Table A6_3-2. Results of repeated dose toxicity study on rats (recovery group)

Parameter	Control 0 mg/kg bw		Low dose 30 mg/kg bw		Medium dose 100 mg/kg bw		High dose 300 mg/kg bw		Dose-response +/-	
	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m	f
Number of animals examined	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]		
Mortality	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Organ: liver										
Microscopic pathology	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Organ: spleen										
Microscopic pathology	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Organ: adrenals										
Microscopic pathology	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

[a] number of animals affected/total number of animals

↑ increase

↓ decrease

— not different from control

Section A6.3.2 — 6.3 Short-term repeated dose toxicity (dermal)

Annex Point IIA6.3 — Subacute dermal toxicity study in rabbits

Official use only

1 REFERENCE

1.1	**Reference**	[redacted], 1988, HWG 1608 techn. – Subacute dermal study of toxicity to rabbits (Addendum to Report no. [redacted] of 8.5.1984), [redacted], Report No. [redacted], 1988-03-03
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes OECD-Guideline 410
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]

3 MATERIALS AND METHODS

3.1	**Test material**	HWG 1608 techn.
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	technical grade
3.1.2.1	Description	yellowish white powder
3.1.2.2	Purity	[redacted]
3.1.2.3	Stability	The stability of test compound formulation over the study period, the homogeneity and active ingredient content in the formulations were confirmed by analysis.
3.2	**Test Animals**	
3.2.1	Species	Rabbit
3.2.2	Strain	New Zealand White, strain HC:NZW
3.2.3	Source	[redacted]
3.2.4	Sex	males and females (1:1)
3.2.5	Age/weight at study initiation	age: ten to seventeen weeks mean weight: 3.23 kg (males) and 3.15 kg (females)
3.2.6	Number of animals per group	five per sex per group
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Dermal

Section A6.3.2 — 6.3 Short-term repeated dose toxicity (dermal)

Annex Point IIA6.3 — Subacute dermal toxicity study in rabbits

3.3.1	Duration of treatment	3 weeks
3.3.2	Frequency of exposure	5 days per week
3.3.3	Postexposure period	No
3.3.4	**Dermal**	
3.3.4.1	Area covered	11 cm x 12 cm
3.3.4.2	Occlusion	Occlusive
3.3.4.3	Vehicle	Cremophor EL 2% v/v (castor oil, ethoxylated)
3.3.4.4	Concentration in vehicle	25 % formulation
3.3.4.5	Total volume applied	2 ml/kg bw for the control group 4 ml/kg bw for the treatment group; dose: 1000 mg/kg bw
3.3.4.6	Duration of exposure	6 h
3.3.4.7	Removal of test substance	soap and water
3.3.4.8	Controls	solvent
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	yes (daily)
3.4.1.2	Mortality	yes (daily)
3.4.2	Body weight	yes (weekly)
3.4.3	Food consumption	no
3.4.4	Water consumption	no
3.4.5	Ophthalmoscopic examination	no
3.4.6	Haematology	yes number of animals: all animals time points: before the start and at the end of study Parameters: erythrocyte and leukocyte count, haemoglobin concentration, haematocrit, platelet count, mean corpuscular volume of erythrocytes, mean haemoglobin content of erythrocytes, differential blood count
3.4.7	Clinical Chemistry	yes number of animals: all animals time points: before the start and at the end of study Parameters: glucose, urea, creatinine, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase

		in liver tissue: N-demethylase, O-demethylase, Cytochrome P-450
3.4.8	Urinalysis	yes number of animals: all animals time points: before the start and at the end of study Parameters: pH, urobilinogen, blood, protein, glucose, sediment microscopically examined (bacteria, epithelia, erythrocytes, leucocytes, amorphous salts, triple phosphates and calcium oxalates)
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	yes organs: thyroid, heart, lung, liver, kidneys, adrenals, spleen, testicles, ovaries
3.5.2	Gross and histopathology	yes all dose groups organs: skin (untreated and treated specimens), thyroid, heart, lungs, liver, kidneys, spleen, adrenals, testicles, epididymes, ovaries, uterus
3.5.3	Other examinations	Local skin irritation according to Draize; skin fold measurement
3.5.4	Statistics	The arithmetic group means, standard deviations, upper and lower confidence limits were calculated. For the comparison of the control group with the treatment group, MANN, WHITNEY and WILCOXON´s significance test (U-test) was used.
3.6	**Further remarks**	
		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	no systemic effects Macroscopic examination of the skin in the region of the test substance-treated area revealed isolated and temporary very slight redness in the males between the fifth and fourteenth treatment day. One female produced a comparable finding between the sixth and eleventh treatment day. A further female reacted with very slight redness on the fourteenth treatment day only. Skin fold measurements revealed slightly thicker skin folds of treated skin in the males but not in the females.
4.1.2	Mortality	no mortalities at any dose
4.2	**Body weight gain**	no test-substance related effects
4.3	**Food consumption and compound intake**	not determined
4.4	**Ophtalmoscopic examination**	not determined
4.5	**Blood analysis**	
4.5.1	Haematology	no test-substance related effects

Section A6.3.2 — 6.3 Short-term repeated dose toxicity (dermal)

Annex Point IIA6.3 — Subacute dermal toxicity study in rabbits

4.5.2	Clinical chemistry	no test-substance related effects
4.5.3	Urinalysis	no effects
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	no test-substance related effects
4.6.2	Gross and histopathology	no test-substance related effects
4.7	**Other**	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The study was done according to OECD Guideline 410. As in a previous dermal repeated dose study no toxicity was noted at 50 and 200 mg/kg bw (see [redacted], Report No. 12669), only one dose of 1000 mg/kg bw was used in this study.
5.2	**Results and discussion**	The dermal treatment with the test substance did not produce any systemic effects on male and female rabbits which would have to be associated with the active ingredient. The slight alterations in the skin revealed by the clinical and histopathological examinations are presumably attributable to mechanical irritation of the skin, since the test compound formulation was a suspension of viscous consistency or slurry, and the pressure of the occlusive dressing presumably resulted in skin friction.
5.3	**Conclusion**	
5.3.1	LO(A)EL	
5.3.2	NO(A)EL	1000 mg/kg bw for systemic effects
5.3.3	Other	Local minimal alterations of the treated skin were noted, which are most likely to be attributable to mechanical irritation.
5.3.4	Reliability	[redacted]
5.3.5	Deficiencies	[redacted]

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	July 2005
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

Table A6_3-1. Results of clinical chemistry haematology and urinalysis

Not needed due to results of study

Table A6_3-2. Results of repeated dose toxicity study

Not needed due to results of study

Section A6.3.3

Annex Point IIA6.3

6.3 Short-term repeated dose toxicity (inhalation)

Subacute inhalation toxicity study in rats

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted], 1985, HWG 1608 – Study for subacute inhalation toxicity to the rat for three weeks (exposure 15x6 hours[redacted], Report No. [redacted], 1985-02-22 [redacted], 1987, HWG 1608 – Addendum – Study for subacute inhalation toxicity to the rat for three weeks (exposure 15x6 hours), [redacted], Report No. [redacted], 1987-07-23
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes OECD-Guideline 412
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]
		3 MATERIALS AND METHODS
3.1	**Test material**	HWG 1608
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	technical grade
3.1.2.1	Description	
3.1.2.2	Purity	[redacted]
3.1.2.3	Stability	The stability of stock solutions was checked twice a week by chemical analysis.
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Wistar, strain Bor:WISW (SPF-Cpb)
3.2.3	Source	[redacted]
3.2.4	Sex	males and females (1:1)
3.2.5	Age/weight at study initiation	weight: approximately 160 to 200 g
3.2.6	Number of animals per group	ten per sex per group
3.2.7	Control animals	Yes (air and vehicle)

Section A6.3.3

Annex Point IIA6.3

6.3 Short-term repeated dose toxicity (inhalation)

Subacute inhalation toxicity study in rats

3.3	**Administration/ Exposure**	Inhalation	
3.3.1	Duration of treatment	3 weeks	
3.3.2	Frequency of exposure	5 days per week	
3.3.3	Postexposure period	No	
3.3.4	**Inhalation**		
3.3.4.1	Concentrations	Nominal concentration	0, 5, 50 and 500 mg/m³
		Analytical concentration	0, 1.2, 10.6 and 155.8 mg/m³
3.3.4.2	Particle size	MMAD (mass median aerodynamic diameter) for the aerosols:	
		0 mg/m³ (vehicle): MMAD = 2.0 µm (± 2.1)	
		5 mg/m³: MMAD = 2.1 µm (± 2.0)	
		50 mg/m³: MMAD = 2.0 µm (± 2.0)	
		500 mg/m³: MMAD = 2.1 µm (± 2.1)	
3.3.4.3	Type or preparation of particles	not applicable	
3.3.4.4	Type of exposure	nose/head only	
3.3.4.5	Vehicle	ethanol / polyethylene glycol E 400 (1:1)	
3.3.4.6	Concentration in vehicle	0.025 % (5 mg/m^3), 0.25 % (50 mg/m^3) or 2.5 % (500 mg/m^3)	
3.3.4.7	Duration of exposure	6 h per day	
3.3.4.8	Controls	controls were exposed to air or vehicle under the same conditions as the treatment groups	
3.4	**Examinations**		
3.4.1	Observations		
3.4.1.1	Clinical signs	yes (several times on the exposure days, but not during exposure in tubes)	
3.4.1.2	Mortality	yes (several times on the exposure days, but not during exposure in tubes)	
3.4.2	Body weight	yes (weekly)	
3.4.3	Food consumption	no	
3.4.4	Water consumption	no	
3.4.5	Ophthalmoscopic examination	no	

Section A6.3.3 — 6.3 Short-term repeated dose toxicity (inhalation)

Subacute inhalation toxicity study in rats

Annex Point IIA6.3

3.4.6	Haematology	Yes number of animals: all animals time points: end of study Parameters: haematocrit, haemoglobin concentration, erythrocyte count, total and differential leucocyte count, platelet count, MCV (mean corpuscular volume), MCHC (mean corpuscular haemoglobin concentration), MCH (mean corpuscular haemoglobin), thromboplastin time
3.4.7	Clinical Chemisty	yes number of animals: all animals time points: end of study Parameters: glucose, urea, creatinine, alanine aminotransferase, aspartate aminotransferase, ALP (alkaline phosphatase), LDH (lactate dehydrogenase), GLDH (glutamate dehydrogenase), creatine phosphokinase in liver tissue: Cytochrome P-450, N-demethylase, O-demethylase
3.4.8	Urinalysis	yes number of animals: all animals time points: end of study Parameters: blood, protein, glucose, pH, sediment microscopically examined (bacteria, erythrocytes, epithelia, leucocytes, amorphous salts, triple phosphate, calcium oxalate)
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	yes organs: heart, testes, ovaries, liver, lung, spleen, adrenals, kidneys and thyroids
3.5.2	Gross and histopathology	yes all dose groups and controls organs: eyes, brain (cerebellum and cerebrum), duodenum, lung hilus lymph node, heart, testes, ovaries, head, nasal area (nasal cavities with paranasal sinuses), liver, lung, stomach (fore- and glandular stomach), spleen, skeletal musculature, nervus ischiadicus, adrenals, kidneys, oesophagus, pharynx, larynx, trachea, thyroids with parathyroids
3.5.3	Other examinations	no
3.5.4	Statistics	The arithmetic group means, standard deviations, upper and lower confidence limits were calculated. For the comparison of the two control groups (air and vehicle) with the three groups exposed to the test substance, MANN, WHITNEY and WILCOXON´s significance test (U-test) was used.
3.6	**Further remarks**	

Section A6.3.3 | **6.3 Short-term repeated dose toxicity (inhalation)**

Annex Point IIA6.3 | Subacute inhalation toxicity study in rats

		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	The treatment was tolerated without ill-effect by the rats in the control groups and in the low and medium dose groups. At the highest dose group, both sexes showed bristling coats (piloerection) after each exposure.
4.1.2	Mortality	One female rat in the control group (air) died due to broken neck on insertion into exposure tube. No other death occurred.
4.2	**Body weight gain**	There were no toxicologically significant variations in body weight gain in any of the groups.
4.3	**Food consumption and compound intake**	not determined
4.4	**Ophtalmoscopic examination**	not determined
4.5	**Blood analysis**	
4.5.1	Haematology	no effects
4.5.2	Clinical chemistry	There was an increase in N-demethylase activity in the liver tissue of both sexes in the highest dose group. The males of this group furthermore exhibited a marginal increase in O-demethylase activity. These findings are considered to be causally connected to a slight liver enzyme induction.
4.5.3	Urinalysis	no effects
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	No toxicologically relevant alterations were noted.
4.6.2	Gross and histopathology	No test substance-related alterations were found at gross or histopathological examination.
4.7	**Other**	
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The study was conducted according to OECD-Guideline 412 with no major deviations.
5.2	**Results and discussion**	The treatment was tolerated without toxic signs, apart from piloerection in animals of the highest dose group. A toxicologically relevant effect on the body weights could not be found in any of the groups. The clinical chemical and haematological examinations did not reveal any toxicologically significant indications pointing to specific organ damage. The animals in the highest dose group exhibited slight enzyme induction. There were however no signs of liver damage. The analysis of the organ weight – body weight correlation and the histomorphological examinations did not reveal any indications of specific organ or liver alterations.

Section A6.3.3

Annex Point IIA6.3

6.3 Short-term repeated dose toxicity (inhalation)

Subacute inhalation toxicity study in rats

5.3	**Conclusion**		
5.3.1	LO(A)EL	155.8 mg/m^3	*
5.3.2	NO(A)EL	10.6 mg/m	*
5.3.3	Other		
5.3.4	Reliability	[redacted]	
5.3.5	Deficiencies	[redacted]	

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Give date of action
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

Table A6_3-1. Results of clinical chemistry haematology and urinalysis

Not needed due to results of study

Table A6_3-2. Results of repeated dose toxicity study

Parameter	Control air		Control vehicle		low dose 1.2 mg/m³		medium dose 10.6 mg/m³		high dose 155.8 mg/m³		dose-response +/-	
	m	f	m	f	m	f	m	f	m	f	m	f
number of animals examined	10	10	10	10	10	10	10	10	10	10		
Mortality		[redacted]										
clinical signs piloerection									[redacted]	[redacted]	[redacted]	[redacted]
body weight												
clinical chemistry N-demethylase (liver) O-demethylase (liver)									[redacted]	[redacted]	[redacted]	[redacted]

Section A6.4.1 — 6.4 Subchronic toxicity (90 days)

Annex Point IIA6.4 — Subchronic oral toxicity test in dogs

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted], HWG 1608 – Subchronic study of toxicity to dogs with oral administration, [redacted], Report No. [redacted], 1987-05-06	
1.2	**Data protection**	[redacted]	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	[redacted]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes OECD-Guideline 409	
2.2	**GLP**	[redacted]	
2.3	**Deviations**	[redacted]	
		3 MATERIALS AND METHODS	
3.1	**Test material**	HWG 1608	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	technical grade	
3.1.2.1	Description	colourless crystals (solid)	
3.1.2.2	Purity	[redacted] symmetrical isomer	*
3.1.2.3	Stability	Before start of the study it had been established that the test substance was stable for a minimum of fourteen days in the dry diet and at least twenty-four hours in the wet diet, and was homogeneously distributed in the mix.	
3.2	**Test Animals**		
3.2.1	Species	dog	
3.2.2	Strain	beagle (bor:beag)	
3.2.3	Source	[redacted]	
3.2.4	Sex	males and females (1:1)	
3.2.5	Age/weight at study initiation	age: 24 to 27 weeks weight: between 6.3 and 9.0 kg	
3.2.6	Number of animals per group	4 per sex per group	
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	Oral	

Section A6.4.1 — 6.4 Subchronic toxicity (90 days)

Annex Point IIA6.4 — Subchronic oral toxicity test in dogs

3.3.1	Duration of treatment	90 days (13 weeks)
3.3.2	Frequency of exposure	daily
3.3.3	Post-exposure period	no
3.3.4	**Oral**	
3.3.4.1	Type	in food
3.3.4.2	Concentration	0, 200, 1000 and 5000 ppm food consumption per day: all animals were given the same quantity of food in the morning; the food not eaten until the next feeding time was weighed so that the individual food consumption and consequently the amount of test substance administered were individually determined uptake of test substance: 200 ppm: 6.69 g/animal total and 73.5 mg/animal per day 1000 ppm: 32.77 g/animal total and 360.1 mg/animal per day 5000 ppm: 158.05 g/animal total and 1736.8 mg/animal per day
3.3.4.3	Vehicle	no vehicle, test substance mixed in food
3.3.4.4	Concentration in vehicle	
3.3.4.5	Total volume applied	
3.3.4.6	Controls	plain diet
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	yes (several times daily during feeding, care, stall cleaning and exercising)
3.4.1.2	Mortality	yes (see 3.4.1.1)
3.4.2	Body weight	yes (individual body weights weekly)
3.4.3	Food consumption	yes (daily)
3.4.4	Water consumption	no (only observation, no measurement)
3.4.5	Ophthalmoscopic examination	yes (before start of study and in the third, seventh and thirteenth treatment weeks)
3.4.6	Haematology	yes number of animals: all animals time points: before start of study and in the third, seventh and thirteenth treatment weeks Parameters: haematocrit, haemoglobin concentration, erythrocyte count, total and differential leukocyte count, MCV, MCH, MCHC, platelet count, reticulocyte count, thromboplastin time, blood sedimentation rate
3.4.7	Clinical Chemisty	yes

Section A6.4.1 **6.4 Subchronic toxicity (90 days)**

Annex Point IIA6.4 Subchronic oral toxicity test in dogs

		number of animals: all animals
		time points: before start of study and in the third, seventh and thirteenth treatment weeks
		Parameters: glucose, urea, creatinine, total protein, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, glutamate dehydrogenase, bilirubin, cholesterol, serum protein electrophoresis (separation on acetate foils), sodium, potassium, calcium, chloride
		in liver tissue: cytochrome P450, N-demethylase and triglycerides
3.4.8	Urinalysis	yes
		number of animals: all animals
		time points: before start of study and in the third, seventh and thirteenth treatment weeks
		Parameters: volume, specific gravity, pH, protein, glucose, blood, bilirubin, ketone bodies, urobilinogen, urine sediment was microscopically examined
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	yes
		organs: brain, heart, testicles, liver, lung, spleen, adrenals, kidneys, ovaries, pancreas, prostate, thyroid (including parathyroid)
3.5.2	Gross and histopathology	yes
		all dose groups and controls
		organs: heart, lung, liver (2x), spleen, kidney, cerebrum (incl. cerebral stem), cerebellum (incl. medulla oblongata), adrenals, thyroid (incl. parathyroid), pituitary, eyes (4 sections per eye), nervi optici, nervus ischiadicus, pancreas, aorta, gallbladder, urinary bladder, skeletal muscle, bones (os femoris, sternum), salivary gland (parotis), oesophagus, stomach (2 locations), intestines (5 locations), lymph node, thymus, testicles (2x), epididymes (2x), prostate, ovaries (2x), uterus (2x), skin, mamma, bone marrow
3.5.3	Other examinations	no
3.5.4	Statistics	In view of the low number of animals per group (4 males, 4 females), descriptive statistical methods were employed. The calculations comprised here the determinations of the arithmetic means and standard deviations. In addition maximum and minimum figures have been given.
3.6	**Further remarks**	
		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	no substance-related findings
		vomiting, pasty stool, diarrhoea also appeared in controls; incidence did not show correlation to treatment
4.1.2	Mortality	One female dog in the highest dose group was found dead in its cage on

Section A6.4.1 — 6.4 Subchronic toxicity (90 days)

Annex Point IIA6.4 — Subchronic oral toxicity test in dogs

		the second study day after only a single treatment. Since this was an animal in the highest dose group, a connection with the treatment may not be ruled out. The authors state that the most likely cause of death was an acute circulatory collapse as on autopsy moderate pulmonary oedema was found. This animal was replaced by another female. All the other dogs survived the thirteen weeks of treatment.
4.2	**Body weight gain**	1000 and 5000 ppm led to a retarded weight development in the dogs. The level of 200 ppm was tolerated without effect on the body weight.
4.3	**Food consumption and compound intake**	Concentrations up to and including 1000 ppm did not have any notable effect on the animals´ food consumption. Most of the animals´ food consumption in the high dose group was repeatedly incomplete which was attributed to the treatment.
4.4	**Ophthalmoscopic examination**	In the highest dose group the formation of lens opacity was noted in all the animals during the study (histological diagnosis: lens degeneration).
4.5	**Blood analysis**	
4.5.1	Haematology	After 5000 ppm, an increased thrombocyte level and increased signs of severe anisocytosis were found at the end of the treatment. Concentrations up to and including 1000 ppm did not have any notable effect on the haematological parameters examined.
4.5.2	Clinical chemistry	A retardation of the age-induced fall (1000 ppm) and a rise (5000 ppm) of the mean alkaline phosphatase activity (AP) was measured in the plasma. There were shifts in the percentages of the serum proteins observed at 5000 ppm (slight decrease in albumin, slight increase in beta-globulin). There was a slight (1000 ppm) and distinct (5000 ppm) rise in the mean N-demethylase activities in the liver. Also, a slight increase in the mean cytochrome P-450 concentrations was observed in the liver at 5000 ppm.
4.5.3	Urinalysis	no effects
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	The relative and absolute mean spleen weights were increased in both sexes at 5000 ppm.
4.6.2	Gross and histopathology	Gross pathology: no test substance-related effects Histopathology: Eyes: Degenerative alteration in the posterior wall of the lens was observed in 4/4 males and 1/4 females at 5000 ppm. In 3/4 females of the highest dose group a cataracta lentis was noted. Liver: Slightly increased accumulation of ferriferous pigments in Kupffer cells was noted in all four females and one male at 5000 ppm. Spleen: Slightly increased accumulation of ferriferous pigments in siderocytes of the red spleen pulp was observed in three of four males and in two of four females at 5000 ppm. Adrenals: One female at 5000 ppm exhibited heightened vacuole formation as against control animals in the plasma of cells in the zona fasciculata.

