophagus. There were no test substance related effects on reproduction in this study. Pups of both generations at 300 ppm showed decreased body weight, body weight gain and changes in organ weights. Furthermore, slightly delayed balanopreputial separation and vaginal patency were noted in F1-pups at 300 ppm. The NOAELs in this study were 80 ppm for parental toxicity and offspring effects and 300 ppm for reproductive toxicity.
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 increased mortality could not be reproduced in the second study indicate that these severe findings of the first 2-generation study were rather caused by an infection of the animals than by a systemic effect of spiroxamine. Whether the severe clinical symptoms observed in the first study at 300 ppm were related to a possible infection or to the higher daily dose intake (mg/kg bw/d) cannot be answered.

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).
In the oral developmental toxicity study in rabbits a maternal NOAEL of 20 mg/kg bw/d based on clinical findings, reduced body weight and feed consumption at 80 mg/kg bw/d was established. The NOAEL of 20 mg/kg bw/d for developmental toxicity was determined based on slightly increased spontaneous skeletal malformation at 80 mg/kg bw/d.
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.

B.6.10.1.7 Neurotoxicity

In an acute neurotoxicity screening study in rats, a dose-related increase in unspecific toxicity but no neurotoxicity was observed using standard toxicological testing, a neurotoxicity-related functional observational battery and automated measurement of movement activity. Minimal effects were found at a dose of 30 mg/kg bw (piloerection, incoordinated gait, laboured breathing in one animal; reduced landing foot splay) and severe toxicity at higher doses of 100 and 220 mg/kg bw. Effects were reversible up to day 4. Histopathological

investigations of neural tissues revealed no indication of neurotoxic effects. The NOAEL was 10 mg/kg bw/d.
In a subchronic neurotoxicity screening study in rats doses of 700 and 155 ppm produced clear evidence of unspecific toxicity in both sexes including mortality, reduced body weight development, body-weight-related effects on absolute liver weights, unspecific changes in the functional observational battery (high dose males only), clinical pathology findings (increased activity of alanine-aminotransferase at 700 ppm) and microscopic lesions in non-neural tissues (hyperkeratosis of oesophagus and tongue epithelium). The low dose of 35 ppm (equal to 2.4 or 2.5 mg/kg bw/d in males or females, respectively) was the overall NOAEL for males and females. Neurobehavioural changes indicative of selective neurotoxicity were not evident at any dose level. There were no pathologic findings in neural or muscle tissues.

2.1.5 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

Selected toxicological findings and the criteria for classification for specific target organ toxicity – repeated exposure are summarised in Table 7.

Table 7: Selected toxicological results (at dose levels below the guidance values) in comparison with criteria of specific target organ toxicity – repeated exposure

Toxicological result	**CLP criteria**
28-d feeding study in rats: 100 ppm (10.8 / 12.2 mg/kg bw/d): liver weight ↑; steatosis of hepatocytes; hyperkeratosis of oesophagal mucosa 300 ppm (33.6 / 35.6 mg/kg bw/d): liver enzyme induction; hyperplasia of bladder epithelium **28-d gavage study in rats:** 10 mg/kg bw/d: clinical symptoms 30 mg/kg bw/d: liver enzyme induction; kidney weight ↑ 90 mg/kg bw/d: liver weight ↑; steatosis of hepatocytes; hyperkeratosis fore-stomach mucosa; hyperplasia of bladder epithelium **90-d feeding study in rats:** 125 ppm (9.3 / 13.2 mg/kg bw/d, m/f): hyperkeratosis epithelium oesophagus & fore-stomach, hyperplasia/hypertrophy oesophagus, slight liver enzyme induction 625 ppm (54.9 / 75.14 mg/kg bw/d, m/f):	Category 1 (H372): Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of: reliable and good quality evidence from human cases or epidemiological studies; or observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations. Equivalent guidance values for different study durations: Oral, rat: 28-day: ≤ 30 mg/kg bw/d 90-day: ≤ 10 mg/kg bw/d 1-yr: ≤ 2.5 mg/kg bw/d 2-yr: ≤ 1.25 mg/kg bw/d Dermal, rat or rabbit: 28-day: ≤ 60 mg/kg bw/d 90-day: ≤ 20 mg/kg bw/d Inhalation (dust/mist/fume), rat: 28-day: ≤ 0.06 mg/(L * 6h * d) 90-day: ≤ 0.02 mg/(L * 6h * d)
	Category 2 (H373): Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following repeated exposure.

