TRIFLOXYSTROBIN

First draft prepared by
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Explanation

Trifloxystrobin (methyl(*E*)-methoxyimino-{(*E*)-α-[1-(α,α,α,-trifluoro-*m*-tolyl)-ethylideneaminooxy]-*o*-tolyl}acetate) is a new broad-spectrum foliar fungicide that has not been evaluated previously by the JMPR.

Trifloxystrobin is being evaluated as a foliar fungicide for the control of fungi from the classes *Ascomycetes*, *Deuteromycetes*, *Basidiomycetes* and *Oomycetes*, in particular, for the treatment of powdery mildew and leaf spot diseases of pome fruit, grapes and bananas.

Trifloxystrobin is a synthetic derivative of the naturally occurring strobilurins found in several genera of wood-decaying fungi such as *Strobilurus tenacellus*. They have been shown to inhibit mitochondrial respiration by blocking electron transfer within the respiratory chain. As a consequence, important cellular biochemical processes are severely disrupted and fungal growth ceases. The intended fungicidal effects are derived from the parent molecule (CGA 279202) of the active ingredient, while the acid form is essentially inactive.

Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution, and excretion

There are three studies of biokinetics and metabolism in rats dosed orally with radiolabelled trifloxystrobin. There are two studies of dermal absorption, a study of absorption in vitro in rat and human skin, and a study of dermal absorption in vivo in rats. Also, there are two studies of metabolism in goats given trifloxystrobin radiolabelled in each ring. The position of the ^{14}C radiolabel is shown in Figure 1.

The fate of [glyoxyl-phenyl-U-^{14}C]-labelled and [trifluormethyl-phenyl-U-^{14}C]-labelled trifloxystrobin (radiochemical purity, >97%–>99%) was investigated in several groups of male and female rats after a single oral administration of ^{14}C-labelled compound at 0.5 or 100 mg/kg bw. An additional group received a single oral dose of [glyoxyl-phenyl-U-^{14}C]-labelled compound after 14 daily oral doses of unlabelled trifloxystrobin at 0.5 mg/kg bw. Urine, faeces, bile, and expired air were individually and separately collected. Blood was taken from three animals of each sex from each group by amputating the tip of the tail.

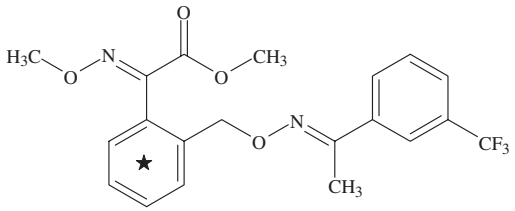
After administration of the trifloxystrobin at a dose of 0.5 mg/kg bw, 57% and 66% of the dose was absorbed (% of administered radiolabel present in urine + cage wash + bile + tissues) from the gastrointestinal tract in bile-duct cannulated male and female rats, respectively (Table 1). Absorption was slightly less after administration of trifloxystrobin at a dose of 100 mg/kg, based on decreased urinary and biliary elimination and a non-proportional increase of the area under the curve of concentration–time (AUC) (Tables 1 and 2).

Seven days after administration of [glyoxyl-phenyl-U-^{14}C]-labelled trifloxystrobin, 19% and 36% of the 0.5 mg/kg bw dose and 12% and 27% of the 100 mg/kg bw dose was excreted in the urine (including cage wash) of male and female rats, respectively (Table 1). The amount eliminated in the faeces was 79% and 63% at 0.5 mg/kg bw and 82% and 64% at 100 mg/kg bw in males and females, respectively (Table 1). Prior repeated dosing did not seem to significantly alter the pattern of excretion (Table 1).

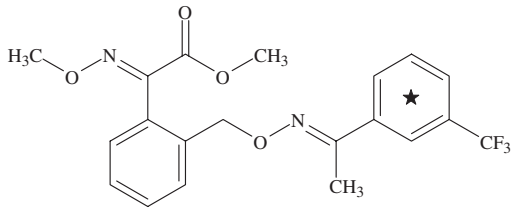
Experiments with bile-duct cannulated rats demonstrated that biliary excretion was the major route of elimination as 12%, 41%, 27% and 15%, 47% and 15% of the 0.5 mg/kg

Figure 1. Position of the ^{14}C radiolabel on trifloxystrobin used in studies of absorption, distribution and excretion

[Glyoxyl-phenyl-U-^{14}C]-trifloxystrobin



[Trifluormethyl-phenyl-U-^{14}C]-trifloxystrobin



★ = position of radiolabel

Table 1. Summary of data on excretion (% of administered dose) in rats given a single oral dose of ^{14}C-labelled trifloxystrobin

Radiolabel	[Glyoxyl-phenyl-U-^{14}C]										[Trifluormethyl-phenyl-U-^{14}C]	
Group	Lower dose		Repeated doses[a]		Higher dose		Bile-duct cannulated rats				Higher dose	
							Lower dose		Higher dose			
Sex	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Dose (mg/kg bw)	0.48	0.50	0.42	0.48	105.5	101.7	0.46	0.50	113.7	99.9	97.5	105.2
Urine:												
0–24 h	13.9	30.0	13.9	36.2	8.4	19.6	6.5	8.6	2.5	5.3	6.2	15.8
24–48 h	3.2	3.9	2.9	4.0	2.7	5.8	5.7	6.0	1.6	0.8[b]	2.4	8.8
48–168 h	1.7	1.3	1.6	1.5	1.0	1.2	—	—	—	—	1.0	2.2
Subtotal	18.8	35.2	18.4	41.7	12.1	26.6	12.2	14.6	4.1	6.1	9.6	26.8
Bile:												
0–48 h	—	—	—	—	—	—	41.0	46.5	34.7	19.1[c]	—	—
Faeces:												
0–24 h	57.1	50.3	54.5	41.0	45.9	32.6	7.6	5.4	8.6	20.8	52.7	25.8
24–48 h	19.0	11.5	20.1	11.8	33.2	26.9	19.7	9.2	35.6	8.3[b]	26.6	29.0
48–72 h	2.2	1.0	3.5	2.4	2.3	4.2	—	—	—	—	3.2	10.4
72–169 h	1.1	0.5	1.2	0.8	0.7	0.5	—	—	—	—	1.5	1.2
Subtotal	79.4	63.3	79.3	56.0	82.1	64.2	27.3	14.6	44.2	29.1	84.0	66.4
Expired air	—	—	—	—	<0.01	<0.01	—	—	—	—	0.08	0.05
Cage wash	0.3	0.5	0.1	0.3	0.2	0.4	1.0	0.9	0.3	0.4	0.4	0.7
Tissues	0.4	0.4	0.5	0.4	0.3	0.3	3.2	4.2	2.1	1.4	0.3	0.4
Total excretion	98.4	98.9	97.9	98.0	94.4	91.2	81.5	76.7	83.4	54.8	94.1	94.0

From Muller (1996)
[a] Daily oral doses (0.5 mg/kg) of unlabelled trifloxystrobin for 14 days
[b] 24–42 h
[c] 0–42 h

Table 2. Summary of blood kinetics in rats given radiolabelled trifloxystrobin as a single oral dose

Radiolabel	[Glyoxyl-phenyl-U-^{14}C]				[Trifluormethyl-phenyl-U-^{14}C]	
Group	Lower dose		Higher dose		Higher dose	
Sex	Male	Female	Male	Female	Male	Female
Dose (mg/kg bw)	0.48	0.49	105.0	99.4	96.5	105.5
C_{max} (ppm trifloxystrobin equivalents)	0.07	0.07	9.34	6.52	6.09	5.94
t_{cmax} (h)	12	12	24	12	24	12
$t_{cmax/2}$ (h)	48	23	50	44	67	52
AUC_{0-48h} (mg.h/kg)	2.7	1.6	334.6	214.3	229.7	214.8
AUC_{0-96h} (mg.h/kg)	3.8	2.3	—	—	375.1	331.6

From Muller (1996)
AUC, area under the curve

bw dose was excreted in the urine, bile and faeces of male and female rats, respectively (Table 1). The urinary and biliary excretion of bile-duct cannulated male and female rats was lower at 100 mg/kg bw than at 0.5 mg/kg bw. The decreased urinary excretion of [glyoxyl-phenyl-U-^{14}C]-labelled trifloxystrobin in bile-duct cannulated rats, especially in females, may be indicative of the involvement of an enterohepatic shunt mechanism in the elimination process. However, poor recoveries of excreted radiolabel may be responsible for the apparent decrease in urinary excretion (Table 1).

After administration of [trifluormethyl-phenyl-U-^{14}C]-labelled trifloxystrobin at 100 mg/kg bw, elimination in the urine within 7 days was 10% in males and 27% in females, and in faeces was 84% in males and 66% in females, indicating that elimination of [trifluormethyl-phenyl-U-^{14}C]-labelled trifloxystrobin was similar to that of [glyoxyl-phenyl-U-^{14}C]-labelled trifloxystrobin. The amount of the dose eliminated in expired air was insignificant and independent of the site of the radiolabel.

Maximum blood concentrations of residues were reached between 12 h and 24 h after a single oral administration, independent of the dose, the sex of the animals and the position of the radiolabel. The half-life ranged from 48–67 h and 23–52 h after dosing in male and female rats, respectively. The areas under the blood concentration–time curve (AUC) were increased by 129-fold at the 100 mg/kg bw when the dose was increased 200-fold, but were not influenced by the sex of the animals.

Assuming first-order kinetics, the half-lives of residues in all tissues ranged from 13 h to 33 h except for blood and spleen, which showed a retarded depletion of 30–82 h and 38–68 h, respectively (Table 3).

Seven days after a single oral dose of [glyoxyl-phenyl-U-^{14}C] trifloxystrobin at 0.5 mg/kg bw, the tissue concentration of residues did not exceed 0.014 ppm trifloxystrobin equivalents. Pre-treatment with unlabelled trifloxystrobin at a dose of 0.5 mg/kg bw for 14 consecutive days did not influence the pattern of distribution of tissue residues. At 100 mg/kg bw, the tissue concentrations of residues were 108–126 times higher than at the lower dose. Differences related to sex and label were apparent in the tissue residues (fat, kidneys, liver, and plasma). Concentrations of residues were generally higher in females than in males. The residues in the blood were associated predominantly with the blood cells and the extent of binding depended on the label and sex of the animals. The blood cell to plasma ratio was 4 : 1 and 18 : 1 for the [glyoxyl-phenyl-U-^{14}C] label and 11 : 1 and 17 : 1 for the [trifluormethyl-phenyl-U-^{14}C] label in male and female rats, respectively (Muller, 1996).

Table 3. Depletion of residual radioactivity (half-life [h]) from selected tissues in rats given [trifluormethyl-phenyl-U-^{14}C]-labelled trifloxystrobin as a single oral dose

Tissue	Dose (mg/kg bw)			
	0.55	0.54	101.9	104.7
	Male	Female	Male	Female
Blood	38	30	40	82
Bone	30	13	26	28
Brain	15	27	31	33
Fat (abdominal)	18	18	18	33
Heart	23	19	26	26
Kidneys	23	21	31	30
Liver	21	15	28	23
Lungs	28	15	28	29
Muscle (skeletal)	20	18	24	25
Ovaries	NA	22	NA	24
Plasma	24	14	23	18
Spleen	39	38	42	68
Testes	26	NA	23	NA
Uterus	NA	22	NA	22

From Muller (1996)
NA, not applicable

In the earlier study, some label-related differences in the extent of tissue residues were observed at a dose of 100 mg/kg bw in rats. Therefore, another study was performed to investigate the disposition of [trifluormethyl-pheny-U-^{14}C]-trifloxystrobin (radiochemical purity, >98%), i.e. absorption, distribution and excretion at 0.5 mg/kg bw and the tissue depletion kinetics at 0.5 and 100 mg/kg bw. In addition, the experiment with bile-duct cannulated female rats dosed with [glyoxyl-phenyl-U-^{14}C]-trifloxystrobin (radiochemical purity, >99%) at 100 mg/kg bw was repeated because of inappropriate low recovery of radioactivity in the previous study. Urine, faeces, bile, and expired air were individually and separately collected. For the study of blood kinetics, blood was taken from three animals of each group and each sex by amputating the tip of the tail.

After administration of [trifluormethyl-phenyl-U-^{14}C]-trifloxystrobin at a dose of 0.5 mg/kg bw, the amount of radiolabel recovered in the urine and tissues within 7 days was twice as high in females (34%) as in males (17%). These data confirmed the findings of the first study.

After oral administration of a low dose of [trifluormethyl-phenyl-U-^{14}C]-labelled trifloxystrobin, the radiolabel was rapidly and completely eliminated, predominantly in the faeces. Within 48 h, 93% of the administered dose was excreted in males and females, and the administered dose was completely eliminated within 7 days after administration. The route of elimination was influenced by the sex of the animals: female rats excreted twice as much radiolabel in the urine (33% of the administered dose) as the males (16%). The faeces contained 80% and 62% of the administered dose in male and female rats, respectively. These data confirmed the findings of the first study.

After a single oral administration of [trifluormethyl-phenyl-U-^{14}C]-trifloxystrobin at a dose of 0.5 mg/kg bw, two blood concentration maxima were observed, at approximately 0.5 h and 12 h. In females, the first maximum concentration exceeded the second, while in males the second exceeded the first. Thereafter, the amount of radioactivity in the blood depleted at a moderate rate. Assuming monophasic first-order kinetics, a half-life of 40 h was determined that was independent of the sex of the animals. The areas under the blood concentration–time curve (AUC_{0-96h}) were in the same range for male and female rats, indicating a similar bioavailability. These data confirmed the findings of the first study.

Independent of the dose administered or the sex of the animal, the highest tissue concentrations of residues were generally found between 12 h and 24 h after administration. The terminal concentrations of residues were very low at 7 days after a single oral administration of [trifluormethyl-phenyl-U-^{14}C]-trifloxystrobin at 0.5 mg/kg bw, irrespective of the sex of the animal. The highest concentrations of radiolabel were found in the blood, kidneys and liver amounting to 0.014/0.009 ppm (male/female), 0.010/0.012 mg/kg, and 0.012/0.007 mg/kg, respectively. All the other tissue concentrations of residues did not exceed 0.006 mg/kg. No significant differences were found between this data and that for the first study using the glyoxyl-phenyl radiolabel.

The residual radioactivity was depleted from tissues and organs with a half-life of 12–37 h independent of the dose and sex of the animals, except for blood (25–41 h) and spleen (22–99 h). These data confirmed the findings of the first study.

On the basis of urinary and biliary excretion and the radioactivity retained in the tissues of female rats dosed with [glyoxyl-phenyl-U-^{14}C]-trifloxystrobin at 100 mg/kg bw,

approximately 22% of the administered dose was absorbed into the systemic circulation. Within 48 h, the bile-duct cannulated female rats excreted approximately 18%, 3% and 20% in the bile, urine and faeces, respectively. The sponsor stated that the low recovery determined in the previous study was caused by the incorrect determination of the dose remaining in the gastrointestinal tract (Stampf, 1998).

Dermal absorption of trifloxystrobin was investigated in vitro in isolated rat and human epidermis. A study of absorption in vitro compared the dermal absorption of trifloxystrobin, as a 125 EC formulation, in human and rat skin. The blank formulation (purity, 88.3%) was mixed with 11.7% (w/w) radiolabelled (radiochemical purity, >95%) or unlabelled (purity, 99.9%) trifloxystrobin at three different concentrations. The resulting mixtures were either applied undiluted (10.26 mg/cm^2) or after dilution with water (1.478 and 0.236 mg/cm^2). Composition of the blank formulation is shown in Table 4.

Epidermal membranes of human skin from a female Caucasian donor aged 43 years were prepared immediately before the start of the study by immersion of the pre-frozen skin in water heated to approximately 60 °C. Skin was also taken from male Sprague-Dawley rats aged 26 days. Epidermal membranes were prepared immediately before the start of the study by overnight immersion in 2 mol/l sodium bromide, containing 0.01% sodium azide. Sterile glass rings (internal area, approximately 0.64 cm^2) were glued onto the epidermis. The epidermis was then transferred onto a Netwell insert (200 µm mesh) in a six-well plate, allowing contact of the basal membrane with the receptor fluid, while the stratum corneum remained exposed to air. In all test groups, 50 µl of test solutions were applied into the glass rings. [4-^{14}C]-Labelled testosterone was used as a reference compound. Final concentrations of trifloxystrobin and applied doses in the three samples are shown in Table 5.

The concentration of trifloxystrobin at the higher dose corresponds to that of the undiluted product, while the concentration at the lower dose corresponds to that of a typical

Table 4. Composition of blank formulation used in a study of dermal penetration in vitro

Component	Proportion (% w/w)
Copolymer butanol 34 PO/22 EO	11.7
Tristyrylphenol 16 EO	9.3
Styrylphenol polyethoxyester phosphate	2.3
1-Methyl-2-pyrrolidone	65.0

From Van de Sandt (1997)

Table 5. Dose and concentration of ^{14}C-labelled trifloxystrobin applied to rat or human skin membranes in a study of dermal penetration in vitro

Group	Concentration		Dose (mg/cm^2)
	mg/ml	MBq/ml	
A1 (low)	3.023	0.31	0.236
A2 (intermediate)	18.924	0.32	1.478
A3 (high)	131.388	0.34	10.265
B (testosterone)	0.299	0.74	0.015

From Van de Sandt (1997)

spray solution of the respective formulation. Samples of receptor fluid (200 µl) were collected at 1-h intervals for the first 12 h, then at 2-h intervals until 24 h after application. Thereafter, samples were collected at 4-h intervals until the end of the study (48 h). Test compound remaining was removed from the membrane with cotton swabs soaked in ethanol, and epidermal membranes were solubilized in 1.5 mol/l potassium hydroxide and 20% ethanol. The remaining receptor fluid was collected and wells were washed with ethanol.

The absorption of trifloxystrobin was non-linear and was faster in the rat epidermis than in human epidermis (see Tables 6 and 7). The penetration of testosterone was similar to that described in the data on historical controls for the laboratory, according to the sponsor (Van de Sandt, 1997).

In a study of absorption in vivo, trifloxystrobin, as a 125 EC formulation, was applied to the shaved backs of male rats aged 8 weeks at a higher (1.12–1.14 mg/cm^2) or lower (0.024–0.026 mg/cm^2) dose to replicate exposure to either diluted or concentrated product. The blank formulation (purity, 88.3%) was identical to that described above (Table 4) in the study of dermal penetration in vitro. The blank formulation was mixed with either: (a) 11.7% (w/w) of radiolabelled trifloxystrobin (purity, >95%) for the lower dose; or (b) a mixture of radiolabelled and unlabelled trifloxystrobin (purity, >99.9%) for the higher dose. The resulting mixtures were either applied undiluted (higher dose) or after dilution with water (lower dose). The final concentrations of trifloxystrobin and radiolabel, and applied doses for the two dosing solutions are shown in Table 8.

Table 6. In-vitro percutaneous absorption of trifloxystrobin in rat epidermis

	Dose (mg/cm^2)		
	0.236	1.478	10.265
Penetration (% of dose (µg/cm^2)):			
Within 8 h	0.58 (1.37)	0.50 (7.32)	0.43 (44.50)
Within 24 h	1.66 (3.93)	1.35 (19.93)	0.72 (74.19)
Within 48 h	3.52 (8.32)	2.18 (32.25)	1.24 (127.69)
Flux constant (µg/cm^2 per h)	0.28	1.35	13.88
K_p value (cm/h.10^{-5})	9.20	7.14	10.56
Lag time (h)	3.6	4.0	0.2

From Van de Sandt (1997)

Table 7. In-vitro percutaneous absorption of trifloxystrobin in human epidermis

	Dose (mg/cm^2)		
	0.236	1.478	10.265
Penetration (% of dose (µg/cm^2)):			
Within 8 h	0.06 (0.15)	0.03 (0.51)	0.05 (4.72)
Within 24 h	0.26 (0.62)	0.11 (1.56)	0.13 (13.56)
Within 48 h	0.61 (1.44)	0.23 (3.35)	0.26 (26.43)
Flux constant (µg/cm^2 per h)	0.03	0.07	0.77
Kp value (cm/h.10^{-5})	1.09	0.38	0.59
Lag time (h)	5.6	1.6	2.2

From Van de Sandt (1997)

Table 8. Dose and concentration of ^{14}C-labelled trifloxystrobin applied to male rat skin in a study of absorption in vivo

Group	Concentration		Dose (mg/cm^2)
	mg/g	MBq/g	
Lower dose	3.30	2.90	0.024–0.026
Higher dose	116.93	4.23	1.12–1.14

From De Bie (1997)

Table 9. Blood kinetics of radiolabel after dermal application of ^{14}C-labelled trifloxystrobin in male rats

Time-point (h)	Concentration (µg parent compound equivalents/g blood)	
	Lower dose—24 µg/cm^2	Higher dose—130 µg/cm^2
0.5	0.009	0.07
1	0.011	0.10
2	0.007	0.13
4	0.012	0.10
6	0.009	0.09
8	0.008	0.12
12	0.011	1.65
24	0.013	0.30
48	0.013	0.39

From De Bie (1997)

Table 10. Summary of dermal absorption (expressed as % of applied dose) of trifloxystrobin in male rats

Sample	Lower dose (24 µg/cm^2)			Higher dose (1130 µg/cm^2)		
	8 h	24 h	48 h	8 h	24 h	48 h
Urine (total)	0.18	0.51	1.43	0.09	0.51	1.43
Faeces (total)	0.01	3.34	9.47	0.01	1.06	3.49
Cage wash	0.04	0.20	0.46	0.01	0.09	0.36
Control skin + blood	0.49	0.07	0.13	0.18	0.13	0.16
Carcass	4.39	3.73	4.81	9.85	4.19	4.54
Absorbed	5.11	7.85	16.30	10.14	5.70	9.25
Application site	28.11	19.05	21.57	12.18	7.08	5.58
Not absorbed	61.92	67.67	75.72	78.86	89.06	85.80

From De Bie (1997)

Concentrations of radiolabel found in the blood were generally low, close to the limit of determination. For the lower dose (0.024–0.026 mg/cm^2), the concentration remained constant throughout the entire observation period. For the higher dose (1.12–1.14 mg/cm^2), the highest concentration was obtained at 12 h, and was most probably induced by the washing procedure, according to the sponsor (Table 9).

Trifloxystrobin was absorbed to a moderate extent by rat skin. After 8 h and 24 h, 5–10% of the applied dose was absorbed, independent of the dose level (Table 10). It should be noted that in animals given the higher dose, the absorption value of 10.14% at 8 h is too high and is likely to be inaccurate because of the time-course trends in absorption at the lower and higher doses. In rats given the lower dose, 28% of the applied dose remained in the skin after washing. Depletion of this radioactivity was slow, dropping to approximately 20% after 48 h (Table 10). A similar pattern was observed in animals given the higher dose;

about 12% remained in the skin after washing and this amount decreased to about 5% after 48 h. The rate of dermal absorption over 48 h was considered to be 16% on the basis of continued absorption of trifloxystrobin following the 8 h wash and removal from the application site in rats given the lower dose (De Bie, 1997).

1.2 Metabolism

Specimens from the main study of toxicokinetics (Muller, 1996) and the supplementary study of toxicokinetics (Stampf, 1998) were analysed to determine the metabolic pathway of trifloxystrobin in male and female rats. Radioactivity in urine and other liquid specimens was measured by liquid scintillation counting (LSC). The radioactivity in aliquots of faeces and other solid specimens was determined after combustion. Fractions of extracted specimens were separated and analysed by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). The pattern of radioactivity on TLC plates was detected with a spark chamber camera or a biomaging analyser and quantified by scraping off the radioactive fractions and analysing by LSC. Non-radioactive fractions on TLC plates were located under ultraviolet light at 254 nm. Extracts of urine, bile, and faeces were pre-purified by solid-phase extraction (SPE). Mass spectrometry (MS) and nuclear magnetic resonance (NMR)–spectroscopy as well as high-voltage electrophoresis were used to elucidate the structures of metabolites. In addition, CGA 347242 and CGA 373463 were characterized as metabolites by comparison with authentic reference substances.