Section A6.4.1 6.4 Subchronic toxicity (90 days)

Annex Point IIA6.4 Subchronic oral toxicity test in dogs

4.7 Other

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The study was done according to OECD-Guideline 409, though not stated in the study report. There are no relevant deviations from current guidelines for subchronic toxicity studies. Briefly, groups of each 4 male and female Beagles were fed with diets containing 200, 1000 or 5000 ppm of test substance. Examinations were done on all animals.

5.2 Results and discussion

One female in the highest dose group was found dead on the second study day after only a single treatment, without having previously shown clinical abnormalities. The cause of the death was probably an acute circulatory collapse. However, a connection with the treatment cannot be completely ruled out but seems to be unlikely as all the other dogs survived during the end of study and were normal in behaviour and appearance.

Most of the animals´ food consumption at 5000 ppm was repeatedly incomplete. At 1000 ppm and above the mean weight development was retarded. These findings are attributed to the treatment.

The ophthalmoscopic examination revealed alterations in the high dose animals which are attributable to the treatment, due to their correlation with dose and their increasing trend during the study. Initially in only a few animals lens opacity was noted, but at the end all the animals of the high dose groups were concerned. These results were confirmed by histopathology where morphological degeneration was observed.

At 5000 ppm, thrombocyte counts slightly rised and marked anisocytosis was observed. Histology revealed slight siderosis in the spleen and liver at 5000 ppm, which points to an increased level of breakdown of the red blood cells. This increased level of iron pigment accumulation in conjunction with adaptation mechanisms to the increased metabolic rate are considered to be the reason for the mean absolute and relative higher spleen weights in the highest dose group. The findings described are associated with administration of the test substance, due to their increased incidence in animals in the highest dose group and assessed as a manifestation of a compensated anaemic process. Nevertheless these are marginal effects, since the erythrocyte counts and the haematocrit and haemoglobin figures were unchanged at all the examination times. The increased breakdown of the red blood cells, which was apparent as slightly increased siderosis of liver and spleen, was not apparent during the study at laboratory examinations. This points to complete compensation.

Signs of treatment-induced effects on the liver were detected by the clinical chemical examination at 1000 ppm and above. At 1000 ppm the age-induced (physiological) fall in alkaline phosphatase activity was slightly retarded while at 5000 ppm a distinct rise of activity was observed sometimes. The liver N-demethylase activity was slighly (1000 ppm) or distinctly (5000 ppm) increased at the end of the study. In addition, the high dose group animals also exhibited higher cytochrome P-450 concentrations in the liver. At 5000 ppm there was also a decrease of mean albumin content and a simultaneous increase of beta-globulin fraction in serum proteins.

Histological examination of the adrenals revealed slight to moderate increases in vacuole formation in the zona fasciculata cells in one

Section A6.4.1 — 6.4 Subchronic toxicity (90 days)

Annex Point IIA6.4 — Subchronic oral toxicity test in dogs

		female of the high dose group. The intensity of the finding and the fact that this was an animal in the high dose group points to a treatment-induced effect. However, this is not a manifestation of cytotoxic damage, the alteration is most likely to be a non-specific adaptive reaction to the treatment.	
5.3	**Conclusion**		
5.3.1	LO(A)EL	1000 ppm	*
5.3.2	NO(A)EL	200 ppm	*
5.3.3	Other		
5.3.4	Reliability	[redacted]	
5.3.5	Deficiencies	[redacted]	

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	July 2005
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
	[redacted]
	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

Table A6_3-1. Results of clinical chemistry haematology and urinalysis

Not needed due to results of study

(Use this or similar table, if relevant effects occur and if time sequence is important.Give either symbols for increases or decreases (↑↓) or abbreviations inc., dec. Only if more information is needed, give figures or percentages.)

parameter changed	Unit	Controls			low dose			medium dose			high dose		
weeks after start of treatment													
males													
females													

* $p < 0,05$

Give only those parameters which are changed in at least one dose group compared to control. Usually only statistically significant effects

Depending on number of parameters changed one table each for Haematology, Clinical Chemistry, Urinalysis

Table A6_3-2. Results of repeated dose toxicity study

Parameter	Control		low dose 200 ppm		Medium dose 1000 ppm		high dose 5000 ppm		dose-response +/-	
	m	f	m	f	M	f	m	f	m	f
number of animals examined	4	4	4	4	4	4	4	4		
mortality								■		■
clinical signs										
body weight					■	■	■	■	■	■
food consumption							■	■	■	■
ophthalmology							■	■	■	■
clinical chemistry										
alkaline phosphatase					■	■	■	■	■	■
albumin							■	■	■	■
beta-globulin							■	■	■	■
N-demethylase (liver)					■	■	■	■	■	■
CYP 450 (liver)							■	■	■	■
haematology										
thrombocyte count							■	■	■	■
signs of anisocytosis							■	■	■	■
Spleen:										
organ weight							■	■	■	■
histopathology										
siderosis							■	■	■	■
Liver:										
histopathology										
siderosis							■	■	■	■
Eyes:										
histopathology										
lens degeneration							■	■	■	■
Adrenals:										
histopathology										
vacuole formation								■		■

[1] this female was replaced by another female on the second study day

Section A6.4.2

Annex Point IIA6.4

6.4 Subchronic toxicity (90-day)

Subchronic oral toxicity study in rats

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted], Subchronic toxicological study with rats, [redacted], Report No. [redacted], 1986-10-27	
1.2	**Data protection**	[redacted]	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	[redacted]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes OECD 408 and "Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals", 1982 (US EPA)	
2.2	**GLP**	[redacted]	
2.3	**Deviations**	[redacted]	
		3 MATERIALS AND METHODS	
3.1	**Test material**	HWG 1608	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	technical grade	
3.1.2.1	Description	whitish-yellow powder	
3.1.2.2	Purity	[redacted]	*
3.1.2.3	Stability	Stability of test material was proven by analysis before and three times during the study for all three test concentrations.	
3.2	**Test Animals**		
3.2.1	Species	rat	
3.2.2	Strain	Wistar (BOR:WISW)	
3.2.3	Source	[redacted]	
3.2.4	Sex	males and females (1:1)	
3.2.5	Age/weight at study initiation	age: about 6 weeks weight: 79 g (mean males) and 77 g (mean females)	
3.2.6	Number of animals per group	ten per sex per group	
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	Oral	

Section A6.4.2

Annex Point IIA6.4

6.4 Subchronic toxicity (90-day)

Subchronic oral toxicity study in rats

3.3.1	Duration of treatment	90 days (13 weeks)
3.3.2	Frequency of exposure	daily
3.3.3	Postexposure period	no
3.3.4	**Oral**	
3.3.4.1	Type	in food
3.3.4.2	Concentration	control: 0 ppm
		low dose: 100 ppm (= males: 8.6 mg/kg bw, females: 10.8 mg/kg bw)
		medium dose: 400 ppm (= males: 34.8 mg/kg bw, females: 46.5 mg/kg bw)
		high dose: 1600 ppm (= males: 171.7 mg/kg bw, females: 235.2 mg/kg bw)
		food consumption per day ad libitum
3.3.4.3	Vehicle	no vehicle, test substance mixed with food
3.3.4.4	Controls	plain diet
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	yes (twice daily; once at weekend and holiday times)
3.4.1.2	Mortality	yes (twice daily; once at weekend and holiday times)
3.4.2	Body weight	yes (weekly)
3.4.3	Food consumption	yes (periodical weighting of the food, calculations for uptake per day)
3.4.4	Water consumption	yes (periodical weighting of the water, calculations for uptake per day)
3.4.5	Ophthalmoscopic examination	yes (after four weeks and at the end of study: animals from control group and 1600 ppm dose group)
3.4.6	Haematology	yes
		number of animals and time points: 5 males and 5 females from each group after one month; all surviving animals at the end of the study
		Parameters: erythrocyte count, total and differential leukocyte count, platelet count, haemoglobin, haematocrit, MCV (mean corpuscular volume), MCH (mean corpuscular haemoglobin), MCHC (mean corpuscular heamoglobin concentration), thromboplastin time
3.4.7	Clinical Chemisty	yes
		number of animals and time points: 5 males and 5 females from each group after one month; all surviving animals at the end of the study
		Parameters: AP (alkaline phosphatase), ALT/GPT (alanine aminotransferase), AST/GOT (aspartate aminotransferase), creatinine, urea, glucose, total cholesterol, bilirubin, total protein, triglycerides, ferrum
		in liver: Cytochrome P-450, N-demethylase

Section A6.4.2 **6.4 Subchronic toxicity (90-day)**

Annex Point IIA6.4 Subchronic oral toxicity study in rats

3.4.8	Urinalysis	yes number of animals and time points: 5 males and 5 females from each group after one month; all surviving animals at the end of the study Parameters: volume, bilirubin, specific gravity, pH, protein, glucose, blood, ketone bodies, urobilinogen, urine sediment (microscopical examination)
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	yes organs: liver, kidneys, heart, adrenals, testes, spleen, lungs
3.5.2	Gross and histopathology	yes gross pathology: all dose groups histopathology: high dose group and controls all organs; other dose groups: liver and adrenals organs: heart, lungs, liver, spleen, kidney, brain, pituitary gland, thyroid, adrenals, pancreas, testes, epididymes, salivary gland, oesophagus, stomach, gastro-intestinal tract (duodenum, jejunum, ileum, caecum, colon, rectum), urinary bladder, trachea, eyes, lymph nodes (mesenteric and cervical), thymus, aorta, skeletal muscle, sternum (with bone marrow), skin, prostate, seminal vesicle, measurement of femur diameter (with bone marrow), nervus ischiadicus, ovaries, uterus
3.5.3	Other examinations	
3.5.4	Statistics	The arithmetic group means, standard deviations, upper and lower confidence limits were calculated from individual results of the clinical laboratory data and the animal and organ weight determinations. The test collective's figures were compared to the control collectives with the two sided significance test (U Test, Mann, H.B. and Whitney, D.R., Ann. Math. Stat. 18, 1947, 50) and by F.Wilcoxon's method (Biometrics 1, 1945, 80).
3.6	**Further remarks**	
		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	no effects
4.1.2	Mortality	During the study two males died in the control group (associated with blood sampling). Because one male and one female in the 1600 ppm group died spontaneously, it is concluded that mortality was slightly increased after 1600 ppm).
4.2	**Body weight gain**	The 400 and 1600 ppm females and the 1600 ppm males had significantly lower body weights than the controls
4.3	**Food consumption and compound intake**	Food consumption was about the same in the control and the 100 and 400 ppm dose groups. In the 1600 ppm dose group increased food intake was observed in both sexes.
4.4	**Ophtalmoscopic examination**	The ophthalmological (as well as histopathological examinations) did not detect any indications of substance-induced damage to the eye.

Section A6.4.2

Annex Point IIA6.4

6.4 Subchronic toxicity (90-day)

Subchronic oral toxicity study in rats

4.5	**Blood analysis**	
4.5.1	Haematology	No toxicologically relevant differences of the haematology related data between the treated animals (up to 1600 ppm) and the controls were observed.
4.5.2	Clinical chemistry	No toxicologically relevant differences between the treated animals (up to 1600 ppm) and the controls were observed, except an increase of liver enzyme induction in the 1600 ppm males group at the end of the study.
4.5.3	Urinalysis	The results of the urinalyses did not show toxicologically relevant differences between the dose groups and the controls.
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	In relation to body weight slightly increased liver weights were observed in the females at 1600 ppm.
4.6.2	Gross and histopathology	A significantly reduced diameter of femur was observed in both sexes at 1600 ppm and was regarded secondary to reduced body weight in these dose groups. Histopathology revealed very slight increased siderin accumulation in the red spleen pulp in some females of the 1600 ppm dose group. The results of the gross and histopathology did not reveal any liver damage in male and female rats in the groups up to and including 1600 ppm. The histopathological examination did not detect any indications of effects on the adrenals of the males in the 100 and 400 ppm groups or the females in the 100 ppm group. The increased intra-plasmatic vacuoles in the zona fasciculata of the adrenal cortex observed in females at 400 and 1600 ppm and in males at 1600 ppm were regarded to be induced by treatment.
4.7	**Other**	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The study was performed according to OECD-Guideline 408. Groups of 10 male and female Wistar rats each were administered to 0, 100, 400 and 1600 ppm tebuconazole in the diet during 13 weeks.
5.2	**Results and discussion**	Appearance, general behaviour, food and water consumption and mortality rate were unaffected in the groups up to and including 400 ppm. After 1600 ppm food consumption and mortality were increased. Females' growth in the 400 ppm dose group and the males' and females' growth in the 1600 ppm group was retarded. No substance induced damages to the eyes were found in ophthalmological and histopathological examinations. The haematological examination did not detect any adverse effects on the blood. Histopathology revealed very slightly increased siderin accumulation in the red spleen pulp in some females in the 1600 ppm dose group. The results of the clinical, gross pathological and histopathological examinations did not reveal any liver damage for males and females in the groups up to and including 1600 ppm. At 1600 ppm however

Section A6.4.2 **6.4 Subchronic toxicity (90-day)**

Annex Point IIA6.4 Subchronic oral toxicity study in rats

		indications of induction of microsomal enzyme systems were noted. The results of the urinalyses, the clinical chemical analyses and the pathomorphological examination did not reveal toxicologically significant functional or morphological alterations in the dose range investigated. The histopathological examination did not detect any indications of effects on the adrenals of the males in the 100 and 400 ppm groups or the females in the 100 ppm group. The increased intra-plasmatic vacuoles in the zona fasciculata of the adrenal cortex observed in females from 400 and in males in the 1600 ppm dose group are to be regarded as induced by the treatment.	
5.3	**Conclusion**	Under the described conditions tebuconazole was tolerated without adverse effects in male rats administered doses up to 400 ppm, and in female rats administered 100 ppm.	
5.3.1	LO(A)EL	1600 ppm (males) / 400 ppm (females)	*
5.3.2	NO(A)EL	400 ppm (males) / 100 ppm (females)	*
5.3.3	Other		
5.3.4	Reliability	[redacted]	
5.3.5	Deficiencies	[redacted]	

Evaluation by Competent Authorities

Use separate "evaluation boxes" to provide transparency as to the comments and views submitted

EVALUATION BY RAPPORTEUR MEMBER STATE

Date July 2005

Materials and Methods [redacted]

Results and discussion [redacted]

Conclusion [redacted]

Reliability [redacted]

Acceptability [redacted]

Remarks [redacted]

Table A6_3-1. Results of clinical chemistry haematology and urinalysis

Not needed due to results of study

Table A6_3-2. Results (specify) of repeated dose toxicity study

Parameter	Control		low dose 100 ppm		Medium dose 400 ppm		high dose 1600 ppm		dose-response +/-	
	m	f	m	f	M	f	m	f	m	f
number of animals examined	10	10	10	10	10	10	10	10		
Mortality	■						■	■	▮	▮
body weight						▮	▮	▮	▮	▮
food consumption							▮	▮	▮	▮
clinical chemistry N-demethylase (liver) Cytochrom P-450 (liver)							 ▮ ▮		 ▮ ▮	
Liver organ weight								▮		▮
Spleen										
histopathology siderin content ↑								 ■		 ■
Adrenals										
histopathology vacuoles in cells of zona fasciculata						 ▮	 ▮	 ▮	 ▮	 ▮

Section 6.4 **Annex Point IIA 6.4**	**Subchronic dermal and inhalation toxicity** 6.4.2 & 6.4.3 Subchronic dermal and inhalation toxicity	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [...] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	According to BPD, Annex IIA, and the TNsG on data requirements subchronic toxicity studies are only required for oral route. For tebuconazole for both the dermal and inhalation route repeated dose studies are submitted which cover 3-5 weeks. These studies are assumed to deliver sufficient information for the related exposure routes. In addition dermal studies were submitted with respect to reproductive toxicity. Therefore is justified to submit no data on subchronic toxicity with regard to the dermal and inhalation route.	
Undertaking of intended data submission [ ]	–	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	July 2005	
Evaluation of applicant's justification	[redacted]	
Conclusion	[redacted]	
Remarks		

Section A6.5(01) 6.5 Chronic toxicity

Annex Point IIA6.5 Chronic oral toxicity to the dog I

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1 REFERENCE

1.1	**Reference**	[redacted], HWG 1608 – Study of chronic toxicity to dogs after oral administration (twelve month feeding study), [redacted], Report No. [redacted], 1987-11-11
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes OECD-Guideline 452
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]

3 MATERIALS AND METHODS

3.1	**Test material**	HWG 1608
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	technical grade
3.1.2.1	Description	colourless to slightly yellowish crystals
3.1.2.2	Purity	[redacted]
3.1.2.3	Stability	Regular analytical inspections throughout the study ensured that the food-substance mixes actually contained the specified concentrations of HWG 1608. Before start of study it was established that the test substance was stable for at least fourteen days in the dry feed, and at least 24 hours in the wet feed, and was homogeneously distributed in the mixture.
3.2	**Test Animals**	
3.2.1	Species	dog
3.2.2	Strain	beagle (Bor:Beag)
3.2.3	Source	[redacted]
3.2.4	Sex	males and females (1:1)
3.2.5	Age/weight at study initiation	At the time of randomisation (one week before study initiation): age: 24 to 28 weeks weight: 7.1 to 10.5 kg
3.2.6	Number of animals per group	four per sex per group