body weight ↓; hyperkeratosis epithelium of tongue, hyperplasia of bladder epithelium, liver hyaline droplets **90-d feeding study in mice:** 320 ppm (88.4 / 126.6 mg/kg bw/d, m/f): epidermal hyperplasia of auricles, hepatocellular hypertrophy & fatty changes **90-d gavage study in mice:** 60 mg/kg bw/d: liver enzyme induction **90-d feeding study in dogs:** 750 ppm (20.02 / 21.29 mg/kg bw/d, m/f): liver weight ↑, serum albumin ↓, ALP ↑, triglycerides ↓; minimal diffuse hepatocytomegaly **110-d feeding study in dogs:** Up to 500 ppm (16.2 / 15.1 mg/kg bw/d, m/f, highest dose tested): No adverse effects reported **1-yr feeding study in dogs:** 1000 ppm (28.03 / 25.84 mg/kg bw/d, m/f): cataracts, hepatocytomegaly, albumin ↓; triglycerides ↓ **2-yr study in rats:** Effect levels were above guidance values **18-mo feeding studies in mice:** Effect levels were above guidance values **2-generation feeding study in rats (Pickel, 1993):** 80 ppm (9.19 / 10.59 mg/kg bw/d, m/f): Hyperkeratosis, feed intake ↓ 300 ppm (35.88 / 41.85 mg/kg bw/d): Additionally, clinical chemistry alterations, organ wt changes, bw ↓, clinical signs in offspring **2-generation feeding study in rats (Milius, 2008):** 300 ppm (21.0 / 24.5 mg/kg bw/d): bw gain ↓, APTT ↑, hyperkeratosis in oesophagus, delayed	Substances are classified in category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations. Guidance dose/concentration values are provided below in order to help in classification. In exceptional cases human evidence can also be used to place a substance in Category 2. Equivalent guidance values for different study durations: Oral, rat: 28-day: $30 < C \leq 300$ mg/kg bw/d 90-day: $10 < C \leq 100$ mg/kg bw/d 1-yr: $2.5 < C \leq 25$ mg/kg bw/d 2-yr: $1.25 < C \leq 12.5$ mg/kg bw/d Dermal, rat or rabbit: 28-day: $60 < C \leq 600$ mg/kg bw/d 90-day: $20 < C \leq 200$ mg/kg bw/d Inhalation (dust/mist/fume), rat: 28-day: $0.06 < C \leq 0.6$ mg/(L * 6h * d) 90-day: $0.02 < C \leq 0.2$ mg/(L * 6h * d)

development in offspring **Developmental gavage studies in rats (main and range-finding):** 100 mg/kg bw/d: Feed intake ↓, bw gain ↓, clinical signs 150 mg/kg bw/d: clinical signs, mortality (21/25, GD 11 to 16) 250 mg/kg bw/d: mortality (5/5, GD 10 and 13) **Developmental gavage studies in rabbits (main and range-finding):** 80 mg/kg bw/d: Feed intake ↓, bw gain ↓, mortality (1/15) 100 mg/kg bw/d: mortality (1/3, gastric ulcer) **90-d neurotoxicity study in rats:** 155 ppm (10.6 / 11.1 mg/kg bw/d, m/f): hyperkeratosis epithelia 700 ppm (48.5 / 50.6 mg/kg bw/d, m/f): hyperkeratosis epithelia, bw gain ↓, urothelial hyperplasia in urinary bladder, 1 mortality (wk 13)	
3-wk dermal studies in rats: 0.5 mg/kg bw/d, 1 mg/kg bw/d, 5 mg/kg bw/d: irritation related skin findings (erythema, swelling, hardening, cracking); no adverse systemic effects reported up to 5 mg/kg bw/d (highest dose tested) **Dermal developmental study in rats:** 5 mg/kg bw/d and above: Skin irritation 80 kg bw/d: bw gain ↓	
4-wk inhalation study in rats: 87.0 mg/m^3 air: irritation-related findings in the respiratory tract; increase of polymorphonuclear granulocytes; haemoglobin ↓ 518.4 mg/m^3 air: irritation-related findings in respiratory tract and eyes; liver weight ↑, body weight ↓, clinical chemistry (liver related), urinary	

bladder (hyperplasia)	