The patterns of metabolites in the urine were complex; they were qualitatively independent of pretreatment and slightly dependent on dose, but showed significant differences dependent on sex and position of the radiolabel. In total, there were about 26 urinary metabolite fractions, most of which represented about 1% or less of the administered radiolabel in addition to an unresolved fraction that comprised 9–40% of the administered radiolabel. In the radioactivity extracted from the faeces, there were about 10 resolved metabolite fractions whose patterns were qualitatively independent of sex, dose, pretreatment, and position of radiolabel, with some quantitative variations. In addition, some of the faecal-extracted radiolabel was unresolved (9–24% of the administered dose) while the non-extractable fraction represented 5–15% of the administered radiolabel. Also, the patterns of metabolites in the bile were complex and qualitatively independent of sex and dose, with some quantitative variations. The patterns in the urine, faeces, and bile were essentially distinct from each other.

Thirty-five metabolites were isolated from urine, faeces, and bile of the male and female rats at the highest dose and were identified by spectroscopy. In addition, CGA 347242, CGA 373463, NOA 414412, and NOA 417076 were characterized as metabolites by co-chromatography with authentic reference substances. Some of the metabolites (mainly urinary) were unique to one of the two sites of the radiolabel indicating cleavage between the glyoxyl-phenyl and trifluoromethyl-phenyl moieties. The proposed metabolic pathway is shown in Appendix 1.

On the basis of the structures of the metabolites, the following metabolic pathways for trifloxystrobin were derived:

— Hydrolysis of the methyl ester to the corresponding acid (e.g. CGA 321113) (major pathway);

— *O*-Demethylation of the methoxyimino group yielding a hydroxyimino compound (e.g. NOA 405637) (major pathway);
— Oxidation of the methyl side-chain to a primary alcohol (e.g. Met 2U), followed by partial oxidation to the respective carboxylic acid (e.g. Met 13U) (major pathway);
— Hydrolysis of the imino group of the glyoxyl-phenyl moiety to yield a ketone, with subsequent chain shortening by oxidative decarboxylation ultimately producing a benzoic acid derivative (minor pathway);
— Chain shortening of the glyoxyl moiety by oxidative decarboxylation, giving rise to a benzoic acid amide (minor pathway);
— Hydroxylation of the phenyl rings (minor pathways);
— Oxidation of the hydroxyimino group to produce a nitro group (minor pathway);
— Cleavage between the glyoxyl-phenyl and trifluoromethyl-phenyl moiety

Cleavage between the glyoxyl-phenyl and trifluoromethyl-phenyl moiety accounted for about 10% of the applied dose. The primary cleavage products were prone to further degradation mainly by the above mentioned processes. For the trifluoromethyl-phenyl part, these included oxidation of the hydroxyimino group leading to a nitro compound, oxidation of the methyl group resulting ultimately in a carboxylic acid, hydrolysis of the imino group producing a ketone, followed by oxidation of the methyl group to an intermediary carboxylic acid. This α-keto acid can either be reduced to an α-hydroxy acid or may undergo chain shortening by oxidative decarboxylation to trifluoromethyl-benzoic acid. The other fragment (glyoxyl-phenyl part) is transformed by oxidation of the benzylic substituent to a benzoic acid. *O*-Demethylation of the methoxyimino group yielding a hydroxyimino compound, hydrolysis of the imino group to an α-keto acid and subsequent chain shortening by oxidative decarboxylation ultimately yields phthalic acid.

Glucuronic and, to a lesser extent, sulfuric acid conjugates were generated from metabolites containing a hydroxy group. The majority of metabolites resulted from more than one of the above mentioned transformations. Approximately 4–7% and 31–47% of the lower and higher dose, respectively, was eliminated in the faeces as unchanged trifloxystrobin. The oxidation of the methyl side-chain to a primary alcohol was more pronounced in female rats, resulting in sex-specific major metabolites mainly in the urine. The degradation resulted in metabolites that were eliminated at a moderate rate. The absorbed portion of the administered dose was almost completely degraded and eliminated mainly via the bile and to a lesser extent via urine. Bile metabolites were mostly glucuronic and tentatively sulfuric acid conjugates. After hydrolysis by the gut microflora, these metabolites were ultimately eliminated via faeces, together with unchanged trifloxystrobin escaping absorption, or via urine after enterohepatic circulation and further transformation (Thanei, 1997).

Two studies were carried out in lactating goats to investigate the metabolic fate of [trifluormethyl-phenyl-U-^{14}C]-labelled trifloxystrobin or [glyoxyl-phenyl-U-^{14}C]-labelled trifloxystrobin.

In the first study, two lactating female goats received gelatin capsules containing [trifluormethyl-phenyl-U-^{14}C]-labelled trifloxystrobin (radiochemical purity, >99%) at doses equivalent to 103.8 mg/kg feed or 4.24 mg/kg bw on 4 consecutive days. Milk, urine, faeces, bile, and cage wash were collected. The animals were sacrificed 6 h after the last dose. Blood, muscle, fat, liver, and kidney were taken from both animals.

During the in-life period, 0.08%, 44.5% and 17.4% of the total dose were eliminated via milk, faeces and urine, respectively. Tissue concentrations of residues were: leg muscle, 58 ppb trifloxystrobin equivalents; tenderloin, 59 ppb; omental fat, 182 ppb; perirenal fat, 209 ppb; liver, 4815 ppb; and kidneys, 1830 ppb. Tissue concentrations of residues in milk were 85 ppb (interval 0–78 h), and blood and bile contained 248 ppb and 71 315 ppb, respectively. The extractability of the milk and tissue samples was good (>90%) with the exception of liver (66.5%) where microwave-assisted extraction was used to release additional radioactive residues thereby increasing the extractability to 95.0%.

Trifloxystrobin was found to be the major compound in fat (79.0%) and in milk (51.6%) and to a smaller extent in muscle (20.6%), liver (2.8%), kidneys (1.8%) and faeces (21.7%). Hydrolysis of trifloxystrobin produced CGA 321113, i.e. methoxyimino-{2-[1-(3-trifluoromethy-phenyl)-ethylidene-aminooxymethyl]-phenyl}-acetic acid, being the major metabolite excreted via urine (70.4% of the urine radioactivity) and faeces (35.5%). This hydrolysis product was also the major metabolite in muscle (57.2%) and kidneys (54.3%) and was also found in all other samples, i.e. fat (10.4%), liver (20.0%) and milk (3.6%). The major metabolite in liver (27.8%) was metabolite L7a, i.e. the taurine conjugate of CGA 321113 also present in muscle (1.2%), kidneys (12.7%) and milk (13.0%). Metabolite L7b was identified as the glycine conjugate of CGA 321113. This conjugate was found in muscle (1.2%), liver (10.7%), kidneys (5.2%) and faeces (2.9%).

Demethylation of the methoxyimino group of trifloxystrobin to metabolite 2F, i.e. hydroxyimino-(2-[1-(3-trifluoromethyl-phenyl)-ethylideneaminooxymethyl]-phenyl}-acetic acid methyl ester was only found in faeces (10.2%). Metabolite 1U, the demethylation product of CGA 321113, i.e. hydroxyimino-{2-[1-(3-trifluoromethyl-phenyl)-ethylidene-aminooxymethyl]-phenyl}-acetic acid, was identified in muscle (1.3%), fat (0.5%), kidneys (1.9%), milk (0.9%), urine (7.2%) and faeces (3.0%).

Hydroxylation of the aminooxymethyl group was observed in the monohydroxylated metabolite 2U, i.e. {2-[2-hydroxy-l-(3-trifluoromethyl-phenyl)-ethylidene-aminooxymethyl]phenyl}-methoxyimino-acetic acid in muscle (2.0%), fat (0.4%), kidneys (3.1%), milk (1.9%), urine (3.2%), faeces (4.1%) and in the dihydroxylated metabolite 6U, i.e. hydroxyimino-{2-[2-hydroxy-1-(3-trifluoromethyl-phenyl)-ethylidene-aminooxymethyl]phenyl)-acetic acid in liver (4.4%) and milk (4.0%).

Hydroxylation of the glyoxyl-phenyl ring in position four was found in metabolite 7F, i.e. {4hydroxy-2-[1-(3-trifluoromethyl-phenyl)-ethylideneaminooxymethyl]-phenyl}-methoxyiminoacetic acid methyl ester. This metabolite was only characterized in faeces (8.0%).

Cleavage of the molecule between the two phenyl rings was a minor pathway, as shown by the presence of metabolite 12 U, i.e. hydroxy-(3-trifluoromethyl-phenyl)-acetic acid in muscle (1.7%), fat (0.7%), milk (3.1%) and urine (3.7%) and metabolite 11 U, i.e. sulfuric acid mono-[1-(3-trifluoromethyl-phenyl)-ethanone oxime]ester in kidneys (0.3%) and urine (4.5%) (Rumbeli, 1997a).

In the second study, two lactating female goats received gelatin capsules containing [glyoxyl-phenyl-U-^{14}C]-labelled trifloxystrobin (radiochemical purity, >98%) at daily doses equivalent to 100.4 mg/kg feed or a dose of 4.13 mg/kg bw on 4 consecutive days. The animals were sacrificed 6 h after the last dose.

During the study, 0.06%, 36.0% and 18.9% of the total administered dose was eliminated via milk, faeces, and urine, respectively. Tissue concentrations of residues were: leg muscle, 77 ppb trifloxystrobin equivalents; tenderloin, 74 ppb; omental fat, 364 ppb; perirenal fat, 343 ppb; liver, 3913 ppb; and kidneys, 2331 ppb. Milk contained 89 ppb (interval 0–78 h) and blood and bile contained 330 ppb and 40 813 ppb, respectively. The extractability of the milk and tissue samples was good with the exception of liver (68.7%) where microwave-assisted extraction was used to release additional radioactive residues, thereby increasing the extractability to 97.9%.

Trifloxystrobin was found to be the major compound in fat (82.0%) and in milk (73.8%) and to a smaller extent in muscle (26.5%), liver (2.5%) and kidneys (1.8%). Trifloxystrobin was the major residue eliminated via the faeces (48.2%). Findings on metabolites, tissue distribution, and residue concentrations were very similar to those described earlier for [trifluormethyl-phenyl-U-^{14}C]-labelled trifloxystrobin.

In this study there was not a significant amount of label-specific metabolites, i.e. cleavage of the molecule between the two phenyl rings did not seem to be a major reaction in the metabolism of trifloxystrobin. In conclusion, the metabolism of trifloxystrobin in the goat follows the same major pathways as in the rat (Figure 2) (Rumbeli, 1997b).

2. Toxicological studies

2.1 Acute toxicity

The results of studies of acute toxicity with trifloxystrobin, performed in compliance with OECD guidelines and good laboratory practice (GLP), are summarized in Table 11.

Trifloxystrobin has low acute oral toxicity in rats and mice (LD_{50} > 5000 mg/kg), low acute dermal toxicity in rats and rabbits (LD_{50} > 2000 mg/kg), low acute inhalational toxicity in rats (LC_{50} > 4650 mg/m^3), slight skin irritancy in rabbits, is a moderate eye irritant in unwashed rabbit eyes but a non-irritant in washed rabbit eyes, and is a skin sensitizer in guinea-pigs by the maximization test but not by the Buehler test.

Table 11. Acute toxicity of trifloxystrobin

Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	LC_{50} (mg/l air)	Reference
Mouse	Tif:MAG	Male & female	Oral	>5000	—	Winkler (1996)
Rat	Crl:CD (SD)	Male & female	Oral	>5000	—	Glaza (1994a)
Rat	HSD: (SD)	Male & female	Inhalation	—	>4.65	Holbert (1995)
Rat	Tif: RAI f (SD)	Male & female	Dermal	>000	—	Marty (1995)
Rabbit	Hra: (NZW)	Male & female	Dermal	>2000	—	Glaza (1994b)
Rabbit	Hra: (NZW)	Male & female	Skin irritation	Slightly irritating	—	Glaza (1994c)
Rabbit	Hra: (NZW)	Male & female	Eye irritation	Moderately irritating (unwashed)	—	Glaza (1994d)
		Female		Non-irritating (washed)		
Guinea-pig	Tif:DHP (White)	Male & female	Skin sensitization: maximization	Sensitizing	—	Marty (1994)
	Crl: (HA)BR	Male	Skin sensitization: Buehler	Non-sensitizing	—	Glaza (1994e)

Figure 2. Proposed metabolism of trifloxystrobin (CGA 279202) in rats, goats and hens

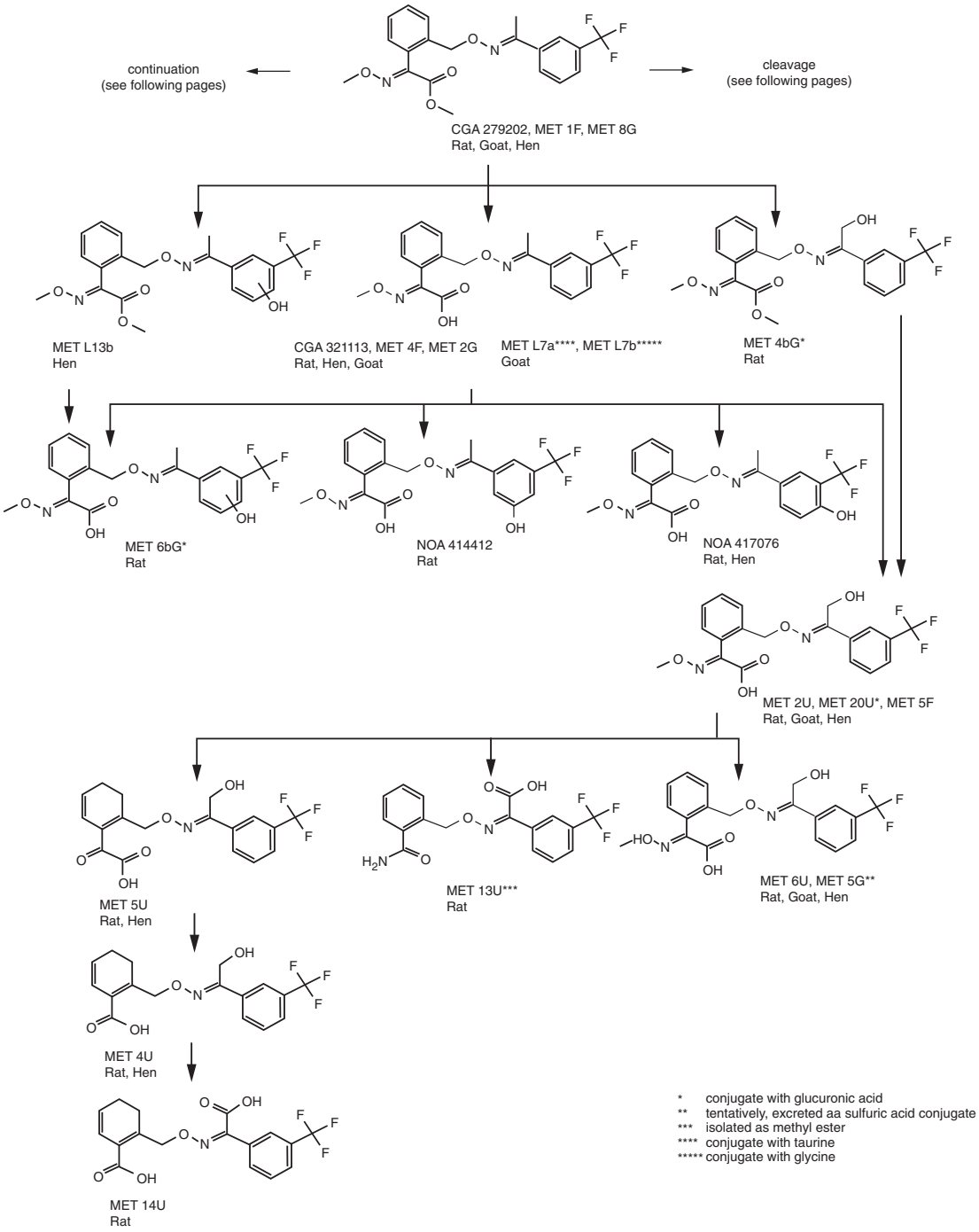


Figure 2. Continued

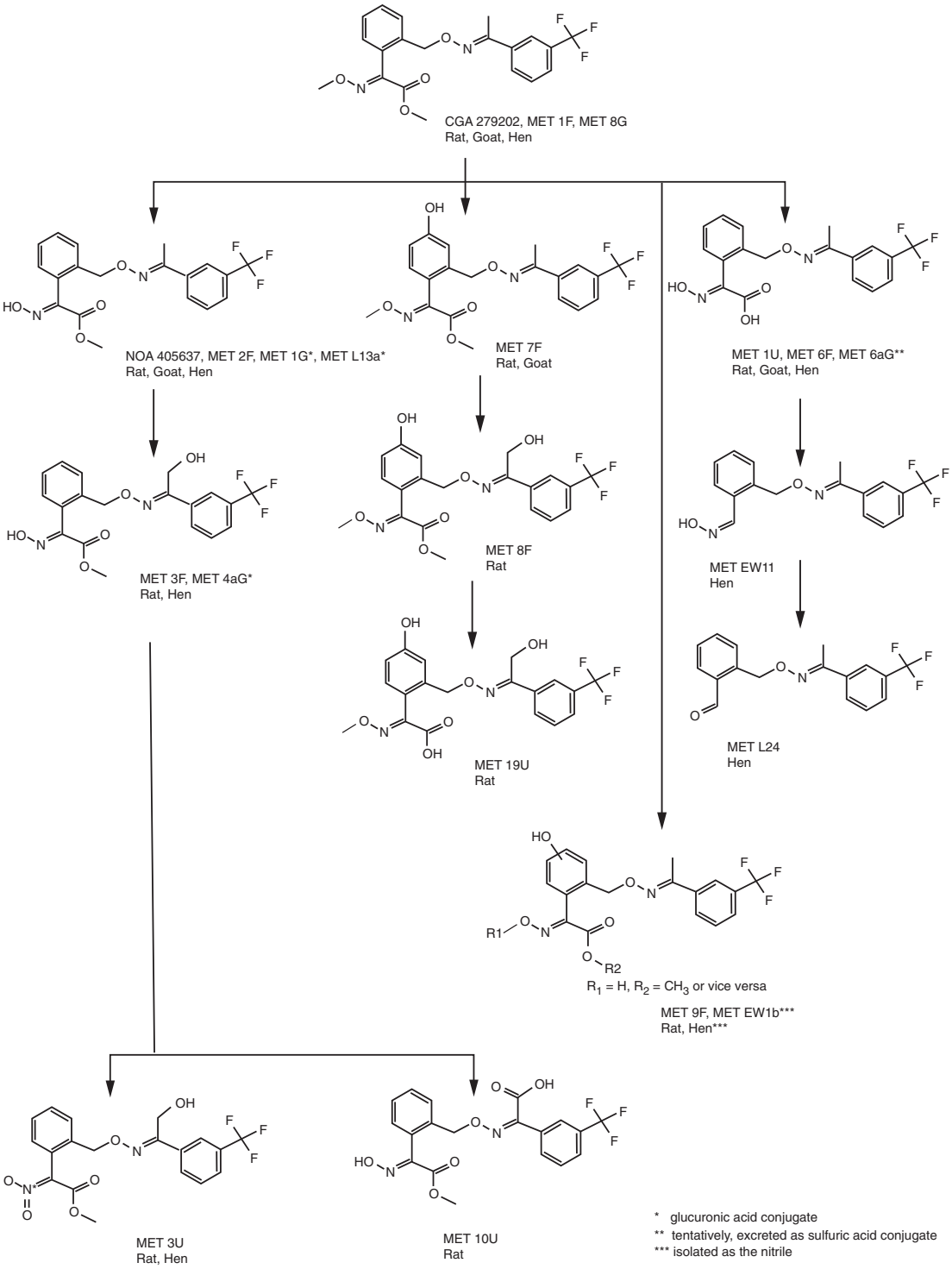
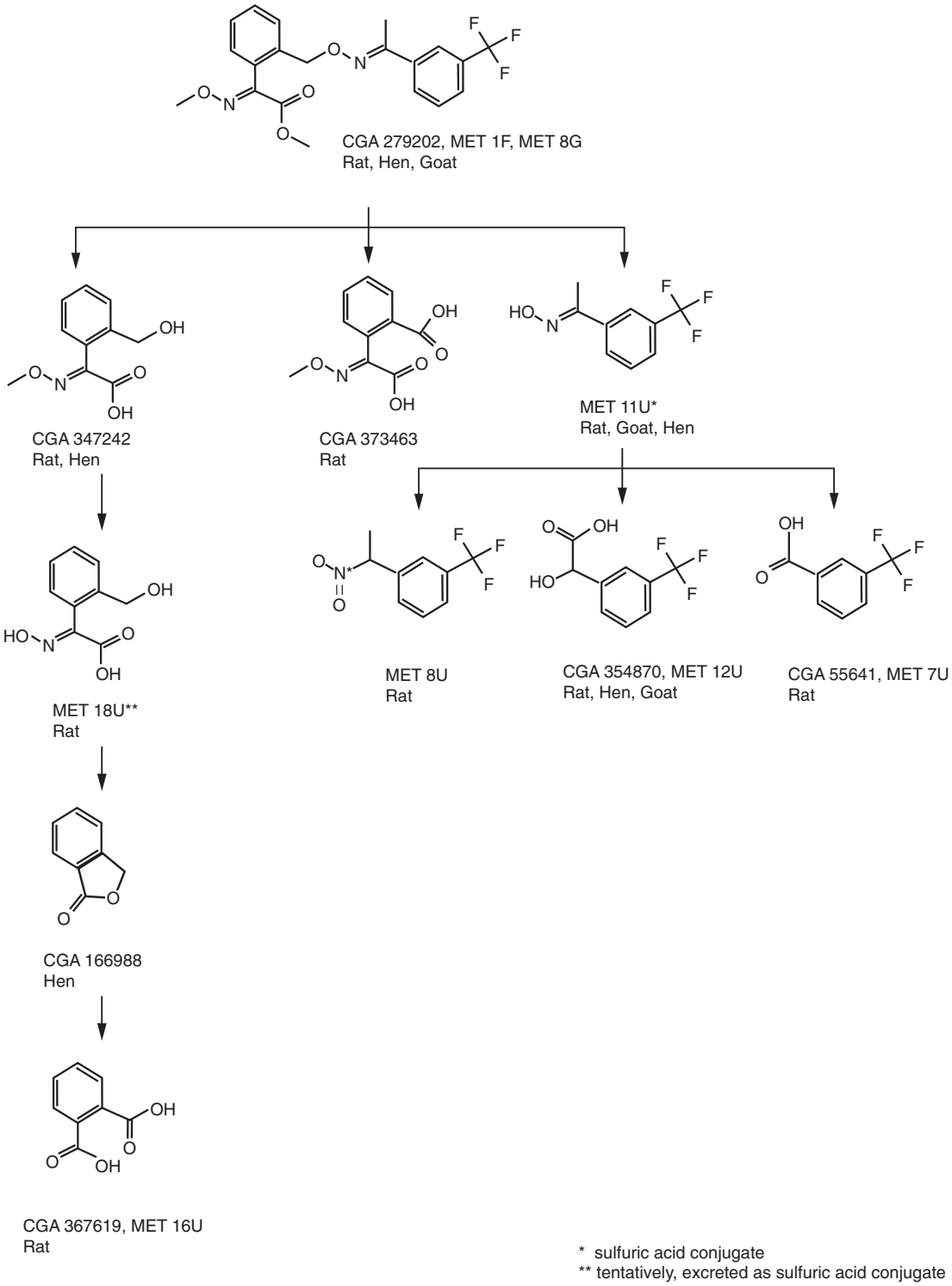


Figure 2. Continued



* sulfuric acid conjugate
** tentatively, excreted as sulfuric acid conjugate

2.2 Short-term studies of toxicity

Mice

In a study conducted in compliance with the principles of GLP (with QA certification), groups of 10 male and 10 female mice (Tif: MAGf) were continuously fed with diets containing trifloxystrobin (purity, 96.2%) at a concentration of 0, 500, 2000 or 7000 ppm (equal to 0, 76.9, 315.1 and 1275 mg/kg bw per day in males and 0, 110.4, 425.1 and 1649 mg/kg bw per day in females, respectively) for 3 months. Concentrations of trifloxystrobin in the diet were analysed twice during the treatment period for all doses. Mortality was checked twice per day and clinical signs were checked daily; body weight, food and water consumption were recorded weekly. Because this study was designed as a dose range-finding study, the extent of laboratory examinations do not meet the OECD 408/EPA (OPPTS) 870-3100 requirements for a 90-day study of oral toxicity in rodents. At the end of treatment, all animals were subjected to standard haematology and all surviving animals were subjected to a detailed necropsy, including collection of organs and tissues. Weights of the adrenals, kidneys, liver, ovaries, spleen, testes, and thymus were recorded. Although all organs were sampled, microscopic evaluations were limited to liver, spleen, and gross lesions. Food, water and the housing environment were controlled and monitored.