Section A6.5(01) 6.5 Chronic toxicity

Annex Point IIA6.5 Chronic oral toxicity to the dog I

3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of treatment	12 months
3.3.2	Frequency of exposure	daily
3.3.3	Postexposure period	no
3.3.3.1	Type	in food
3.3.3.2	Concentration	control: 0 ppm; low dose: 40 ppm; medium dose: 200 ppm; high dose: 1000 ppm (from 1st to 39th week) and 2000 ppm (from 40th to 52nd week) All the animals were given the same quantity of food in the morning. The food not consumed until the next feeding time was weighed, so that the amount of food consumed, and consequently the amount of test substance administered, were individually determined. uptake of test substance: 40 ppm: 5.6 g/animal total and 108.4 mg/animal per week 200 ppm: 28.1 g/animal total and 541 mg/animal per week 1000 ppm/2000 ppm: 175.8 g/animal total and 3380.2 mg/animal per week
3.3.3.3	Vehicle	no vehicle, test substance mixed in food
3.3.3.4	Concentration in vehicle	
3.3.3.5	Total volume applied	
3.3.3.6	Controls	plain diet
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	yes (several times daily, in the cages and during exercising)
3.4.1.2	Mortality	yes (several times daily, in the cages and during exercising)
3.4.2	Body weight	yes (weekly)
3.4.3	Food consumption	yes (daily)
3.4.4	Water consumption	no (only observation, no measurement)
3.4.5	Ophthalmoscopic examination	yes (2 weeks before treatment and at week 13, 26, 32, 39, 46 and 52 during study)
3.4.6	Haematology	yes number of animals: all animals time points: 2 weeks before treatment and at week 6, 13, 26, 39, 46 and 52 during study (at week 46 only controls and high dose group were examined) Parameters: haematocrit, haemoglobin concentration, erythrocyte count,

		total and differential leukocyte count, MCV (mean corpuscular volume), MCH (mean corpuscular haemoglobin), MCHC (mean corpuscular heamoglobin concentration), platelet count, reticulocyte count, thromboplastin time, blood sedimentation rate
3.4.7	Clinical Chemisty	yes number of animals: all animals time points: 2 weeks before treatment and at week 6, 13, 26, 39, 46 and 52 during study (at week 46 only controls and high dose group were examined) Parameters: sodium, potassium, calcium, chloride, glucose, total cholesterol, urea, total bilirubin, creatinine, total protein, serum protein electrophoresis, ALT/GPT (alanine aminotransferase), AST/GOT (aspartate aminotransferase), AP (alkaline phosphatase), GLDH (glutamate dehydrogenase) in liver: Cytochrome P-450, N-demethylase, triglycerides
3.4.8	Urinalysis	yes number of animals: all animals time points: 2 weeks before treatment and at week 6, 13, 26, 39, 46 and 52 during study (at week 46 only controls and high dose group were examined) Parameters: volume, specific gravity, pH, protein, glucose, blood appearance, bilirubin, ketone bodies, urine sediment (microscopical examination)
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	yes organs: brain, heart, testicles, liver, lung, spleen, adrenals, kidneys, ovaries, pancreas, prostate and thyroid (including parathyroid)
3.5.2	Gross and histopathology	yes all dose groups organs: bone, bone marrow (os femoris, sternum), adrenals (2x), thyroid (incl. parathyroid, 2x), nervus ischiadicus, nervi optici (2x), pituitary, stomach (2 locations), oesophagus, aorta, urinary bladder, gallbladder, intestines (5 locations), skin, mamma, skeletal musculature, salivary gland (parotis), pancreas, lymph node (mesenterium), thymus, tonsils (2x), brain (cerebrum, cerebellum, brain stem), testicles (2x), epididymes (2x), prostate, ovaries (2x), uterus (3x), liver (2x), lung, spleen, heart (2x), eyes (2x), kidney, altered organs of organ parts
3.5.3	Other examinations	body temperature, pulse rate and reflex tests number of animals: all animals time points: 2 weeks before treatment and at week 6, 13, 26, 39, and 52 during study
3.5.4	Statistics	Due to the low number of animals per group (4 animals/sex), the statistical procedure was descriptive. The arithmetic means and standard deviations were calculated, and maximums and minimums are given.

Section A6.5(01) 6.5 Chronic toxicity

Annex Point IIA6.5 Chronic oral toxicity to the dog I

3.6	**Further remarks**	
		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	The animals in all groups did not differ from each other in appearance and behaviour. Common findings such as vomiting, pasty faeces and diarrhoea were found in controls and all dose groups with no dose correlation.
4.1.2	Mortality	no mortalities at any dose
4.2	**Body weight gain**	no effects
4.3	**Food consumption and compound intake**	no effects
4.4	**Ophthalmoscopic examination**	Lens alterations (opacity) were noted in two dogs in the medium dose group at the examinations in week 26 or 32, respectively. From then on, these alterations were apparent at the same intensity at all the following examination times. In case of one dog of the high dose group, a fine stellar lens opacity was seen for the first time in the 26th week, and then only at the following examination time in the 32nd week. A connection of these findings with treatment cannot be ruled out. Clinical observation did not reveal impairment in any animal's vision.
4.5	**Blood analysis**	
4.5.1	Haematology	no effects
4.5.2	Clinical chemistry	The mean activity of the AP fell between start and end of study in all groups, however this physiological (age-induced) fall in activity was retarded in the high dose animals. This was at most a slight effect, since even after the raise in dose to 2000 ppm a clear rise in AP activity above the normal range was not noted. Nevertheless it must be assumed that the test substance has a slight effect on AP. The N-demethylase activity and the triglyceride concentration were on average slightly higher in the animals in the high dose group than in the animals in the other groups at the end of study which is attributed to the treatment.
4.5.3	Urinalysis	no effects
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	no effects
4.6.2	Gross and histopathology	There was a dose-related incidence of livers with increased lobulation noted at autopsy (2 of 8 dogs in the medium dose group, 5 of 8 dogs in the high dose group). According to the histopathological examination, these alterations were however not the result of morphological apparent liver lesions. Intra-cytoplasmatic vacuoles in cells of zona fasciculata of adrenals were observed in 2 females of the medium dose group and 2 females of the high dose group. These findings were regarded as induced by test

Section A6.5(01) 6.5 Chronic toxicity

Annex Point IIA6.5 Chronic oral toxicity to the dog I

		substance, since no similar alterations were found in controls and low dose group. The slightly increased siderin content in the spleen is regarded as a marginal test substance-related effect, and was detected in 5 of 8 dogs in the high dose group.	
4.7	**Other**	The reflex tests did not detect any pathological findings at any examination time. The measurements of body temperature and pulse rates did not show any notable variations between the animals in any of the groups.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The study was done according to OECD-Guideline 452, though not stated in the study report. There are no relevant deviations from current guidelines for chronic toxicity studies. Beagles were treated with three dose levels of test substance by oral feed. During the study, the high dose was increased from 1000 ppm to 2000 ppm in order to test tolerability of higher substance concentrations and to produce a clear toxic effect.	
5.2	**Results and discussion**	At ophthalmoscopic examinations, two dogs of the medium and one dog of the high dose group exhibited alterations in the lenses (opacity) during study. A connection with treatment may not be ruled out. The clinical chemical examinations provided indications of a slight effect of the test substance on the liver at 1000/2000 ppm (reduction of age-induced fall of AP activity; slight rise of N-demethylase activity; tendency towards higher triglyceride contents). The higher triglyceride concentrations could be responsible for the more marked lobulation of the liver, which was particularly apparent in the animals in the high dose group. As there were no signs of degenerative alterations and the liver weights also were normal, the effects on the liver caused by the test substance are assessed as slight. The findings of the haematological examination did not reveal any damage to the red blood cells. Nevertheless the histopathological examination at the end of study detected a slightly increased siderin level in the spleen in five of eight animals at 1000/2000 ppm. The incidence of this finding may point to an increased rate of breakdown of the red blood cells, which, however, was so marginal that it was not apparent from the haematological data. The histopathological examination revealed alterations in the adrenals (increased levels of intra-cytoplasmatic vacuoles in cells of the zona fasciculata) in two females at 200 ppm and two females at 1000/2000 ppm, which are likely to be the result of the treatment with the test substance.	
5.3	**Conclusion**		
5.3.1	LO(A)EL	200 ppm	*
5.3.2	NO(A)EL	40 ppm	*
5.3.3	Other		
5.3.4	Reliability	[redacted]	
5.3.5	Deficiencies	[redacted]	

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	July 2005
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

Table A6_3-1. Results of clinical chemistry haematology and urinalysis

Not needed due to results of study

Table A6_3-2. Results *(specify)* of repeated dose toxicity study

Parameter	Control		low dose 40 ppm		medium dose 200 ppm		high dose 1000/2000 ppm		dose-response +/-	
	m	f	m	f	m	f	m	f	m	f
number of animals examined	4	4	4	4	4	4	4	4		
ophthalmology										
lens opacity						■		■		■
clinical chemistry										
ALP activity							■	■	■	■
							■	■		
N-demethylase (liver)							■	■	■	■
triglycerides (liver)							■	■	■	■
liver										
gross pathology										
distinct lobulation						■	■	■	■	■
adrenals										
histopathology										
vacuoles in cells of										
zona fasciuculata						■		■		■
spleen										
histopathology										
siderin content ↑	■	■		■	■	■	■	■	■	■

Section A6.5(02) 6.5 Chronic toxicity

Annex Point IIA6.5 Chronic oral toxicity to the dog II

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted]., Safety evaluation of HWG 1608: chronic (1 year) feeding study in dogs, [redacted]., Report No. [redacted], 1989-06-28	
		[redacted], Supplemental submission to [redacted]: Safety evaluation of HWG 1608: chronic (1 year) feeding study in dogs, [redacted]., Report No. [redacted], 1993-11-4	
1.2	**Data protection**	[redacted]	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	[redacted]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes FIFRA § 83-1	
2.2	**GLP**	[redacted]	
2.3	**Deviations**	[redacted]	*
		3 MATERIALS AND METHODS	
3.1	**Test material**	HWG 1608	
3.1.1	Lot/Batch number	16013/86	
3.1.2	Specification	technical grade	
3.1.2.1	Description	colourless to yellow crystalline solid	
3.1.2.2	Purity	[redacted]	
3.1.2.3	Stability	HWG 1608 was found to be stable in dog feed while stored in closed containers at ambient temperature for eight weeks. The test substance was also stable during the 24 hour feeding period in the wet food.	
3.2	**Test Animals**		
3.2.1	Species	dog	
3.2.2	Strain	beagle (purebred)	
3.2.3	Source	[redacted]	
3.2.4	Sex	males and females (1:1)	
3.2.5	Age/weight at study initiation	age: approximately 6 months weight: approximately 6-9 kg	
3.2.6	Number of animals per group	four per sex per group	
3.2.7	Control animals	Yes	

Section A6.5(02) 6.5 Chronic toxicity

Annex Point IIA6.5 Chronic oral toxicity to the dog II

3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of treatment	12 months
3.3.2	Frequency of exposure	daily
3.3.3	Postexposure period	no
3.3.4	**Oral**	
3.3.4.1	Type	in food
3.3.4.2	Concentration	control: 0 ppm
		low dose: 100 ppm (= males: 2.69 mg/kg bw; females: 2.94 mg/kg bw)
		high dose: 150 ppm (= males: 4.39 mg/kg bw; females: 4.45 mg/kg bw)
3.3.4.3	Vehicle	no vehicle, test substance mixed in food
3.3.4.4	Concentration in vehicle	
3.3.4.5	Total volume applied	
3.3.4.6	Controls	plain diet
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	yes (daily prior to feeding, approx. one hour after feeding, and additionally as deemed necessary)
3.4.1.2	Mortality	yes (daily prior to feeding, approx. one hour after feeding, and additionally as deemed necessary)
3.4.2	Body weight	yes (twice during pretreatment time, once weekly during the first 6 months of the study, every two weeks during the last 6 months of the study)
3.4.3	Food consumption	yes (daily)
3.4.4	Water consumption	no
3.4.5	Ophthalmoscopic examination	yes (prior to study, at the end of 3 and 6 months, at the end of study)
3.4.6	Haematology	yes
		number of animals: all animals
		time points: three times during pretreatment time, at the end of the 3rd and the 6th month, at the end of study
		Parameters: haematocrit, haemoglobin concentration, erythrocyte count, total and differential leukocyte count, platelet count, reticulocyte count, prothrombin time, activated partial thromboplastin time, MCV (mean corpuscular volume), MCH (mean corpuscular haemoglobin), MCHC (mean corpuscular haemoglobin concentration), erythrocyte appearance (morphology)

Section A6.5(02) 6.5 Chronic toxicity

Annex Point IIA6.5 Chronic oral toxicity to the dog II

3.4.7	Clinical Chemistry	yes number of animals: all animals time points: three times during pretreatment time, at the end of the 3rd and the 6th month, at the end of study Parameters: sodium, potassium, glucose, total cholesterol, urea nitrogen, total bilirubin, creatinine, total protein, albumin, ALT/GOT (alanine aminotransferase), AST/GPT (aspartate aminotransferase), AP (alkaline phosphatase), GGT (gamma glutamyl transferase), globulin, A/G ratio, chloride, calcium, inorganic phosphates, triglycerides in liver: cytochrom P-450, N-demethylase, O-demethylase, triglycerides
3.4.8	Urinalysis	yes number of animals: all animals time points: three times during pretreatment time, at the end of the 3rd and the 6th month, at the end of study Parameters: volume, specific gravity, pH, protein, glucose, blood, ketones, urobilinogen, bilirubin, sodium, potassium, chloride, microscopic solids
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	yes organs: liver, kidneys, adrenals, testes, ovaries, thymus, spleen, brain, heart, pituitary, thyroid with parathyroid
3.5.2	Gross and histopathology	yes all dose groups organs: brain (cerebrum, cerebellum, medulla), spinal cord (cervical, thoracic, lumbar), pituitary, thyroid with parathyroid, thymus, salivary glands (submaxillary), stomach (fundic and pyloric), liver (4x), pancreas, kidneys, adrenals, spleen, heart (septum and both ventricular walls), trachea, lung (left posterior), aorta (thoracic), uterus, mammary gland with skin, prostate, urinary bladder, gall bladder, lymph nodes (cervical and mesenteric), nerve (sciatic), bone and marrow, eyes with optic nerve, caecum, colon, ileum, jejunum, duodenum, rectum, oesophagus, epididymis, cervix uteri, ovaries, vagina, testes, skeletal muscle (biceps femoris)
3.5.3	Other examinations	
3.5.4	Statistics	body weight, food consumption and organ weight: Dunnett´s test (C.W. Dunnett: J. Am. Stat. Assoc. 50, 1096-1121, 1955 and Biometrics 20, 482-491, 1964) Clinical pathology data: analyzed using a computerized system described by Bare et al. (Drug Inf. J., July/September, 141-152, 1978), based on the methods of Harris (Clin. Chem. 22, 1343-1350, 1976)
3.6	**Further remarks**	

Section A6.5(02) 6.5 Chronic toxicity

Annex Point IIA6.5 Chronic oral toxicity to the dog II

4 RESULTS AND DISCUSSION

4.1	**Observations**	
4.1.1	Clinical signs	no effects Sporadic incidences of soft stools/diarrhoea and rare incidences of emesis were considered spontaneous.
4.1.2	Mortality	no mortalities in any treated animals One control female stopped eating during the 8th week of study and showed a body temperature of 40.6°C and elevated white blood cell count. The animal was isolated in a separate room and sacrificed during the 10th week of study. Another female from the same shipment of animals replaced this female on day 70.
4.2	**Body weight gain**	no effects
4.3	**Food consumption and compound intake**	no effects
4.4	**Ophthalmoscopic examination**	no effects
4.5	**Blood analysis**	
4.5.1	Haematology	no effects (none of the variations occurred in a dose-related fashion and generally represented a low-magnitude change, so are not considered biologically significant)
4.5.2	Clinical chemistry	no effects (none of the variations occurred in a dose-related fashion and generally represented a low-magnitude change, so are not considered biologically significant)
4.5.3	Urinalysis	no effects (none of the variations occurred in a dose-related fashion and generally represented a low-magnitude change, so are not considered biologically significant)
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	no effects
4.6.2	Gross and histopathology	no effects observed at gross pathology In all high dose animals, a hypertrophy of adrenal zona fasciculata cells was observed. This alteration was not accompanied by a change in adrenal weight and appeared to be due to an increase in the size and/or number of lipid vacuoles.
4.7	**Other**	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The study was done according to FIFRA § 83-1-Guideline. This study was designed as a supplementary chronic feeding study with HWG 1608 in dogs to establish a higher no-effect level as observed in an earlier chronic study (see [redacted], 1987). Therefore, only two dose levels were used (100 and 150 ppm).