2.1.6 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

Some (groups of) findings were reported consistently in several studies. Their relevance for classification is discussed in the following.

Mortality was described in several dose groups of the dose-range finding developmental toxicity studies in rats after oral exposure and in the 90-d neurotoxicty study in rats. In the developmental toxicity studies in rats, the test material was formulated in 0.5 % v/v aqueous Cremophor and administered in a volume of 10 mL/kg bw. In the 28-d gavage study in rats, the test material was formulated in 2 % v/v aqueous Cremophor EL and administered in a volume of 10 mL/kg bw. It is noted that the highest dose tested in the 28-d gavage study in rats (i.e., 90 mg/kg bw/d) was lower than the dose levels which induced mortality in gravid rats (i.e., 150 and 250 mg/kg bw/d). In the 90-d neurotoxicty study in rats, one male treated with 700 ppm (48.5 mg/kg bw/d) died in study week 13.

Dams in dose-range finding developmental toxicity studies were treated from gestation days (GD) 6 to 15 and found dead as listed below:

Dose level:	150 mg/kg bw/d	250 mg/kg bw/d
Females in dose group	25	5
Dams died on:		
GD 10	0	3
GD 11	2	0
GD 12	3	0
GD 13	6	2
GD 14	3	0
GD 15	6	0
GD 16	1	0
Total number	21	5

Acute oral LD50 values determined in rats were in the range of 500 and 600 mg/kg bw/d in females or males, respectively (for details, see section 2.2). In this study, mortality was observed at dose levels of 500 mg/kg bw and above and occurred within hours after administration. At the next lower dose level of 100 mg/kg bw, no mortality was reported. Similarly, in the acute neurotoxicity study in rats (range-finding part), no mortality was reported after administration of 200 mg/kg bw but at 300 mg/kg bw (1 male and 1 female of 5 animals per sex) and 400 mg/kg bw (3 males and 3 females of 5 animals per sex).

Hence a specific mechanism leading to mortality after repeated exposure can neither be excluded nor confirmed. Dose levels inducing mortality were below the guidance values for category 2 but above those for category 1 (when adjusted for 28-d study duration). Mortality is considered to be a severe finding which may lead to classification with STOT-RE.

Ocular findings in dogs were reported at dose levels of 1000 ppm (28.03 / 25.84 mg/kg bw/d) and above. At study termination after 1 year of treatment, two males had evidence of bilateral sub

capsular clouding and cataracts and one female had bilateral lens opacity (cataracts). At the higher dose level, such findings were observed earlier. In this study, four animals per sex and group were treated.

These findings might be considered as "significant functional changes in the central or peripheral nervous systems or other organ systems, including signs of central nervous system depression and effects on special senses (e.g., sight, hearing and sense of smell)" or as "significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination" (as listed in the classification criteria, section 3.9.2.7.1). The dose level of 1000 ppm is at the upper range of the guidance values for category 2 (i.e., 25 mg/kg bw/d, when adjusting for 1-yr study duration). However, it is noted, that these guidance values are not intended as strict demarcation values (classification criteria, section 3.9.2.9.8).

Hyperkeratosis in tongue, oesophagus or fore-stomach was reported in several repeat dose studies after oral or inhalation exposure in dose levels compatible with both category 1 or category 2. Hyperplasia/hypertrophy was also observed in some of these organs and in urinary bladder (90-d feeding study in rats and 90-d neurotoxicity study in rats).