Trifloxystrobin was found to be homogeneously distributed and stable in the diet for at least 5 weeks at room temperature. One female at the lowest dose (500 ppm) died prematurely. Histopathological examination revealed no treatment-related cause of death (see below). There were no clinical observations that were related to the treatment. Depressed body-weight gain was recorded in males treated at 7000 ppm, resulting in a terminal weight which was 5.3% below control and an overall weight gain which was reduced by 20% (not statistically significant). The depression became particularly obvious towards the end of treatment. Body weights were not affected in other groups. Food consumption was slightly increased compared with that of controls for male mice at 500, 2000 and 7000 ppm. The increase in food consumption was evident by week 6, but increases became significantly different at weeks 9, 11 and 12 at all doses, and at week 13 at 500 ppm. Mean increases compared with values for controls for weeks 9–13 ranged from 6.5% to 23.8% at 500 ppm, 8.3% to 28.2% at 2000 ppm, and 26.4% to 46.0% at 7000 ppm. A slight increase was also observed in females at the highest dose (mean, 17.9%; range, 3.4–39.4% over weeks 1–13). The sponsor states that exact determinations were biased by food spillage in several groups. Food consumption ratios were not calculated for the group at 7000 ppm because of the food spillage, but no deviations were recorded for animals of both sexes for the groups at 500 and 2000 ppm compared with values for controls. Overall mean water consumption (weeks 1–13) was markedly increased (50%) in females at the highest dose, but water consumption in males was not affected.

No treatment-related effects on the haematological profile were found. Mean carcass weight for the males at 7000 ppm was slightly decreased (by 8%) compared with values for controls. Mean absolute weights of the liver were increased in males at 2000 ppm (23%) and 7000 ppm (30%), and in females at 2000 ppm (39%) and 7000 ppm (51%) (Table 12). Mean relative weights of the liver were increased in males at 2000 ppm (15%) and 7000 ppm (40%), and in females at 2000 ppm (30%) and 7000 ppm (49%). Elevated absolute weights of the spleen were found in males at 2000 ppm (11%) and 7000 ppm (8.5%), and in females at 2000 ppm (15.8%) and 7000 ppm (36.8%). Increased relative weights of the spleen were found in males at 7000 ppm (17.5%), and in females at 500 ppm (7.2%),

2000 ppm (8.2%) and 7000 ppm (35.3%) (Table 12). However, the increased relative weight of the spleen in females at 500 ppm was not correlated with histopathology and was not considered to be toxicologically relevant by the consulting pathologist.

An increased incidence of enlarged liver (two out of ten livers examined) and spleen (six out of ten spleens examined) was found in females at 7000 ppm. In the female (in the group at 500 ppm)that died on day 92 of the study, a mass in the small intestine, scarring of the liver and an enlarged spleen were observed. However, the consulting pathologist stated that the cause of death was probably invagination of a length of intestine into an adjacent portion producing obstruction of the bowel. The lesions of the liver in this animal were likely to be caused by enterotoxins associated with the ileus (obstruction of the intestines), and the extramedullary haematopoiesis of the spleen was considered to be concomitant to the intestinal changes, according to the consulting pathologist.

Microscopic findings in the 10 livers examined from each sex per group included hypertrophy of hepatocytes, predominantly of the centrilobular region, in males (minimal, one; moderate, two; marked, four) and females (minimal, three; moderate, seven) at 7000 ppm, and necrosis of single hepatocytes or small groups of hepatocytes in males at 2000 ppm (minimal, three; moderate, three) and 7000 ppm (minimal, two; moderate one) and females at 2000 ppm (minimal, two) and 7000 ppm (minimal, one; moderate, three). An increased incidence of haemosiderosis in the spleen was noted in males (minimal, five) and females (minimal, seven; moderate, one) treated at 7000 ppm. Extramedullary haematopoiesis was found at an increased incidence in the spleen of males at 2000 ppm (minimal, two) and in males (minimal, six; moderate, one) and females (six minimal, three moderate) at 7000 ppm. Other tissues were not examined. However, no other treatment-related microscopic findings were noted in all examined tissues from the 18-month study of carcinogenicity at a dietary concentration of 500 ppm or below.

The no-observed-adverse-effect level (NOAEL) in this dose range-finding study in mice was 500 ppm, equal to 77 and 110 mg/kg bw per day in males and females, respectively, on the basis of increased absolute and relative weights of the liver, and increased incidences of liver necrosis in both sexes, in addition to increased incidence of extramedullary haematopoiesis in the spleen of males (Gerspach, 1994a).

Table 12. Organ weights in mice given diets containing trifloxystrobin for 3 months

Organ	Dietary concentration (ppm)							
	0		500		2000		7000	
	Males	Females	Males	Females	Males	Females	Males	Females
Liver								
Absolute weight (g)	2.40	1.82	2.54	2.00	2.96[b]	2.53[a]	3.11[a]	2.75[a,b]
Relative weight (%)	5.90	5.90	6.00	6.20	6.80[a,b]	7.70[a,b]	8.30[a,b]	8.80[a,b]
Spleen								
Absolute weight (g)	0.082	0.095	0.082	0.105	0.091	0.110	0.089	0.130[b]
Relative weight (%)	0.201	0.308	0.195	0.330	0.209	0.333	0.236	0.416[b]

From Gerspach (1994a)
[a] $p < 0.01$, Lepage two-sample test
[b] $p < 0.01$, Jonckheere trend test

Rats

In a study conducted in compliance with the principles of GLP (with QA certification), groups of five male and five female Sprague-Dawley derived rats (Tif: RAIf (SPF) hybrids) were given diets containing trifloxystrobin (purity, 96.2%) at a concentration of 0, 200, 1000, 4000 or 12 000 ppm (equal to 0, 16.5, 84.4, 337 and 1074 mg/kg bw per day in males and 0, 16.4, 84.1, 327 and 1005 mg/kg bw per day in females, respectively) for 28 days. All animals were checked daily for mortality, health, and behaviour. Body weight, and food and water consumption were recorded weekly. Laboratory investigations (haematology, blood chemistry and urine analysis) were carried out on all surviving animals at each dose at the end of the treatment period. At scheduled sacrifice, all animals were subjected to macroscopic examination, including collection of organs and tissues. Organ weights were recorded for adrenals, brain, heart, kidneys, liver, ovaries, spleen, testes, and thymus, but because this was a dose range-finding study, no microscopic evaluations were performed. Food, water and the housing environment were controlled and monitored.

There were no deaths. During the treatment period, soft faeces were observed for all animals at 4000 and 12 000 ppm. The finding was reversible within the treatment period for females at 4000 ppm. Diarrhoea was observed in one male at 4000 ppm and in all males and one female at 12 000 ppm. Body-weight gain over the entire 4-week period was decreased in male rats at 1000 (13%), 4000 (22%) and 12 000 ppm (34%) as well as in females at 12 000 ppm (27%). There was a slight reduction in mean food consumption in weeks 1–4 of 4–6% below the respective control values for males at 1000 and 4000 ppm and for both sexes at 12 000 ppm. The mean food consumption ratios of animals at 12 000 ppm were lower than those of the control group at week 1 of treatment. Water consumption was not affected. There were no treatment-related effects on haematological parameters. Blood chemistry results, which were statistically significantly different from control values and were considered to be treatment-related, included: increased blood concentrations of glucose in males at 4000 ppm (17.8%) and 12 000 ppm (13.6%), and in females at 12 000 ppm (34.9%); increased serum concentrations of albumin in males at 4000 ppm (4.8%), and in animals of both sexes at 12 000 ppm (males, 7.8%; females, 5.9%); increased serum concentrations of cholesterol in animals of both sexes at 4000 ppm (males, 29.0%; females, 32.9%) and 12 000 ppm (males, 69.1%; females, 31.3%); increased serum concentrations of urea in females at 4000 ppm (22.9%) and 12 000 ppm (55.7%). All other minor fluctuations in blood chemistry were incidental and not related to treatment. There were no treatment-related effects on urine analysis parameters. Compared with the control group, mean carcass weights were 12% and 20% lower in males at 4000 and 12 000 ppm respectively, and 15% lower in females at 12 000 ppm. Mean relative weights of the liver were 13% and 31% higher in males at 4000 and 12 000 ppm and 15% higher in females at 12 000 ppm. Mean relative weights of the kidney were increased in males (15%) and females (9%) at 12 000 ppm. Mean relative weight of the adrenals was 20% higher in males at 12 000 ppm than in the controls. There were no treatment-related necropsy findings.

The NOAEL was 1000 ppm, equal to 84 mg/kg bw per day, on the basis of decreased body weight and body-weight gain and increased relative weight of the liver in males, and clinical signs and changes in clinical chemistry parameters in both sexes (Gerspach, 1994b). The study was considered to be supplementary, as it is a range-finding study and no histopathological examination was performed.

In a 28-day study of dermal toxicity, conducted in compliance with the principles of GLP with QA certification, groups of five male and five female Sprague-Dawley-derived rats (Tif:RAIf(SPF) were given trifloxystrobin (purity, 96.4%) administered dermally on 5 days per week for 4 weeks. Fur was clipped from the dorsal area of the rats' trunks over an area of at least 10% of the body surface on the day before the first application and weekly thereafter. Trifloxystrobin was applied as a suspension in 0.5% (w/v) carboxymethylcellulose in 0.1% (w/v) aqueous polysorbate 80 at a dose of 0, 10, 100 or 1000 mg/kg bw to the right side of the clipped area, and the vehicle only was applied to the left side of the clipped area, evenly dispersed on gauze patches, loosely covered with aluminium wrap and fastened to the body with adhesive tape. Dressings were removed after 6 h and the application areas were cleaned with lukewarm water. Food, water, and the housing environment were controlled and monitored. Animals were checked daily for clinical signs and mortality. Skin reactions were assessed at the application site approximately 17 h after removal of the gauze patches. Body weight and food consumption were recorded once weekly (on study days −7, 1, 8, 15, 22 and 28). Blood chemistry and haematological investigations were carried out on all surviving animals at each dose at the end of the treatment period. All control and treated animals were subjected to a detailed necropsy at the end of treatment, including collection of organs and tissues. Organ weights were recorded for adrenals, brain, heart, kidneys, liver, ovaries, spleen, testes, and thymus. Although all organs were sampled, microscopic evaluations were limited to kidneys, liver, pancreas, skin (both treated and untreated), spleen, thymus, and thyroid/parathyroid.

No treatment-related clinical signs or changes to behaviour were noted. One female animal at 10 mg/kg bw presented with transient crust and slight erythema, and two females at 1000 mg/kg bw presented with blisters on the skin application site. Since two females in the control group also had a similar skin reaction, these signs were likely to be caused by physical irritation. No other signs of local irritation were observed during the treatment period. There were no deaths during the study. No treatment-related changes were observed for mean body weights, food consumption and food consumption ratios. In females, there was a dose-related tendency to higher neutrophil and monocyte counts compared with values for controls and this was significant at 1000 mg/kg bw (neutrophils, 68.2%; monocytes, 73.0%). However, these findings were probably not toxicologically relevant given the absence of any histopathological abnormalities, and were likely to be incidental in nature. No other haematological parameters were affected by treatment with trifloxystrobin. There were minor changes in some of the blood chemistry parameters which were not dose-dependent and were within the normal range for historical controls. In males at 1000 mg/kg bw, the mean absolute and relative weights of the liver were increased by 17% and 15%, respectively, compared with controls. Mean absolute and relative weights of the kidney were also increased at 1000 mg/kg bw by 17% and 15%, respectively, compared with controls. No treatment-related macroscopic findings were noted. A lobular necrosis caused by incidental torsion of a hepatic lobe was observed but, according to the consulting pathologist, this lesion occurred spontaneously in the colony of rats used. No remarkable treatment-related microscopic changes were revealed. A low incidence of very slight local reactive lesions resulting from mechanical irritation caused by clipping of the fur was observed in both control and treated application skin sites.

In conclusion, repeated dermal treatment with trifloxystrobin did not result in irritation or any dermal toxicity. The NOAEL for systemic effects in male rats was 100 mg/kg bw per day on the basis of increased absolute and relative weights of the kidney and liver as supported by evidence of toxicity in the kidney and liver after oral administration of

trifloxystrobin. The NOAEL in female rats was 1000 mg/kg bw per day, since dermal application of trifloxystrobin at doses of up to and including 1000 mg/kg bw per day (a limit dose) resulted in no treatment-related toxicity (Gerspach, 1996).

In a 90-day study conducted in compliance with the principles of GLP and with quality assurance (QA) certification, groups of 15 or 25 Sprague-Dawley derived rats (Tif:RAlf, hybrids) of each sex were given diets into which trifloxystrobin (purity, 96.2%) had been homogeneously incorporated at a concentration of 0, 100, 500 or 2000 ppm (equal to 6.4, 30.6, and 127 mg/kg bw per day) in males and 0, 100, 500, 2000 or 8000 ppm (equal to 6.8, 32.8, 133 and 618 mg/kg bw per day) in females. The control group and the group receiving the highest dose (females, 8000 ppm; and males, 2000 ppm) included an additional 10 rats of each sex (i.e. total group size was 25 of each sex) that were kept on a control diet for a 4-week recovery period after 13 weeks of treatment. All other groups contained 15 animals of each sex. Trifloxystrobin concentrations in the diet were analysed twice during the treatment period for all doses. Mortality was checked twice per day and clinical signs daily; body weight, food, and water consumption were recorded weekly. Before the test, towards the end of the treatment period, and after recovery, all animals from the control group and from the group receiving trifloxystrobin at 8000 ppm were subjected to ophthalmology examinations (appearance of eye and periocular region, pupillary reflex). Observations and neurological examinations including functional observational battery (FOB) and motor activity were performed on 10 animals of each sex per group (15 animals in the control group and in the group at 8000 ppm) at weeks 4, 9, 13 and 17 (recovery animals only). The neurological examinations covered the functional domains of centra nervous sytem (CNS) activity, CNS excitation, sensorimotor functions (approach, touch, vision, audition, pain, vestibular), autonomic functions (pupillary reflex, body temperature), sensorimotor coordination (grip strength, landing foot splay) and physiological functions. At the end of the treatment and recovery periods, animals were subjected to haematology, clinical chemistry and urine analyses. At necropsy, the weights of the adrenal glands, brain, heart, kidneys, liver, ovaries, spleen, testes, thymus and thyroid/parathyroid were recorded and gross findings were noted. Microscopic evaluations were conducted on a range of tissues from all animals, including the animals that died during the test period or that had to be sacrificed in a moribund condition. In addition, a range of organs and tissues were specifically prepared for neuropathological assessment (glutaraldehyde perfusion and fixation) that was limited to animals in the control group and at the highest dose (five rats of each sex per group). Food, water and the housing environment were controlled and monitored.

Trifloxystrobin was found to be homogeneously distributed and stable in the diet for at least 5 weeks at room temperature. Some animals in the groups at 8000 ppm, 2000 ppm and the control group were found dead or had to be sacrificed. Five females in the group at 8000 ppm were found dead or had to be sacrificed in a moribund condition between days 28 and 34. At 2000 ppm, one female was found dead at day 16 and one male had to be sacrificed on day 35. In the control group, one male had to be sacrificed in a moribund condition on day 69 and one female was found dead on day 43. All deaths in the groups receiving trifloxystrobin at 2000 and 8000 ppm were considered to be treatment-related. Transient piloerection and soft faeces at week 1 were noted in all females at 8000 ppm. Hunched posture or hypoactivity were observed with moribund animals. There were no differences in ophthalmology findings between animals in the control group and animals at the highest dose.

Body-weight loss was associated with being in a moribund condition. At the end of dosing, body weights were statistically significantly decreased in males at 2000 ppm (13%) and in females at 8000 ppm (20%). The mean terminal body-weight gain was reduced by 9% and 20% in males at 500 and 2000 ppm and by 17% and 40% in females at 2000 and 8000 ppm, respectively (Table 13). At the end of the recovery period, body weights were similar in the control group and in the group receiving the highest dose owing to achieving a greater body-weight gain (23% and 60% above that in males and females in the control groups, respectively) after the high-dose animals were switched to control diets. Effects on body weight were partly associated with reduced food consumption. During the treatment period, food intake in males at 500 and 2000 ppm and in females at 2000 and 8000 ppm was lowered by approximately 5–10%. The resulting food consumption ratio was increased in females at 8000 ppm except for week 1 (5.8% at week 2 to 13.8% at week 13). During recovery, food intake of animals at the highest dose was 25–53% higher than that of animals in the control group. Water consumption was slightly reduced in males at 2000 ppm during weeks 1–4 (10.3–23.7%). Thereafter, the water intake was similar to values for animals in the control group. Overall, the mean water consumption of females at 8000 ppm was 11% lower than that of the control group, while it was similar to that of the control group during the recovery period.

FOB testing revealed no indications for a potential neurological or behavioural effect of trifloxystrobin. No changes of toxicological relevance were observed in any of the parameters associated with motor activity. Macro- and microscopic examination of tissues of the central and peripheral nervous system did not reveal any treatment-related neuropathological changes. Trifloxystrobin was considered to be nonneurotoxic in rats treated continuously for 90 days. There were minimal increases in erythrocyte parameters, including erythrocyte counts (4%) and haemoglobin concentration (3%) and a tendency to eosinophilia among females at 8000 ppm. These minor alterations were reversible within the recovery period.

There were some changes in clinical chemistry parameters consistent with a marginal effect on liver and kidney functions in males at 2000 ppm and in females at 2000 and 8000 ppm. Compared with values for controls, males at 2000 ppm had slightly increased plasma concentrations of creatinine (13.8% at week 14 and 3.7% at week 18), increased total bilirubin (44.8% at week 18 only), and increased cholesterol (28% at week 14), while

Table 13. Mean body-weight gain and weights of liver and kidneys in rats given diets containing trifloxystrobin for 90 days

	Dietary concentration (ppm)						
	Males			Females			
	100	500	2000	100	500	2000	8000
Body-weight gain (% of control)	104	91	80	96	104	83	60
Organ weight (% of control):							
Liver							
Absolute	107	105	106	94	98	107	109
Relative	102	113*	122*	95	96	113	139*
Kidney							
Absolute	105	102	97	96	97	95	89
Relative	100	108	112*	96	95	100	114*

From Gerspach (1995)

small reductions were noted in plasma globulin (9.6%) and protein concentrations (4.4%). Females at 2000 and 8000 ppm also had decreased plasma concentrations of globulin (8.7 and 11.5% respectively) and protein concentrations (5.3% and 4.3% respectively). In addition, females treated at 8000 ppm had increased concentrations of glucose (13.1%), urea (17.8%), potassium (by 10.1%), serum glutamic pyruvic transaminase (SGPT) (11.7%), and alkaline phosphatase activity (47.4%). These changes were partly reversible during recovery. Also, the urine excreted by females at 8000 ppm was slightly acidic. The most notable clinical chemistry change was the increased alkaline phosphatase activity, which may point to an adverse change in the hepatobiliary function of females at 8000 ppm.

One male at 2000 ppm and three females at 8000 ppm were emaciated at termination of treatment. Macroscopic examination revealed a small thymus in three out of 13 female animals at 8000 ppm. The consulting pathologist considered that the observation of a small thymus in the male animal was not toxicologically relevant. At 8000 ppm, one out of eight females had a small thymus after recovery. The mean relative weights of the liver were increased in males at 500 ppm (13%) and 2000 ppm (22%), and in females at 2000 ppm (13%) and 8000 ppm (39%). Mean relative weights of the kidney were also above control values in males at 500 ppm (8%) and at 2000 ppm (12%) and in females at 8000 ppm (14.2%). Liver and kidney weight changes were partly reversible after recovery. Mean relative weights of the heart were also increased in females at 8000 ppm both at 14 weeks (26%) and 18 weeks (21%).

Histopathological examination of moribund or dead animals revealed minimal perilobular hepatocyte hypertrophy in the livers of four out of five females and minimal to moderate acute tubular lesion in the kidneys in five out of five females at 8000 ppm; minimal to moderate atrophy of the pancreas (exocrine and endocrine) in one out of one male and female animals each at 2000 ppm, and minimal to marked pancreatic atrophy in five out of five females at 8000 ppm (two out of five associated with oedema); minimal to marked atrophy of the spleen in one out of one and three out of five females at 2000 and 8000 ppm (although one out of eleven control males showed a minimal splenic atrophy as well); minimal to moderate hypocellularity of the bone marrow, associated with haemorrhage in one out of one male and female animals at 2000 ppm and in five out of five females at 8000 ppm; minimal to moderate atrophy of the lymphatic tissue in one out of one female and five out of five females at 2000 and 8000 ppm, respectively; minimal to moderate atrophy of the salivary gland in one out of one male and female animals each at 2000 ppm, and in five out of five females at 8000 ppm; minimal to moderate mucosal atrophy affecting the small intestine in one out of five female animals and the large intestine in three out of five females at 8000 ppm; moderate atrophy of the uterus in one out of one female at 2000 ppm and three out of five females at 8000 ppm; minimal to moderate atrophy of the ovary in one out of one and three out of five females at 2000 and 8000 ppm, respectively; moderate atrophy of the adenohypophysis in one out of one female at 2000 ppm and three out of five females at 8000 ppm; moderate to marked atrophy of the thymus in one out of one male and female animals each at 2000 ppm and moderate to marked thymic atrophy in five out of five females at 8000 ppm.

At scheduled sacrifice, there were a few histopathology findings among females at 8000 ppm and among both sexes at 2000 ppm, but no changes were seen at the lower dose. Among the changes observed were minimal hepatocellular hypertrophy in males dosed at 2000 ppm (five out of 10) and in females dosed at 8000 ppm (seven out of eight), minimal to moderate atrophy of the pancreas in two out of 10 males, in one out of nine females at

2000 ppm, and in seven of eight females at 8000 ppm. In addition, one female treated at 8000 ppm had minimal atrophy of the salivary gland. In the recovery group there were minimal to moderate atrophy of the endocrine pancreas in two out of 10 males at 2000 ppm and minimal atrophy of the thymus and the uterus in one out of eight females each at 8000 ppm. The liver pathology findings among males at 2000 ppm and females at 8000 ppm are consistent with some of the changes in clinical chemistry parameters and the increased relative weight of the liver.