Section A6.5(02) 6.5 Chronic toxicity

Annex Point IIA6.5 Chronic oral toxicity to the dog II

5.2	**Results and discussion**	The only test substance-related change observed in this study was a subtle hypertrophy of adrenal zona fasciculata cells in all animals of the 150 ppm group compared to a similar finding in only 1 control animal. The slight enlargement was not accompanied by a change in adrenal weight and appeared to be due to an increase in the size and/or number of lipid vacuoles.	
5.3	**Conclusion**		
5.3.1	LO(A)EL	150 ppm	*
5.3.2	NO(A)EL	100 ppm	*
5.3.3	Other		
5.3.4	Reliability	[redacted]	
5.3.5	Deficiencies	[redacted]	

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	July 2005
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

Table A6_3-1. Results of clinical chemistry haematology and urinalysis

Not needed due to results of study

Table A6_3-2. Results *(specify)* of repeated dose toxicity study

Parameter	Control		low dose 100 ppm		high dose 150 ppm		dose-response +/-	
	m	f	m	f	m	f	m	f
number of animals examined	4	4	4	4	4	4		
mortality		[redacted]						
Adrenals								
microscopic pathology								
hypertrophy of cells of zona fasciculata		[redacted]			[redacted]	[redacted]	[redacted]	[redacted]

[1] female was replaced by another female on day 70

Section A6.5.1/6.7.1(01)

Annex Point IIA6.5/6.7

6.5/6.7 Chronic toxicity/carcinogenicity study

Chronic toxicity and carcinogenicity study in mice – Part I

Official use only

1 REFERENCE

1.1 Reference

[redacted], 1988, HWG 1608 – Study for cancerogenicity in NMRI mice (Administration in diet for up to twenty-one months[redacted], Report No. [redacted], 1988-01-25 (unpublished),

[redacted], 1991, HWG 1608 – Toxic dose-range carcinogenicity study in NMRI mice (Supplement to study [redacted] (= Report no. [redacted]) with administration in diet over a 21-month period), [redacted], Report No. [redacted], 1991-12-18 (unpublished),

[redacted], 1992, HWG 1608 – Oncogenicity study in NMRI-mice in a toxic dose range (Additional to study no. [redacted] (= Report no. [redacted]) with application in diet feed over a 21 months), Addendum: the frequency of histiocytic sarcomas in NMRI-mice – a compilation of historical data, [redacted], Report No. [redacted], 1992-06-30 (unpublished)

1.2 Data protection

[redacted]

1.2.1 Data owner

[redacted]

1.2.2 Companies with letter of access

[redacted]

1.2.3 Criteria for data protection

[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study

Yes

The conduct of the study conformed to the recommendations laid down in the OECD-Guideline 453

2.2 GLP

[redacted]

2.3 Deviations

[redacted]

Section A6.5.1/6.7.1(01)

Annex Point IIA6.5/6.7

6.5/6.7 Chronic toxicity/carcinogenicity study

Chronic toxicity and carcinogenicity study in mice – Part I

3 MATERIALS AND METHODS

3.1	**Test material**	As given in section 2 of dossier.
3.1.1	Lot/Batch number	Batch no.: mixed batch; [redacted] Single samples: [redacted]
3.1.2	Specification	As given in section 2 of dossier.
3.1.2.1	Description	Colourless crystals
3.1.2.2	Purity	Approx [redacted] active substance
3.1.2.3	Stability	During the study period the test compound content in the administered formulations was checked at regular intervals (approx. three month). To ensure homogeneity and stability of the test substance in the formulation, sample mixes were analytically examined before start of test. Mixes were accordingly stable and showed homogeneous distribution in the concentration range used and over period of use.
3.2	**Test Animals**	
3.2.1	Species	Mouse
3.2.2	Strain	Bor:NMRI (SPF-Han)
3.2.3	Source	[redacted]
3.2.4	Sex	Males and females
3.2.5	Age/weight at study initiation	Age: 5-6 weeks Weight: males mean weight: 29 g (24 g – 34 g); females mean weight: 24 g (18 g – 31 g)
3.2.6	Number of animals per group	60/sex/group
3.2.6.1	at interim sacrifice	10 animals/group/sex
3.2.6.2	at terminal sacrifice	50 animals/group/sex
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of treatment	Mice 21 months
3.3.2	Interim sacrifice(s)	After 12 months
3.3.3	Final sacrifice	After 21 months
3.3.4	Frequency of exposure	Daily
3.3.5	Post-exposure period	None.
3.3.6	Type	In food

Section A6.5.1/6.7.1(01)

Annex Point IIA6.5/6.7

6.5/6.7 Chronic toxicity/carcinogenicity study

Chronic toxicity and carcinogenicity study in mice – Part I

3.3.7	Concentration	Food: 0, 20, 60 or 180 ppm (= 0, 5.9, 18.2 or 53.1 mg/kg bw/day for males and 0, 9.0, 26.1 or 80.5 mg/kg bw/day for females) Food consumption per day ad libitum.	
3.3.8	Vehicle	Wessalon (highly dispersed silicates), was added to the powdered food, to improve homogeneity and stability at a ratio of 1:1 (test compound: wessalon)	
3.3.9	Concentration in vehicle	—	
3.3.10	Total volume applied	—	
3.3.11	Controls	Plain diet.	
3.4	**Examinations**		
3.4.1	Body weight	Yes, before start of administration, then weekly up to and including week 12, and at two-weekly intervals from week 15 until end of study (before necropsy).	
3.4.2	Food consumption	Yes, at study initiation, then weekly up to and including week 13, and at two-weekly intervals from week 15 until end of study.	
3.4.3	Water consumption	Yes, at study initiation, then weekly up to and including week 13, and at two-weekly intervals from week 15 until end of study.	
3.4.4	Clinical signs	Yes, twice daily (once at weekend and public holidays). Detailed individual inspections: once a week.	
3.4.5	Macroscopic investigations	Palpable masses	
3.4.6	Ophthalmoscopic examination	—	
3.4.7	Haematology	Yes	
		Number of animals:	10 animals/sex/group
		Time points:	After 12 and 21 months of treatment
		Parameters:	Haematocrit, haemoglobin concentration, erythrocyte count, erythrocyte morphology, total and differential leukocyte count, reticulocyte count, platelet count, mean corpuscular haemoglobin (MCH), mean corpuscular cell volume (MCV), mean corpuscular haemoglobin concentration (MCHC)
		Other:	—

Section A6.5.1/6.7.1(01)

Annex Point IIA6.5/6.7

6.5/6.7 Chronic toxicity/carcinogenicity study

Chronic toxicity and carcinogenicity study in mice – Part I

3.4.8	Clinical Chemistry		Yes
		Number of animals:	10 animals/sex/group
		Time points:	After 12 and 21 months of treatment
		Parameters:	total cholesterol, urea, total bilirubin, creatinine, total protein, alanine aminotransferase, aspartate amino-transferase, alkaline phosphatase
			Other: —
3.4.9	Urinalysis	No	
		Number of animals:	—
		Time points:	
		Parameters:	
			Other: —
3.4.10	Pathology	Yes	
3.4.10.1	Organ Weights	Yes	
		from:	10 animals at interim sacrifice, 50 animals at terminal sacrifice
		Organs:	Liver, kidneys, adrenals, testes, spleen, brain, heart, lung
			Other: —
3.4.11	Histopathology	Yes	
		from:	All animals of all dose groups
		from:	at interim sacrifice
			at terminal sacrifice
		Organs:	Aorta, brain, spinal cord, pituitary, thyroid, thymus, gall bladder, oesophagus, salivary glands, stomach, small and large intestines, liver, pancreas, kidneys, adrenals, spleen, heart, lungs, gonads, peri-anal glands, epididymides, seminal vesicle, uterus, oviduct, vagina, mammary gland, prostate, urinary bladder, lymph node (mandibular and mesenteric), peripheral nerve, femur, sternum, bone marrow in femur and sternum, skin, eyes, musculature, Harder's glands, trachea, tongue
			Other: all tissues exhibiting alterations
3.4.12	Other examinations	None.	

Section A6.5.1/6.7.1(01)

Annex Point IIA6.5/6.7

6.5/6.7 Chronic toxicity/carcinogenicity study

Chronic toxicity and carcinogenicity study in mice – Part I

3.5	**Statistics**	Body weight, medical laboratory tests, food consumption, organ weight: Arithmetic group means, standard deviation, and for the organ weights and part of the medical laboratory findings the upper and lower confidence limits on the confidence level of 1 - α = 95% and 1 - α = 99%. The test groups were compared with the control groups by using the "U test" of H.B. Mann and D. R. Whitney, Ann. Math. Stat. 18, 50, 1947 or F. Wilcoxon, Biometrics 1, 80, 1945 at significance levels of α = 5 % and α = 1 %. Incidence data: mortality, clinical signs, etc.: Fisher's exact test (statistical methods for research workers, Stuchart Verlag, New York, 1946) at significance levels of α = 5 % and α = 1 % (two-tailed).
3.6	**Further remarks**	—
		4 RESULTS AND DISCUSSION
4.1	**Body weight**	No effects.
4.2	**Food consumption**	No effects.
4.3	**Water consumption**	No effects.
4.4	**Clinical signs**	No effects.
4.5	**Macroscopic investigations**	No effects.
4.6	**Ophthalmoscopic examination**	—
4.7	**Haematology**	No effects up to and including 60 ppm. At 180 ppm decreased erythrocytes, haemoglobin and haematocrit concentration in females of the satellite group. These findings no longer existed after 90 weeks. The male mice showed significantly lower erythrocyte counts at the end of study.
4.8	**Clinical Chemistry**	The total bilirubin concentration was statistically significantly increased in dose concentration in the 53rd week for the females in the 180 ppm group, and in the 92nd week, females also, from 20 ppm. In addition males and females in the 180 ppm satellite group exhibited statistically significant and distinctly lower cholesterol concentrations in the plasma. At the end of study the males figures' at 180 ppm were still abnormally low, the females' however not.
4.9	**Urinalysis**	—
4.10	**Pathology**	No effects.
4.11	**Organ Weights**	The male female mice absolute and relative liver weights in the 180ppm group were increased after 12 and 21months. At final autopsy after 24 month these effects were no longer present. However the variations were only statistically significant for the males' relative weights at the end of study. The females' means in this dose group were greatly affected by three extreme figures at the end of study, while the other animals' were largely in the range for control females.

Section A6.5.1/6.7.1(01)

Annex Point IIA6.5/6.7

6.5/6.7 Chronic toxicity/carcinogenicity study

Chronic toxicity and carcinogenicity study in mice – Part I

4.12	**Histopathology**	Non-neoplastic alterations: Male and female mice in the 180 ppm dose group and females in the 60 ppm dose group exhibited a largely minimal periportal vacuolisation in the liver after 12 month of treatment. The vacuoles were shown to be lipid-containing. After 2 years, the males in the 180 ppm dose group exhibited an increased incidence of periportal vacuolisation of the liver, this effect only being marginal in the case of the females. In addition the number of animals with centrilobular fine vacuolisation in the case of the males in the 60 and 180 ppm dose groups was higher than in the control group. Neoplastic changes: No treatment-related effects in animals of the satellite and the main groups. Dose-related incidences of benign and/or malignant blastomas in all the locations were not present. There was no increased frequency of unusual tumours in the treatment groups.
4.13	**Other examinations**	—
4.14	**Time to tumours**	Not applicable.
4.15	**Other**	—

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The objective of the study was to establish toxic effects, including a dose-effect relationship, and a no-effect dose for a mammalian species under conditions of exposure relevant to practice. In particular potential effects requiring a long latency period and/or accumulation were to be detected under the test conditions. To establish the potential presence of chronic toxic effects, animals were interim-autopsied after one year and additional haematological and clinical examinations were carried out. The conduct of the study conformed to the recommendations laid down in the OECD-Guideline 453. The tebuconazole doses were based on the results of two previous feeding studies lasting four and eight weeks, respectively in NMRI-mice of the same strain ([redacted], Report no. [redacted], 1986-07-21, unpublished[redacted], Report no. [redacted], 1986-12-04, unpublished).

Section A6.5.1/6.7.1(01)

Annex Point IIA6.5/6.7

6.5/6.7 Chronic toxicity/carcinogenicity study

Chronic toxicity and carcinogenicity study in mice – Part I

5.2 Results and discussion

The appearance, general behaviour, food and water intakes, growth and mortality were unaffected in the groups up to and including 180 ppm.

The haematological examination did not provide any indication of damage to the blood up to and including 60 ppm.
Temporarily reduced erythrocyte counts (males and females) and haemoglobin and haematocrit values (females) after 180 ppm may be a result of the treatment with test substance.

Histopathology revealed an increased incidence of periportal vacuolisation of the liver, within the satellite groups in the case of the 180 ppm dose group males and the 60 ppm and 180 ppm group females, and also in the main groups in the males and females treated with 180 ppm. Moreover the number of animals with centrilobular fine vacuolisation was higher than in the control group for the males in the main groups after 60 and 180 ppm. Further examination showed that the vacuoles were filled with lipids. Accordingly the animals in these groups were subject to treatment-induced fatty degeneration of the liver. The significantly lower cholesterol concentrations in the plasma, which were noted in males and females in the 180 ppm dose group at interim sacrifice, possibly also the significantly increased concentrations of total bilirubin in the plasma noted in females from 20 ppm onward at study termination and from 180 ppm (both examination times) may possibly represent clinical chemical correlation of this effect on liver. The effects for the bilirubin effect are however so slight and the normal range of variation, that toxicological relevance should not to be assumed after 20 ppm, and after 60 ppm and 180 ppm relevance is at least questionable. The tendency towards higher liver weights, which was also statistically significant after 180 ppm in the main groups, might likewise be assessed as a result of the test substance's effect on this organ.

Indications of a carcinogenic action of tebuconazole were not provide by the spectrum of the various types of neoplastic alterations and their distribution over the organs and tissues concerned, or by the distribution of tumour hosts throughout the study groups.

5.3 Conclusion

Under the condition described above, tebuconazole was tolerated without adverse effects by the male and female rats at doses up to and including 20 ppm. *

NOEL for carcinogenicity: 180 ppm for males and females (= 53.1 mg/kg bw/day for males and 80.5 mg/kg bw/day for females)

NOEL systemic: 20 ppm for males and females (= 5.9 mg/kg bw/day for males and 9.0 mg/kg bw/day for females)

LOEL systemic: 60 ppm for males and females (= 18.2 mg/kg bw/day for males and 26.1 mg/kg bw/day for females)

5.3.1 Reliability [redacted]

5.3.2 Deficiencies [redacted]

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	July 2005
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

Table A6.5/6.7-1. Table for Clinical Chemistry and Haematology

Clinical Chemistry	Sex	Unit	Control	Low dose 20 ppm	Medium dose 60 ppm	High dose 180 ppm
Bilirubin		µmol/l	**53 weeks after start of treatment**			
	male		[redacted]	[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]	[redacted]
			92 weeks after start of treatment			
	male		[redacted]	[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]	[redacted]
Cholesterol		mmol/l	**52 weeks after start of treatment**			
	male		[redacted]	[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]	[redacted]
			93 weeks after start of treatment			
	male		[redacted]	[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]	[redacted]

↓ decrease

↑ increase

— not different from control

* significantly different from controls, $p \leq 0.05$

** significantly different from controls, $p \leq 0.01$

Table A6.5/6.7-1. Table for Clinical Chemistry and Haematology, continued

Haematology	Sex	Unit	Control	Low dose 20 ppm	Medium dose 60 ppm	High dose 180 ppm
Erythrocyte count		10^{12}/l	**51 weeks after start of treatment**			
	male		[redacted]	[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]	[redacted]
			90 weeks after start of treatment			
	male		[redacted]	[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]	[redacted]
Haemoglobin		g/l	**51 weeks after start of treatment**			
	male		[redacted]	[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]	[redacted]
			90 weeks after start of treatment			
	male		[redacted]	[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]	[redacted]
Haematocrit		l/l	**51 weeks after start of treatment**			
	male		[redacted]	[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]	[redacted]
			90 weeks after start of treatment			
	male		[redacted]	[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]	[redacted]

↓ decrease

↑ increase

— not different from control

* significantly different from controls, $p \leq 0.05$

** significantly different from controls, $p \leq 0.01$

Table A6_5/7-2.B Results of the combined chronic toxicity/carcinogenicity study in mice (main groups)

Parameter	Control data				Low dose 20 ppm		Medium dose 60 ppm		High dose 180 ppm		Dose-response +/-	
	historical		study									
	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m	f
Number of animals examined			50	50	50	50	50	50	50	50		
Mortality			■	■	■	■	■	■	■	■	▌	▌
Clinical signs			■	■	■	■	■	■	■	■	▌	▌
Body weight gain			■	■	■	■	■	■	■	■	▌	▌
Food consumption			■	■	■	■	■	■	■	■	▌	▌
Water consumption			■	■	■	■	■	■	■	■	▌	▌
Clinical chemistry			Effects described in table A 6_5/7-2.A above								▌	▌
Haematology											▌	▌
Number of animals examined			■	■	■	■	■	■	■	■		
Overall tumour incidence:												
No. of animals with neoplasms			■	■	■	■	■	■	■	■	▌	▌
No. of animals with benign neoplasms			■	▌	■	▌	■	■	■	■	▌	▌
No. of animals with malignant neoplasms			▌	■	■	■	▌	■	■	■	▌	▌
No. of animals with multiple neoplasms			▌	▌	▌	■	▌	▌	▌	▌	▌	▌

[a] number of animals affected/total number of animals

↑ increase

↓ decrease

— not different from control

* significantly different from controls, $p \leq 0.05$

** significantly different from controls, $p < 0.01$

Table A6_5/7-2.B Results of the combined chronic toxicity/carcinogenicity study in rats (main groups), continued

Parameter	Control data				Low dose 20 ppm		Medium dose 60 ppm		High dose 180 ppm		Dose-response +/-	
	historical		study									
	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m	f
Organ: liver												
Non-neoplastic changes:												
Incidence of periportal vacuolisation			■	■	■	■	■	■	▮	■	▮	■
Incidence of centrolobular vacuolisation			■	■	■	■	▮	■	▮	■	▮	▮

[a] number of animals affected/total number of animals

↑ increase

↓ decrease

— not different from control

* significantly different from controls, $p \leq 0.05$

** significantly different from controls, $p \leq 0.01$

Section A6.5.1/6.7.1(02)

Annex Point IIA6.5/6.7

6.5/6.7 Chronic toxicity/carcinogenicity study

Chronic toxicity and carcinogenicity study in mice- Part II

Official use only

1 REFERENCE

1.1 Reference

[redacted], 1988, HWG 1608 – Study for cancerogenicity in NMRI mice (Administration in diet for up to twenty-one months), [redacted], Report No. [redacted], 1988-01-25 (unpublished),

[redacted], 1991, HWG 1608 – Toxic dose-range carcinogenicity study in NMRI mice (Supplement to study [redacted] (= Report no. [redacted]) with administration in diet over a 21-month period), [redacted], Report No. [redacted], 1991-12-18 (unpublished),

[redacted], 1992, HWG 1608 – Oncogenicity study in NMRI-mice in a toxic dose range (Additional to study no [redacted] (= Report no. [redacted]) with application in diet feed over a 21 months), Addendum: the frequency of histiocytic sarcomas in NMRI-mice – a compilation of historical data, [redacted], Report No. [redacted], 1992-06-30 (unpublished)

1.2 Data protection

[redacted]

1.2.1 Data owner

[redacted]

1.2.2 Companies with letter of access

[redacted]

1.2.3 Criteria for data protection

[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study

Yes

The conduct of the study conformed to the recommendations laid down in the OECD-Guideline 453 and EPA Pesticide Assessment Guideline, Subdivision F

2.2 GLP

[redacted]

2.3 Deviations

[redacted] *

[redacted]

Section A6.5.1/6.7.1(02)

Annex Point IIA6.5/6.7

6.5/6.7 Chronic toxicity/carcinogenicity study

Chronic toxicity and carcinogenicity study in mice- Part II

3 MATERIALS AND METHODS

3.1	**Test material**	As given in section 2 of dossier.
3.1.1	Lot/Batch number	Batch no.: [redacted]
3.1.2	Specification	As given in section 2 of dossier.
3.1.2.1	Description	Crystalline
3.1.2.2	Purity	Approx. [redacted] active substance
3.1.2.3	Stability	The batch used had been analysed and approved. The stability and homogeneity of the test substance in the food mixes were tested before the study was initiated. During the study period the test compound content in the administered formulations was checked at regular intervals (approx. three month). Mixes were accordingly stable and showed homogeneous distribution in the concentration range used and over period of use.
3.2	**Test Animals**	
3.2.1	Species	Mouse
3.2.2	Strain	Bor:NMRI (SPF-Han)
3.2.3	Source	[redacted]
3.2.4	Sex	Males and females
3.2.5	Age/weight at study initiation	Age: 6 - 7 weeks Weight: males: approx. 34 g ; females: approx. 29 g
3.2.6	Number of animals per group	60/sex/group
3.2.6.1	at interim sacrifice	10 animals/group/sex
3.2.6.2	at terminal sacrifice	50 animals/group/sex
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of treatment	Mice: 91 weeks
3.3.2	Interim sacrifice(s)	After 52 weeks
3.3.3	Final sacrifice	After 91 weeks
3.3.4	Frequency of exposure	Daily
3.3.5	Post-exposure period	None.
3.3.6	Type	In food
3.3.7	Concentration	Food: 0, 500 or 1500 ppm (= 0, 84.9 or 279.0 mg/kg bw/day for males and 0, 103.1or 356.5 mg/kg bw/day for females) Food consumption per day ad libitum.