These findings might be discussed to be related to the skin irritant effect of spiroxamine, which was observed in a skin irritation study in rabbits (exposure to 500 µL/6 cm², Märtins, 1990, for details, see section 2.2). According to another report (Shelanski, 2001, for details, see section 2.2), gross skin changes were observed in humans after application of spiroxamine solutions in concentrations of 0.3 % or 1.02 % under occlusive conditions. Under these conditions, concentrations of 0.2 % (i.e., 75 µg/cm²) did not induce gross skin changes. However, the mechanistic correlation of bladder findings with irritating properties was not demonstrated.

Hyperplasia of urinary bladder epithelium did not progress to bladder carcinoma in the submitted long-term studies.

Observed findings of hyperkeratosis and hyperplasia/hypertrophy might be considered a "significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination" (as listed in the classification criteria, section 3.9.2.7.1) as the barrier function of the respective epithelia may be weakened by these effects.

Considering that the test material was formulated in feed, it is conceivable that the concentration was lower than the area doses that induced dermal irritation. Guidance on Application on the CLP Criteria (Version 4.0, November 2013) advises in chapter 3.9.2.5.1 on the classification of irritating/corrosive substances for STOT-RE. Regarding evaluation of corrosive substances, it is stated there:

> "… In such cases, it has to be evaluated whether the severe effect is a reflection of true repeated exposure toxicity or whether it is in fact just acute toxicity (i.e. corrosivity). One way to distinguish between these possibilities is to consider the dose level which causes the toxicity. If the dose is more than half an order of magnitude lower than that mediating the evident acute toxicity (corrosivity) then it could be considered to be a repeated-dose effect distinct from the acute toxicity. In this case, classification as specific target organ toxicant (repeated exposure) would be warranted even if the substance (or mixture) is also classified as acutely toxic and/or corrosive.
>
> In assessing non systemic effects caused by irritating/corrosive substances it should be kept in mind, that the guidance values /criteria for R48 in the DSD and later on those for

> STOT-RE of the CLP were derived from acute toxicity criteria (lethality based) assuming that systemic effects show a time dependent increase of severity due to accumulation of toxicity and taking also adaptive and detoxification processes into account. The effect considered in this context was lethality. This indicates that classification was intended for the presence of severe health damage, only."

I.e., regarding the evaluation of irritating substances, the guidance is less clear.

Liver findings were described in several studies in rats, mice and dogs after oral or inhalation exposure in dose levels compatible with both category 1 or category 2. However, the findings were considered to be not severe enough to support classification with STOT-RE.

In summary, mortality was observed in oral developmental toxicity studies in rats at dose levels compatible with STOT-RE category 2. Ocular findings were observed in dogs at dose levels compatible with STOT-RE category 2. Hyperkeratosis in tongue, oesophagus or fore-stomach was reported in several repeat dose studies after oral or inhalation exposure in dose levels compatible with both category 1 or category 2.

2.1.7 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

Considering the induction of mortality in rats and ocular findings in dogs, a classification with STOT-RE 2; H373 is proposed for spiroxamine..

It is less clear, whether the findings summarised above under "hyperkeratosis" were sufficiently severe to lead to classification. If regarded as sufficiently severe, , these hyperkeratosis-related findings may lead to a classification with STOT-RE 1 (H372).

Considering the available species-route-duration combinations of the various studies, it seems to be not possible to conclude on specific routes for classification.

As outlined in the initial CLH proposal, malformations (cleft palates) were observed in a developmental toxicity rat study at a dose level (100 mg/kg/day) which also caused slight maternal toxicity (reduced feed intake and decreased corrected body weight gain). Cleft palates were also noted in two range-finding studies in rats: at 100 mg/kg/day which also caused a slight reduction in maternal body weight gain as well as clinical signs; at 150 mg/kg/day in 2 out of the 4 surviving mothers.

The induction of mortality in rat dams in the second range finding study, cannot explain the observed induction of malformations nor render them to be irrelevant, non-specific findings secondary to severe maternal toxicity. It is highlighted that the dose levels inducing malformations were similar to and lower than the dose levels inducing mortality.

Similarly, there is no known correlation between observed hyperkeratosis (and related findings) and the observed induction of malformations. Hence, it is unlikely that hyperkeratosis (and related findings) would render the malformations as irrelevant, non-specific findings secondary to severe maternal toxicity.