The NOAEL was 500 ppm, corresponding to 31–33 mg/kg bw per day, on the basis of statistically significantly decreased body-weight gains, increased relative liver weights, changes in clinical chemistry, and liver histopathology findings in addition to pancreatic atrophy at the next higher dose of 2000 ppm. In males at 500 ppm, the decrease in body-weight gain was minor (<10%) and not statistically significant; also the slightly increased relative weight of the liver was not corroborated by liver histopathology or clinical chemistry findings (Gerspach, 1995).

Dogs

In a study conducted in compliance with the principles of GLP and with QA certification, groups of two male and two female beagle dogs were given gelatin capsules containing trifloxystrobin (purity, 96.2%) at a dose of 0, 20, 50 or 150 mg/kg bw per day orally once daily, 7 days per week for 28 days. At day 29, dogs in the control group and at 20 and 50 mg/kg bw per day were sacrificed. Owing to a lack of toxic effects, the dose for dogs in the group receiving trifloxystrobin at 150 mg/kg bw per day was increased to 500 mg/kg per day for an additional 21 days. Food, water and the housing environment were controlled and monitored. All animals were checked daily for behavioural signs and mortality. The body weight and food consumption of all animals was recorded at weekly weighing sessions. Food consumption ratios were calculated as: weekly food consumption (g)/body weight (kg) × 7 (g food/kg bw per day). Eye examinations were performed in all animals before the test and at week 4. Laboratory investigations (haematology, blood chemistry and urine analysis) were carried out on all surviving animals at each dose at the beginning of the study and at weeks 4 and 7. All animals were subjected to a detailed necropsy at the end of the test period and organ samples were taken for microscopic examination. Only the following organs were microscopically examined: brains, heart, liver, kidneys, testes, ovaries, spleen, thymus adrenal gland, thryoid, parathyroid gland, and any tissues with gross lesions.

There were no deaths during the study. During the treatment period, vomiting was observed in the males and females at 150/500 mg/kg bw per day on several occasions. There was also increased incidence and severity of diarrhoea noted at 500 mg/kg bw per day. Mean body weights were lower (by 3.1%) in male animals given trifloxystrobin at 150/500 mg/kg bw per day at week 4 compared with before the start of treatment. A slight body-weight loss was noted for one male at 50 mg/kg bw per day at the end of the treatment (2.2%), and for one male at 150/500 mg/kg bw per day (6.25%), and a transient loss was observed at weeks 1 and 2 (by 3.1%) for one male at 150/500 mg/kg bw per day, compared with before the test. Mean body-weight gains of treated females were similar to those of the controls. Compared with before the test, mean food consumption was reduced maximally at week 3 by 25% for one male at 50 mg/kg bw per day, and at week 1 by 25% for one female and by 55% at week 2 for one male at 150/500 mg/kg bw per day. Food intakes were partially returned to values recorded before the test by the end of treatment. Lower food

consumption ratios were noted for males at 50 mg/kg bw per day (week 1, by 4.8%; week 2, by 10.6%; week 3, by 14.1%; and week 4, by 5.3%) and 150/500 mg/kg bw per day (week 1, by 21.9%; week 2, by 24.0%; week 3, by 23.5%; week 4, by 15.5%) during the treatment period. There were no treatment-related findings in the conjunctivae, sclera, cornea, lens, and fundus and no alterations of the pupillary reflex.

Treatment with trifloxystrobin had no effect on the haematology, blood chemistry or urine analysis parameters investigated. Mean absolute and relative weights of the liver were slightly increased in animals of both sexes at 150/500 mg/kg bw per day (Table 14), while mean absolute and relative weights of the spleen were increased in females only at this dose. The last finding in the spleen was confirmed by histological changes consisting of moderate congestion of the splenic red pulp in three out of four animals in the at the highest dose. The sponsor considered the splenic congestion to be an agonal condition known to occur as a result of the method of euthanasia, and was not considered of toxicological relevance. However, the correlation between congestion and increased spleen weight in the females at the highest dose and the lack of similar findings at the other doses suggest that the histopathology changes are treatment related. There were no other microscopic treatment-related effects and no remarkable findings on necropsy.

The NOAEL was 50 mg/kg bw per day on the basis of clinical signs (diarrhoea and vomiting), decreased body-weight gain, and measures of liver and spleen weights in both sexes in addition to increased splenic congestion in females at the highest dose (Altmann, 1994).

In a study conducted in compliance with the principles of GLP with QA certification, four male and four female beagle dogs were fed gelatin capsules containing trifloxystrobin (purity, 96.2%) once per day at a dose of 5, 30, 150 and 500 mg/kg bw per day for 91 days. After experiencing significant vomiting and decreased food consumption during the first 10 days, dogs in the group receiving trifloxystrobin at 500 mg/kg bw per day were given two capsules per day (each equivalent to 250 mg/kg bw per day), one at 2 h after feeding and one at approximately 3 h thereafter. Owing to severe body-weight loss in the group at

Table 14. Organ weights and organ-to-body-weight ratios in dogs given capsules containing trifloxystrobin for 28 days

	Dose (mg/kg bw per day)			
	0 (control)	20	50	150/500
Males				
Liver:				
Organ weight (g)	323.3	321.2	317.7	344.4
Organ-to-body-weight ratio (%)	2.98	2.94	3.41	3.85*
Kidney:				
Organ weight (g)	53.80	50.62	46.38	49.89
Organ-to-body-weight ratio (%)	0.49	0.45	0.50	0.56*
Females				
Liver:	261.1	280.1	247.1	345.5
Organ weight (g)	2.92	3.34	3.34	3.91*
Organ to body-weight ratio (%)				
Spleen:				
Organ weight (g)	24.31	27.87	25.09	41.19
Organ to body-weight ratio (%)	0.27	0.34	0.34	0.47*

From Altmann (1994)

* Statistical significance using the Jonckheere test ($p < 0.05$)

500 mg/kg bw per day, treatment for certain animals had to be discontinued for a few days at around the middle of the treatment period. Concentrations in the capsule were adjusted to maintain appropriate dosages according to body weight-gain/-loss of the animals. Mortality was checked twice per day and clinical signs daily; body weights were recorded weekly; food consumption was determined daily and reported as weekly means. Before the test and towards the end of the treatment period, all animals were subjected to ophthalmology examinations. Before the test, and at week 7 and week 13, all animals were subjected to haematology, clinical chemistry and urine analyses. After 90 days of treatment, all control and surviving treated animals were subjected to a detailed necropsy, including collection and microscopic evaluations of organs and gross lesions. Organ weights were recorded for adrenals, brain, heart, kidneys, liver, ovaries, spleen, testes, thymus, and thyroid/parathyroid.

One male at 500 mg/kg bw per day dose had to be sacrificed on day 66 owing to reduced food consumption, body-weight loss, and reduced locomotor activity. Vomiting was increased in a dose-related manner in animals of both sexes in week 1 (150 mg/kg bw per day: 15 occurrences observed in males, 12 in females; 500 mg/kg bw per day: 28 occurrences observed in males, 23 in females), followed by moderate to severe diarrhoea throughout the whole treatment period. Traces of blood were observed twice in the faeces of one male at 500 mg/kg bw per day at week 13. There were no differences in ophthalmology findings between control and treated animals.

A dose-dependent loss in body weight was recorded for males and females at 150 and 500 mg/kg bw per day. Normal body-weight development returned for animals in the group receiving trifloxystrobin at 150 mg/kg bw per day after about 4 weeks of treatment in males and after 6 weeks in females, but pre-test values were not reached until the end of the treatment period. Compared with values obtained before the test, mean body-weight loss at study termination for males and females was 0.4 kg and 0.38 kg at 150 mg/kg bw per day and 3.37 kg and 2.21 kg at 500 mg/kg bw per day, respectively. Body-weight development was not affected in animals at 5 or 30 mg/kg bw per day.

Food consumption of animals of both sexes at 500 mg/kg bw per day was markedly reduced during the first 2 weeks. To avoid excessive weight loss, feeding periods (normally about 3 h) were partly prolonged and food consistency was changed to a 1 : 1 mixture of pellets and powder. In males, force-feeding and partial discontinuation of treatment was also necessary. Food consumption in animals of both sexes at 500 mg/kg bw per day was reduced by as much as 15.7–43.7% in males and 18.9–37.8% in females during weeks 1–13. Mean food consumption was also moderately reduced at 150 mg/kg bw per day in males (by 30% at week 1 returning to control levels by week 9) and females (by 23% at week 1 and 45% at week 2, returning to control levels by week 10). Food consumption in the groups receiving the two higher doses returned to normal levels during the second half of the treatment period. During the first 3 weeks, a slight and transient reduction (by less than 12%) was also noted in females at 30 mg/kg bw per day dose, mainly owing to one animal. As body-weight development was not affected and no other toxicological findings were noted in this group, the transiently reduced food consumption in females at 30 mg/kg bw per day was not considered to be an adverse effect.

A slight hypochromic anaemia, in the form of lowered erythrocyte count, haemoglobin and erythrocyte volume fraction values (each by about 20–23%), was noted in all males and in one female at 500 mg/kg bw per day. Slightly reduced numbers of eosinophils (by

65% and 40% at weeks 7 and 13, respectively) and increased platelet counts (by 67% at week 13) were noted in males at 500 mg/kg bw per day. One male at 500 mg/kg bw per day had a leukocytosis with markedly increased numbers of neutrophils and monocytes. On the basis of increases in platelets, decreases in basophils, and increases in monocytes, it is likely that some of the males given trifloxystrobin at 500 mg/kg bw per day had an acute infection, possibly resulting from the overall poor health status of this group.

In the males and females at 500 mg/kg bw per day, there were significant decreases in several serum clinical chemistry parameters including creatinine, bilirubin, protein, albumin, and cholesterol, in addition to increases in concentrations of triglycerides (Tables 15 and 16). At 150 mg/kg bw per day, concentrations of triglycerides were increased in males and females, while concentrations of creatinine and cholesterol were decreased in females.

Table 15. Selected clinical chemistry changes (mean and % of control) in male dogs given capsules containing trifloxystrobin for 90 days

	Dose (mg/kg bw per day)				
	0	5	30	150	500
Creatinine (µmol/l)	76.15	73.73	69.73	67.48*	40.28*
Change at week 7		97%	92%	89%	53%
Creatinine (µmol/l)	74.93	72.55	77.78	78.30	46.08*
Change at week 13		97%	104%	104%	61%
Total bilirubin (µmol/l)	2.38	2.02	1.73*	1.96	1.51*
Change at week 13		85%	73%	82%	63%
Protein (g/l)	59.0	59.60	57.90	59.70	48.30*
Change at week 13		101%	98%	101%	82%
Albumin (g/l)	33.19	33.11	33.30	31.49	27.18*
Change at week 13		100%	100%	95%	82%
Cholesterol (mmol/l)	4.12	3.40*	3.72	4.09	2.84*
Change at week 13		83%	90%	99%	69%
Triglycerides (mmol/l)	0.38	0.35	0.58*	0.68*	0.65*
Change at week 13		92%	153%	179%	171%

From Altmann (1996)

* Statistical significance using the Wilcoxon test ($p < 0.05$)

Table 16. Selected clinical chemistry changes (mean and % of control) in female dogs given capsules containing trifloxystrobin for 90 days

	Dose (mg/kg bw per day)				
	0	5	30	150	500
Creatinine (µmol/l)	81.13	87.20	80.88	67.03*	53.83*
Change at week 13		107%	100%	83%	66.5%
Total bilirubin (µmol/l)	2.85	2.73	2.25	2.14	1.90*
Change at week 7		96%	79%	75%	67%
Protein (g/l)	55.84	58.68	59.71	57.78	48.64*
Change at week 13		105%	107%	103%	87%
Albumin (g/l)	33.16	34.46	34.86	33.35	28.02*
Change at week 13		104%	105%	101%	84%
Cholesterol (mmol/l)	3.66	4.08	3.93	2.83*	2.49*
Change at week 13		111%	107%	77%	68%
Triglycerides (mmol/l)	0.36	0.46	0.40	0.64*	0.59
Change at week 13		128%	111%	178%	164%

From Altmann (1996)

* Statistical significance using the Wilcoxon test ($p < 0.05$)

The only notable change in the group at 30 mg/kg bw per day was a slight increase in concentrations of triglycerides among males; however, this increase is considered to be incidental since pre-test levels were also high in this group. There were other changes (not shown here) including small decreases (generally <20%) in plasma concentrations of calcium, potassium, and phospholipids. Collectively, these changes may reflect a state of perturbed metabolism secondary to gastrointestinal problems, dehydration, lack of nutrition, and partial starvation. There were also isolated incidences of statistically significantly decreases in activities of serum liver enzymes that are not considered to be toxicologically relevant because a decrease was observed rather than the anticipated increase associated with liver toxicity; also, the decreases may be incidental, owing to unusually high levels in the control animals. In females at 500 mg/kg bw per day, there was a slight decrease in urine pH. Urine analysis profiles in other treatment groups were not altered.

Several organ weights and organ: body-weight ratios were affected. Compared with controls, carcass weights were moderately reduced in the group receiving trifloxystrobin at 500 mg/kg bw per day (males, by 35.7%; females, by 23.3%) and slightly lowered (by 5%) in females at 150 mg/kg bw per day. Mean relative weights of the liver increased dose-dependently at 150 mg/kg bw per day (males, by 33.6%; females, by 18.3%) and 500 mg/kg bw per day (males, by 60.9%; females, by 40.3%). Absolute weights of the liver were also increased (32.6%) in males at 150 mg/kg bw per day. The following changes were also seen in males and females at 500 mg/kg bw per day: increased relative weights of the kidney, by 36% and 21%; decreased absolute weights of the heart, by 50% and 31%; and decreased absolute (by 73% and 44%) and relative (by 61% and 30%) thymus weights, respectively. In addition, males at the same dose had increased relative weights of the adrenals (by 77%) and decreased absolute (by 68%) and relative (by 45%) testis weights. The consulting pathologist considered that the weight/relative weight changes in heart, kidneys, and adrenals were incidental, not dose-related, and not associated with histopathology findings. Therefore, the changes were considered to reflect the poor nutritional status and emaciation of the respective animals rather than a toxic effect; it should be noted, however, that relative weights of the kidney were also increased in the 3-month study in rats.

At necropsy, three out of four males and two out of four females at 500 mg/kg bw per day presented with emaciation of the body. Hair loss was also noted in one female in the same group. The male dog which was prematurely sacrificed in a moribund state had an enlarged gall bladder, which correlated with a moderate hyperplasia of the epithelium. Additional findings in this animal included enlargement of the adrenal glands, mottled stomach, and dilatation of the large intestines. There were no other treatment-related gross pathology findings.

There were several microscopic pathology findings that seemed to reflect both exposure to trifloxystrobin and effects of emaciation in the group at 500 mg/kg bw per day. The following observations probably resulted from emaciation: myopathy of skeletal muscle and atrophy of the cervical, mesenteric, and popliteal lymph nodes. Minimal hypertrophy of hepatocytes was observed in three out of four males at 150 and 500 mg/kg bw per day and in all females at 500 mg/kg bw per day. Minimal to moderate hyperplasia of the epithelium of the gall bladder was also noted in two out of four males and three out of four females at 500 mg/kg bw per day. In addition, a marked cytoplasmic vacuolization of hepatocytes, minimal erosion of the small intestine mucosa, moderate inflammatory cell infiltration and a moderate and focal dilatation of the intestinal glands was seen in the prematurely sacrificed male at 500 mg/kg bw per day. Furthermore, a minimal hypocellularity of the bone

marrow and a moderate atrophy of the white pulp of the spleen were detected in this animal. Atrophy was considered by the consulting pathologist to be secondary to the reduced food intake and the resulting emaciation in the animals at 500 mg/kg bw per day causing increased incidences of the following: minimal atrophy in the mesenteric lymph nodes of one out of four females and minimal to moderate atrophy in the thymus of three out of four females; atrophy of the skeletal muscle, reported as myopathy, in three out of four males (minimal to moderate) and in two out of four females (minimal); and moderate prostatic atrophy and moderate tubular atrophy of the testes (associated with a secondary moderate or marked reduction of spermatozoa in the lumen of epididymides) in all male dogs. All other changes were considered by the consulting pathologist to be incidental and/or occurring commonly in the colony of beagle dogs, and were not treatment-related.

The NOAEL was 30 mg/kg bw per day in both sexes on the basis of clinical signs of vomiting, body-weight loss, and increased absolute and relative weights of the liver, accompanied by hepatocyte hypertrophy (Altmann, 1996).

In a study conducted in compliance with the principles of GLP with QA certification, groups of beagle dogs were given gelatin capsules containing trifloxystrobin (purity, 96.4%) at a dose of 0, 2, 5, 50 or 200 mg/kg bw per day orally, once daily, 7 days per week for 52 weeks. The dogs received the capsules approximately 1–2 h after feeding. Capsules were prepared in about weekly intervals adjusted to body weights of the preceding week. Urine was collected by catheterization. Checks for mortality were made twice per day and for clinical signs daily; body weights were recorded weekly; food consumption was determined daily and reported as weekly means. At pre-test and towards the end of the treatment period, all animals were subjected to ophthalmology examinations (external inspection, examination of lens, iris and fundus with ophthalmoscope, pupillary reflex, examination of third eye lid after local anaesthesia). At pre-test, week 13, 26 and 52, all animals were investigated for haematology, clinical chemistry and urine analyses. Animals were fasted 16 h before blood collections. At scheduled sacrifices, the surviving control and treated animals were subjected to detailed necropsy and histopathology. Organ weights were recorded for adrenal glands, brain, heart, kidneys, liver, ovaries/testes, spleen, thymus, and thyroid/parathyroid.

Trifloxystrobin was found to be stable under the conditions of the test. There were no deaths during the study. Diarrhoea was increased in frequency and severity among animals of both sexes at 200 mg/kg bw per day and to a lesser degree in males at 50 mg/kg bw per day (Table 17). Slightly loose stools were also noted in females at 50 mg/kg bw per day. Vomiting occurred in animals of both sexes at 200 mg/kg bw per day, but more severely in females. Brownish discolouration of hair and skin of the paws, thorax and abdomen was noted in all animals at 200 mg/kg bw per day and in six out of eight animals at 50 mg/kg bw per day (Table 17). Discolouration was observed first on the paws of animals at the highest dose at week 15 and was seen throughout the entire observation period. Towards the end of the study the intensity of staining decreased and discolouration was mainly restricted to the paws. In the group at 50 mg/kg bw per day, the finding was generally restricted to paws and occurred in a transient manner or at the end of the study. The stain could not be removed by washing. There were no treatment-related changes in ophthalmology findings.

In females at 50 and 200 mg/kg bw per day, mean overall body-weight gain was slightly depressed, with the reduction being statistically significant (from weeks 2–7 inclusive) and was more pronounced earlier in the study. For instance, in females at 50 and 200 mg/kg bw per day, body-weight gain was reduced by 35.3% and 97.5% at week 2, by

Table 17. Incidences of clinical signs in dogs given capsules containing trifloxystrobin for 12 months

Clinical sign	Dose (mg/kg bw per day)									
	0		2		5		50		200	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
Diarrhoea[a]										
Moderate	<5	<10	0	0	0	0	>70	<20	>350	>350
Severe	0	0	0	0	0	0	<20	<5	>180	>80
Vomiting[a]	0	0	0	0	0	0	0	4	18	113
Dark discolouration[b] of coat of:										
Paws	0	0	0	0	0	0	3	3	4	4
Thorax	0	0	0	0	0	0	0	2	4	4
Abdomen	0	0	0	0	0	0	0	1	4	4
Dark discolouration[b] of skin of above regions	0	0	0	0	0	0	1	3	4	4

From Altmann (1997)

[a] Number of occurrences

[b] Number of animals affected within each group ($n = 4$)

18.5% and 26.1% at week 26, and by 22.8% and 19.9 at week 52, respectively. Body-weight gain in males was unimpaired by treatment.

Mean food consumption was slightly and transiently reduced in males and moderately reduced in females at 200 mg/kg bw per day with the largest decrease being seen at the beginning of the study. For instance, during week 2, food intake in males and females at 200 mg/kg bw per day was below values for controls by 10.3% and 27.3%, respectively, and remained slightly depressed until the end of treatment in females (by 6.9% at week 52). A slightly reduced food consumption was also noted in females at 50 mg/kg bw per day (by 10.3% at week 52). Food consumption ratios fluctuated in both sexes at 200 mg/kg bw per day and in females at 50 mg/kg bw per day, reflecting the reduced food intake observed in these animals.

Lower eosinophil counts were recorded for males at 200 mg/kg bw per day from week 13 onwards, with a statistically significant difference compared with those of controls at week 52 (by 42.6%). Compared with controls, females at 200 mg/kg bw per day had statistically significantly higher platelet counts at weeks 26 (by 6.5%) and 52 (by 9.0%). However, these platelet counts did not differ appreciably from those recorde before the start of the test and were therefore judged by the sponsor to be unrelated to treatment. Other differences between the means that attained a level of statistical significance were not likely to be related to treatment as the magnitude of the changes was too small to be toxicologically relevant and/or the changes occurred without any relation to the dose administered or to the duration of treatment.

Some of the clinical chemistry parameters were changed in males at 50 mg/kg bw per day and in males and females at 200 mg/kg bw per day; the changes in males were consistent throughout the study. Compared with controls, males at 50 mg/kg bw per day had lower plasma concentrations of albumin (by 3–9%) and higher activities of alkaline phosphatase (by 13%, 29% and 53% at weeks 13, 26 and 52, respectively). Compared with controls, males at 200 mg/kg bw per day also had a lower plasma concentrations of albumin (by 7–13%), higher activities of alkaline phosphatase activity (by 28%, 72%, and 117.0%), and higher plasma concentrations of of triglycerides (by 46%, 51% and 97%) throughout the study (at weeks 13, 26 and 52, respectively); in addition, there was a slight increase in

concentrations of chloride at week 52 (by 4.8%). Some treatment-related changes were also seen among females throughout the study, but were noted only at 200 mg/kg bw per day and were limited to a higher plasma concentrations of triglycerides (by 70%, 66% and 43% at weeks 13, 26 and 52, respectively) and a higher alkaline phosphatase activity (by 26%, 70%, and 82% at weeks 13, 26 and 52 respectively). Other sporadic statistically significant differences were not dose-related, were observed only at a single time-point, and/or the magnitude of the changes were small. No treatment-related effects were found in any of the qualitative or quantitative urine analysis parameters.

No treatment-related gross lesions were found. The findings of "lung nodule" and "lung mottled" were not associated with microscopic findings and therefore were not considered treatment-related by the consulting pathologist.

Mean absolute and relative weights of the liver were increased by nearly 15–39% in males and females at 50 or 200 mg/kg bw per day (Table 18). The effects on liver weights correlated with some of the clinical chemistry and histopathology findings.

At 50 and 200 mg/kg bw per day, there was a slight increase in mean absolute (by 21.8% and 25.4%) and relative (by 18.7% and 31.6%) testes weights. However, the testes weights in the treated groups were within the range for historical controls, while the testes weights in the control group were at the lower end of the range for historical controls. Additionally, there were no pathology findings in the testes and the increased weight was therefore not likely to be treatment-related. In the absence of histological changes and because absolute weights were similar to those of the controls, the significant increase in relative weight of the adrenals (by 24.4%) in males at 200 mg/kg bw per day was probably incidental in nature and unrelated to treatment. Also, the increased weight of the spleen (by 58.15%) in females at 2 mg/kg bw per day was not dose-dependent and not likely to be treatment-related.