Section A6.5.1/6.7.1(02)

Annex Point IIA6.5/6.7

6.5/6.7 Chronic toxicity/carcinogenicity study

Chronic toxicity and carcinogenicity study in mice- Part II

3.3.8	Vehicle	—	
3.3.9	Concentration in vehicle	—	
3.3.10	Total volume applied	—	
3.3.11	Controls	Plain diet.	
3.4	**Examinations**		
3.4.1	Body weight	Yes, before start of administration, then at weekly intervals.	
3.4.2	Food consumption	Yes, at study initiation, then weekly up to and including week 13, and thereafter at four-week intervals.	
3.4.3	Water consumption	Yes, at study initiation, then weekly up to and including week 13, and thereafter at four-week intervals.	
3.4.4	Clinical signs	Yes, twice daily (once at weekend and public holidays).	
		Detailed individual inspections: once a week.	
3.4.5	Macroscopic investigations	Palpable masses	
3.4.6	Ophthalmoscopic examination	—	
3.4.7	Haematology	Yes	
		Number of animals:	10 animals/sex/group
		Time points:	After weeks 50/51and 90/91of treatment.
		Parameters:	Haematocrit, haemoglobin concentration, erythrocyte count, erythrocyte morphology, total and differential leukocyte count, platelet count, thromboplastin time, mean corpuscular haemoglobin (MCH), mean corpuscular cell volume (MCV), mean corpuscular haemoglobin concentration (MCHC)
			Other: —
3.4.8	Clinical Chemistry		Yes
		Number of animals:	10 animals/sex/group
		Time points:	After weeks 50/51and 90/91of treatment.
		Parameters:	Glucose, albumin, total cholesterol, urea, total bilirubin, creatinine, total protein, serum electrolytes (inorganic phosphate, calcium, potassium, sodium, chloride), alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase
			Other: —

Section A6.5.1/6.7.1(02)
Annex Point IIA6.5/6.7

6.5/6.7 Chronic toxicity/carcinogenicity study

Chronic toxicity and carcinogenicity study in mice- Part II

3.4.9	Urinalysis	No	
		Number of animals:	—
		Time points:	
		Parameters:	
			Other: —
3.4.10	Pathology	Yes	
3.4.10.1	Organ Weights	Yes	
		From:	10 animals at interim sacrifice, 50 animals at terminal sacrifice
		Organs:	Liver, kidneys, adrenals, testes, brain, heart
			Other: —
3.4.11	Histopathology	Yes	
		From:	All animals of all dose groups
		From:	at interim sacrifice
			at terminal sacrifice
		Organs:	Aorta, brain, spinal cord, pituitary, thyroid, thymus, gall bladder, oesophagus, salivary glands, stomach, small and large intestines, liver, pancreas, kidneys, adrenals, spleen, heart, trachea, lungs, gonads, epididymides, seminal vesicle, uterus, gland, prostate, urinary bladder, lymph node (mandibular and mesenteric), peripheral nerve, femur with marrow, sternum with bone marrow, skin, eyes, musculature, Harder's glands, tongue
			Other: all tissues exhibiting alterations
3.4.12	Other examinations	None.	

3.5 Statistics

Body weight, medical laboratory tests, food consumption, organ weight:

Arithmetic group means, standard deviation were calculated. The test groups were compared with the control groups by using the "U test" of H.B. Mann and D. R. Whitney, Ann. Math. Stat. 18, 50, 1947 or F. Wilcoxon, Biometrics 1, 80, 1945 at significance levels of $\alpha = 5$ % and $\alpha = 1$ %.

Incidence data: mortality, clinical signs, etc.:

Fisher's exact test (statistical methods for research workers, Stuchart Verlag, New York, 1946) at significance levels of $\alpha = 5$ % and $\alpha = 1$ % (two-tailed).

3.6 Further remarks —

Section A6.5.1/6.7.1(02)

Annex Point IIA6.5/6.7

6.5/6.7 Chronic toxicity/carcinogenicity study

Chronic toxicity and carcinogenicity study in mice- Part II

4 RESULTS AND DISCUSSION

4.1	**Body weight**	The growth of the males in both treatment groups underwent dose-related retardation over lengthy periods during the study. At 1500 ppm, the difference in body weights were slightly greater than 10% at times, whereas they were around 6 –7% at maximum at 500 ppm. The females exhibited reduced body weight after dosing with 1500 ppm. The deviations from the control group were less than 10%. The body weights were subsequently comparable to those of the controls.
4.2	**Food consumption**	A tendency to dose-related increases in the food intakes was present in the male and female mice of both treatment groups, particularly in the data relative to body weight.
4.3	**Water consumption**	No effects.
4.4	**Clinical signs**	No effects up to and including 500 ppm. At 1500 ppm, the incidence of animals exhibiting a distended abdomen was elevated.
4.5	**Macroscopic investigations**	No effects.
4.6	**Ophthalmoscopic examination**	—
4.7	**Haematology**	Males of the 500 ppm dose group showed depressed haematocrit values at both examination time points. The MCH value in week 90 was decreased, whereas the MCHC values were elevated at both time points. The erythrocyte count was slightly depressed at both times in males of the 1500 ppm dose groups, whereas the haemoglobin content and haematocrit value were greatly reduced at both times. Although the 1500 ppm dose group females exhibited strikingly reduced erythrocyte counts, and haemoglobin and haematocrit values at both times, most of these could not be statistically verified due to the relatively wide scatter range. The thrombocyte count in females of the 500 ppm was slightly elevated at both times. Both males and females displayed increased thrombocyte counts at the 1500 ppm level. The thromboplastin time determined with the Hepatoquick test exhibited dose-related reduction in males and females treated with 1500 ppm at both times. The leukocyte count showed markedly elevated values in the 1500 ppm group males and females at interim examination, and less highly increased values at week 90. However, no evidence for substance-related effects may be inferred from the differential blood count. Despite the presence of several statistically significant deviations, the other red blood cell parameters exhibit no uniform trend.

Section A6.5.1/6.7.1(02)

Annex Point IIA6.5/6.7

6.5/6.7 Chronic toxicity/carcinogenicity study

Chronic toxicity and carcinogenicity study in mice- Part II

4.8	**Clinical Chemistry**	The activities of the alanine aminotransferase (ALAT)and aspartate aminotransferase (ASAT) and that of alkaline phosphatase underwent dose-related increases in the males and females of both dose-groups at both times, at 1500 ppm to an extremely large extent in some cases. However, the differences could not always be statistically verified at the 500 ppm level due to the relatively wide range of biological scatter. Marked and statistically significant reductions in the cholesterol levels were found at both times in the 500 ppm dose group males and females. Although the 1500 ppm dosed animals similarly exhibited significantly depressed plasma cholesterol levels in week 52, these generally corresponded to those of the control animals in week 91. A more or less marked trend to lower bilirubin values was also found at both times in the 500 ppm dose group. This only applies to week 52 at the 1500 ppm level. In contrast, elevated mean values were observed in week 91, but were not found to be statistically significant because of wide scattering and the presence of isolated extreme values. The blood phosphate levels underwent no noteworthy change at 500 ppm. In contrast, they were significantly elevated at both times in both sexes at the 1500 ppm level.
4.9	**Urinalysis**	—
4.10	**Pathology**	Evidence for substance-related effects on the liver was determined in both treatment groups of both sexes at interim and terminal necropsy. <u>Interim sacrifice:</u> In most of the animals, the liver appeared pale, and was also enlarged at the 1500 ppm level, particularly in the males. Isolated animals in both treatment groups exhibited enhanced lobulation. Altered areas of the liver, capsular thickening and swelling were additionally observed in isolated 1500 ppm dose group animals. <u>Terminal sacrifice:</u> Changes were observed at a low incidence at 500 ppm, mainly enhanced lobulation in males and females, and enlargement and hepatic pallor in females. The liver was enlarged and exhibited an irregular surface structure in most animals at 1500 ppm. In addition, nodular masses and discoloration were determined in several animals.
4.11	**Organ Weights**	The liver weights exhibited dose-related increases at both necropsy dates. The liver weight increases were moderately great (weight differences ≤ 25%) in males and females at the 500 ppm level, but were extremely pronounced at 1500 ppm. In the latter group, the liver was slightly more than twice as heavy as in the control animals at the time of interim necropsy, and was more than three times as heavy as the time of terminal necropsy. Elevated absolute and relative adrenal weights could be determined in the 1500 ppm dose group females at both times.

Section A6.5.1/6.7.1(02)

Annex Point IIA6.5/6.7

6.5/6.7 Chronic toxicity/carcinogenicity study

Chronic toxicity and carcinogenicity study in mice- Part II

4.12	**Histopathology**	Non-neoplastic alterations: Interim sacrifice: Numerous treatment-related findings were determined in the livers of both dose groups at a dose-related incidence and/or intensity: single cell and focal necroses, inflammation, bile duct hyperplasia, steatosis, pigment accumulation in the Kupffer stellate cells, periportal fibrosis and others. A dose-related increase in the number of animals exhibited hyperkeratosis and acanthosis of the forestomach mucosa was observed after 500 ppm and above. Terminal sacrifice: Test substance-related liver findings were observed in both treatment groups, dose-proportional accumulation not always being present. Particular striking incidences of the following were observed: single cell necroses (500 ppm males), focal necroses (females at 500 ppm and above), focal hyperplasia of the hepatocytes (1500 ppm males and females), panacinal, fine-droplet fatty vacuolation (males and females at 500 ppm and above), centriacinal coarse-droplet fatty vacuolation (500 ppm females), periacinal hypertrophy of the hepatocytes (1500 ppm females), extramedullary haematopoiesis (males and females at 1500 ppm), pigment accumulation in the Kupffer stellate cells (males at 1500 ppm and females at 500 ppm and above). No significant increase of animals with hyperkeratosis and acanthosis of the forestomach mucosa was observed. Neoplastic changes: No treatment-related effects in animals of the satellite groups. Increased numbers of tumour hosts were observed among the 1500 ppm males, the incidence of animals exhibiting benign tumours and those malignant tumours both being elevated. The incidence of hepatocellular was elevated to a marked statistically highly significant extent in the 1500 ppm group males and females.
4.13	**Other examinations**	—
4.14	**Time to tumours**	Not applicable.
4.15	**Other**	—

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The objective of the study was to recognise a possible oncogenic potential in the range of elevated (more or less toxic) dosages. A prior study involving a dose range from 20 – 180 ppm ([redacted], 1988[redacted], Report No. [redacted], 1988-01-25, unpublished) had shown no evidence for an oncogenic potential, but the effects on the liver at doses of 60 ppm and above, and those detected in the haematology at 180 ppm were not very marked in intensity, posing the question as to whether a maximum tolerated dose (MTD) had been reached. The conduct of the study conformed to the recommendations laid down in the OECD-Guideline 453. To establish the potential presence of chronic toxic effects, animals were interim-autopsied after one year and additional haematological and clinical tests as well as histopathology

examinations were carried out.

The tebuconazole doses were based on the results of two previous feeding studies lasting four and six weeks, as well as on those of the carcinogenicity study in NMRI-mice of the same strain ([redacted], Report no. [redacted], 1986-07-21, unpublished; [redacted], Report no. [redacted], 1986-12-04, unpublished, [redacted], 1988, [redacted], Report No. [redacted], 1988-01-25, unpublished).

5.2 Results and discussion

The appearance, general behaviour, water intakes, and mortality were unaffected in the groups up to and including 500 ppm. The incidence of animals exhibiting increases in the abdominal girth was elevated at the 1500 ppm level. The food intakes underwent dose-related increases. Retarded growth was observed in the 1500 ppm groups.

The haematological examination did not provide any indication of damage to the blood up to and including 500 ppm dose group females. Marginal effects on the haematocrit value (reduced), and the MCH and MCHC figures (elevated) were present in the male at 500 ppm. The erythrocyte count, haemoglobin content, haematocrit value and thromboplastin time were generally reduced at 1500 ppm, whereas the thrombocyte and leukocyte counts were elevated, in some cases to a marked extend. The clinical laboratory tests, gross pathology, organ gravimetry and histopathology afforded evidence for marked and dose-related liver damage in both treatment groups. The main findings included a marked increase in the activities of the alanine and aspartate aminotransferases, in some cases major enlargement of the liver, single cell and focal necroses, inflammation, bile duct hyperplasia and steatoses. The rate of hepatocellular tumours was unaffected at the 500 ppm level. The conclusion arrived at in the prior study ([redacted], 1988, [redacted], Report No. [redacted], 1988-01-25, unpublished) was thus confirmed.
In that study it had been argued that a certain tendency to an increase in the rate of hepatocellular tumours in male mice at 60 ppm and 180 ppm fell within the range of spontaneous variation. In contrast, the rates of hepatocellular tumours in males and females were elevated to a highly significant extend at 1500 ppm, and lied markedly above the range of spontaneous incidences observed in this mice strain. Especially in mice, hepatocellular tumours caused by a variety of chemical substances at hepatotoxic doses occur frequently, and it is thought that under these circumstances, elevated incidences of spontaneous, relatively frequent tumours in rodents have no relevance for humans if the exposure lies in a non-toxic range.
In addition, the histopathology of the interim necropsy animals showed a dose-related increase in the incidence of hyperkeratosis and acanthosis of the forestomach mucosa. The incidence of these findings underwent no treatment-related increase in the animals of the main groups. The effects may possibly have experienced regression despite continuation of the treatment.

No evidence for disturbances in the electrolyte balance was found in the 500 ppm dose group. A significant increase in the blood phosphate levels was determined in the 1500 ppm males and females, but no morphological correlative was found for this observation. In addition, the levels of the other electrolytes did not deviate from the values in the control animals in this group.

Section A6.5.1/6.7.1(02)

Annex Point IIA6.5/6.7

6.5/6.7 Chronic toxicity/carcinogenicity study

Chronic toxicity and carcinogenicity study in mice- Part II

5.3	**Conclusion**	The relatively major effects, particularly those on the liver, represent unequivocal evidence that the maximum tolerated dose (MTD) has been exceeded, and underscore the correctness of dose selection in the prior study ([redacted], 1988, [redacted], Report No. [redacted], 1988-01-25, unpublished). No evidence for carcinogenic effects by the test substance on the other organs may be inferred from the incidence, type, location or distribution among the study groups of the other neoplasms observed.
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	[redacted]

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	July 2005
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

Table A6.5/6.7-1. Table for Clinical Chemistry and Haematology

Clinical Chemistry	Sex	Unit	Control	Low dose 500 ppm	High dose 1500 ppm
ALAT		U/l	**51 weeks after start of treatment**		
	male		[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]
			90 weeks after start of treatment		
	male		[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]
ASAT		U/l	**51 weeks after start of treatment**		
	male		[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]
			90 weeks after start of treatment		
	male		[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]
Alkaline phosphatase		U/l	**51 weeks after start of treatment**		
	male		[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]
			90 weeks after start of treatment		
	male		[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]
Cholesterol		mmol/l	**51 weeks after start of treatment**		
	male		[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]
			90 weeks after start of treatment		
	male		[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]

↓ decrease

↑ increase

— not different from control

* significantly different from control $p \leq 0.05$

** significantly different from control $p \leq 0.01$

Table A6.5/6.7-1. Table for Clinical Chemistry and Haematology, continued

Clinical Chemistry	Sex	Unit	Control	Low dose 500 ppm	High dose 1500 ppm
Bilirubin		µmol/l	**51 weeks after start of treatment**		
	male		[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]
			90 weeks after start of treatment		
	male		[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]
Inorganic phosphate		mmol/l	**51 weeks after start of treatment**		
	male		[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]
			90 weeks after start of treatment		
	male		[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]

↓ decrease

↑ increase

— not different from control

* significantly different from control $p \leq 0.05$

** significantly different from control $p \leq 0.01$

Table A6.5/6.7-1. Table for Clinical Chemistry and Haematology, continued

Haematology	Sex	Unit	Control	Low dose 500 ppm	High dose 1500 ppm
Leucocyte count		10^9/l	**51 weeks after start of treatment**		
	male		[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]
			90/91 weeks after start of treatment		
	male		[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]
Erythrocyte count		10^{12}/l	**51 weeks after start of treatment**		
	male		[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]
			90/91 weeks after start of treatment		
	male		[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]
Haemoglobin		g/l	**51 weeks after start of treatment**		
	male		[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]
			90/91 weeks after start of treatment		
	male		[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]
Haematocrit		l/l	**51 weeks after start of treatment**		
	male		[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]
			90/91 weeks after start of treatment		
	male		[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]

↓ decrease

↑ increase

— not different from control

* significantly different from control $p \leq 0.05$

** significantly different from control $p \leq 0.01$

Table A6.5/6.7-1. Table for Clinical Chemistry and Haematology, continued

Haematology	Sex	Unit	Control	Low dose 500 ppm	High dose 1500 ppm
MCHC		g/l erythrocytes	**51 weeks after start of treatment**		
	male		[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]
			90/91 weeks after start of treatment		
	male		[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]
MCH		pg	**51 weeks after start of treatment**		
	male		[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]
			90/91 weeks after start of treatment		
	male		[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]
Thrombocyte count		10^9/l	**51 weeks after start of treatment**		
	male		[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]
			90/91 weeks after start of treatment		
	male		[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]
Clotting time (Hepatoquick)		sec	**51 weeks after start of treatment**		
	male		[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]
			90/91 weeks after start of treatment		
	male		[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]

↓ decrease

↑ increase

— not different from control

* significantly different from control $p \leq 0.05$

** significantly different from control $p \leq 0.01$

Table A6_5/7-2.B Results of the combined chronic toxicity/carcinogenicity study in mice (main groups)

Parameter	Control data				Medium dose 500 ppm		High dose 1500 ppm		Dose-response +/-	
	historical		study							
	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m	f
Number of animals examined			50	50	50	50	50	50		
Mortality			[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Clinical signs			[redacted]	[redacted]	[redacted]	[redacted]	[redacted]		[redacted]	[redacted]
Body weight gain			[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Food consumption			[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Haematology			Effects were described in detail in table A6.5/6.7-1						[redacted]	[redacted]
Clinical chemistry									[redacted]	[redacted]
Water consumption			[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Overall tumour incidence:										
No. of animals with primary neoplasms			[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
No. of animals with benign neoplasms			[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
No. of animals with malignant neoplasms			[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

↓ decrease

↑ increase

— not different from control

* significantly different from control $p \leq 0.05$

** significantly different from control $p \leq 0.01$

Table A6_5/7-2.B Results of the combined chronic toxicity/carcinogenicity study in mice (main groups), continued

Parameter	Control data				Low dose 500 ppm		High dose 1500 ppm		Dose-response +/-	
	historical		study							
	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m	f
Organ: liver										
Organ weight, relative and (absolute)			↑ ↑	↑ ↑	↑ ↑	— ↑	↑ ↑	↑ ↑	↑	↑
Pathology										
enlarged			↑	↑	↑	↑	↑	↑	↑	↑
irregular surface			↑	↑	↑	↑	↑	↑	↑	↑
masses			↑	↑	↑	↑	↑	↑	↑	↑
Non-neoplastic changes:										
Necrosis of single hepatocytes			↑	↑	↑	↑	↑	↑	↑	↑
Focal hyperplasia of hepatocytes			↑	↑	↑	↑	↑	↑	↑	↑
Panacinar fine fatty vacuolation			↑	↑	↑	↑	↑	↑	↑	↑
Centriacinar fatty vacuolation			↑	↑	↑	↑	↑	↑	↑	↑
Periacinar hepato-cytic hypertrophy			↑	↑	↑	↑	↑	↑	↑	↑
Oval cell proliferation			↑	↑	↑	↑	↑	↑	↑	↑
Extramedullary Haemopoiesis			↑	↑	↑	↑	↑	↑	↑	↑
Hepatocellular alteration			↑	↑	↑	↑	↑	↑	↑	↑
Pigment laden Kupffer cells			↑	↑	↑	↑	↑	↑	↑	↑
Neoplastic changes:										
Hepatocellular adenoma			↑	↑	↑	↑	↑	↑	↑	↑
Hepatocellular carcinoma			↑	↑	↑	↑	↑	↑	↑	↑

[a] number of animals affected/total number of animals [1] not relevant for humans

↑ increase

↓ decrease

— not different from control

* significantly different from controls, $p \leq 0.05$;** significantly different from controls, $p \leq 0.01$