In the available toxicity studies with repeat administration, no effects were reported which would be sufficient for classification as a reproductive toxicant. Similarly, no reproducible findings sufficient for classification as a reproductive toxicant were reported in the reproductive toxicity studies.

2.2 Additional information: Acute toxicity studies

To support the evaluation of effects seen in repeat dose toxicity studies and their distinction from findings reported in acute toxicity studies, the following information was extracted from the toxicology chapter of the assessment report prepared for the Annex-I-renewal procedure (i.e., Vol. 3, chapter B.6). This chapter was also part of the technical dossier initially submitted by the DS.

B.6.2 Acute toxicity including irritancy and skin sensitisation (OECD Annex IIA 5.2)

The acute toxicity data of spiroxamine after various routes of application (oral, dermal, i.p., inhalative) including irritancy and skin sensitisation are summarised in Table B.6.2-1.

Table B.6.2-1: Results of acute toxicity studies with spiroxamine

Study	Vehicle	Species	Results
Acute oral	water/cremophor	rat	LD_{50}: ~500 mg/kg bw, f; 595 mg/kg bw, m
Acute oral	water/cremophor	mouse	LD_{50}: 460 mg/kg bw, m; 561 mg/kg bw, f
Acute dermal	cellulose powder	rat	LD_{50}: 1068 mg/kg bw, f; >1600 mg/kg bw, m
Acute inhalation (4 h)	none	rat	LC_{50}: 1982 mg/m^3 air, f; 2772 mg/m^3 air, m
Skin irritation	none	rabbit	severe irritant
Eye irritation	none	rabbit	not irritant
Skin sensitisation	0.9 % NaCl/ cremophor	guinea pig	sensitiser

Spiroxamine has a moderate acute toxicity after single oral, dermal or inhalative application. Following clinical symptoms of acute spiroxamine intoxication in laboratory animals were observed: apathy, piloerection, staggering gait, increased salivation, spasms, vocalisation, reduced motility and laboured breathing.
Spiroxamine is a strong skin irritant, but not an eye irritant. The compound exhibited skin sensitising properties.

On basis of the acute toxicity of spiroxamine technical following classification/labelling is proposed:

Xn	(harmful);
R 20/21/22	(harmful by inhalation, in contact with skin and if swallowed)
R 38	(irritating to the skin);
R 43	(may cause sensitisation by skin contact).

B.6.2.1 Oral studies

B.6.2.1.1 Rat

Reference: KIIA 5.2 (OECD)
Report: Krötlinger, F. (1991), 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 B.6.2-2). Following a dose of 100 mg/kg bw both sexes showed signs of apathy and increased salivation. In higher doses both sexes exhibited pilocrection, 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 B.6.2-2.

Table B.6.2-2: 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:
Spiroxamine in Cremophor El/demineralised water (2 % v/v) was harmful by oral administration to rats and has to be classified accordingly.

Re-evaluation in 2009
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. According to EU Directive 67/548/EEC spiroxamine has to be classified with R22. Considering the criteria laid down in regulation (EC) No. 1272/2008, allocation into acute toxicity category 4 (H302) is considered necessary.

Remark: In the previous DAR, a LD_{50} of 374 mg/kg bw was stated. Most probably this value was calculated when taking into account mortality data out of the acute neurotoxicity study (range-finding part). However, the approach taken was not documented.
When calculating a linear regression with the mortality data (Table B.6.2-3) between 300 and 400 mg/kg bw, a dose of 375 mg/kg bw is reached for a mortality rate of 50 %.

Table B.6.2-3: 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

B.6.2.1.2 Mouse

Reference: KIIA 5.2 (OECD)

Report: Krötlinger, F. (1991) 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, pilocrection, 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 B.6.2-4).
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 B.6.2-4: 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:
Spiroxamine in water/Cremophor El (2 % v/v) was harmful by oral administration to mice and has to be classified accordingly.

Re-evaluation in 2009
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.