An increase in the incidence and/or severity of hepatocellular hypertrophy was found in the livers of animals of both sexes at 200 mg/kg bw per day and in females at 50 mg/kg bw per day (Table 19). Minimal to slight bone marrow hypocellularity occurred at a higher incidence in males and females in the group receiving trifloxystrobin at a dose of 200 mg/kg bw per day (Table 19) with no attendant effect on erythrocyte or leukocyte populations.

A dose-related tan discolouration of the skin was found in animals at 50 and 200 mg/kg bw per day. The author of the study considered it to be a physicochemical "dyeing" effect of the test article. The discolouration of the body surface areas appeared to be resistant to formalin solution, but disappeared after normal histopathological process-

Table 18. Absolute and relative weights of the liver in dogs given capsules containing trifloxystrobin for 12 months

Dose (mg/kg bw)	Males		Females	
	Absolute weight (g)	Relative weight (%)	Absolute weight (g)	Relative weight (%)
0 (control)	317.4	2.81	282.2	2.78
50	375.1	3.22	341.5	3.48
200	421.9*	3.90*	363.3	3.79*

From Altmann (1997)
* $p < 0.05$; Jonckheere's test

Table 19. Incidences of microscopic findings in dogs given capsules containing trifloxystrobin for 12 months

Finding	Males					Females				
	0	2	5	50	200	0	2	5	50	200
Tissues examined	4	4	4	4	4	4	4	4	4	4
Treatment-related incidences										
Liver: hepatocellular hypertrophy	1	1	1	1	3	0	0	0	3	4
Bone marrow: hypocellularity	1	1	1	1	3	2	1	1	2	4

From Altmann (1997)

ing. On microscopic examination, no discolouration and no treatment-related histopathological changes were seen in tissue sections stained in a standard manner with haematoxylin and eosin. In the absence of any pathological consequences, this finding was considered to be of no toxicological relevance.

Cross-sections of testes as well as the seminiferous tubules appeared slightly larger in the groups receiving trifloxystrobin at 50 and 200 mg/kg bw per day than in the control group. However, histological appearance, maturity, cyclic development and spermatogenic stages were normal in all animals. No pathological changes were found in any of the examined testes. The consulting pathologist concluded that the difference in testis size reflected normal physiological variations and was of no toxicological relevance.

The NOAEL was 5 mg/kg bw per day in both sexes based on increased absolute and relative weights of the liver (in both sexes), hepatocellular hypertorphy (in females), biochemical changes (in males), diarrhoea (in both sexes), and reduced body-weight gain (in females) at the next higher dose (Altmann, 1997).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a study conducted in compliance with the principles of GLP with QA certification, groups of 70 male and 70 female mice (Tif:MAGf, hybrids) were continuously fed diets containing trifloxystrobin (purity, 96.2%) at a dose of 0, 30, 300, 1000 and 2000 ppm (equal to 3.9, 39.4, 131.1 and 274 mg/kg bw per day in males and 3.5, 35.7, 124.1 and 246 mg/kg bw per day in females, respectively) for 18 months. For the carcinogenicity evaluation, 50 mice of each sex per dose were maintained for 18 months. The remaining animals were subjected to haematological evaluations at weeks 53 (10 of each sex per group) and 79 (10 of each sex per group). In addition, blood smears were prepared from all surviving animals that were involved in the carcinogenicity evaluation at terminal sacrifice. Concentrations of trifloxystrobin in the diet were analysed periodically throughout the study. Samples of the diet prepared for the first 4 weeks of the study were analysed for stability (for 5 weeks) and homogeneity of trifloxystrobin. Mortality was checked twice per day (once at weekends and holidays) and clinical signs daily; body weight and food consumption were recorded weekly for the first 3 months and monthly thereafter; water consumption was recorded monthly. At scheduled sacrifices the surviving control and treated animals were subjected to detailed necropsy and histopathology. Organs weights were recorded for adrenal glands, brain, kidneys, liver, ovaries/testes, and spleen.

Table 20. Survival to termination necropsy in mice given diets containing trifloxystrobin for 18 months

Dietary concentration (ppm)	Males		Females	
	No.	%	No.	%
0 (control)	43	86	42	84
30	45	90	36	72
300	40	80	45	90
1000	45	90	45	90
2000	46	92	42	84

From Gerspach (1997a)

Table 21. Summary of cumulative body-weight gain (% of control) in mice fed diets containing trifloxystrobin for 18 months

Week	Dietary concentration (ppm)							
	Males				Females			
	30	300	1000	2000	30	300	1000	2000
Week 13	100	101	96	90**	96	92	87**	86**
Week 40	98	90	92	93	89	81	83**	85**
Week 78	95	95	92	91	92	86	98	89

From Gerspach (1997a)
** Statistically significant ($p < 0.01$)

Trifloxystrobin was found to be homogeneously distributed and stable in the diet for at least 5 weeks at room temperature. Concentrations in the diet ranged from 82% to 116% of the nominal values.

The survival rate was not affected by treatment (Table 20). The relatively higher number of deaths occurring in females at 30 ppm was considered incidental. No treatment-related clinical signs or behavioural changes were observed at any dose.

Relative to control, body-weight gain was slightly but consistently reduced (by 5–10%) in males at 2000 ppm (Table 21). The effect became obvious particularly during the growth phase, i.e. the first 9 months, with statistical significance being achieved only at weeks 7, 10 and 13. After 3 months of treatment, the mean body-weight gains were decreased in females by 10% at 1000 ppm and by 14% at 2000 ppm when compared with controls. The difference between the group at 2000 ppm and the control group exceeded 20% on several occasions. Body-weight development of females at 300 ppm was also affected, but to a lesser extent in magnitude and significance. Decreased body-weight gain in this group started around week 10 and persisted throught the study at a magnitude of nearly 10–20%; however, statistical significance was only achieved at weeks 10, 43, 47 and 67 (by 21.4%, 20.7%, 18.1% and 14.2%). There were no other toxicological findings in the same group (females at 300 ppm). The decreased body-weight gain of females at 300 ppm is most likely to be treatment-related but, on its own, this effect is not sufficiently robust to be considered as adverse. In the groups of males at 30, 300 and 1000 ppm and the group of females at 30 ppm, body-weight development was similar to that in the control group. Minor variations in these groups were incidental.

Cumulative food consumption in females at 2000 ppm throughout the study was 7% below that of the control group. No treatment-related effects were observed at any other

dose. The occasional occurrence of statistically significant differences was influenced by food spillage in the respective groups. Water consumption was not affected by treatment.

At both weeks 53 and 79, no treatment-related effects on haematology parameters were observed at any dose. The trends to lower mean cell haemoglobin concentration (MCHC) values in males at 2000 ppm at weeks 53 (by 2.7% compared with controls) and 79 (by 2.95% compared with controls) and at 1000 ppm at week 79 (by 3.6% compared with controls) in addition to lower haemoglobin concentrations (by 7%compared with controls) at week 53 in females at 2000 ppm were of no toxicological relevance owing to the absence of dose-dependency or the low magnitude of the effect. Lymphatic leukaemia, which was observed with an incidence of one out to three animals per group in all groups (including the control group), is known to occur spontaneously at a low incidence in the colony of mice, according to the sponsor.

Compared with the controls, carcass weight was reduced in females at 2000 ppm by 13% at interim sacrifice (week 39). Probably as a result of this, relative weights of the liver and spleen were increased by 14% and 34%, respectively. Absolute and relative weights of the liver were significantly increased in males at 2000 ppm (by 33% and 27%) and to a lesser extent also at 1000 ppm (by 15% and 13%). At terminal sacrifice (week 79), absolute and relative weights of the liver showed significantly increased values in males at 2000 ppm (by 22% and 25%) and in females (by 7% and 12%). A similar effect was also observed in females at 1000 ppm (by 7% and 10%), but only minimally (by 4–5%) in the respective group of males. There was a minimal trend to increased relative weights of the kidney in females at 2000 ppm at week 79 (11.4%), but this is not likely to be of toxicological relevance owing to the absence of any other relevant findings in this organ.

A slightly increased number of males and females with enlarged liver was present in the group receiving trifloxystrobin at 2000 ppm, which corresponded with hepatocellular hypertrophy in some animals. There were no other treatment-related gross macroscopic findings. The slight decrease in masses and/or nodules of the liver in animals of both sexes at 1000 and 2000 ppm was likely to be incidental. These changes corresponded microscopically to various neoplasias of the liver.

Microscopic evaluation revealed treatment-related findings in liver only (Table 22). The incidence of hepatocellular hypertrophy was significantly increased in males and females at 2000 ppm. The severity of this change, however, was similar in control and treated animals. Incidences of single-cell necrosis (including minute clusters of necrotic hepatocytes) were significantly increased in males at 1000 ppm and in males and females at 2000 ppm. The incidence of focal liver necrosis was slightly increased in females at 2000 mg/kg. The severity of fatty change was increased in males at 2000 ppm. Most of these effects had already been detected at interim sacrifice (9 months). In treated female mice, fatty change and inflammatory cell infiltration of the liver were decreased in incidence but occurred in numerous animals.

Other observations not considered to be toxicologically relevant by the consulting pathologist included: an increased incidence of tubular casts in kidneys in females at 2000 ppm (83.3%) might be indicative of a mild stage of chronic progressive nephropathy, if it were associated with tubular atrophy. However, the incidence of tubular atrophy was not increased in this group. Chronic reactive hyperplasia was increased in mesenteric lymph nodes of females at 1000 and 2000 ppm. At both doses, 17 out of 47 mesenteric lymph

Table 22. Microscopic findings in the livers of mice given diets containing trifloxystrobin for 9 months (interim sacrifice) or 18 months (terminal sacrifice)

Finding	Sacrifice group	Dietary concentration (ppm)									
		Males					Females				
		0	30	300	1000	2000	0	30	300	1000	2000
Tissues examined	Terminal	50	50	50	50	50	50	50	50	50	49
	Interim	10	10	10	10	10	10	10	10	10	10
Hepatocellular hypertrophy	Terminal	36	37	36	41	44	7	7	7	13	21
	Interim	6	8	8	7	10	2	1		2	5
Single-cell necrosis	Terminal	6	4	7	15	22	5	3	3	6	12
	Interim	1	0	2	2	4	2	0	0	1	5
Necrosis	Terminal	3	3	1	3	1	2	5	5	6	9
	Interim	0	0	0	0	1	1	2	0	0	1
Fatty change	Terminal	32	30	35	36	39	42	33	33	30	25
	Interim	9	10	9	10	9	7	9	8	6	5

From Gerspach (1997a)

nodes were affected (36.2%), compared with incidences of 9 out of 44 (20%) in the concurrent control group; however, the incidences in the treated groups were similar to those at the higher end of the range for historical controls (18/50 or 36.0%). The slightly increased incidences of necrosis in the Harderian gland of males at 2000 ppm were considered by the sponsor to be a consequence of the blood sampling procedure performed in all animals for evaluation of carcinogenicity potential (terminal group). Peritoneal, lymphohistiocytic infiltration was slightly increased in females at 1000 and 2000 ppm. The consulting pathologist stated that peritoneal inflammation is known to occur secondarily to inflammatory processes of abdominal organs. The inflammatory process in other organs or tissues occurred incidentally.

Malignant lymphoma, a systemic neoplasia, occurred in variable incidences in animals of either sex in the control group and in the treated groups (2%, 6%, 4%, 8% and 8% in males and 10%, 18%, 18%, 8% and 22% in females, at 0, 30, 300, 1000 and 2000 ppm, respectively). However, no dose–response relationship was evident and the incidences were well within the range for incidence of malignant lymphoma in the historical control groups (range: males, 2%–9.4%; females, 10%–36.5%). In addition, the concurrent control group had incidences of malignant lymphoma (2% in males and 10% in females) which were closer to the lower end of the range for historical controls. Also, the type and number of organs infiltrated by this tumour varied by chance within a wide range of individual animals. Accordingly, no toxicological relevance was attributed to the increased numbers of infiltrations in certain tissues. Ovarian cysts and pressure atrophy of the brain were present in numbers too small to be of any toxicological relevance. There was no evidence for a treatment-related effect on the incidence of tumour-bearing animals.

The NOAEL was 300 ppm in males and females (equivalent to 39.4 and 35.7 mg/kg bw per day, respectively) on the basis of findings at 1000 ppm—increased absolute and/or relative liver weights in both sexes, increased hepatocellular single cell necrosis in males, and impaired body-weight development (by about 15–30%) in females starting at week 5 and persisting throughout the treatment period. Trifloxystrobin was tested at adequate doses, on the basis of decreased body weight/body-weight gain in females and increased liver pathology findings in both sexes at 2000 ppm, and was found to be not carcinogenic in mice (Gerspach, 1997a).

Rats

In a study conducted in compliance with the principles of GLP (with QA certification), rats were continuously fed diets containing trifloxystrobin at a dose of 0, 50, 250, 750 and 1500 ppm (equal to 1.95, 9.8, 29.7 and 62.2 mg/kg bw per day in males, and 2.2, 11.4, 34.5 and 72.8 mg/kg bw per day in females) for 24 months. Concentrations of trifloxystrobin in the diet were analysed periodically throughout the study. Samples of the diet prepared for the first 4 weeks of the study were analysed for stability (for 5 weeks) and homogeneity. Checks were made twice per day for mortality and daily for clinical signs; body weight and food consumption were recorded weekly for the first 3 months and monthly thereafter; water consumption was recorded monthly. Ophthalmological examinations were conducted at pre-test and at 6, 12, 18 and 24 months and included inspection of the surroundings of the eyes, of sclera, cornea, iris and adaptation of the pupil to light. Haematology (20 of each sex per group), clinical chemistry (10 of each sex per group) and urine analysis (10 of each sex per group) investigations were carried out at weeks 13, 27, 53, 79 and 105. At scheduled sacrifices, the surviving control and treated animals were subjected to detailed necropsy and histopathology. At the scheduled terminal and interim sacrifices, the surviving control and treated animals were subjected to detailed necropsy and histopathology. Organ weights were recorded for adrenal glands, brain, heart, kidneys, liver, ovaries/testes, and spleen.

Towards the end of the study, the incidence of diarrhoea in males at 1500 ppm was increased. No other treatment-related findings were noted. There were no apparent treatment-related deaths. However, significantly more males at 750 and 1500 ppm survived to the end of treatment. The survival rates of animals from the carcinogenicity group were 68% at 750 ppm and 80% at 1500 ppm, compared with 34% in the control group. Better survival was also seen in females at 1500 ppm (80%) in comparison with controls (66%), although statistical significance was not reached. In addition, slightly more male rats given trifloxystrobin at 50 and 250 ppm survived to the end of treatment compared with the control group. However, there was no dose–response relationship and the 24-month survival of males in the control group (17 out of 50, or 34%) was in the low range of historical control values. There were no treatment related ophthalmic changes at any time-point.

Body-weight gain was reduced during most of the study in males and females in the groups receiving trifloxystrobin at 750 or 1500 ppm and in females at 250 ppm (Table 23). Cumulative body-weight gain was generally about 5–6% and 11–17% below that of the controls in males treated at 750 and 1500 ppm, respectively. Statistical significance was reached throughout the entire study period for the group at 1500 ppm, but only during the first 9 months of the study in the group at 750 ppm. Compared with the control group, females at 250, 750 and 1500 ppm had reductions of approximately 5–7%, 9–11% and 17–27%,

Table 23. Cumulative body-weight gain (% of control) in rats fed diets containing trifloxystrobin for 2 years

Week	Dietary concentration (ppm)							
	Males				Females			
	50	250	750	1500	50	250	750	1500
12	99.4	98.6	94.7**	87.0**	99.4	94.7	89.6**	80.9**
51	100.1	100.0	94.8	83.7**	98.9	92.6	89.2**	73.8**
103	106.7	106.1	101.2	93.5	93.2	95.5	91.3	74.5**

From Oishi et al. (1995)
** Statistically significant ($p < 0.01$)

respectively. Statistical significance was reached throughout the entire study period in the group at 1500 ppm, and during most of the time at 750 ppm. The marginal deviations in females at 250 ppm were associated with slightly reduced food intake but never reached statistical significance. As no functional change was observed at this dose, the body-weight effect is considered to be not toxicologically relevant.

Cumulative food consumption throughout treatment in males and females at 1500 ppm was 4% and 8% below that of the control group, respectively (Table 24). A tendency to minimally reduced food intake was also seen in females treated at 250 and 750 ppm (approximately 4% lower overall intake each). Food consumption in the remaining groups was similar to that of the controls. While males in the group receiving the highest dose consumed more water (by 4%) over most of the study compared with control animals, female animals in the same group had a reduced water consumption.

There were minor changes (generally less than ±5% of respective control values) in erythrocyte parameters including increased erythrocyte counts, haemoglobin concentrations, and erythrocyte volume fraction, and lower mean cell volume (MCV), mean corpuscular haemoglobin (MCH), MCHC, and haemoglobin concentration distribution width (HDW) in both sexes at ≥750 ppm, predominantly during the first year of the study. These alterations are considered incidental as there was no clear dose–response relationship or time dependency, and most of the variations were minimal and did not attain statistical significance. Even if the changes are considered to be treatment-related, the magnitude of the effect was too low to be of any toxicological relevance. One male at 50 ppm was found to have a myeloid leukaemia and one female at 750 ppm had a blast cell leukaemia. These pathologies were considered by the sponsor to be of spontaneous origin and not related to treatment.

There were no treatment-related findings in any of the examined blood chemistry parameters. Some intergroup statistically significant differences were seen, including increases in serum concentration of urea (by 11–19%), creatinine (by 7–16%), potassium (by 10%), cholesterol (by 14%), and triglycerides (by 33%) or decreases in concentrations of total bilirubin (by 30%), potassium (by 13–18%), and chloride (by 0.9–2.9%). However, these changes are not considered to be toxicologically relevant owing to the lack of dose-

Table 24. Food consumption (g/animal) in rats fed diets containing trifloxystrobin for 24 months

	Dietary concentration (ppm)				
	0	50	250	750	1500
Cumulative food consumption, total for weeks 1–103 (% of controls):					
Males	17455.9	17710.5	17537.8	17116.2	16785.9
	(NA)	(101.5)	(100.5)	(98.1)	(96.2)
Females	12429.1	12339.7	11893.8	11881.8	11453.7
	(NA)	(99.3)	(95.7)	(95.6)	(92.2)
Time-weighted average food consumption per week (% of controls):					
Males	169.5	171.9	170.3	166.2	163.0
	(NA)	(101.4)	(100.5)	(98.1)	(96.2)
Females	120.7	119.8	115.5	115.5	111.2
	(NA)	(99.3)	(95.7)	(95.6)	(92.1)

From Oishi et al. (1995)
NA, not applicable

dependency, occurrence at a single time-point, the very low magnitude of the changes and/or the changes being in the opposite direction from that considered to be a toxic effect.

The quantitative and qualitative tests on urine did not reveal any evidence for a treatment-related effect. There were a few minor differences that, despite attaining statistical significance, were considered to be incidental, not dose-related, and toxicologically irrelevant.

At interim sacrifice (week 53), the mean carcass weights were reduced in males at 1500 ppm (13.3%) and in females at 750 (9.2%) and 1500 ppm (18.7%). Mean heart : body-weight ratios were increased in females at 750 ppm (14.6%) and 1500 ppm (25.5%). Relative weights of the liver were increased (not statistically significantly) in males at 750 (11.1%) and 1500 ppm (10.0%), while females at 1500 ppm had statistically significantly increased mean relative weights of the liver (23.9%) and kidney (20.2%).

At terminal sacrifice, the mean carcass weight of females at 1500 ppm was still significantly lower (16.5%) than that of the control group. Some organs from males and females at 1500 ppm had reduced absolute weights (by about 7–20%), probably commensurate with reduced body-weight development. Significant increases were seen in the mean relative weights of the heart (12%), liver (8.8%), and kidneys (12.4%) in females at 1500 ppm. Increased relative testes weights in the animals at 1500 ppm (21.7%) was attributed to the occurrence of fluid contents in the albugineous tunica of some animals, as was noted at necropsy. Since there were no microscopic correlates, these observations are most likely not treatment-related.

At interim necropsy, there were no macroscopic findings indicating an effect of treatment. At terminal necropsy, there were decreased incidences of masses on body surfaces and decreased incidence of enlarged pituitary gland in males and females of the groups at 1500 and 750 ppm. These observations reflected a decrease in tumour incidences and are not toxicologically relevant.

At interim necropsy, there were no treatment-related, non-neoplastic histopathology findings except for a slightly decreased incidence of fatty liver and pancreas in females at 750 and 1500 ppm. Decreased incidences of fatty liver and pancreas became more prominent at terminal necropsy. These changes possibly reflect the low mean body weight, especially in the females at 1500 ppm.

Owing to significantly increased survival in the treated groups, several age-related spontaneous histopathology findings, which tend to occur in geriatric animals, were seen at higher incidences in treated animals especially among the males at 1500 ppm. Such increased incidences, however, probably reflected the age difference rather than being a direct effect of treatment. In particular, when adjusted for time-dependence, statistical analysis revealed no significant differences, except for the findings of angiomatous hyperplasia in the mesenteric lymph node and of developmental cysts in the pituitary gland. The consulting pathologist stated that these findings were not considered to be treatment-related because angiomatous hyperplasia of the mesenteric lymph node is a characteristic spontaneous age-related finding in rats, and developmental cysts of the pituitary gland represent a developmental change already present in the animals before the beginning of treatment.

Overall, there were no apparent treatment-related neoplastic findings. At interim necropsy, the numbers of observed tumours were low and not affected by treatment. At

terminal necropsy, the overall incidence of tumours, including several specific tumour types, were decreased in a dose-related manner. The decreased incidence of tumours in general probably reflects the development of lower body weight in treated animals when compared with controls, and may not be a direct effect of trifloxystrobin.

Certain tumours, however, appeared to be increased dose-dependently (Table 25). The sponsor explained that the apparent increases were caused by the higher number of survivors to termination in the groups at 750 and 1500 ppm and that the tumour types and incidences were comparable with findings normally occurring in ageing rats. According to the sponsor, the increased incidences of benign adrenal medullary tumours and haemangiomas of the mesenteric lymph node in males at 1500 ppm are a result of random distribution, in view of the common occurrence of these tumour types in ageing male rats (Losco & Harleman, 1992). The sponsor also argued that the microscopic features of the findings in the study were characteristic of spontaneous lesions based on irregularly sized blood-filled spaces lined by elongated cells with oval nuclei, with solid sheets of oval cells in larger tumours. According to the consulting pathologist, the origin of these lesions is unknown and some authors do not consider them to be neoplastic and prefer to characterize them as "mesenteric disease". The observed incidence in the males at 1500 ppm was 5 out of 49 (10.2%), which is above the maximum value for historical controls of 4 out of 59 (6.8%). However, the consulting pathologist stated that it is important to note that all five cases in the group at 1500 ppm were observed in animals surviving until terminal sacrifice and this lesion is age-related, and consequently the relative incidence of this finding in the survivors is the more appropriate index for comparison. Following this approach, the sponsor argues that the finding in this study was not treatment-related since the relative incidence of 5 out of 40 (12.5%) in the study was comparable to the maximum for historical controls of 3 out of 26 (11.5%).