Table A6_5/7-2.B Results of the combined chronic toxicity/carcinogenicity study in mice (main groups), continued

Parameter	Control data				Low dose 500 ppm		High dose 1500 ppm		Dose-response +/-	
	historical		study							
	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m	f
Organ: stomach										
Neoplastic changes:										
Dysplasia			■	■	■	■	■	■	▮	▮
Hyperkeratosis and acanthosis			■	■	■	■	■	■	▮	▮
Organ: adrenals										
Organ weight, relative and (absolute)			■ ■	■ ■	■ ■	■ ■	■ ■	■ ■	▮	▮

[a] number of animals affected/total number of animals

↑ increase

↓ decrease

— not different from control

* significantly different from controls, $p \leq 0.05$

** significantly different from controls, $p \leq 0.01$

Section A6.5.2/6.7.2 6.5/6.7 Chronic toxicity/carcinogenicity study

Annex Point IIA6.5/6.7 Chronic toxicity and carcinogenicity study in rats

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1 REFERENCE

1.1	**Reference**	[redacted], 1988, HWG 1608 – Study for chronic toxicity and cancerogenicity in Wistar rats (Administration in diet for two years), [redacted], Report No. [redacted], 1988-01-25 (unpublished)
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes The conduct of the study conformed to the recommendations laid down in the OECD-Guideline 453 and the EPA Pesticides Assessment Guidelines, subdivision F.
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]

3 MATERIALS AND METHODS

3.1	**Test material**	As given in section 2 of dossier.
3.1.1	Lot/Batch number	Batch no.: mixed batch; Fl. no.: [redacted] Single samples: [redacted]
3.1.2	Specification	As given in section 2 of dossier.
3.1.2.1	Description	Solid/light yellow crystals

Section A6.5.2/6.7.2 6.5/6.7 Chronic toxicity/carcinogenicity study

Annex Point IIA6.5/6.7 Chronic toxicity and carcinogenicity study in rats

3.1.2.2	Purity	Approx. [redacted] active substance
3.1.2.3	Stability	During the study period the test compound content in the administered formulations was checked at regular intervals (approx. three month). To ensure homogeneity and stability of the test substance in the formulation, sample mixes were analytically examined before start of test. Mixes were accordingly stable and showed homogeneous distribution in the concentration range used and over period of use.
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Wistar Bor:WISW (SPF-Cpb)
3.2.3	Source	[redacted]
3.2.4	Sex	Males and females
3.2.5	Age/weight at study initiation	Age: 5-6 weeks Weight: males mean weight: 97 g (80 g – 112 g); females mean weight: 90 g (71 g – 111 g)
3.2.6	Number of animals per group	60/sex/group
3.2.6.1	at interim sacrifice	10 animals/group/sex
3.2.6.2	at terminal sacrifice	50 animals/group/sex
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of treatment	Rats 104 weeks
3.3.2	Interim sacrifice(s)	after 52 weeks
3.3.3	Final sacrifice	104 weeks
3.3.4	Frequency of exposure	Daily
3.3.5	Post-exposure period	None.
3.3.6	Type	In food
3.3.7	Concentration	Food: 0, 100, 300 or 1000 ppm (= 0, 5.3, 15.9 or 55.0 mg/kg bw/day for males and 0, 7.4, 22.8 or 86.3 mg/kg bw/day for females) Food consumption per day ad libitum.
3.3.8	Vehicle	—
3.3.9	Concentration in vehicle	—
3.3.10	Total volume applied	—
3.3.11	Controls	Plain diet.

Section A6.5.2/6.7.2 6.5/6.7 Chronic toxicity/carcinogenicity study

Annex Point IIA6.5/6.7 Chronic toxicity and carcinogenicity study in rats

3.4	**Examinations**		
3.4.1	Body weight	Yes, before start of administration, then weekly up to and including week 12, and at two-weekly intervals from week 15 until end of study (before necropsy).	
3.4.2	Food consumption	Yes, at study initiation, then weekly up to and including week 13, and at two-weekly intervals from week 15 until week 103.	
3.4.3	Water consumption	Yes, at study initiation, then weekly up to and including week 13, and at two-weekly intervals from week 15 until week 103.	
3.4.4	Clinical signs	Yes, twice daily (once at weekend and public holidays).	
		Detailed individual inspections: once a week.	
3.4.5	Macroscopic investigations	Palpable masses	
3.4.6	Ophthalmoscopic examination	Yes, at study initiation, after 12 month and before end of study.	
		10 animals/sex of the control and high dose group	
3.4.7	Haematology	Yes	
		Number of animals:	10 animals/sex/group
		Time points:	After 6, 12, 18 and 24 months of treatment
		Parameters:	Haematocrit, haemoglobin concentration, erythrocyte count, erythrocyte morphology, total and differential leukocyte count, reticulocyte count, platelet count, thromboplastin time, mean corpuscular haemoglobin (MCH), mean corpuscular cell volume (MCV), mean corpuscular haemoglobin concentration (MCHC)
			Other: —
3.4.8	Clinical Chemistry		Yes
		Number of animals:	10 animals/sex/group
		Time points:	After 6, 12, 18 and 24 months of treatment
		Parameters:	Sodium, potassium, calcium, chloride, iron, inorganic phosphate, glucose, total cholesterol, urea, triglycerides, total bilirubin, creatinine, total protein and albumin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, lactate dehydrogenase, creatinine kinase
			Other: —
3.4.9	Urinalysis	Yes	
		Number of animals:	10 animals/sex/group
		Time points:	After 6, 12, 18 and 24 months of treatment.
		Parameters:	Volume, specific gravity, pH, protein, glucose, blood, bilirubin, ketone bodies, urobilinogen, sediment (microscopic examination)

Section A6.5.2/6.7.2 6.5/6.7 Chronic toxicity/carcinogenicity study

Annex Point IIA6.5/6.7 Chronic toxicity and carcinogenicity study in rats

3.4.10	Pathology	Yes	
3.4.10.1	Organ Weights	Yes	
		from:	10 animals at interim sacrifice, 50 animals at terminal sacrifice
		Organs:	Liver, kidneys, adrenals, testes, ovaries, spleen, brain, heart, lung
3.4.11	Histopathology	Yes	
		from:	All animals of all dose groups
		from:	at interim sacrifice
			at terminal sacrifice
		Organs:	Brain, spinal cord, pituitary, thyroid, thymus, oesophagus, salivary glands, stomach, small and large intestines, liver, pancreas, kidneys, adrenals, spleen, heart, trachea, lungs, aorta, gonads, epididymides, seminal vesicle, uterus, oviduct, vagina, mammary gland, prostate, urinary bladder, lymph node (mandibular and mesenteric), peripheral nerve, femur, sternum, bone marrow in femur and sternum, skin, eyes, musculature
			Other: all tissues exhibiting alterations.
3.4.12	Other examinations	None.	
3.5	**Statistics**	Body weight, medical laboratory tests, food consumption, organ weight:	
		Arithmetic group means, standard deviation, and for the organ weights the upper and lower confidence limits on the confidence level of 1 - α = 95% and 1 - α = 99%. The test groups were compared with the control groups by using the "U test" of H.B. Mann and D. R. Whitney, Ann. Math. Stat. 18, 50, 1947 or F. Wilcoxon, Biometrics 1, 80, 1945 at significance levels of α = 5 % and α = 1 %.	
		Incidence data: mortality, clinical signs, etc.:	
		Fisher's exact test (statistical methods for research workers, Stuchart Verlag, New York, 1946) at significance levels of α = 5 % and α = 1 % (two-tailed).	
3.6	**Further remarks**	—	

4 RESULTS AND DISCUSSION

4.1	**Body weight**	No effects up to and including 300 ppm. After 1000 ppm the males and females body weights of the main groups were decreased.
4.2	**Food consumption**	No effects up to and including 300 ppm. At 1000 ppm the females' food intake was increased (mean about 15%).
4.3	**Water consumption**	No effects up to and including 300 ppm. At 1000 ppm the females' water intake was decreased.
4.4	**Clinical signs**	No effects up to and including 1000 ppm.
4.5	**Macroscopic investigations**	No effects.

4.6	**Ophthalmoscopic examination**	No effects.
4.7	**Haematology**	No effects.
4.8	**Clinical Chemistry**	Females of the 1000 ppm dose group showed lower triglyceride concentrations as the control animals at all examination times.
4.9	**Urinalysis**	No effects.
4.10	**Pathology**	No effects.
4.11	**Organ Weights**	After dosing of 1000 ppm the females' lung weights were absolutely and relatively higher than the control females ($p \leq 0.05$). The liver weights of the female satellite rats were statistically significantly lower in the 300 ppm (relative weight) and 1000 ppm (absolute and relative weight) dose group. After 24 months, these effects were not longer observable. The relative liver weight of females at 1000 ppm was increased. The females' absolute and relative spleen weights in the 1000 ppm group were statistically significantly increased after 12 month. At final autopsy after 24 month these effects were no longer present. After the two years' treatment the female adrenal weights (absolute and relative) were lower in all dose groups. The males relative testicle weights in the main 1000 ppm treatment groups were statistically significantly lower than the control males. The males relative testicle weights in the main 1000 ppm treatment groups were statistically significantly lower than the control males. In the latter case the mean was however unusually high due to individual extreme figures (tumours).
4.12	**Histopathology**	No treatment-related effects in animals of the satellite groups. <u>Non-neoplastic alterations:</u> After 2 years, the females in the 1000 ppm dose group exhibited an increased incidence of pigment deposits in the Kupffer star cell and signs of induction of the microsomal enzyme systems in the liver. The incidence of females with haemosiderin accumulation in the spleen was likewise increased after 1000 ppm. The treated females' adrenals showed a reduced incidence of cortical haemorrhagic degeneration in correlation with dose. <u>Neoplastic changes:</u> Dose-related incidences of benign and/or malignant blastomas in all the locations were not present. There was no increased frequency of unusual tumours in the treatment groups. *
4.13	**Other examinations**	—
4.14	**Time to tumours**	Not applicable.
4.15	**Other**	—

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The objective of the study was to establish toxic effects, including a dose-effect relationship, and a no-effect dose for a mammalian species under conditions of exposure relevant to practice. In particular potential effects requiring a long latency period and/or accumulation were to be detected under the test conditions.

The conduct of the study conformed to the recommendations laid down in the OECD-Guideline 453 and the EPA Pesticides Assessment Guidelines, subdivision F.

The tebuconazole doses were based on the results of a previous feeding study with Wistar rats of the same strain lasting thirteen weeks ([redacted], Report no. [redacted], 1986-10-27, unpublished)

5.2 Results and discussion

The appearance, general behaviour, food and water intakes and mortality were unaffected in the groups up to and including 300 ppm. After 1000 ppm the females' water intake was decreased and the males and females growth in this dose groups was retarded. In the case of the 1000 ppm dosed females, food consumption was increased and the nutritive result (food consumption per gram weight increase) was therefore impaired.

The haematological examination did not provide any indication of damage to the blood. Histopathology revealed an increased incidence of female animals with haemosiderin accumulation in the spleen and pigment deposits in the Kupffer star cells in the liver in the 1000 ppm dose group. The results of the clinical chemical, gross pathological and histopathological examinations did not detect any liver damage in the male and female rat up to and including 1000 ppm. After 1000 ppm however the females exhibited signs of induction of microsomal enzyme systems.

The result of urinalyses, the clinical chemical analyses and the patho-morphological examination did not reveal a toxicologically relevant restriction of function or morphological alterations in the kidneys in the examined dose range.

The histopathological examination did not detect any effects on the adrenals in the male animals in all the dose groups or in the females in the dose groups up to and including 300 ppm. The clearly reduced number of females with haemorrhagic degeneration of the adrenal cortex after 1000 ppm is most likely to be a treatment-related functional effect.

The gross pathological and histopathological examinations did not provide any indications of substance-induced organ lesions, and for this reason the weights of lung, spleen, and testicles, which varied significantly in single groups, are not interpreted as a manifestation of substance-induced ill-effect.

Section A6.5.2/6.7.2 6.5/6.7 Chronic toxicity/carcinogenicity study

Annex Point IIA6.5/6.7 Chronic toxicity and carcinogenicity study in rats

5.3	**Conclusion**	Under the condition described above, tebuconazole was tolerated without adverse effects by the male and female rats at doses up to and including 300 ppm. NOEL for carcinogenicity: 1000 ppm for males and females (= 55.0 mg/kg bw/day for males and 86.3 mg/kg bw/day for females) NOEL systemic: 300 ppm for males and females (= 15.9 mg/kg bw/day for males and 22.8 mg/kg bw/day for females) LOEL systemic: 1000 ppm for males and females (= 55.0 mg/kg bw/day for males and 86.3 mg/kg bw/day for females)
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	[redacted]

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	July 2005
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

Table A6.5/6.7-1. Table for Clinical Chemistry, Haematology and Urinalysis

Table for haematology and urinalyses not needed due to results of study.

Clinical Chemistry	Sex	Unit	Control	Low dose 100 ppm	Medium dose 300 ppm	High dose 1000 ppm
Triglyceride		mg/dL	**27 weeks after start of treatment**			
	male		[redacted]	[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]	[redacted]
			52 weeks after start of treatment			
	male		[redacted]	[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]	[redacted]
			79 weeks after start of treatment			
	male		[redacted]	[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]	[redacted]
			104 weeks after start of treatment			
	male		[redacted]	[redacted]	[redacted]	[redacted]
	female		[redacted]	[redacted]	[redacted]	[redacted]

↓ decrease

↑ increase

— not different from control

* significantly different from controls, $p \leq 0.05$

** significantly different from controls, $p \leq 0.01$

Table A6_5/7-2.B Results of the combined chronic toxicity/carcinogenicity study in rats (main groups)

Parameter	Control data				Low dose 100 ppm		Medium dose 300 ppm		High dose 1000 ppm		Dose-response +/-	
	historical		study									
	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m	f
Number of animals examined			50	50	50	50	50	50	50	50		
Mortality			▬	▬	▬	▬	▬	▬	▬	▬	▮	▮
Clinical signs			▮	▮	▮	▮	▮	▮	▮	▮	▮	▮
Body weight gain			▮	▮	▮	▮	▮	▬	▬	▬	▮	▮
Food consumption			▮	▮	▮	▮	▮	▮	▮	▮	▮	▮
Water consumption			▮	▮	▮	▮	▮	▮	▮	▮	▮	▮
Clinical chemistry			Effects described in table A 6_5/7-2.A above								▮	▮
Haematology			▮	▮	▮	▮	▮	▮	▮	▮	▮	▮
Urinalysis			▮	▮	▮	▮	▮	▮	▮	▮	▮	▮
Number of animals examined			▮	▮	▮	▮	▮	▮	▮	▮		
Overall tumour incidence:			▮	▮	▮	▮	▮	▮	▮	▮	▮	▮
No. Of animals with neoplasms			▮	▮	▮	▮	▮	▮	▮	▮	▮	▮
No. Of animals with benign neoplasms			▮	▮	▮	▮	▮	▮	▮	▮	▮	▮
No. Of animals with malignant neoplasms			▮	▮	▮	▮	▮	▮	▮	▮	▮	▮
No. Of animals with multiple neoplasms			▮	▮	▮	▮	▮	▮	▮	▮	▮	▮

[a] number of animals affected/total number of animals

↓ decrease

↑ increase

— not different from control

* significantly different from controls, $p \leq 0.05$

** significantly different from controls, $p \leq 0.01$

Table A6_5/7-2.B Results of the combined chronic toxicity/carcinogenicity study in rats (main groups), continued

Parameter	Control data				Low dose 100 ppm		Medium dose 300 ppm		High dose 1000 ppm		Dose-response +/-	
	historical		study									
	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m	f
Organ: liver												
Non-neoplastic changes: Incidence of pigment deposits in the Kupffer star cell			■	■	■	■	■	■	■	▮	▮	▮
Induction of microsomal enzyme liver system			■	■	■	■	■	■	■	▮	▮	▮
Organ: spleen												
Non-neoplastic changes: Haemosiderin accumulation			■	■	■	■	■	■	■	▮	▮	▮
Organ: adrenals												
Non-neoplastic changes: Cortical haemorrhagic degeneration			■	■	■	■	■	■	■	▮	▮	▮

[a] number of animals affected/total number of animals

↑ increase

↓ decrease

— not different from control

* significantly different from controls, $p \leq 0.05$

** significantly different from controls, $p \leq 0.01$

Section A6.6.1 Genotoxicity in vitro

Annex Point IIA6.6.1 6.6.1. In-vitro gene mutation study in bacteria (Salmonella typhimurium)

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1 REFERENCE

1.1 Reference

[redacted] (1988a), Salmonella/microsome test to evaluate for point mutagenic effects, [redacted], Report No. [redacted], 1988-01-27

[redacted] (1988b), Salmonella/microsome test using TA 1538 to evaluate for point mutagenic effects (Addendum to the final report no. [redacted]), [redacted], Report No. [redacted], 1988-02-08

1.2 Data protection [redacted]

1.2.1 Data owner [redacted]

1.2.2 Companies with letter of access [redacted]

1.2.3 Criteria for data protection [redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study Yes

OECD-Guideline 471

2.2 GLP [redacted]

2.3 Deviations [redacted]

3 MATERIALS AND METHODS

3.1 Test material HWG 1608

3.1.1 Lot/Batch number [redacted]

3.1.2 Specification

3.1.2.1 Description beige powder

3.1.2.2 Purity [redacted]

3.1.2.3 Stability The batch used was analytically examined prior to study initiation and was approved for use during the test period. A stability test in the solvent did not detect a relevant change in the percent active ingredient.

3.2 Study Type Bacterial reverse mutation test

3.2.1 Organism/cell type S. typhimurium:
TA 1535, TA 1537, TA 98, TA 100, TA 1538

3.2.2 Deficiencies / Proficiencies

3.2.3 Metabolic activation system Male Sprague-Dawley-rats, induced by Aroclor 1254, served as source of the S9 fraction.