B.6.2.2 Percutaneous studies

B.6.2.2.1 Rat

Reference: KIIA 5.2 (OECD)
Report: Krötlinger, F. (1991), 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, pilocrection, 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 B.6.2-5). Contrary to female rats observed deaths in males were not dose related.

Table B.6.2-5: 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:
Spiroxamine was harmful to the rat following acute dermal application and has to be classified accordingly.

Re-evaluation in 2009
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. Accordingly to EU Directive 67/548/EEC spiroxamine has to be classified with R21. Considering the criteria laid down in regulation (EC) No. 1272/2008, allocation into acute toxicity category 4 (H312) is considered necessary.

B.6.2.3 Inhalation studies

B.6.2.3.1 Rat

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 B.6.2-6). The inhalative LC_{50} was calculated to be 1982 mg/m^3 air in females and 2772 mg/m^3 air in males.

Table B.6.2-6: 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:
Spiroxamine has a moderate toxicity in the rat as a respirable aerosol. Rats exposed to 869 mg/m³ air tolerated the exposure without noticeable effect.

Re-evaluation in 2009
The inhalative LD_{50} measured in rats was 1982 mg/m³ air (1.982 mg/L air). Accordingly to EU Directive 67/548/EEC spiroxamine has to be classified with R20. Considering the criteria laid down in regulation (EC) No. 1272/2008, allocation into acute toxicity category 4 (H332) is considered necessary [test compound was a liquid, therefore, ATEs for mists apply].

B.6.2.4 Skin irritation

B.6.2.4.1 Rabbit

Reference:	KIIA 5.2 (OECD)
Report:	Märtins, T.(1990), KWG 4168 - Study for skin and eye irritation/corrosion in rabbits - Report no.: 19584 (October 01, 1990); Bayer AG, Institute for Toxicology, Wuppertal, Germany, Dates of exp. work: April 1990 - May 1990 TOX9552593
Guidelines:	OECD TG 404.
Deviations:	None
GLP:	Yes
Acceptability:	The study is considered to be acceptable.

Material and methods:
Test system: Three female albino rabbits (Source: HC:NZW, Interfauna U.K. Ltd., Wyton, Huntingdon) received spiroxamine (batch no.: 17002/90; purity: 94.6 %) via a single dermal administration. The degree of erythema (eschar) and oedema formation was recorded as specified by Draize.

Findings:
Exposure of the skin to 500 µL test substance (exposure time: 4 h) caused marked inflammatory reactions. After 24 h irritation scores were 2 for erythema and 2-3 for oedema. The reactions extended beyond the exposed area in all animals from 24 h to day 8. The skin was covered by a whitish scaly layer in all animals on day 8 and 14.

Table B.6.2-7: Results of skin irritation testing in females

Animal no.			C7	C16	C9
Body weight (kg)			3.0	3.4	3.4
DRAIZE grade	1h	e	1	2	2
		o	1	2	2
	24h	e	2	2	2
		o	3	3	2
	48h	e	2	2	2
		o	2	2	1
	72h	e	2	2	2
		o	2	2	1
	8d	e	1	2	1
		o	3	3	1
	14d	e	1	2	0
		o	2	1	0
Irritation index		e	2.0	2.0	2.0
		o	2.3	2.3	1.7

e = erythema and eschar formation o = oedema formation

Conclusion:
According to these results spiroxamine must be considered to be severely irritating to the skin and has to be classified accordingly.

Re-evaluation in 2009
Spiroxamine is severely irritating to the skin and accordingly to EU Directive 67/548/EEC spiroxamine has to be classified with R38. Considering the criteria laid down in regulation (EC) No. 1272/2008, allocation into skin irritation/corrosion category 2 (H315) is considered necessary.

B.6.2.4.2 Human

The following study was submitted after the previous DAR was finalised. The following summary was prepared in the context of the re-evaluation in 2009.

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 B.6.2-8.

Table B.6.2-8: 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.

B.6.2.5 Eye irritation

B.6.2.5.1 Rabbit

Reference: KIIA 5.2 (OECD)
Report: Märtins, T. (1990), KWG 4168 - Study for skin and eye irritation/corrosion in rabbits - Report no.: 19584 (October 01, 1990); Bayer AG, Institute for Toxicology, Wuppertal, Germany, Dates of exp. work: April 1990 - May 1990.
TOX9552593
Guidelines: OECD TG 405

Deviations: None

GLP: Yes

Acceptability: The study is considered to be acceptable.