There was an indication that in the treated animals a few tumours were observed earlier than in the control groups. For example, an adenocarcinoma of the mammary gland was noted at week 16, and a malignant astrocytoma of the brain in a female was noted at week 26, both of these in the group receiving trifloxystrobin at 1500 ppm. The sponsor placed no toxicological relevance on the occurrence of these two neoplasms owing to their

Table 25. Selected tumours occurring at apparently increased incidences in male rats fed diets containing trifloxystrobin for 24 months

	Dietary concentration (ppm)				
	0	50	250	750	1500
Adrenal medullary tumour					
Organs examined	50	50	50	50	50
Organs with benign tumour	0	2	3	3	5
Organs with malignant tumour	1	1	0	1	0
Organs with either tumour	1	3	3	4	5
Mesenteric lymph node: haemangioma					
Organs examined	49	50	49	49	49
Organs affected	0	1	1	2	5
Organs examined in survivors	17	25	23	34	40
Organs affected in survivors	0	0	1	2	5
% affected in survivors	0	0	4.3	5.9	12.5

From Oishi et al. (1995)

low incidence and because the general occurrence of neoplasms was decreased in a dose-related manner. Moreover, a spontaneous early occurrence of mammary adenocarcinoma in a female Sprague-Dawley rat aged 10 weeks has been reported (Oishi et al., 1995).

A variety of other non-neoplastic and neoplastic changes in the study were marginally increased or were considered to be findings that are known to occur spontaneously in laboratory rats.

The NOAEL was 250 ppm for both sexes (corresponding to 9.8 and 11.4 mg/kg bw per day in males and females, respectively), based on the reduction in body weight and body-weight gain at the next higher dose. Dosing was considered adequate for testing for carcinogenicity in rats on the basis of decreased body-weight gain in males (by 11–17%) and females (by 17–27%) at 1500 ppm, the highest dose, in addition to decreased food consumption in females (by 7.9%) at the same dose. Trifloxystrobin was not carcinogenic in rats (Gerspach, 1997b).

2.4 Genotoxicity

Trifloxystrobin (purity, 96.4%) was evaluated for potential genotoxicity in an adequate range of assays performed in vitro and in vivo (Table 26). The tests performed in vitro included tests for mutagenicity in bacterial and mammalian cells, for chromosome damage (clastogenicity) and for unscheduled DNA synthesis. In all tests carried out in vitro, trifloxystrobin was tested at adequate concentrations resulting in cytotoxicity and/or precipitation. With the exception of the test for forward gene mutation, the results of these studies demonstrated the absence of a genotoxic effect (Table 26). In the teste for forward gene

Table 26. Results of studies of genotoxicity with trifloxystrobin

End-point	Test object	Concentration	Purity (%)	Result	Reference
In vitro					
Reverse mutation[a,b]	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537; *E. coli* WP2 uvrA	62–5000 µg/plate ± S9; in DMSO	96.4	Negative	Hertner (1994a)
Forward mutation[c,d]	Chinese hamster V79 lung cells	11.11–833.5 µg/ml +S9; 0.14–833.5 µg/ml −S9; both in DMSO	96.4	Positive at cytotoxic doses Equivocal	Hertner (1995a)
Chromosomal aberrations[c,e]	Chinese hamster ovary (CHO-K1)	0.78–200 µg/ml +S9 0.049–200 µg/ml −S9; in DMSO	96.4	Negative Negative	Hertner (1994b)
Unscheduled DNA synthesis[f,g]	Rat hepatocytes (male Sprague-Dawley)	0.39–400 µg/ml 0.39–50 µg/ml; in DMSO	96.4	Negative Negative	Hertner (1995b)
In vivo					
Chromosomal aberrations[h]	Mouse (Tif:MAG)	1250–5000 mg/kg bw; in carboxymethyl cellulose	96.4	Negative	Hertner (1995c)

S9, 9000 × *g* supernatant of liver of rats induced with Aroclor 1254; DMSO, dimethylsulfoxide

[a] Test in triplicate; positive control included; GLP and QA statements provided

[b] Cytotoxicity was not seen at 5000 µg/plate with or without S9; test material precipitated at or above 1250 µg/plate

[c] Test in duplicate; positive control included; GLP and QA statements provided

[d] Cytotoxicity observed at doses of >0.41 µg/ml without S9 (by 94–95%) and >6.5 µg/ml with S9 (by 6.4–92.4%); test material precipitated at or above 50 µg/ml without S9 and at or above 150 µg/ml with S9

[e] Cytotoxicity observed at doses of >3.125 µg/ml without S9 and >100 µg/ml with S9

[f] Two independent trials were done; positive control included; GLP and QA statements provided

[g] Cytotoxicity observed at doses of >50 µg/ml; test material precipitated at or above 25 µg/ml

[h] Aberration tested in five animals of each sex per group for each sacrifice time; positive control included; absorption or transport of compound to bone marrow was not tested but limit dose was achieved; GLP and QA statements provided

mutation in Chinese hamster V79 cells, there were slight statistically significant increases in mutant frequencies at cytotoxic doses in the presence of metabolic activation. Results were equivocal in the absence of metabolic activation. Trifloxystrobin was also assessed for induction of micronucleus formation in mice. The result of this study showed that trifloxystrobin does not exhibit a chromosome damaging potential in vivo.

Despite the equivocal mutagenicity findings in the mammalian test system, the overall in findings vitro and in vivo support the conclusion that trifloxystrobin is not likely to be mutagenic or genotoxic.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

In a study conducted in compliance with the principles of GLP with QA certification, male and female Sprague-Dawley (Tif:RAIf) rats were given diets containing trifloxystrobin (purity, 96.4%) at a nominal concentration of 0, 50, 750 or 1500 ppm continuously over two successive generations (F_0 and F_1). After 10 weeks of dietary exposure to trifloxystrobin before mating, animals were paired 1:1 within each dose group (30 of each sex per group) until there was evidence of positive mating or for 19 days, whichever occurred first. Dams were allowed to litter and suckle their pups naturally. On postnatal day 4, litters were culled to four male and four female pups. After weaning of the F_1 pups, the F_0 parental animals were remated to produce a second set of litters. The F_1 generation was selected from the first litters of the F_0 generation. The animals were checked daily for mortality and clinical signs (twice per day if signs were observed); body weights were recorded weekly; food consumption was determined weekly (except during cohabitation for mating) and were reported as daily means. Also, parental mating, fertility, and gestation indices were determined.

Signs of difficult or prolonged parturition were recorded. All animals were necropsied and subjected to a complete macroscopic pathological examination with special attention to the reproductive system. Organ weights from parental animals were recorded for adrenal glands, brain, kidneys, liver, ovaries/testes, spleen, and thymus. Full histopathological examination was performed on selected organs/tissues, including vagina, uterus, ovaries, testes, epididymides, seminal vesicles, prostate, pituitary gland, liver, pancreas, and all gross lesions, from all control and at the highest dose F_0 and F_1 animals that were selected for mating. The liver, spleen, and kidneys from all males and females of both F_0 and F_1 generations were wighed and the same organs were examined histopathologically.

The number of viable and stillborn pups was determined on postnatal day 0. Mortality and clinical signs were checked daily from postnatal day (twice per day if signs observed); body weights were recorded on postnatal days 0, 4, 7, 17 and 21. Righting reflex was tested from postnatal day 2 to 100% occurrence. Eye opening was examined daily from postnatal day 14 to 100% occurrence. Live birth, viability, and lactation indices were also determined.

The F_{1A} pups that were not selected for mating, in addition to F_{1B} and F_2 pups, were killed on or shortly after weaning of the last litter of that mating. All these animals, as well as all culled pups and those found dead or killed in a moribund condition, were subjected

to gross necropsy, which consisted of macroscopic examination of the body, limbs, and organs of the thoracic and abdominal cavities, with special attention to the reproductive system.

Trifloxystrobin was found to be homogeneously distributed and stable in the diet for at least 5 weeks at room temperature. In males/females of the groups at 50, 750 and 1500 ppm, the overall mean intake of test substance was 3.8/4.1, 55.3/58.0 and 110.6/123.1 mg/kg bw per day for the F_0 generation and 4.2/4.4, 65.5/67.0, and 143.0/146.0 mg/kg bw per day for the F_1 generation, respectively.

No treatment-related mortality or clinical signs were seen in parental F_0 animals. At 1500 ppm, food consumption was reduced in both sexes, resulting in a retarded body-weight development from the start of the dosing period with final body weights being 8.6% and 7.1% below that of males and females in the control groups, respectively. A slightly reduced overall body-weight gain was also observed in females at 750 ppm during first gestation (days 7–14, 3.8%; days 14–21, 12.4%), which was also associated with a slightly reduced food consumption (days 7–14, 3.3%; days 14–21, 3.6%). Body-weight gain during the first lactation period was lowered at 750 (27.2%) and 1500 ppm (21.8%), but was significantly increased during the second lactation period at 750 (days 0–7, 141%; days 0–21, 290%) and 1500 ppm (days 0–7, 991%; days 0–21, 355%).

The number of animals mating and the number of females becoming pregnant was not affected by treatment during either mating period for the F_0 generation. Also, there were no treatment-related effects on any of the gestation or parturition indices at the first or second mating. Viability and lactation indices for F_{1A} and F_{1B} litters were comparable in all groups, and the sex ratio among F_{1A} and F_{1B} pups was not changed by treatment with trifloxystrobin.

At 1500 ppm, absolute weights of the spleen in males and adrenals in females were significantly lowered by 13.4% and 8.0%, respectively. Also, relative organ weights were significantly increased in most cases in F_0 males (14.9%, 15.5%, 10.7% and 11.0% at 1500 ppm in liver, kidneys, testes and brain, respectively) and F_0 females (11.1%, 9.7%, 14.5% and 8.4% at 1500 ppm in liver, kidneys, ovaries and brain, respectively). At 750 ppm, a slight statistically significant increase in the relative weights of the liver and kidney (each by 5.7%) in males and liver (by 5.7%) and ovaries (by 12.5%) in females was considered to be of little or no toxicological significance.

Necropsy of F_0 parents revealed no treatment-related macroscopic changes and no histopathological findings in the reproductive organs. Some of the histopathology findings in F_0 parental animals are shown in Table 27. There was increased centrilobular hepatocyte hypertrophy (minimal to moderate) among F_0 males and females at 1500 ppm (Table 27). Also, there was increased incidence of minimal pigmentation of renal tubules in males and females at 1500 ppm and in males at 750 ppm. Decreased incidence of splenic haemosiderosis (pigmentation disturbances) was observed in males and females at 750 and 1500 ppm. Splenic haemosiderosis might correlate with increased iron overload (Klaassen, 1996) or possibly with erythrocyte turnover. However, there was no evidence of decreased erythrocyte turnover to account for the observed decrease in haemosiderosis. Hence, while possibly treatment-related, this observation may not be adverse. There were no treatment-related histopathology findings in the reproductive systems of males or females.

Table 27. Summary of incidence of histopathology findings in F_0 parental rats given diets containing trifloxystrobin

Finding	Dietary concentration (ppm)							
	Males				Females			
	0	50	750	1500	0	50	750	1500
Number of tissues rxamined	30	30	30	30	30	30	30	30
Kidney: pigmentation	1	0	4	7	0	0	0	3
Liver: hepatocellular hypertrophy	3	1	4	10	1	0	1	5
Spleen: haemosiderosis	17	20	12	9	23	22	15	8

From Khalil (1997)

There were no clinical signs in F_{1A} and F_{1B} pups that could be considered to be related to treatment of the dams. Mean pup weights at birth were similar in all groups of F_{1A} and F_{1B} generation. Body-weight development of male and female pups at 750 and 1500 ppm was significantly reduced during lactation to a similar extent. At weaning on postnatal day 21, mean body weights of combined male and female F_{1A} pups at 1500 and 750 ppm were 28.4% and 9.3% below values for the controls, respectively. The respective values for the F_{1B} generation were 27.2% and 10.8% below values for the controls. For F_{1A} and F_{1B} pups at 1500 ppm, mean values for eye opening were delayed by 0.7 and 0.6 days, respectively, compared with control animals. This developmental delay is consistent with the retarded body-weight development. Necropsy of F_{1A} and F_{1B} pups revealed no treatment-related macroscopic findings.

In F_1 parental animals, there were no treatment-related mortality or clinical signs. One male at 750 ppm was sacrificed in a moribund condition on day 85. Piloerection, reduced activity, and respiratory sounds had been noted for 1 day, but no related macroscopic changes were observed at necropsy. Incidental clinical signs observed occasionally included palpable masses, hair loss, various wounds and/or crust/scurf, swelling and chromodacryorrhea.

In F_1 parental animals, food consumption was significantly reduced before day 50 in males treated at 1500 ppm. The decrease ranged from 20.4% for days 1–8 to 8.4% for days 43–50; values were similar to those of controls after day 50 of the study. Food consumption was also significantly reduced in F_1 parental females treated at 750 (ranging from 9.0% for days 1–8 to 6.2% for days 57–64; values were similar to those of the controls after day 64) and 1500 ppm (ranging from 20.6% for days 1–8 to 7.3% for days 57–64) throughout the F_1 generation. Food consumption in the remaning parental groups was similar to that of the control groups throughout the F_1 generation.

Throughout the F_1 generation, body weights in both sexes at 750 and 1500 ppm remained significantly lower than those of the controls. At 750 ppm, the mean decrease in body weight in males was 7.4% over 19 observations; in females, the mean decreases were 9.4% over 12 observations before mating, 8.4% over four observations during gestation, and 7.3% over four observations during lactation. At 1500 ppm, the mean decrease in males was 17.5% over 19 observations; in females, the mean decreases were 18.8% over 12 observations before mating, 15.7% over four observations during gestation, and 14.4% over four observations during lactation. In F_1 males, body-weight gain was decreased for days 1–8 at 750 (by 7.7%) and 1500 ppm (by 16.9%), and for days 8–15 at 1500 ppm (by 13.9%), but

was increased for days 22–29, 68–71 and 71–78 at 1500 ppm (by 14.3%, 554% and 227%, respectively). In F_1 females, body-weight gain was decreased at 1500 ppm before mating for days 68–71 (by 147.8%) and during gestation for days 0–21 at 750 ppm (by 8.2%) and at 1500 ppm (by 19.4%). However, body-weight gain in F_1 females was increased during lacatation for days 0–21 at 750 ppm (by 192%) and at 1500 ppm (by 299%). The sponsor considered that the increased body-weight gain during lactation was related to the marked retardation in body-weight development of the pups in this group, resulting in them being suckled longer and weaned later than usual by the dams.

There were no treatment-related effects on the number of animals mating, the number of females becoming pregnant or on the mean precoital time. Five males (one in the control group, one at 50 ppm, two at 750 ppm and one at 1500 ppm) failed to mate. One mated female was not pregnant. Also, there were no effects on the duration of gestation (from 22.0 to 22.1 days in all groups) or parturition with a total of 29, 28, 28 and 29 pregnant females giving birth to live young in the groups at 0, 50, 750 and 1500 ppm, respectively.

Both the viability index (percentage of pups surviving postnatal days 0–4) and the lactation index (percentage of pups surviving postnatal days 4–21) were similar to those of the control group. Dams (28, 28, 28 and 29 at 0, 50, 750 and 1500 ppm) successfully reared their litters to weaning on postnatal day 21. The sex ratios of the F_2 pups on postnatal days 0 and 21 were similar in all groups.

In F_1 adult animals, both sexes at 1500 ppm group had significantly lower body weights at sacrifice (15.0% and 16.6% in males and females, respectively). In the same group, males had significantly decreased absolute weights of the spleen (by 11.5%) and brain (by 5.7%), while females had reduced absolute weights of kidneys (by 9.1%) and brain (by 3.5%). Compared with controls, relative organ weights were significantly increased in most cases in both the F_1 males (12.4, 11.3, 16.7, 18.5 and 10.8% at 1500 ppm for liver, kidneys, testes, adrenals and brain, respectively) and F_1 females (12.8%, 9.2%, 16.2%, 19.8% and 15.8% at 1500 ppm for liver, kidneys, ovaries, thymus and brain, respectively). At 750 ppm, absolute weights of the brain in males were significantly lowered (4.1%), while, in females, the absolute weights of liver and kidney were decreased (by 10.8% and 9.1%, respectively), but that of the thymus was increased (by 12.5%). The effects on absolute weights of the thymus in females at 750 ppm group were considered to be incidental by the consulting pathologist due to the lack of dose-dependency. At 50 ppm, relative weights of the thymus were significantly increased (11.8%) in females. The relative increase in weight of the thymus is not considered toxicologically to be significant since there were no corroborating histopathology findings in the thymus.

Microscopic findings in the F_1 parental animals were unremarkable but similar to those seen in F_0 parental animals. In the liver, there were increased incidences of minimal to moderate centrilobular hepatocyte hypertrophy in males and females at 750 ppm (14/30 and 7 out of 30, respectively) and at 1500 ppm (24 out of 30 and 9 out of 30, respectively) compared with 1 out of 30 and none out of 30 incidences in males and females in the control group, respectively. Microscopic examination of the spleen in males and females showed decreased incidence of splenic haemosiderosis from 14 out of 30 and 23 out of 30 in the control group to 5 out of 30 and 17 out of 30 at 750 ppm, and 2 out of 30 and 14 out of 30 at 1500 ppm, respectively. As noted for the F_0 parents, splenic haemosiderosis may correlate with turnover of erythrocytes such that decreased splenic hemosiderosis may be caused by decreased turnover of erythrocytes. However, a decrease in erythrocyte turnover was not

observed in any of the studies of toxicity with trifloxystrobin. Although possibly treatment-related, the findings of decreased splenic haemosiderosis are considered not to be adverse.

There were no treatment-related histopathology findings in the reproductive systems of animals of both sexes.

In the F_2 pups, there were no treatment-related clinical signs and the mean pup weights at birth were similar in all groups. During lactation, body weights of the F_2 pups at 750 and 1500 ppm were significantly decreased on lactation days 7 (males: by 9.9% and 15.9%; females: by 8.2% and 17.0%, respectively), 14 (males: by 12.3% and 21.3%; females: 9.2% and 21.8%, respectively), and 21 (males: by 16.7% and 28.5%; females: 12.3% and 27.6%, respectively). Body-weight gains were also significantly reduced in F_2 pups in the same respective groups on lactation days 0–4 (females: by 12.9% and 22.6%), 4–7 (males: by 14.3% and 28.6%; females: by 12.5% and 30.4%), 7–14 (males: by 15.9% and 27.2%; females: by 10.3% and 27.4%) and 14–24 (males: by 22.4% and 37.9%; females: by 16.6% and 35.5%). At 50 ppm, mean pup weights and mean pup body-weight gain were similar to that of the control group after culling on postnatal day 4 until to weaning on postnatal day 21.

For F_2 pups at 1500 ppm, mean values for eye opening were delayed by 0.7 days compared with that of control animals, which is consistent with the retarded body-weight development. Values for eye opening were similar to that of the control group for all other doses. Mean values for surface righting were similar for all groups.

There were no treatment-related macroscopic necropsy changes and no histopathology findings in the reproductive organs of the pups. Conjoined twins with exencephaly were seen in one stillborn pup at 750 ppm, but there was no evidence that this finding was treatment-related.

The NOAEL for parental toxicity was 50 ppm, equal to 3.8 mg/kg bw per day, on the basis of reduced body weights and body-weight gains, reduced food consumption, and histopathology observations in liver and kidneys. The NOAEL for offspring toxicity was 50 ppm, equal to 3.8 mg/kg bw per day, on the basis of decreased pup body weights during lactation. The NOAEL for reproductive toxicity was 1500 ppm, equal to 110.6 mg/kg bw per day, the highest dose tested (Khalil, 1997).

(b) Developmental toxicity

Rats

In a study conducted in compliance with the principles of GLP (with QA certification), groups of 24 pregnant Sprague-Dawley (Tif:RAIf) rats were given trifloxystrobin (purity, 96.4%; in 0.5% aqueous sodium carboxymethylcellulose) at a dose of 0, 10, 100 or 1000 mg/kg bw per day by oral gavage from day 6 to day 15 of gestation. Dams were sacrificed on day 21 of gestation and fetuses were removed. Mortality, clinical signs, and body weights were recorded daily; food intake was determined on days 6, 11, 16 and 21 and daily consumption was calculated. After removal from the uterus, fetuses were numbered, weighed, sexed and examined for external malformations. After killing by subcutaneous injection of barbiturate, fetuses were processed for visceral or skeletal examination (at a ratio of approximately 1:1). Food, water and the housing environment were controlled and monitored.

Dose selection for this main study was based on a preliminary dose-range finding study in which four groups of seven mated virgin female albino Sprague-Dawley rats (Tif:RAIf) aged 2 months were given trifloxystrobin (in sodium carboxymethylcellulose) at a dose of 0, 10, 100 or 1000 mg/kg bw per day from day 6 to day 15 of gestation, inclusive. No treatment-related effects were noted in clinical signs, body weights and body-weight gains, postimplantation loss, fetal weights, mean gravid uterine weights, carcass weights, maternal necropsy or external fetal observations. There was a slight reduction in food consumption at 1000 mg/kg per day (Fitzgerald, 1993).

In the main study, all dams survived until terminal sacrifice. One dam in the group at 10 mg/kg bw per day and one in the group at 100 mg/kg bw per day had hair loss starting on day 11 and 13, and one dam in the group at 1000 mg/kg bw per day had an ear wound on days 13 and 14 and then ear crust/scurf until necropsy. Haemorrhagic discharge in the perineal area was seen in one animal at 100 mg/kg bw per day and six animals at 1000 mg/kg bw per day. This finding was observed for 1 day only, and all these animals had normal pregnancies. Three of these animals had no resorptions and four animals had one to four resorptions. These findings were not regarded as treatment-related by the sponsor. At 1000 mg/kg bw per day, mean absolute maternal body weight was consistently less than that of the controls; this difference were statistically significant on days 8 (by 3.7%) and 16 (by 5.3%), but body weight was fully recovered thereafter. Mean body-weight values for the groups at the lowest dose remained comparable with control values throughout the study. There were statistically significant reductions in mean maternal body-weight gains at 1000 mg/kg bw per day during the entire dosing period (days 6–16 of gestation, 21%) as well as within the dosing period (e.g. days 6–11 of gestation, 37.2%; days 11–16 of gestation, 20.9%). Also, the corrected maternal body-weight gain for the dosing period plus the post-dosing period (i.e. net body-weight change from day 6 of gestation, which equals the carcass weight minus body weight on day 6) was statistically significantly decreased at 100 and 1000 mg/kg bw per day (by 17.8% and 32.5%, respectively). During the post-treatment period (days 16–21 of gestation), there was a slight compensatory increase in body-weight gain noted at 1000 and 100 mg/kg bw per day compared with controls (by 4.1% and 2.1, respectively). Food consumption was significantly reduced at 100 mg/kg bw per day (days 6–11 of gestation, 7.6%; days 11–16 of gestation, 7.7%) and 1000 mg/kg bw per day (days 6–11 of gestation, 29.6%; days 11–16 of gestation, 15.5%) during the treatment period.

Of the 24 mated animals per group, one, two, four and two were not pregnant in the control group, and at 10, 100 and 1000 mg/kg bw per day, respectively. The number of pregnant animals with viable fetuses at scheduled necropsy was 23, 22, 20 and 22, respectively. Preimplantation losses, number of implantation sites, and early and late postimplantation losses were comparable between all groups. No dead or aborted fetuses were noted. Numbers of live fetuses/litter and fetal weights were not affected by treatment.