Section A6.6.1	Genotoxicity in vitro
Annex Point IIA6.6.1	6.6.1. In-vitro gene mutation study in bacteria (Salmonella typhimurium)

3.2.4	Positive control	2-Aminoanthracene (for all strains) Sodium azide (10 µg/plate for TA 1535) Nitrofurantoin (0.2 µg/plate for TA 100) 4-nitro-1,2-phenylene diamine (10 µg/plate for TA 1537; 0.5 µg/plate for TA 98 and TA 1538)
3.3	**Administration / Exposure; Application of test substance**	
3.3.1	Concentrations	for TA 1535, TA 1537, TA 98 and TA 100: first test: 0, 37.5, 75, 150, 300, 600, 1200 and 2400 µg/plate repeat test: 0, 39.5, 59.3, 88.9, 133.3, 200, 300 and 450 µg/plate (due to the substance´s toxicity lower doses were chosen for the repeat test) for TA 1538: first and repeat test: 0, 39.5, 59.3, 88.9, 133.3, 200, 300 and 450 µg/plate
3.3.2	Way of application	dissolved in medium (solvent: DMSO)
3.3.3	Pre-incubation time	-
3.3.4	Other modifications	-
3.4	**Examinations**	for TA 1535, TA 1537, TA 98 and TA 100: first test: with and without activation (30% S9-mix) repeat test: with and without activation (10% and 30% S9-mix) for TA 1538: first and repeat test: with and without activation (10% and 30% S9-mix) Bacteriotoxicity: Bacteriotoxicity was determined by titration. Additionally, background growth on the plates was grossly appraised.
3.4.1	Number of cells evaluated	

4 RESULTS AND DISCUSSION

4.1	**Genotoxicity**	
4.1.1	without metabolic activation	No
4.1.2	with metabolic activation	No

Section A6.6.1 Genotoxicity in vitro

Annex Point IIA6.6.1 6.6.1. In-vitro gene mutation study in bacteria (Salmonella typhimurium)

4.2	**Cytotoxicity**	Yes Doses up to and including 39.5 µg/plate did not cause any bacteriotoxic effects in all strains. At higher doses strong strainspecific bacteriotoxic effects were observed, so that this range could only be used to a limited extent up to 600 µg/plate for evaluation purposes.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The mutagenicity of the test substance was evaluated with the Salmonella / microsome test, also termed the Ames Test, as described by Ames et al. (Proc. nat. Acad. Sci. 70: 2281-2285, 1973 and Mutation Res. 31: 347-364, 1975) and Maron and Ames (Mutation Res. 113: 173-215, 1983), respectively. The study was done according to OECD-Guideline 471, though not stated in the study report.
5.2	**Results and discussion**	Doses up to and including 39.5 µg/plate did not cause any bacteriotoxic effects. At higher doses the substance had a strong – strain specific-bacteriotoxic effect. Evidence of mutagenic activity for tebuconazole was not found. Neither a dose-related doubling nor a biologically relevant increase of mutant count, in comparison with the negative controls, were observed. The positive controls had a marked mutagenic effect, as it was seen by a biologically relevant increase of mutagenic colonies compared with the negative controls.
5.3	**Conclusion**	Under the stated test conditions, the test substance is not mutagenic in Salmonella typhimurium.
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	[redacted]

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	August 2005
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	

Table A6_6_1-1.A: Table for Gene Mutation Assay: First Test for TA 1535, TA 100, TA 1537, TA 98

Concentration [µg/plate]	Number of mutant cells							
	TA 1535		TA 100		TA 1537		TA 98	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
0								
37.5								
75								
150								
300								
600								
1200								
2400								
positive control								
2-Amino-anthracene								

*) bacteriotoxic effects observed by titre determination

b) rcduction in background growth

Table A6_6_1-1.B: Table for Gene Mutation Assay: Repeated Tests for TA 1535, TA 100, TA 1537, TA 98

Concent-ration [µg/plate]	Number of mutant cells											
	TA 1535			TA 100			TA 1537			TA 98		
	-S9	+S9 10%	+S9 30%	-S9	+S9 10%	+S9 30%	-S9	+S9 10%	+S9 30%	-S9	+S9 10%	+S9 30%
0												
39.5												
59.3												
88.9												
133.3												
200												
300												
450												
positive control												
2-Amino-anthracene												

*) bacteriotoxic effects observed by titre determination

Table A6_6_1-1.C: Table for Gene Mutation Assay: First and Repeated Tests for TA 1538

Concent-ration [µg/plate]	Number of mutant cells							
	TA 1538							
	First test				Repeated test			
	-S9	+S9 10%	-S9	+S9 30%	-S9	+S9 10%	-S9	+S9 30%
0								
39.5								
59.3								
88.9								
133.3								
200								
300								
450								
positive control								
2-Amino-anthracene								

*) bacteriotoxic effects observed by titre determination

b) reduction in background growth

Section A6.6.2 Genotoxicity in vitro

Annex Point IIA6.6.2 6.6.2. In-vitro cytogenicity study in human lymphocytes

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] (1988), In vitro cytogenetic study with human lymphocytes for the detection of induced clastogenic effects [redacted], Report No. [redacted], 1988-02-02
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes OECD-Guideline 473
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]

3 MATERIALS AND METHODS

3.1	**Test material**	HWG 1608
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	
3.1.2.1	Description	beige powder
3.1.2.2	Purity	[redacted]
3.1.2.3	Stability	A stability test in the solvent did not detect a relevant change in the percent active ingredient.
3.2	**Study Type**	In Vitro mammalian chromosome aberration test
3.2.1	Organism/cell type	primary cultures: human lymphocytes
3.2.2	Deficiencies / Proficiencies	
3.2.3	Metabolic activation system	Male Sprague-Dawley-rats, induced by Aroclor 1254, served as source of the S9 fraction.
3.2.4	Positive control	without S9 mix: Mitomycin C (0.15 µg/ml) with S9 mix: Cyclophosphamide (15 µg/ml)

Section A6.6.2 Genotoxicity in vitro

Annex Point IIA6.6.2 6.6.2. In-vitro cytogenicity study in human lymphocytes

3.3	**Application of test substance**	
3.3.1	Concentrations	concentrations used for treatment without S9 mix: 0, 3, 10 and 30 µg/ml culture medium concentrations used for treatment with S9 mix: 0, 30, 100 and 300 µg/ml culture medium The treatments used were based on a pilot study in which the concentrations were 1, 10, 100, 1000 and 5000 µg/ml. The results indicated a higher cytotoxicity of test substance when applied without S9 mix.
3.3.2	Way of application	dissolved in medium (solvent: DMSO)
3.3.3	Pre-incubation time	
3.3.4	Other modifications	
3.4	**Examinations**	
3.4.1	Number of cells evaluated	The mitotic index was determined by counting 1000 cells per culture (4000 per concentration). Approximately 100 metaphases per sex and test group were evaluated (200 per concentration). The structural chromosome damage was assessed by using the terminology defined by Rieger and Michaels (Die Chromosomen-mutation, VEB Gustav Fischer Verlag, Jena, 1967)
	4 RESULTS AND DISCUSSION	
4.1	**Genotoxicity**	
4.1.1	without metabolic activation	No
4.1.2	with metabolic activation	No
4.2	**Cytotoxicity**	Yes 100 and 300 µg/ml of test substance with S9 mix showed cytotoxic effects on lymphocytes

Section A6.6.2

Annex Point IIA6.6.2

Genotoxicity in vitro

6.6.2. In-vitro cytogenicity study in human lymphocytes

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The study was done according to OECD-Guideline 473, though not stated in the study report. Human lymphocytes were stimulated to divide by the plant lectin, phytohaemagglutinin, in the culture medium and then were treated with the test compound. After addition of the spindle inhibitor colcemid, both the mitotic index and the chromosome aberration rate were determined.

Human lymphocytes were gained from the blood of one male and one female healthy donor. There were two cultures per donor per concentration. The ratio of number of cells or metaphases evaluated was always 1:1 for cultures from male or female donor, respectively.

5.2 Results and discussion

After treatment of lymphocytes at concentrations of up to 30 µg/ml without S9 mix and 300 µg/ml with S9 mix, respectively, the test substance produced a fall in mitotic index in human lymphocyte cultures only with S9 mix. Without S9 mix no such effect was noted.

Evaluation of the individual groups with respect to parameters relevant for evaluating clastogenicity detected no variations of biological relevance between the groups.

The results for the positive controls mitomycin C and cyclophosphamide indicated a clear clastogenic effect and documented the system's sensitivity.

5.3 Conclusion

Under the stated test conditions, the test substance did not show a clastogenic effect on human lymphocytes.

5.3.1 Reliability [redacted]

5.3.2 Deficiencies [redacted]

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	August 2005
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	

Table A6_6_1-2. Table for Cytogenetic In-Vitro-Test: Chromosomal Analysis

		Treatment without S9 mix					
		number evalutated	negative control	low dose 3 µg/ml	mid dose 10 µg/ml	high dose 30 µg/ml	Mito-mycin C
cytotoxicity (mitoses)		4000					
mitoses absolute and in % of negative control		4000					
mitotic index		4000					
chromatid aberrations	gaps	200					
	breaks	200					
	fragments	200					
	deletion	200					
	exchange	200					
	multiple aberrations	200					
polyploidy		400					

		Treatment with S9 mix					
		number evalutated	negative control	low dose 30 µg/ml	mid dose 100 µg/ml	high dose 300 µg/ml	Cyclo-phospha-mide
cytotoxicity (mitoses)		4000					
mitoses absolute and in % of negative control		4000					
mitotic index		4000					
chromatid aberrations	gaps	200					
	breaks	200					
	fragments	200					
	deletion	200					
	exchange	200					
	multiple aberrations	200					
polyploidy		400					

* p ≤0.01 in chi-square test

Section A6.6.3 Genotoxicity in vitro

Annex Point IIA6.6.3 6.6.3. In-vitro gene mutation assay in CHO-cells (HPRT-test)

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] (1988), Mutagenicity study for the detection of induced forward mutations in the CHO-HGPRT assay in vitro, [redacted], Report No. [redacted], 1988-05-31
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes OECD-Guideline 476
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]

3 MATERIALS AND METHODS

3.1	**Test material**	HWG 1608
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	
3.1.2.1	Description	white powder
3.1.2.2	Purity	[redacted]
3.1.2.3	Stability	A stability test in the solvent did not detect an indication of a relevant change in the active ingredient.
3.2	**Study Type**	In vitro mammalian cell gene mutation test
3.2.1	Organism/cell type	mammalian cell lines: Chinese hamster Ovary (CHO)
3.2.2	Deficiencies / Proficiencies	
3.2.3	Metabolic activation system	Male Sprague-Dawley-rats, induced by Aroclor 1254, served as source of the S9 fraction.
3.2.4	Positive control	without S9 mix: Ethylmethanesulfonate (907 µg/ml) with S9 mix: 3-Methylcholanthrene (5 µg/ml)

Section A6.6.3 Genotoxicity in vitro

Annex Point IIA6.6.3

6.6.3. In-vitro gene mutation assay in CHO-cells (HPRT-test)

3.3	**Application of test substance**	
3.3.1	Concentrations	concentrations used for treatment without S9 mix: 80, 90, 92.5, 95, 97.5 and 100 µg/ml culture medium concentrations used for treatment with S9 mix (5%): 12.5,25, 50, 100, 150 and 200 µg/ml culture medium The test concentrations were based on a pilot study in which the dose ranged from 5 to 125 µg/ml without S9 mix and from 3.9 to 1000 µg/ml with S9 mix.
3.3.2	Way of application	dissolved in medium (solvent: DMSO)
3.3.3	Pre-incubation time	
3.3.4	Other modifications	
3.4	**Examinations**	
3.4.1	Number of cells evaluated	

4 RESULTS AND DISCUSSION

4.1	**Genotoxicity**	
4.1.1	without metabolic activation	No
4.1.2	with metabolic activation	No
4.2	**Cytotoxicity**	Yes Without S9 mix, a dose-related decrease in relative population growth was observed only in the third trial over the whole treatment range. With S9 mix, all cells were lost at a concentration of 200 µg/ml in all three trials. In addition, cells were also killed in the third trial at 150 µg/ml. In all assays, high toxicities were induced so that the treated cultures showed dose-related decreases in both relative survival to treatment and relative population growth.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The study was done according to OECD-Guideline 476, though not stated in the study report. Three trials were performed each for treatment with and without activation. In the first two trials, no duplicates were used. The third trial was performed employing duplicates.
5.2	**Results and discussion**	Under both treatment conditions (with and without S9 mix), the test substance induced cytotoxic effects. There were neither dose-related nor reproducible increases in mutant frequency, which were significantly elevated over the negative controls. In contrast, the positive controls revealed a clear mutagenic effect in this assay.
5.3	**Conclusion**	Under the stated test conditions, the test substance is considered non-mutagenic in the CHO-HPRT assay.
5.3.1	Reliability	[redacted]
5.3.2	Deficiencies	[redacted]

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	August 2005
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	

Table A6_6_1-1. Table for Gene Mutation Assay

Treatment without S9 mix					
Concentration [µg/ml]	Mutant Frequency (Thioguanin-resistant mutants per 10^6 clonable cells)				Comments
	1st trial	2nd trial	3rd trial with duplicates		
Negative control	[redacted]	[redacted]	[redacted]	[redacted]	
Vehicle control	[redacted]	[redacted]	[redacted]	[redacted]	
80	[redacted]	[redacted]	[redacted]	[redacted]	
90	[redacted]	[redacted]	[redacted]	[redacted]	
92.5	[redacted]	[redacted]	[redacted]	[redacted]	
95	[redacted]	[redacted]	[redacted]	[redacted]	
97.5	[redacted]	[redacted]	[redacted]	[redacted]	
100	[redacted]	[redacted]	[redacted]	[redacted]	
Positive control	[redacted]	[redacted]	[redacted]	[redacted]	

* significant increase, $p<0.05$

Table A6_6_1-1. Table for Gene Mutation Assay

Treatment with 5% S9 mix				
Concentration [µg/ml]	Mutant Frequency (Thioguanin-resistant mutants per 10^6 clonable cells)			Comments
	1st trial	2nd trial	3rd trial with duplicates	
Negative control	[redacted]	[redacted]	[redacted]	
Vehicle control	[redacted]	[redacted]	[redacted]	
12.5		[redacted]	[redacted]	[redacted]
25	[redacted]	[redacted]	[redacted]	
50	[redacted]	[redacted]	[redacted]	
100	[redacted]	[redacted]	[redacted]	
150	[redacted]	[redacted]		[redacted]
200				[redacted]
Positive control	[redacted]	[redacted]	[redacted]	

* significant increase, $p<0.05$

Section 6.6.4 - 6.6.6 Annex Point IIA 6.6.4-6.6.6.	*In vivo* studies on mutagenicity	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [...]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	According to BPD, Annex IIA, *in vivo* studies on mutagenicity are only required if there would be a positive *in vitro* test on mutagenicity. Tebuconazole was not positive in any of the required mutagenicity tests. Therefore no *in vivo* test must be supplied.	
Undertaking of intended data submission [ ]	–	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	July 2005	
Evaluation of applicant's justification	[redacted]	
Conclusion	[redacted]	
Remarks	[redacted]	

Section A6.8.1 (01) Teratogenicity Study

Annex Point IIA6.8.1

[redacted]., 1985. HWG 1608 – Study for embryotoxic effects on **rats** after oral administration.

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1 REFERENCE

1.1	**Reference**	[redacted] 1985, HWG 1608. Study for Embryotoxic Effects on Rats after Oral Administration. [redacted]), Report no. [redacted], 1985-02-08 (unpublished).
1.1	**Data protection**	[redacted]
1.1.1	Data owner	[redacted]
1.1.2	Companies with letter of access	[redacted]
1.1.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No. Method used is comparable to OECD guideline 414 "Teratogenicity".
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]

3 MATERIALS AND METHODS

3.1	**Test material**	HWG 1608
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	
3.1.2.1	Description	No information given in the report
3.1.2.2	Purity	[redacted] active ingredient
3.1.2.3	Stability	Checked and accepted is what the report tells
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	WISW
3.2.3	Source	[redacted]
3.2.4	Sex	Female – inseminated
3.2.5	Age/weight at study initiation	Sexually mature – 184 – 216 g at study start
3.2.6	Number of animals per group	25
3.2.7	Control animals	Yes (vehicle controls)
3.2.8	Mating period	Until sperm positive vaginal smear
3.3	**Administration/ Exposure**	Oral

Section A6.8.1 (01)	Teratogenicity Study
Annex Point IIA6.8.1	[redacted]., 1985. HWG 1608 – Study for embryotoxic effects on **rats** after oral administration.

3.3.1	Duration of exposure	Rat: day 6-15 post mating
3.3.2	Postexposure period	Days 16 – 20 of gestation
		Oral
3.3.3	Type	Gavage
3.3.4	Concentration	Gavage 0, 10, 30, 100 mg/kg bw
3.3.5	Vehicle	0.5 % Cremophor solution
3.3.6	Concentration in vehicle	0, 0.1, 0.3 or 1 %
3.3.7	Total volume applied	10 ml/kg bw
3.3.8	Controls	Vehicle
3.4	**Examinations**	
3.4.1	Body weight	Yes
3.4.2	Food consumption	No
3.4.3	Clinical signs	Yes
3.4.4	Examination of uterine content	Gravid uterine weight – not recorded Number of corpora lutea - No Number of implantations - Yes Or other – Placental weights recorded
3.4.5	Examination of foetuses	
3.4.5.1	General	Litter Size, Nr. of dead Foetuses, Foetal Weight, Sex Ratio – are recorded
3.4.5.2	Skeleton	Yes
3.4.5.3	Soft tissue	Yes
3.5	**Further remarks**	–
		4 RESULTS AND DISCUSSION
4.1	**Maternal toxic Effects**	30 and 100 mg/kg bw – reduced weight gains. Faecal alteration - only 100 mg/kg group.
4.2	**Teratogenic / embryotoxic effects**	Only in 100 mg/kg bw group: Significantly ($P < 0.5$) increased numbers of malformations and runts/ losses. Significantly ($P < 0.1$) decreased foetal weight.
4.3	**Other effects**	Significantly increased number of implants ($P < 0.5$) in high dose group Most probably incidental.

Section A6.8.1 (01) Teratogenicity Study

Annex Point IIA6.8.1

[redacted]., 1985. HWG 1608 – Study for embryotoxic effects on **rats** after oral administration.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Groups of 25 inseminated Wistar rats were given by stomach tube on day 6 to day 15 of gestation daily doses of 0, 10, 30 or 100 mg/kg bw HWG 1608 ([redacted] pure). The test substance was suspended in 0.5 % Cremophor solution and was administered at 10 ml/kg bw (0, 0.1, 0.3 or 1 %, respectively).

The dams were examined daily for clinical signs, appearance and behaviour and weights were recorded regularly (intervals not stated). Foetuses were delivered by Caesarian section on day 20 of gestation and were weighed, sex determined, and examined for either visceral malformations (Wilson staining) or skeletal deviations and malformations (Dawson staining). The uteri were examined for number of resorptions and placental weights.

No test guideline is given – and small deviation from OECD guideline 414 "Teratogenicity" is recorded.

There is no record of food consumption – may not be very important. In the OECD guideline 414 is stated that *up to 50 % of the foetuses* should be examined for skeletal deviations and malformations and the rest for visceral malformations but in this study on average 70 % were examined with skeletal staining procedure – it may not be important either. The foetuses are very small and are most probably delivered one day early.

5.2 Results and discussion

The dams' weight gains in the 30 and 100 mg/kg bw groups were reduced (dose related) compared to controls and there were other signs (appearance and light faeces) in the high dose group indicating maternal toxicity.