Material and methods:
Test system: Three female albino rabbits (Source: HC:NZW, Interfauna U.K. Ltd., Wyton, Huntingdon) received spiroxamine (batch no.: 17002/90; purity: 94.6 %) via instillation (100 µL) into the conjunctival sac of one eye of each of three rabbits. The other eye was not treated and served as control. After exposure time of 24 h the eye was rinsed with saline.

Findings:
No manifestations of irritation were observed in 2 out of 3 animals. In one animal, a slight reaction of the conjunctivae was recorded 1 h and 24 h after exposure (Table B.6.2-9).

Table B.6.2-9: Results of eye irritation testing

Animal	Sex	bw (kg)	Tissue	Signs	DRAIZE grades					Irrit.
no.					1h	24h	48h	72h	8d	index
C13	f	3.1	conjunctivae	r	1	1	0	0	0	0.3
				s	0	1	0	0	0	0.3
C8	f	3.6	conjunctivae	r	1	0	0	0	0	0.0
				s	0	0	0	0	0	0.0
C12	f	3.6	conjunctivae	r	1	0	0	0	0	0.0
				s	0	0	0	0	0	0.0

r redness, s swelling

Conclusion:
The results indicate that spiroxamine could be regarded as 'not irritating to the eye'.

Re-evaluation in 2009
According to EU Directive 67/548/EEC spiroxamine is considered to be not irritating to eyes. Considering the criteria laid down in regulation (EC) No. 1272/2008, allocation into and eye damage/eye irritation category is considered not necessary.

B.6.2.6 Skin sensitisation

B.6.2.6.1 Maximisation test

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: intra-dermal induction: 5 %; topical induction: 6 %; 1st topical challenge: 1 % and 0.5 %; 2nd topical challenge: 0.1 % and 0.05 %.

Findings:
Range finding for intra-cutaneous induction: One guinea pig was injected intra-dermally 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 B.6.2-10.

Table B.6.2-10: Number of animals exhibiting skin reddening in the range finding test for topical induction (48 and 72 hours after application)

	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 B.6.2-11). Body weight gain amongst the treatment group animals corresponded to that of the control groups.

Table B.6.2-11: Number of animals exhibiting skin reactions in the maximisation test (48 and 72 hours after initiation of challenge)

	Test substance group				1st and 2nd control group			
	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:
Spiroxamine showed a skin sensitising effect under the conditions of the Maximisation Test and has to be classified accordingly.

Re-evaluation in 2009
Under conditions of the Maximisation Test spiroxamine showed sensitising effects and accordingly to EU Directive 67/548/EEC this substance has to be classified with R43. Considering the criteria laid down in regulation (EC) No. 1272/2008, allocation into skin sensitisation category 1 (H317) is considered necessary.

B.6.2.6.2 Buehler Patch Test

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 acceptabel.

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 were 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 B.6.2-12).

Table B.6.2-12: Number of animals exhibiting skin reactions in the Buehler patch test (24, 48 and 72 hours after initiation of challenge)

	Test substance group						1st and 2nd control group					
	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:
Spiroxamine exhibited a slight skin sensitising potential at irritant concentrations.

Re-evaluation in 2009
Under conditions of the Buehler Test spiroxamine exhibited a slight skin sensitising potential.

B.6.2.7 Acute intraperitoneal toxicity

B.6.2.7.1 Rat

Reference: KIIA 5.2 (OECD)
Report: Krötlinger, F. (1991) 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 B.6.2-13: 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:
After intra-peritoneal application to rats, spiroxamine was moderately to low toxic.

Re-evaluation in 2009
The LD_{50} after intraperitoneal administration was calculated to be 114 mg/kg bw in male rats and 150 mg/kg bw in females.

3 ENVIRONMENTAL HAZARD ASSESSMENT

Not evaluated in this report, see the 2014 CLH report.