There was a reduction in maternal carcass weight at 1000 mg/kg bw per day (by 6.0%) as well as in mean net body-weight change from day 6 in the groups receiving trifloxystrobin at 100 (by 17.8%) and 1000 mg/kg bw per day (by 32.5%) relative to the control group (also discussed above). The reduction in mean net body weight was dose-related. Necropsy of all maternal animals showed no macroscopic changes. External examination of fetuses revealed no treatment-related abnormalities. An umbilical hernia was found in a control fetus and a generalized oedema in one fetus at 10 mg/kg bw per day. Upon visceral examination, the same two animals showed an umbilical hernia and pulmonary hyperplasia, respectively (Table 28). Additionally, renal pelvic dilatation was found in three fetuses of

Table 28. Summary of observations in rat fetal viscera

	Dose (mg/kg bw per day)			
	0	10	100	1000
Total fetuses examined/litters examined	149/23	135/22	139/20	146/22
Umbilical hernia	1/1	ND	ND	ND
Enlarged thymus	3/3	3/1	3/3	11*/7
Pulmonary hyperplasia	ND	1/1	ND	ND
Accessory lobulets in liver	1/1	2/2	1/1	2/2
Renal pelvic dilitation	3/3	1/1	ND	1/1
Total visceral observations	8/8	7/4	4/4	14/10

ND, none detected
* Chi-squared test + Fisher's Exact test, $p < 0.05$

the control group and in one fetus each at 10 and 1000 mg/kg bw per day. One to two fetuses in each group had accessory liver lobulets. None of these effects were considered to be treatment-related by the consulting pathologist. Enlarged thymus was seen in three, three, three and eleven fetuses in the control group and at 10, 100 and 1000 mg/kg bw per day, respectively. The increased incidence of enlarged thymus at 1000 mg/kg bw per day (7.5% of fetuses/32% of litters) exceeded both the mean (0.7%/4.0%) and maximum (6.0%/29.2%) fetal/litter incidences for historical controls and was likely to be treatment-related.

No skeletal malformations were noted. The incidence of skeletal anomalies included fused or asymetric sternebrae, irregular ossification of cranial bones, poor ossification of the fifth metacarpal, additional cervical vertebral arches, and bipartite thoracic vertebral centres. Skeletal variations consisted of poor or absent ossification of sternebrae, calcaneus first metatarsal, cervical and thoracic vertebral centres, ribs and phalanges, bipartite or dumbell-shaped cervical or thoracic vertebral centres, and shortened thirteenth rib. None of the skeletal abnormalities were considered to be treatment-related.

Trifloxystrobin was not teratogenic in rats. The NOAEL for maternal toxicity was 10 mg/kg bw per day on the basis of reduced body-weight gain and food consumption at 100 mg/kg bw per day. The NOAEL for embryotoxicity was 100 mg/kg bw per day on the basis of increased incidence of enlarged thymus at 1000 mg/kg bw per day. There was no evidence of teratogenic potential (Khalil, 1995).

Rabbits

In a study conducted in compliance with the principles of GLP (with QA certification), groups of 19 pregnant Russian (Chbb : HM) rabbits were given trifloxystrobin (purity, 96.4%; in 0.5% aqueous sodium carboxymethylcellulose) at a dose of 0, 10, 50, 250 or 500 mg/kg bw per day by oral gavage from day 7 to day 19 of gestation. Dams were sacrificed on day 29 of gestation and fetuses were removed. Mortality, clinical signs, and body weights were recorded daily; food intake was determined on days 4, 7, 12, 16, 20, 24 and 29 and daily food consumption was calculated. Dams were killed on day 29 and the following observations were recorded at necropsy: macroscopic pathological examination of the main organs of the thoracic and abdominal cavities, in particular the genitals; number of corpora lutea in each ovary; weight of the uterus including contents; uterine contents for dams at scheduled necropsy (number and location of live and dead fetuses, number and location of early and late embryonic/fetal losses, total postimplantation and/or abortion sites) and for dams sacrificed or dying before scheduled necropsy (number and location of implantation and/or abortion sites). After removal from the uterus, fetuses were numbered,

weighed, sexed and examined for external malformations. After killing by subcutaneous barbiturate injection, fetuses were processed for visceral or skeletal examination (at a ratio of approximately 1 : 1). Food, water and the housing environment were controlled and monitored.

Dose selection for this main study was based on a preliminary dose-range finding study in which five groups of five artificially inseminated virgin female rabbits (Russian Chbb : HM) aged 3 months were given trifloxystrobin at a dose of 0, 20, 100, 500 or 1000 mg/kg bw per day (in sodium carboxymethylcellulose) from days 7 to 19 of gestation, inclusive. No animals died. There was reduced activity in all animals at the highest dose and two animals exhibited haemorrhagic discharge in the perineal area on several occasions. At 500 mg/kg bw per day or above, animals had reduced body weight, body-weight gain, and food consumption; reduced food consumption was also noted in the group at 100 mg/kg bw per day. All animals at the highest dose in addition to one animal at 500 mg/kg bw per day had total resorptions. At 500 mg/kg bw per day, animals had reduced gravid uterine weights, the number of fetuses was decreased, and postimplantation loss was increased. Mean fetal weight was also reduced in the group at 500 mg/kg bw per day. There were no treatment related effects in the number of corpora lutea, implantation sites, preimplantation loss, external fetal observations, or findings on maternal necropsy (Khalil, 1994a).

In the main study, there was no treatment-related mortality or clinical signs. One dam at 50 mg/kg bw per day died spontaneously on day 27 of gestation without having exhibited any clinical signs before death. At necropsy, this animal was found to have haemorrhagic contents of the uterus. Other incidental findings included hair loss and a palpable mass in the head of one animal at 10 mg/kg bw per day.

Maternal body weights were retarded in the animals at 250 (day 12, 5.4%; day 21, 5.4%) and 500 mg/kg bw per day (day 12, 5%; day 21, 6.3%) from the start of the dosing period until day 21. Body weights were unaffected by treatment at 10 and 50 mg/kg bw per day. During the treatment period (days 7–19 of gestation), there was a significant net weight loss of 83 g and 152 g (compared with controls) in the groups receiving trifloxystrobin at a dose of 250 and 500 mg/kg per day, respectively (Table 29).

As shown in Table 30, food consumption was also significantly reduced during the treatment period, namely during days 7–12, 12–16, and 16–20 by 65.5%, 47.2%, and 34.8%,

Table 29. Maternal body-weight gain in a study of developmental toxicity in rabbits

Days	Dose (mg/kg bw per day)				
	0	10	50	250	500
0–4	24	25	43	30	36
4–7	8	−9	−8	−13	−8
7–12	2	10	−22	−120**	−150**
12–16	59	55	54	55	21**
16–20	2	−5	2	−18	−23
20–24	25	31	36	128**	146**
24–29	83	97	76	68	75
7–20	64	59	34	−83**	−152**

From Khalil (1994b)
** Statistically significant ($p < 0.01$)

Table 30. Food consumption (g/animal per day) in a study of developmental toxicity in rabbits

Days	Dose (mg/kg bw per day)				
	0	10	50	250	500
0–4	127.0 ± 27.6	122.6 ± 18.1	130.8 ± 19.6	123.9 ± 15.8	136.0 ± 48.2
4–7	121.8 ± 25.8	109.3 ± 19.8	123.4 ± 17.3	112.0 ± 21.0	116.6 ± 17.0
7–12	109.5 ± 22.0	103.9 ± 16.5	93.7 ± 16.0	37.8** ± 9.1	37.6** ± 5.2
12–16	106.7 ± 24.5	83.5 ± 31.6	91.9 ± 26.2	56.3** ± 16.2	52.0** ± 9.2
16–20	98.8 ± 18.9	91.4 ± 26.3	102.3 ± 18.0	64.4** ± 26.4	58.7** ± 26.4
20–24	92.8 ± 26.5	93.6 ± 30.6	107.2 ± 18.9	122.4 ± 39.0	109.4 ± 34.5
24–29	90.6 ± 23.4	90.6 ± 25.8	98.5 ± 21.3	107.4 ± 27.6	118.1** ± 25.0

From Khalil (1994b)
** $p < 0.01$ by ANOVA + Dunnett test

Table 31. Food efficiency[a] (%) in a study of developmental toxicity in rabbits

Days	Dose (mg/kg bw per day)				
	0	10	50	250	500
0–7	3.7	2.1	3.9	2.0	3.1
7–20	4.7	4.6	2.7	NV	NV
20–29	13.1	15.4	12.2	19.2	21.4
7–29	9.9	9.1	6.7	6.7	4.1
0–29	7.8	7.1	5.9	5.2	3.8

From Khalil (1994b)
NV, negative value for food efficiency because of loss in body-weight gain during the dosing period
[a] Food efficiency was calculated as % body-weight change relative to food intake during a specified period

respectively, at 250 mg/kg bw per day and by 65.7%, 51.5% and 40.6%, respectively, at 500 mg/kg bw per day. A recovery in food consumption was noted during the post-dosing period (after day 20). At 10 and 50 mg/kg bw per day, food consumption was comparable to that of the control group throughout the study.

Food efficiency (calculated by reviewer from data on means) was also reduced during the dosing period for the groups at 250 and 500 mg/kg bw per day (Table 31).

There were no dead or aborted fetuses in any of the groups, and the number of live fetuses per litter and fetal weights (both sexes) were unaffected by treatment. The numbers of corpora lutea, preimplantation losses, numbers of implantation sites, and postimplantation losses were comparable between groups.

Gravid uterus weights and carcass weight did not differ significantly between groups, although carcass-weight change from day 7 was slightly but not significantly reduced (by 35.3%). One dam in the control group had hypoplasia of the left uterus horn, one animal at 10 mg/kg per day had a palpable mass on the head and one animal at 50 mg/kg bw per day had haemorrhagic contents of the uterus. These findings are not considered to be related to treatment. There were no other remarkable observations at maternal necropsy.

Fetal external and visceral examinations revealed no treatment-related abnormalities. A single fetus in the group at 10 mg/kg bw per day showed craniocele (brain hernia may be

associated with a skull defect). Several limb and gastric malformations were seen in only one animal at 250 mg/kg bw per day, but were considered to be unrelated to treatment because of lack of dose-dependency. Forelimb position anomaly was evenly distributed among all treated groups and was within the range of incidence for historical controls; therefore, it is not considered to be a treatment-related malformation.

Findings in the fetal viscera included aplasia (lack of development) of the gall bladder occurred in one fetus at 50 mg/kg bw per day and in two at 500 mg/kg bw per day; in addition, one to two small gall bladders were found in all treated groups. These findings are considered to be developmental variations that are unrelated to treatment since no statistical significance or dose-dependency were found and since a variety of gall bladder findings were present in the data for historical controls.

Skeletal malformations were observed in one fetus at 10 mg/kg bw per day (reduced interparietal, parietal, frontal and nasal bones), one fetus at 50 mg/kg bw per day (reduced interparietal bone), one fetus at 250 mg/kg bw per day (forelimb, absent ossification of the ulna; forepaw, adactyly) and one fetus at 500 mg/kg bw per day (absent ossification of the pubis) (Table 32). Skeletal anomalies consisted mainly of fused, fragmented or asymmetric sternebrae, irregular ossification of scapula, and displaced cervical and caudal vertebral centers. The incidence of these anomalies was not affected by treatment. The incidence of fused third and fourth sternebrae was slightly higher at 500 mg/kg bw per day than in the controls and was likely to be treatment-related (fetal incidence, 10.3%; litter incidence, 33.3%; range of incidences in historical controls, 0–5.4% and 0–29.4%, respectively). Skeletal variations occurred in about two-thirds of fetuses from almost all litters at all doses. They consisted mainly of poor or absent ossification of the first, fifth and sixth sternebrae, cranial findings (sutural bones, slot or hole in parietal bone), absent ossification of the first

Table 32. Fetal skeletal observations in a study of developmental toxicity in rabbits

	Dose (mg/kg bw per day)				
	0	10	50	250	500
Total fetuses examined/litters examined	116/19	130/18	90/16	97/17	97/18
Skeletal malformations (fetal incidence/litter incidence)					
Reduced interparietal bone	NF	1/1	1/1	NF	NF
Reduced parietal bone	NF	1/1	NF	NF	NF
Reduced frontal bone	NF	1/1	NF	NF	NF
Reduced nasal bone	NF	1/1	NF	NF	NF
Forelimb—absent ossification ulna	NF	NF	NF	1/1	NF
Fore paw—adactyly	NF	NF	NF	1/1	NF
Pelvic girdle—absent ossification pubis	NF	NF	NF	NF	1/1
Total skeletal malformations	NF	1/1	1/1	1/1	1/1
Treatment-related skeletal anomalies (fetal incidence/litter incidence)					
Asymmetrically shaped first sternebra	NF	1/1	1/1	2/1	3/1
Fused second and third sternebra	1/1	1/1	1/1	4/4	4/4
Asymmetrically shaped second sternebra	NF	1/1	1/1	2/2	4/3
Fused third and fourth sternebra	2/2	2/1	1/1	5/4	10*/6
Asymmetrically shaped third sternebra	NF	1/1	NF	2/2	3/3
Fused fourth and fifth sternebra	4/4	2/2	4/4	7/6	8/6
Asymmetrically shaped fourth sternebra	NF	1/1	NF	4/4	2/2
Total skeletal anomalies	12/8	9/7	7/6	21/12	21/
Total skeletal variations	98/19	107/18	74/16	80/16	77/17

From Khalil (1994b)
NF, not found
* $p < 0.05$; chi-squared test plus Fisher's Exact test

metacarpal, tail bone variations (poor or absent ossification of or additional caudalvertebral centres), additional ribs, and poor ossification of the medial phalanx of the fifth anterior digit. Poor ossification of the caudal vertebral centres was statistically significantly increased at 50 mg/kg bw per day (by 112.5% compared with controls). However, since there was no dose–response relationship, this result was not likely to be treatment-related. The incidence of fetuses with additional caudal vertebral centres was significantly lower at 10, 250 and 500 mg/kg bw per day when compared with controls, but this finding was not considered treatment-related by the consulting pathologist.

No teratogenic potential of trifloxystrobin was detected in rabbits. The NOAEL for maternal toxicity was 50 mg/kg bw per day on the basis of effects on body weight, food consumption, and food efficiency at the next higher dose. The NOAEL for developmental toxicity was 250 mg/kg bw per day on the basis of marginally increased incidences of skeletal anomalies of fused third and fourth sternebrae at the next higher dose (Khalil, 1994b).

2.6 Special studies

(a) Acute neurotoxicity

Rats

In a study of acute neurotoxicity conducted in compliance with the principles of GLP (with QA certification), groups of 10 male and 10 female Sprague-Dawley (Tif:RAIf) rats aged 5–7 weeks were given trifloxystrobin (purity, 96.4%; in 0.5% carboxymethylcellulose, 0.1% aqueous polysorbate 80) as a single oral dose at 2000 mg/kg bw by gavage. Animals in the control group received the vehicle alone. The design of this study was based on a previously conducted range-finding study in which no signs of toxicity were observed at doses of up to 2000 mg/kg bw. At 3500 mg/kg bw, reduced activity and piloerection were noted, being most prominent 6–8 h after treatment. Based on these data, a limit-test study using 2000 mg/kg bw, with a time of peak effect of 6 h, was considered adequate. The animals were checked twice per day for mortality and daily for clinical signs. Body weight was recorded at pre-test, day 1, and twice weekly, thereafter; food consumption was measured at pre-test and twice weekly, thereafter. FOB tests were conducted before the assessment of motor activity on randomized animals. Animals were observed in the home cage, during handling and in an open field. Tests of neurological function were performed at pre-test, day 1 (time of peak effect), day 8, and day 15 and included sensorimotor functions (approach, touch, vision, audition, pain, vestibular), autonomic functions (pupillary reflex, body temperature), and sensorimotor coordination (grip strength, landing foot splay). After conducting the FOB tests, motor activity was assessed using an automated open-field device to measure horizontal activity, vertical activity, and other parameters. At the end of the observation period, all animals were sacrificed by in-situ perfusion and submitted to macroscopic examination and tissue sampling of brain, spinal cord, and major peripheral nerves and ganglia. Histopathological examination of nervous system tissue was conducted on five animals of each sex per group.

A single male animal in the treatment group was found recumbent on day 2, had respiratory sounds and had to be sacrificed in a moribund condition. This incident was not considered to be related to treatment since the oral LD_{50} of the compound is known to be >5000 mg/kg bw. No other clinical signs or changes in behaviour were observed at any time during the study. Body-weight development and food consumption were not affected in treated animals.

Table 33. Motor activity in female rats at day 1 after receiving a single dose of trifloxystrobin

Parameter	Dose (mg/kg bw)		% decrease
	0 (control)	2000	
Total distance	2842 ± 805	1728 ± 890	39
Number of movements	193 ± 50	144 ± 72	36
Movement time	219 ± 50	136 ± 73	38
Vertical activity	554 ± 273	357 ± 200	35
Number of rearing	82 ± 37	57 ± 29	30
Vertical time	296 ± 159	188 ± 121*	36
Centre time	139 ± 95	34 ± 31*	75

From Classen (1997a)
* Significantly different from control at $p \leq 0.05$ (ANOVA)

The FOB tests revealed no neurological or behavioural effects of trifloxystrobin. Histopathological examination of tissues of the central and peripheral nervous system, the eyes, optic nerve and skeletal muscle did not show any treatment-related neuropathic changes. On day 1 (time of peak effect), female rats had statistically significantly decreased motor activity for both vertical and centre time (Table 33). There were no decreases in motor activity in males. The effect in females was considered to be treatment-related and likely to be caused by systemic toxicity rather than neurotoxicity.

The NOAEL was <2000 mg/kg bw on the basis of decreased motor activity in females (Classen, 1997a).

In a range-finding study of acute neurotoxicity conducted in compliance with the principles of GLP (with QA certification) to estimate the time of peak effect, groups of three male and three female Sprague-Dawley (Tif:RAIf) rats aged 5 weeks were given trifloxystrobin (purity 96.4%; in 0.5% carboxymethyl-cellulose, 0.1% aqueous polysorbate 80) as a single oral dose at 0, 1000, 2000 or 3500 mg/kg bw. Animals were observed for 4 days and measurements were recorded for body weight, food consumption, clinical signs, abbreviated FOB, and neurological assessment including sensorimotor function tests. Piloerection was seen in all animals at the highest dose at study day 1 and ended on study day 2. Reduced activity was observed at 2–4 h after administration of 2000 or 3500 mg/kg bw, reaching a maximum at 6–8 h after dosing. The reduced activity of males at the highest dose lasted for 3 days, while the remaining groups recovered by day 2. At 2000 mg/kg bw, low activity was noted only in two out of three males and not at all in the females. The effects, in general, were seen more clearly and started earlier and lasted longer in males than in females. On the basis of findings of this study, 2000 mg/kg bw was chosen as a limit dose for the actual study of acute oral neurotoxicity in rats (Classen, 1997b).

(b) Mechanistic studies

In the 3-month study of toxicity in rats (Gerspach, 1995), relative weights of the liver were increased in the males fed diets containing trifloxystrobin at a concentration of 500 or 2000 ppm with minimal hepatocyte hypertrophy at 2000 ppm (see section 2.2). The present study was conducted to assess possible induction of replicative DNA synthesis in the liver of male rats given diets containing trifloxystrobin for 3 months. For this purpose, formalin-fixed tissues from male rats in the same 3-month guideline feeding study were embedded and subjected to immunohistochemical analysis for proliferative cell nuclear antigen

(PCNA). Groups of five male rats were treated with trifloxystrobin admixed to the feed at dietary concentrations of 0, 100, 500 or 2000 ppm for 3 months, corresponding to target doses of 0, 6.44, 30.6 and 127 mg/kg bw per day. In order to test for the reversibilty of potential treatment-related changes, two additional groups of 10 animals received diets containing trifloxystrobin at 0 or 2000 ppm, corresponding to target doses of 0 and 127 mg/kg bw per day for 3 months followed by a recovery period of 28 days. Cells in S-phase of the cell cycle were identified by uniform nuclear staining for PCNA. Hepatocytes but not sinusoidal cells were evaluated for PCNA-positive nuclei using a microscope connected to a Vidas image analysis system. Cells and PCNA-positive nuclei were counted in 10 microscopic fields per animal, giving a total area of 4.44 mm^2. A total of approximately 800 cells were counted per animal.

For each animal, a labelling index for hepatocytes was calculated as follows:

$$\text{Labelling index (\%)} = 100 \times \frac{\text{PCNA positive nuclei} / \text{mm}^2 \text{ investigated area}}{\text{Total number of nuclei} / \text{mm}^2 \text{ investigated area}}$$

Treatment with trifloxystrobin at all doses investigated did not increase the mean hepatocyte nuclear labelling indices (Table 34).

In conclusion, there was no evidence for induction of replicative DNA synthesis in hepatocytes of male rats after 3 months of treatment with trifloxystrobin. However this study is of limited value as proliferation may only occur within the first few days of exposure (Persohn, 1995a).

PCNA-dependent eplicative DNA synthesis was also investigated in livers of male mice from a 3-month range-finding dietary study of toxicity (Gerspach, 1994a). In that study, males and females in groups receiving trifloxystrobin at dietary concentrations of 2000 or 7000 ppm had increased absolute and relative weights of the liver; in addition, there were increased incidences of centrilobular hepatocyte hypertrophy at 7000 ppm and hepatocyte necrotic changes at 2000 and 7000 ppm (see above section 2.2; Gerspach, 1994a). The present study was conducted to assess possible induction of replicative DNA synthesis in livers of male mice after dietary administration of trifloxystrobin for 3 months. Liver samples from groups of 10 male mice fed diets containing trifloxystrobin at a concentration of 0, 500, 2000 or 7000 ppm, corresponding to target doses of 0, 76.9, 315 and 1275 mg/kg bw per day, respectively, were embedded and subjected to immunohistochemical analysis for PCNA, as described above. The same procedure as used in the study in rats

Table 34. Labelling index as measured by PCNA-positive nuclei in livers of male rats fed diets containing trifloxystrobin for 3 months

Dietary concentration (ppm)	Mean labelling index index (% PCNA-positive nuclei)	
	After termination of treatment	After 4 weeks of recovery[a]
0	1.58 ± 0.51	1.05 ± 0.55
100	0.92 ± 0.24	ND
500	0.85 ± 0.33	ND
2000	1.22 ± 0.17	1.24 ± 0.29

From Persohn (1995a)
ND, not determined
[a] Mean of 5 or 10 animals each ± standard deviations

was also used to identify and count uniformly-stained PCNA-positive nuclei, except that a total of 600 S-phase cells per animal were counted, rather than 800. The labelling index was also counted in the same manner.

Immunohistochemical staining of sections of male mouse liver for PCNA did not reveal any increase in the fraction of DNA-synthesizing hepatocytes in S-phase in cells from mice at all doses investigated (Table 35).

In conclusion, there was no evidence for induction of replicative DNA synthesis in hepatocytes of male mice after 3 months of treatment with trifloxystrobin. However, this study is of limited value as proliferation may only occur within the first few days of exposure (Persohn, 1995b).

Trifloxystrobin and its carboxylic acid metabolite, CGA 321113, were tested for cytotoxicity in cultures of hepatocytes, and for inhibition of mitochondrial function.

Hepatocytes from a young adult male Wistar rat (Crl(WI)BR) were isolated by liver perfusion with collagenase and, using standard procedures, were cultured in 24-well plates. To the hepatocyte cultures were added (for 1–24 h) different concentrations of trifloxystrobin (10, 30, 60, 100, 300 and 600 nmol/l) and its carboxylic acid metabolite, CGA 321113, (1000, 10 000, 30 000 nmol/l). Both test chemicals were added in DMSO at a final concentration of 0.1% DMSO in culture. After 1, 4 and 24 h of treatment, the following morphological changes were recorded and graded (1–3): irregular cell surface, formation of blebs, cell spreading, intracellular granulation or vacuolization and cell disaggregation. Cell death and/or complete detachment of the monolayer were also recorded. At 4 h and 24 h after initiation of treatment, lactate dehydrogenase (LDH) activity was determined in the culture medium spectrophotometrically. Total intracellular LDH activity was determined from three additional cultures that were sonicated just before starting treatment. LDH release was expressed as a percentage of total intracellular activity.