5.3 Conclusion

5.3.1	LO(A)EL maternal toxic effects	Reduced weight gain at 30 mg/kg
5.3.2	NO(A)EL maternal toxic effects	10 mg/kg
5.3.3	LO(A)EL embryotoxic / teratogenic effects	Foetal anomalies increased at 100 mg/kg
5.3.4	NO(A)EL embryotoxic / teratogenic effects	30 mg/kg
5.3.5	Reliability	[redacted]
5.3.6	Deficiencies	Yes – very small foetuses due to early Caesarean operation Reporting is not sufficient – no raw data presentation

Section A6.8.1 (01)

Annex Point IIA6.8.1

Teratogenicity Study

[redacted]., 1985. HWG 1608 – Study for embryotoxic effects on **rats** after oral administration.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	July 2005
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

Table A6_8-1. Table for Teratogenic effects (separate data for all dosage groups)

Maternal effects

Modify if necessary and give historical data if available

Parameter	control data		low dose 10 mg/kg	medium dose 30 mg/kg	high dose 100 mg/kg	dose-response + / -
	Historical	study				
Number of dams examined		**22**	**18**	**21**	**24**	
Clinical findings during application of test substance		[redacted]	[redacted]	[redacted]	[redacted]	
Mortality of dams *state %*		[redacted]	[redacted]	[redacted]	[redacted]	
Abortions		[redacted]	[redacted]	[redacted]	[redacted]	
Body weight gain *During pregnancy, during treatment*		[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Food consumption						
Water consumption *if test substance is applied with drinking water*						
Pregnancies *%*		[redacted]	[redacted]	[redacted]	[redacted]	
Necropsy findings in dams dead before end of test		[redacted]	[redacted]	[redacted]	[redacted]	

* Significantly different from study controls ($p \leq 0.05$)

** Significantly different from study controls ($p \leq 0.01$)

Table A6_8-2. Table for Teratogenic effects (separate data for all dosage groups)

Litter response (Caesarean section data)

Modify if necessary and give historical data if available

Parameter	Control data		low dose 10 mg/kg	medium dose 30 mg/kg	high dose 100 mg/kg	dose-response + / -
	Historical	Study				
Corpora lutea *state total/number of dams*						
Implantations *state total/number of dams*		[redacted]	[redacted]	[redacted]	[redacted]	
Resorptions *state total/number of dams*		[redacted]	[redacted]	[redacted]	[redacted]	
total number of fetuses (calculated- no raw data given)		[redacted]	[redacted]	[redacted]	[redacted]	
pre-implantation loss *state %*						
post-implantation loss *state %*						
total number of litters		[redacted]	[redacted]	[redacted]	[redacted]	
fetuses / litter		[redacted]	[redacted]	[redacted]	[redacted]	
live fetuses / litter *state ratio*		[redacted]	[redacted]	[redacted]	[redacted]	
dead fetuses / litter *state ratio*		[redacted]	[redacted]	[redacted]	[redacted]	
fetus weight (mean) *[g]*		[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
placenta weight (mean) *[g]*		[redacted]	[redacted]	[redacted]	[redacted]	
crown-rump length (mean) *[mm]*						
Fetal sex ratio *[state ratio m/f]*		[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

* Significantly different from study controls ($p \leq 0.05$)

** Significantly different from study controls ($p \leq 0.01$)

Table A6_8-3. Table for Teratogenic effects (separate data for all dosage groups)

Examination of the fetuses

Modify if necessary and give historical data if available

Parameter	Control data		low dose 10 mg/kg	Medium dose 30 mg/kg	high dose 100 mg/kg	dose-response + / -
	Historical	Study				
External malformations[*1] *[%]*		[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
External anomalies[*] *[%]*						
Skeletal malformations[*] *[%]*						
Skeletal anomalies[*] *[%]*						
Skeletal variants[*] *[%]*		[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Visceral malformations[*] *[%]*						
Visceral anomalies[*] *[%]*						
Variants visceral[*] *[%]*						

*1: [redacted]

* Significantly different from study controls ($p \leq 0.05$)

** Significantly different from study controls ($p \leq 0.01$)

Section A6.8.1(02) Teratogenicity Study

Annex Point IIA6.8.1

[redacted] 1988, Embryotoxicity study (including teratogenicity) with HWG 1608 technical in the **rat** (oral).

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] 1988, Embryotoxicity Study (including Teratogenicity) with HWG 1608 Technical in the Rat, [redacted], Project [redacted], 1988-04-28 (unpublished).
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]

3 MATERIALS AND METHODS

3.1	**Test material**	HWG 1608 Technical
3.1.1	Lot/Batch number	Batch No: [redacted]
3.1.2	Specification	
3.1.2.1	Description	Colourless crystals
3.1.2.2	Purity	[redacted] active substance
3.1.2.3	Stability	Stored at room temperature, in the dark stable until February 2. 1988
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Wistar/HAN (outbred, SPF quality)
3.2.3	Source	[redacted]
3.2.4	Sex	Mated females
3.2.5	Age/weight at study initiation	Minimum 12 weeks/ 180-235 g
3.2.6	Number of animals per group	25
3.2.7	Control animals	Yes
3.2.8	Mating period	Until sperm-positive vaginal smear or copulation plug identified
3.3	**Administration/ Exposure**	Oral by intubation

Section A6.8.1(02) **Teratogenicity Study**

Annex Point IIA6.8.1 [redacted] 1988, Embryotoxicity study (including teratogenicity) with HWG 1608 technical in the **rat** (oral).

3.3.1	Duration of exposure	rat: day 6-15 post mating
3.3.2	Postexposure period	Day 16 to 21 post mating
		Oral
3.3.3	Type	Gavage
3.3.4	Concentration	Gavage 0, 30, 60, 120 mg/kg bw
3.3.5	Vehicle	Suspended in Cremophor EL 0.5 % solution
3.3.6	Concentration in vehicle	0, 0.3, 0.6 or 1.2 %, respectively
3.3.7	Total volume applied	10 ml/kg bw
3.3.8	Controls	Vehicle
3.4	**Examinations**	
3.4.1	Body weight	Yes
3.4.2	Food consumption	Yes
3.4.3	Clinical signs	Yes, minimum twice daily
3.4.4	Examination of uterine content	Gravid uterine weight Number of corpora lutea Number of implantations Position of foetuses
3.4.5	Examination of foetuses	
3.4.5.1	General	Litter Size, Nr. of dead Foetuses, Foetal Weight, Sex Ratio
3.4.5.2	Skeleton	Yes – modified Dawson staining techniques used – 50 % of foetuses
3.4.5.3	Soft tissue	Yes – Wilson's slicing technique – 50 % of foetuses
3.5	**Further remarks**	

4 RESULTS AND DISCUSSION

4.1	**Maternal toxic Effects**	Toxic effects related to dosing: Food consumption was reduced during treatment in 60 and 120 mg/kg bw dose groups. Slight weight loss occurred at initiation of dosing in the 60 and 120 mg/kg bw dose groups. Significantly lower weight gains from day 7 to day 21 occurred in the 120 mg/kg bw dose group, but the values for body weight gain corrected for uterus weight were not significantly lower. Of necropsy findings only black/brown coloured fluid in the uterus in 9/25 dams in the 120 mg/kg bw dose group was significantly different from controls. Liver weights and liver weight/body weight ratios were significantly increased in the 60 and 120 mg/kg bw dose groups.

Section A6.8.1(02) **Teratogenicity Study**

Annex Point IIA6.8.1 [redacted] 1988, Embryotoxicity study (including teratogenicity) with HWG 1608 technical in the **rat** (oral).

4.2	**Teratogenic / embryotoxic effects**	There were significantly increased number of resorptions (both embryonic and foetal) and the number of live foetuses significantly decreased in the 120 mg/kg bw dose group. Mean foetal body weight was decreased in the 120 mg/kg bw dose group. Skeletal examination revealed statistically significant increases in the following areas in the 120 mg/kg bw dose group: Super-numary ribs, non-ossified cervical vertebrae nos. 1-6, sacral vertebral arches nos. 6 and 7, various phalangeal nuclei and incompletely ossified sternebra no. 2.
4.3	**Other effects**	No other effects were reported.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The study was performed according to OECD guideline 414 for the testing of chemicals – Teratogenicity – and EPA Pesticide Assessment Guidelines Paragraph 163.83-3 (Teratogenicity Study) and in accordance with Swiss, EPA (US) and OECD principles of GLP. Four groups of each 25 mated female Wistar/HAN rats were given on day 6-15 post mating daily doses of HWG 1608 Technical ([redacted] pure) of 0, 30, 60 and 120 mg/kg, respectively. The substance was suspended in 0.5 % Cremophor EL solution. The dosed volumes of 10 mg/kg bw were corrected each day according to the actual body weight of each animal. The animals were weighed each day of the study and food consumption was recorded on days 6, 11, 16 and 21 post mating. The animals were inspected for deaths and clinical signs at least twice daily. The dams were killed by CO_2 asphyxiation on day 21 after mating and the uteri were removed by Caesarean section. The livers of all females were weighed and stored for possible processing and histological examination. The post mortem examination performed included gross macroscopic examination of all internal organs, with emphasis on the uterus, uterine contents, position of foetuses in the uterus and number of corpora lutea. All results were recorded. The foetuses were removed from the uterus, sexed, weighed individually, examined for gross external abnormalities and allocated to either Wilson's slicing technique for examination of viscera and brain or to a modified Dawson stain technique for examination of skeletal abnormalities. Descriptions of all abnormalities were recorded. The uteri (and contents) were weighed. Non pregnant uteri were placed in aqueous ammonium sulphide to accentuate possible haemorrhagic areas of implantation sites.
5.2	**Results and discussion**	Maternal toxic effects related to dosing: Food consumption was reduced during treatment in 60 and 120 mg/kg bw dose groups. Slight weight loss occurred at initiation of dosing in the 60 and 120 mg/kg bw dose groups. Significantly lower weight gains from day 7 to day 21 occurred in the 120 mg/kg bw dose group, but the values for body weight gain corrected for uterus weight were not significantly lower. Of necropsy findings only black/brown coloured fluid in the uterus in 9/25 dams in the 120 mg/kg bw dose group was significantly different from controls. Liver weights and liver weight/body weight ratios were significantly increased in the 60 and 120 mg/kg bw dose groups. Thus, maternal toxicity is seen at 60 mg HWG 1608 Technical/kg bw in this study. Teratogenic effects: There were significantly increased number of resorptions (both embryonic and foetal) and the number of live foetuses significantly decreased in the 120 mg/kg bw dose group. Mean foetal body weight was decreased in the 120 mg/kg bw dose group. Skeletal examination revealed statistically significant increases in the following

Section A6.8.1(02) **Teratogenicity Study**

Annex Point IIA6.8.1 [redacted] 1988, Embryotoxicity study (including teratogenicity) with HWG 1608 technical in the **rat** (oral).

		areas in the 120 mg/kg bw dose group: Super-nummary ribs, non-ossified cervical vertebrae nos. 1-6, sacral vertebral arches nos. 6 and 7, various phalangeal nuclei and incompletely ossified sternebra no. 2. Embryotoxic effects are seen after dosing with 120 mg HWG 1608 Technical/kg (increased number of resorptions, decreased no. of live foetuses and decreased foetal body weight). Effects on foetuses are also seen after dosing with 120 mg HWG 1608 Technical/kg (skeletal malformations).
5.3	**Conclusion**	
5.3.1	LO(A)EL maternal toxic effects	Decreased food consumption during substance administration and increased liver weight and relative liver weight in the 60 mg HWG 1608 Technical/kg bw group.
5.3.2	NO(A)EL maternal toxic effects	30 mg HWG 1608 Technical/kg
5.3.3	LO(A)EL embryotoxic / teratogenic effects	120 mg HWG 1608 Technical/kg for both embryotoxic (increased no. of resorptions, decreased no. of live foetuses and decreased foetal body weight) and teratogenic effects (increased no. of skeletal abnormalities).
5.3.4	NO(A)EL embryotoxic / teratogenic effects	60 mg HWG 1608 Technical/kg bw
5.3.5	Reliability	[redacted]
5.3.6	Deficiencies	[redacted]

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	July 2005
Materials and Methods	[redacted]
Results and discussion	[redacted]
	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

Table A6_8-1. Table for Teratogenic effects (separate data for all dosage groups)

Maternal effects

Modify if necessary and give historical data if available

Parameter	control data		low dose 30 mg/kg	medium dose 60 mg/kg	high dose 120 mg/kg	dose-response + / -
	historical	Study				
Number of dams examined	**505**	**24**	**24**	**22**	**24**	
Clinical findings during application of test substance		[redacted]	[redacted]	[redacted]	[redacted]	
Mortality of dams *state %*	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	
Abortions	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	
Body weight gain *day 6-end of test,*		[redacted]	[redacted]	[redacted]	[redacted]	
Food consumption *Day 6-16*		[redacted]	[redacted]	[redacted]	[redacted]	
Water consumption *if test substance is applied with drinking water*						
Pregnancies *%*	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	
Necropsy findings in dams dead before end of test		[redacted]	[redacted]	[redacted]	[redacted]	

* Significantly different from study controls ($p \leq 0.05$)

** Significantly different from study controls ($p \leq 0.01$)

Table A6_8-2. Table for Teratogenic effects (separate data for all dosage groups)

Litter response (Caesarean section data)

Modify if necessary and give historical data if available

Parameter	Control data		low dose 30 mg/kg	medium dose 60 mg/kg	High dose 120 mg/kg	dose-response + / -
	historical	Study				
Corpora lutea *state total/number of dams*	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	
Implantations *state total/number of dams*	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	
Resorptions *state total/number of dams*		[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
total number of fetuses	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
pre-implantation loss *state %*	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	
post-implantation loss *state %*	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
total number of litters	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	
fetuses / litter	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
live fetuses / litter *state ratio*	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
dead fetuses / litter *state ratio*	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	
fetus weight (mean) *[g]*	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
placenta weight (mean) *[g]*						
crown-rump length (mean) *[mm]*						
Fetal sex ratio *[state ratio m/f]*	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

* Significantly different from study controls ($p \leq 0.05$)

** Significantly different from study controls ($p \leq 0.01$)

Table A6_8-3. Table for Teratogenic effects (separate data for all dosage groups)

Examination of the fetuses

Modify if necessary and give historical data if available

Parameter	Control data		low dose 30 mg/kg	medium dose 60 mg/kg	high dose 120 mg/kg	dose-response + / -
	historical	Study				
External malformations[*1] *[%]*	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	
External anomalies[*] *[%]*		[redacted]	[redacted]	[redacted]	[redacted]	
Skeletal malformations[*] *[%]*						
Skeletal anomalies[*2] *[%]*	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
Skeletal variants[*3] *[%]*						
Visceral malformations[*4] *[%]*	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	
Visceral anomalies[*5] *[%]*		[redacted]	[redacted]	[redacted]	[redacted]	
Variants visceral[*] *[%]*						

*1: [redacted]

*2: [redacted]

*3: [redacted]

*4: Same as *1

*5: [redacted]

Section A6.8.1(03) Teratogenicity Study

Annex Point IIA6.8.1

[redacted] **1988**, HWG 1608 – Study for embryotoxic effects on **rats** after dermal administration

Official use only

1 REFERENCE

1.1	**Reference**	[redacted]: 1988, HWG 1608 – Study for embryotoxic effects on rats after dermal administration, [redacted] Report No: [redacted], 1988-08-30 (unpublished).
1.2	**Data protection**	[redacted]
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
1.2.3	Criteria for data protection	[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes Pesticide Assessment Guidelines Subdivision F. Hazard Evaluation: Human and Domestic Animals, EPA, 83-3, Teratogenicity Study, revised edition, November 1984
2.2	**GLP**	[redacted]
2.3	**Deviations**	[redacted]

3 MATERIALS AND METHODS

3.1	**Test material**	HWG 1608
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	
3.1.2.1	Description	Grey-white powdery crystals
3.1.2.2	Purity	[redacted] active substance
3.1.2.3	Stability	Approved until 20.09.1987 – stored in refrigerator at approx. 4 °C
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	WISW (SPF Cph)
3.2.3	Source	[redacted]
3.2.4	Sex	mated females
3.2.5	Age/weight at study initiation	Sexually mature/194-242 g
3.2.6	Number of animals per group	25
3.2.7	Control animals	Yes
3.2.8	Mating period	Until sperm was found in vaginal smear

Section A6.8.1(03) Teratogenicity Study

Annex Point IIA6.8.1 [redacted] **1988**, HWG 1608 – Study for embryotoxic effects on **rats** after dermal administration

3.3	**Administration/ Exposure**	Dermal
3.3.1	Duration of exposure	Rat: day 6-15 post mating
3.3.2	Postexposure period	Days 16-20
		Dermal
3.3.3	Type	Applied to shaved skin on gauze dressing with aluminium foil base for six hours
3.3.4	Concentration	0, 100, 300, or 1000 mg/kg bw
3.3.5	Vehicle	Suspended in 1 % aqueous Cremophor EL emulsion
3.3.6	Concentration in vehicle	0, 5, 15, or 50 %
3.3.7	Total volume applied	2 ml/kg bw
3.3.8	Controls	Vehicle
3.4	**Examinations**	
3.4.1	Body weight	Yes
3.4.2	Food consumption	Yes
3.4.3	Clinical signs	Yes – at least once daily
3.4.4	Examination of uterine content	Gravid uterine weight – yes Number of corpora lutea – yes Number of implantations – yes
3.4.5	Examination of foetuses	
3.4.5.1	General	Litter Size, Nr. of dead Foetuses, Foetal Weight, Sex Ratio, and head/trunk length
3.4.5.2	Skeleton	Yes – 122 controls, 119 from 100 mg/kg group, 127 from 300 mg/kg group, and 120 from 1000 mg/kg group.
3.4.5.3	Soft tissue	Yes –108 controls, 107 from 100 mg/kg group, 115 from 300 mg/kg group, and 105 from 1000 mg/kg group.
3.5	**Further remarks**	
		4 RESULTS AND DISCUSSION
4.1	**Maternal toxic Effects**	No effects
4.2	**Teratogenic / embryotoxic effects**	No effects
4.3	**Other effects**	None recorded except damages to the skin – similar in controls and dosed animals.

Section A6.8.1(03) **Teratogenicity Study**

Annex Point IIA6.8.1 [redacted] **1988**, HWG 1608 – Study for embryotoxic effects on **rats** after dermal administration

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	In accordance with the EPA Guidelines 83-3, "Teratology Study" and OECD Principles of Good Laboratory Practice were groups of 25 mated female Wistar rats applied daily doses of 0, 100, 300, or 1000 mg/kg [redacted] pure HWG 1608 on days 6 to 15 of gestation. The substance suspended in 1 % aqueous Cremophor EL emulsion was applied evenly to the shorn skin of the backs and were held in position using occlusive bandage. Six hours after the application the bandage was removed and the skin was washed with lukewarm water. The animals were inspected at least once daily for mortality, clinical signs, changed appearance and behaviour. The animals were weighed at regular intervals and food consumption was recorded. On day 20 of gestation caesarean section was performed on all dams. The following examinations were performed: Necropsy findings on dams, determination of implantation sites, number of corpora lutea and of uterus weight. Determination of number of live and dead foetuses or embryos, determination of the sex and weight of each live foetus, and recording of number of runts, determination of individual placenta weights. Examination of all foetuses for external malformations, a number of the foetuses for visceral malformations (modified Wilson's method) and the rest of the foetuses for bone alterations (alizarin red S stained) after exenteration and appraisal of the abdominal and thoracic organs.
5.2	**Results and discussion**	None of the parameters examined, whether with respect to gestation index, reproduction toxicity or teratogenicity as well as toxicity to the dams were significantly different between any dose group and the controls.
5.3	**Conclusion**	HWG 1608 was not toxic to any of the parameters examined in this test. No maternal toxicity was obtained so most probably the doses tested were too low.
5.3.1	LO(A)EL maternal toxic effects	No maternal toxicity was obtained.
5.3.2	NO(A)EL maternal toxic effects	1000 mg/kg bw when administered as in this study
5.3.3	LO(A)EL embryotoxic / teratogenic effects	No toxic effects were recorded.
5.3.4	NO(A)EL embryotoxic / teratogenic effects	1000 mg/kg bw when administered as in this study
5.3.5	Reliability	[redacted]
5.3.6	Deficiencies	[redacted]

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	July 2005
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

Table A6_8-1. Table for Teratogenic effects (separate data for all dosage groups)

Maternal effects

Modify if necessary and give historical data if available

Parameter	control data		Low dose 100 mg/kg	medium dose 300 mg/kg	high dose 1000 mg/kg	dose-response + / -
	historical	study				
Number of dams examined	**260**	**25**	**25**	**25**	**25**	
Clinical findings during application of test substance						
Mortality of dams *state %*		[redacted]	[redacted]	[redacted]	[redacted]	
Abortions		[redacted]	[redacted]	[redacted]	[redacted]	
Body weight gain *Day 6-15/ day 0-end of test,*		[redacted]	[redacted]	[redacted]	[redacted]	
Food consumption		[redacted]	[redacted]	[redacted]	[redacted]	
Water consumption *if test substance is applied with drinking water*						
Pregnancies *pregnancy rate or %*		[redacted]	[redacted]	[redacted]	[redacted]	
Necropsy findings in dams dead before end of test						