Freshly prepared liver mitochondria from an overnight fasted young adult male Tif:RAlf (SPF) rat were used for analysis of oxygen consumption using a biological oxygen monitor connected to a recorder and a polarographic oxygen probe, calibrated at 100% with air-saturated water. Reaction chambers were maintained at 30 °C. Five seconds after addition of a sample of mitochondrial fraction to a Tris-potassium phosphate buffer (pH 7.4) reaction medium, succinate (10 mmol/l) was added as site II metabolic substrate in the presence of rotenone (30 µmol/l), an inhibitor of site I. Subsequent addition of ADP (300 nmol/l) generated state 3 (ADP-stimulated) respiration. After returning to state 4 respiration (resting respiration), the test article dissolved in DMSO was added (0.1% final concentration of DMSO) and state 3 and 4 respiration were again measured.

Table 35. Labelling index as measured by PCNA-positive nuclei in livers of male mice fed diets containing trifloxystrobin for 3 months

Dietary concentration (ppm)	Mean labelling index (% PCNA-positive nuclei)[a]
0	0.68 ± 0.26
500	1.11 ± 0.65
2000	0.66 ± 0.36
7000	0.71 ± 0.38

From Persohn (1995b)
[a] Mean of 10 animals each ± standard deviations

Incubation of cultured rat hepatocytes with trifloxystrobin at a concentration range of 5–100 µmol/l resulted in a rapid and marked degenerative change of the cell structure at 30 and 100 µmol/l with cell death occurring after 4 h and 24 h after treatment. CGA 321113 was much less toxic and caused no morphological changes at a concentration of less than 600 µmol/l. Accordingly, significant LDH leakage was measured after treatment with trifloxystrobin at concentrations of 30 µmol/l and greater, while treatment with CGA 321113 caused an increased LDH leakage at 600 µmol/l only. CGA 321113 was shown to be 20 times less cytotoxic than trifloxystrobin in cultured rat hepatocytes.

Rates of mitochondrial respiration were assessed before and after the addition of the trifloxystrobin or CGA 321113 in the presence of succinate as a substrate. The respiratory control ratio, RCR (state 3 respiration/state 4 respiration) provides a measure of mitochondrial integrity and is an indicator of the "tightness of coupling" in mitochondria. The ADP : O ratio (or P : O ratio), which is equal to moles of ADP phosphorylated per mole of atomic oxygen consumed, is calculated as an index of oxidative phosphorylation. Trifloxystrobin inhibited state 3 and state 4 mitochondrial respiration in a concentration-dependent manner, with IC_{50} values of 68 and 154 nmol/l, respectively. Concentration-dependent decreases of the RCR and P : 0 were observed with trifloxystrobin at concentrations of between 10 and 100 nmol/l. In contrast, CGA 321113 did not inhibit mitochondrial respiration at concentrations of up to 30 000 nmol/l.

In conclusion, trifloxystrobin was cytotoxic in cultures of rat hepatocytes and inhibited mitochondrial respiration and oxidative phosphorylation in the mammalian liver; CGA 321113, a major metabolite found in rats and goats, was far less cytotoxic and did not inhibit mitochondrial function at concentrations of up to three orders of magnitude higher than trifloxystrobin (Bouis, 1997).

(c) Studies with metabolites

(i) Acute oral toxicity

Studies of acute oral toxicity (limit test) in rats were conducted using CGA 357261 (the *Z*,*E*-isomer of trifloxystrobin) or one of four metabolites, namely CGA 373466, NOA 414412, NOA 413161, or NOA 413163. A single oral dose of 2000 mg/kg bw was administered to five rats of each sex, and the animals were observed for 14 days. All animals survived to the scheduled sacrifice. A summary of the results is presented in Table 36.

(ii) Genotoxicity

Trifloxystrobin's metabolites CGA 373466 and NOA 414412, NOA 413161, and NOA 413163, in addition to CGA 357261 (*Z*,*E*-isomer of trifloxystrobin), were tested in assays for reverse gene mutation in bacteria in the presence or absence of a metabolic activation

Table 36. Acute oral toxicity of metabolites of trifloxystrobin in male and female rats

Metabolite	Strain	LD50 (mg/kg bw)	Reference
CGA 357261	Sprague Dawley (Tif : RAI)	>2000	Winkler (1997)
CGA 373466	Wistar Han	>2000	Cantoreggi (1997a)
NOA 414412	Wistar Han	>2000	Cantoreggi (1997b)
NOA 413161	Wistar Han	>2000	Cantoreggi (1998a)
NOA 413163	Wistar Han	>2000	Cantoreggi (1998b)

Table 37. Results of assays for reverse mutation in vitro with metabolites of trifloxystrobin

Metabolite	Test object[a]	Concentration (solvent)	Result	Reference
CGA 357261	*S. typhimurium*; *E. coli*	312.5–5000 µg/plate, ±S9 (DMSO)	Negative	Deparade (1997a)
CGA 373466	*S. typhimurium; E. coli*	20.6–5000 µg/plate, ±S9 (DMSO)	Negative	Deparade (1997b)
NOA 414412	*S. typhimurium; E. coli*	312.5–5000 µg/plate, ±S9 (DMSO)	Negative	Deparade (1997c)
NOA 413161	*S. typhimurium; E. coli*	312.5–5000 µg/plate, ±S9 (DMSO)	Negative	Deparade (1998a)
NOA 413163	*S. typhimurium; E. coli*	312.5–5000 µg/plate, ±S9 (DMSO)	Negative	Deparade (1998b)

[a] The same strains were used in all tests: *S. typhimurium* TA 98, TA 100, TA 102, TA 1535 and TA 1537, and *Escherichia coli* WP2uvrA

system (S9). All results were negative up to the limit tested dose of 5000 µg/plate (Table 37).

3. Observations in humans

At present there are very few data on human exposure to trifloxystrobin, thus no firm conclusions can be drawn. In a recent update by the sponsor, there are no new data on human exposure.

3.1 Literature search

According to a recent open search of various international databases of medical literature conducted by Bayer, no reports of poisoning cases have been recorded (Heimann, 2004).

3.2 Occupational health surveillance

Manufacturing employees in Switzerland are medically examined by a company physician at the beginning of their employment and then routinely once per year according to the criteria of the Swiss Accident Insurance Institution (SUVA).

Routine medical examinations include: anamnesis; physical examination including blood pressure; blood analysis (including haemoglobin, erythrocytes, leukocytes, thrombocytes, leukocyte differentiation, blood sedimentation rate, blood sugar, cholesterol, triglycerides, alanine aminotransferase, aspartate amino transferase, alkaline phosphatase, bilirubin, creatinine, urea, uric acid); and urine analysis.

Trifloxystrobin has been formulated in a pilot plant (EZA) at Münchwilen (Switzerland) since 1996. Manufacturing is performed in campaigns with a total of about 10 campaigns per year. The average duration of a campaign is 1–2 days. The annual rate of production of different formulations is in the range of 1–100 kg per formulation. Four to five formulations had a production volume of 500 kg. The total formulation volume was 2.5 tonnes/year.

Annually, a total of 10 workers are involved in the formulation campaigns of trifloxystrobin. Questionnaires filled in by the head of the manufacturing site and by the responsible occupational physician revealed that no adverse health effect which could be related to trifloxystrobin was observed during this period.

A recent update by Bayer indicated that, during the production period from August 1, 2000 to May 17, 2004, there were no accidents or undesirable symptoms (based on the

above listed laboratory and medical tests) among 34 plant employees (Fehling Voigt & Gatz, 2004).

3.3 Cases reported to the company (European Union dossier)

During field-trial applications in South Africa in March 1996, one case of skin and eye irritation was reported. The symptoms occurred while weighing the product WG-type formulation) into plastic bags. The person experienced a burning sensation in the eyelids and nose tissue and also slightly in the chest; these symptoms started within 5 min after beginning of work and lasted up to 30–45 min after termination of weighing. Washing of hands and face several times during work did not reduce the severity or duration of the symptoms. The effects occurred for the first time during weighing the product for the second application and thereafter every time when weighing the product. No symptoms were noted during spraying in the field (using a knapsack). Protective clothing consisted of overalls but no gloves and no eye/face protection. The person had never experienced similar symptoms before.

In June 1996, two other cases were reported from Germany. Two persons started working in vineyards at two different locations 1–2 h after application of different products, including WG-type formulations of trifloxystrobin. Both workers went home after work with considerable irritation of the eyes and the skin (in one case). Further details of these two cases are not known. Which of the applied products might have been responsible for the observed effects had not been carefully evaluated. There is insufficient evidence for a major contribution of trifloxystrobin to the reported effects.

To learn more about the irritating potential of formulations containing trifloxystrobin, a questionnaire was distributed at the end of July 1996 to all locations worldwide where field trials with these products were ongoing. A total of 13 countries were contacted, and replies were obtained between August and October 1996 from 11 of these. More than 120 people were involved in field trials in these 11 countries. None of these people had ever experienced any irritating effects during their work with formulations of trifloxystrobin.

In conclusion, based on experience of more than 120 persons involved in field trials in 11 countries all over the world with different formulations of trifloxystrobin, these products were considered to have no intrinsic irritation potential to humans. This is in agreement with data from testing in animals. The significane of two reported cases from Germany is inconclusive because of the broad spectrum of products applied in these trials. For the case reported from South Africa, an allergic reaction of this particular individual to the dry product cannot be excluded. Testing in animals has revealed that the active ingredient has sensitizing potential.

Comments

After oral administration, radiolabelled trifloxystrobin was rapidly and appreciably absorbed (66% of the administered dose) in rats of both sexes. The major route of elimination (63–84%) was in the faeces; some of the faecal elimination was via bile (30–45%) while only one-third or less of the administered dose was excreted in the urine, and none through expired air. There was almost complete degradation of trifloxystrobin after single low dose at 0.5 mg/kg bw, but up to 45% was eliminated unchanged in the faeces after administration of the highest dose at 100 mg/kg bw. The pattern of metabolites in rats is very

complex; about 35 metabolites were identified in the urine, faeces, and bile. The major steps in the metabolic pathway include hydrolysis of the methyl ester to the corresponding acid, *O*-demethylation of the methoxyimino group yielding a hydroxyimino compound, and oxidation of the ethylideneamino methyl group to a primary alcohol, and then to the corresponding carboxylic acid. These steps are followed by a complex pattern of further, minor reactions. Cleavage between the glyoxylphenyl and trifluormethylphenyl moieties accounted for about 10% of the administered dose.

The metabolism of trifloxystrobin in plants is similar to that in animals, and occurs primarily via cleavage of the methyl ester group to form CGA 321113 (*E*,*E*)-methoxyimino-{2-[1-(3-trifluoro methyl-phenyl)-ethylideneaminooxymethyl]-phenyl}-acetic acid. In the rat, this metabolite undergoes further hydroxylation and conjugation (glucuronide and sulfate) at the trifluoromethyl phenyl ring. In goat liver, taurine and glycine conjugates of CGA 321113 were the principal residue components (up to 28% of the total radioactive residues). Conjugated metabolites are generally less toxic and more rapidly excreted than the unconjugated parent compound. Being biotransformation products in the rat, CGA 321113 and its metabolites are assumed to have been adequately tested and accounted for in rats given trifloxystrobin. Also, CGA 321113 is not likely to be more toxic than trifloxystrobin.

Dermal absorption of trifloxystrobin in rats was low and decreased slightly with increasing dose. In a test in vitro, rat epidermis was nine and 19 times more permeable to trifloxystrobin at a dose of 0.24 and 10.27 mg/cm^2, respectively, than was human epidermis. In a study of absorption in vivo in which a low or a high dose of radiolabelled trifloxystrobin was applied to the shaved backs of male rats, the amount of recovered radioactivity in the blood was low, but the overall absorption was moderate, ranging from 5% to 10% in 24 h and increasing to 16% at 48 h.

Trifloxystrobin has low acute oral toxicity in rats and mice (LD_{50} > 5000 mg/kg), low acute dermal toxicity in rats and rabbits (LD_{50} > 2000 mg/kg), low acute inhalation toxicity in rats (LC_{50} > 4.65 mg/l), is not a skin irritant in rabbits, is a moderate eye irritant in unwashed rabbit eyes but is not irritating in washed rabbit eyes. It is a skin sensitizer in guinea-pigs, according to the Magnusson & Kligman maximization test, but is not a skin sensitizer in guinea-pigs according to the Buehler test.

In studies of toxicity with repeated doses, slight decreases (5–10%) in body weight and/or body-weight gain were regarded as non-adverse in the absence of other effects.

In studies of repeated doses in mice, the liver and spleen were the principal target organs at the same or higher doses than those affecting body weight and food efficiency. In the 90-day study in male and female mice, liver weight was increased and there were findings on microscopy, including hepatocyte hypertrophy and focal or single cell necrosis. There were also increased incidences of extramedullary haematopoiesis in the spleen at doses of ≥315 mg/kg bw per day. The NOAEL for these effects was 77 mg/kg bw per day.

In a 90-day dietary study in rats, the NOAEL was 31 mg/kg bw per day on the basis of statistically significantly decreased body-weight gain of 20% and 40% in males and females, respectively, increased relative liver weights, changes in clinical chemistry, and liver histopathology findings (mainly hepatocellular hypertrophy), in addition to atrophy of the pancreas at the next higher dose of 127 mg/kg bw per day.

At or above a daily dose of trifloxystrobin at 150 mg/kg bw per day for 3 months or 50 mg/kg bw per day for 1 year, dogs had episodes of diarrhoea, vomiting, reduced food intake, increased relative weight of the liver, and hepatocyte hypertrophy, in addition to changes in clinical chemistry parameters indicative of liver toxicity and/or perturbed metabolism, dehydration, poor nutrition, and possible starvation. Body weights were also affected. In the 3-month study, animals of both sexes had body-weight loss of about 0.4 kg and 2.8 kg at 150 and 500 mg/kg bw per day, respectively. In the 1-year study, body-weight gain in females at 50 and 200 mg/kg bw per day was decreased throughout the study, and at week 52 body-weight gain was about 20% below control values. The NOAELs were 30 and 5 mg/kg bw per day in the 3-month and 1-year studies, respectively.

Long-term study of toxicity and carcinogenicity with trifloxystrobin were carried out in mice and rats. In the 18-month dietary feeding study in mice, the NOAEL was 36 mg/kg bw per day on the basis of liver effects, including increased weight of the liver (both sexes) and increased single-cell necrosis (males), in addition to impaired body-weight gain (females). There was no evidence of carcinogenicity in mice tested at adequate doses.

In the 2-year study in rats, the NOAEL was 30 mg/kg bw per day on the basis of statistically significantly retarded body-weight gain in males (11–17%) and females (17–27%) and decreased food consumption (by 4% and 8%, respectively) and increased relative weights of heart, liver, and kidneys (each by about 20%) in females at the highest dose of 62 mg/kg bw per day. The overall incidence of tumours was lower in the treated animals. Benign adrenal medullary tumours (10% versus 0% in controls) and haemangioma in the mesenteric lymph nodes (10.2% versus 0% in controls) were increased in male rats at the highest dose tested. Incidences of the adrenal medullary tumours were within the range of incidences for historical controls. The incidence of haemangioma in the mesenteric lymph nodes in males at the highest dose group was outside the range of incidences for historical controls. There was markedly reduced mortality in the group receiving the highest dose tested, and this may have contributed to the higher incidence of tumours in this group compared with controls. In ageing male rats of this strain, degenerative lesions associated with the mesenteric lymph nodes are common and are hard to distinguish from neoplastic lesions (haemangiomas). Some age-associated non-neoplastic findings, such as angiomatous hyperplasia of the mesenteric lymph nodes, were increased in males at the highest dose and the increases were correlated with decreased food intake and a lower body-weight development.

The Meeting concluded that trifloxystrobin had no treatment-related carcinogenicity of any toxicological concern.

A wide range of assays for genotoxic potential with trifloxystrobin were conducted in vitro and in vivo, including testing for gene mutation, chromosomal damage and DNA repair. Trifloxystrobin was weakly mutagenic at cytotoxic doses in the test for forward gene mutation in Chinese hamster V79 cells. Results were equivocal in the absence of metabolic activation. Metabolites of trifloxystrobin [CGA 357261 (*Z*, *E*-isomer), CGA 373466, and NOA 414412] were not mutagenic in the Ames test. The Meeting concluded that trifloxystrobin and its metabolites are not genotoxic.

Because of the absence of findings indicative of genotoxicity or carcinogenicity, the Meeting concluded that trifloxystrobin is unlikely to pose a carcinogenic risk to humans.

In the two-generation study in rats given trifloxystrobin at a dose of 55 or 111 mg/kg bw per day, pups in the F_1 and F_2 litters had retarded body-weight development during lactation. The NOAEL for parental toxicity was 3.8 mg/kg bw per day on the basis of findings at 55 mg/kg bw per day, i.e. reduced body weight and food consumption, in addition to histopathology findings in the liver and kidneys. The NOAEL for offspring toxicity was 3.8 mg/kg bw per day on the basis of retarded body-weight development during lactation. The NOAEL for reproductive toxicity was 111 mg/kg bw per day.

Trifloxystrobin was not teratogenic in rats and rabbits when tested at doses of up to 1000 and 500 mg/kg bw per day, respectively. In rats, the NOAEL for developmental toxicity was 100 mg/kg bw per day on the basis of increased incidences of enlarged thymus. In rabbits, the NOAEL for developmental toxicity was 250 mg/kg bw per day on the basis of increased incidences of skeletal anomalies in the form of fused sternebrae 3 and 4. Maternal toxicity in rats and rabbits was limited to reduced food consumption and body-weight loss at 100 and 250 mg/kg bw per day with NOAELs of 10 and 50 mg/kg bw per day, respectively. The developmental effects were considered to be a consequence of overall maternal toxicity.

The Meeting concluded that the existing database on trifloxystrobin was adequate to characterize the potential hazards to fetuses, infants, and children.

In a study of acute oral neurotoxicity in rats given a single dose of trifloxystrobin at 2000 mg/kg bw, the functional observational battery revealed no indications for potential neurological or behavioural effects.

Toxicological evaluation

The Meeting established an ADI of 0–0.04 mg/kg bw based on the parental NOAEL of 3.8 mg/kg bw per day in a multigeneration study of reproductive toxicity in rats and a 100-fold safety factor. The lowest-observed-adverse-effect level (LOAEL) was 55 mg/kg bw per day on the basis of effects on body weight and food consumption, in addition to liver and kidney histopathology findings. This value is supported by the NOAEL of 5 mg/kg bw per day in the 1-year study in dogs.

The Meeting concluded that it was unnecessary to establish an ARfD for trifloxystrobin on the basis of its low acute toxicity and the fact that developmental effects were considered to be a result of severe maternal toxicity, which is related to decreased food intake rather than systemic toxicity. Also, the vomiting and diarrhoea observed in dogs were clearly related to local irritation, rather than systemic acute toxicity.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	18-Month study of toxicity and carcinogenicitya	Toxicity	300 mg/kg, equal to 36 mg/kg bw per day	1000 mg/kg, equal to 124 mg/kg bw per day
		Carcinogenicity	2000 mg/kg, equal to 246 mg/kg bw per dayb	—
Rat	2-Year studies of toxicity and carcinogenicity[a]	Toxicity	750 mg/kg, equal to 30 mg/kg bw per day	1500 mg/kg, equal to 62 mg/kg bw per dayb
		Carcinogenicity	1500 mg/kg, equal to 62 mg/kg bw per dayb	—
	Two-generation reproductive toxicity[a]	Parental toxicity	50 mg/kg, equal to 3.8 mg/kg bw per day	750 mg/kg, equal to 55 mg/kg bw per day
		Offspring toxicity	50 mg/kg, equal to 3.8 mg/kg bw per day	750 mg/kg, equal to 55 mg/kg bw per day
	Developmental toxicity[c]	Maternal toxicity	10 mg/kg bw per day	100 mg/kg bw per day
		Embryo- and fetotoxicity	100 mg/kg bw per day	1000 mg/kg bw per day
Rabbit	Developmental toxicity[c]	Maternal toxicity	50 mg/kg bw per day	250 mg/kg bw per day
		Embryo- and fetotoxicity	250 mg/kg bw per day	500 mg/kg bw per day
Dog	3-Month study of toxicity[d,e]	Toxicity	30 mg/kg bw per day	150 mg/kg bw per day
	12-Month study of toxicity[d]	Toxicity	5 mg/kg bw per day	50 mg/kg bw per day

[a] Diet
[b] Highest dose tested
[c] Gavage
[d] Gelatin capsule
[e] Two or more studies combined

Estimate of acceptable daily intake for humans

0–0.04 mg/kg bw

Estimate of acute reference dose

Unnecessary

Studies that would provide information useful for continued evaluation of the compound

Further observations in humans

Summary of critical end-points for trifloxystrobin

Absorption, distribution, excretion and metabolism in animals	
Rate and extent of absorption	66% in 48 h
Distribution	Widely distributed; highest concentrations in blood, liver, and kidneys
Potential for accumulation	No potential for accumulation.
Rate and extent of excretion	Within 48 h, 72–96% of the administered dose is eliminated in the urine and faeces
Metabolism in animals	Extensive: hydrolysis, *O*-demethylation, oxidation, conjugation, chain shortening, and cleavage between glyoxylphenyl and trifluoromethyl moieties
Toxicologically significant compounds (plants, animals and the environment)	Parent compound, major acid metabolite is CGA 321113
Acute toxicity	
Rat, LD_{50}, oral	>5000 mg/kg bw
Rat, LD_{50}, dermal	>2000 mg/kg bw
Rat, LC_{50}, inhalation:	>4.6 mg/l air
Rabbit, skin irritation:	Not irritating
Rabbit, eye irritation:	Not irritating
Skin sensitization	Sensitizer (Magnusson & Kligman test)
Short-term studies of toxicity	
Target/critical effect	Body weight, food consumption, clinical signs, liver (pathology), kidney (weight), pancreas (atrophy), spleen (weight and pathology)
Lowest relevant oral NOAEL	5 mg/kg bw per day (1-year study in dogs)
Lowest relevant dermal NOAEL	≥1000 mg/kg bw per day (28-day study in rats)
Lowest relevant inhalation NOAEC	No relevant study
Genotoxicity	No genotoxic potential, negative results in vivo, one positive result in study in vitro at cytotoxic doses.
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Body weight (mouse, rat), food consumption (rat), liver (mouse, rat)
Lowest relevant NOAEL	30 mg/kg bw per day (2-year study in rats)
Carcinogenicity	Unlikely to pose a carcinogenic risk to humans
Reproductive toxicity	
Target/critical effect	Decreased body-weight gain of pups accompanied by delayed eye opening at parental toxic doses
Lowest relevant reproductive NOAEL	50 ppm (3.8 mg/kg bw per day)s
Developmental target/critical effect	Enlarged thymus (rat) and skeletal effects (rabbit) at maternally toxic doses
Lowest relevant developmental NOAEL	100 mg/kg bw per day (rat)
Neurotoxicity	No evidence of acute neurotoxicity in rats
Other toxicological studies	No evidence of replicative DNA synthesis in rat or mouse heptocytes after 3-months administration in diet A range of metabolites had low acute oral toxicity and there was no evidence of genotoxic activity
Medical data	New active substance; limited data; some evidence of skin and eye irritation in three people during field trials (but 120 people without effects)

Summary

	Value	*Study*	*Safety factor*
ADI	0–0.04 mg/kg bw	Rat, reproduction study, reduced body weight, liver and kidney effects	100
ARfD	Unnecessary	—	—

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