Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

EC Number: not assigned

CAS Number: 141517-21-7

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CONTENTS

This Annex has been compiled based on the study summaries in the RAR, with additions/corrections made by the Applicant.

1 PHYSICAL HAZARDS

1.1 Explosives

Not relevant for this proposal.

1.2 Flammable gases (including chemically unstable gases)

Not relevant for this proposal.

1.3 Oxidising gases

Not relevant for this proposal.

1.4 Gases under pressure

Not relevant for this proposal.

1.5 Flammable liquid

Not relevant for this proposal.

1.6 Flammable solids

Not relevant for this proposal.

1.7 Self-reactive substances

Not relevant for this proposal.

1.8 Pyrophoric liquids

Not relevant for this proposal.

1.9 Pyrophoric solid

Not relevant for this proposal.

1.10 Self-heating substances

Not relevant for this proposal.

1.11 Substances which in contact with water emit flammable gases

Not relevant for this proposal.

1.12 Oxidising liquids

Not relevant for this proposal.

1.13 Oxidising solids

Not relevant for this proposal.

1.14 Organic peroxides

Not relevant for this proposal.

1.15 Corrosive to metals

Not relevant for this proposal.

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Note: Unless otherwise stated, all studies were GLP compliant. Test diets/dosing solutions were all checked for the content, homogeneity and stability, and the actual concentrations compared to nominal. The methods employed to confirm these parameters are not validated according to current standards but are fit for purpose (see section B.5.) as the data was within an acceptable range of variation and was consistent between the information provided by the sponsor and the designated laboratory.

Unless otherwise stated the NOAELs proposed within the document are the same as those proposed by the applicant.

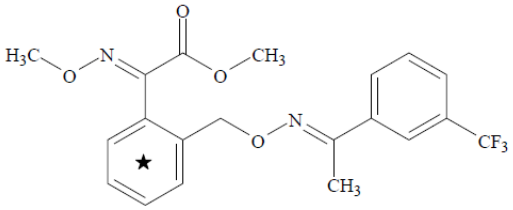
Absorption distribution excretion and metabolism (toxicokinetics) (IIA 5.1)

The applicant has submitted five studies with trifloxystrobin,

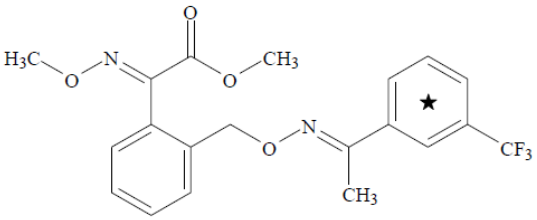
- An absorption, distribution and excretion study to OECD guideline 417 in the rat using both [Glyoxyl-Phenyl-U-^{14}C] and [Trifluoromethyl-Phenyl-U-^{14}C] labelled trifloxystrobin. This study includes investigations of biliary excretion.
- A follow up absorption, distribution and excretion study investigating some apparent label related differences in tissue distribution at high doses.
- A study investigating the metabolites present in the urine, faeces and bile samples taken from animals in the main absorption, distribution and excretion and its follow-up study
- An *in vivo* dermal absorption study in rats using trifloxystrobin as an EC formulation.
- An *in vitro* study comparing the dermal absorption study of the trifloxystrobin EC

formulation in isolated rat and human epidermis.

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



[Trifluoromethyl-Phenyl-U-^{14}C]-trifloxystrobin



★= position of label

2.1.1 [Study 1]

Absorption, distribution and excretion of [Glyoxyl-Phenyl-U-^{14}C] trifloxystrobin and [Trifluoromethyl-Phenyl-U-^{14}C] labelled trifloxystrobin (Anonymous, 1998a)

In a 1994-6 study the absorption, distribution and excretion of [Glyoxyl-Phenyl-U-^{14}C] trifloxystrobin (5 batches used radiochemical purity >97%) and [Trifluoromethyl-Phenyl-U-^{14}C] labelled trifloxystrobin (radiochemical purity >99%) were investigated in Sprague Dawley derived rats.

The vehicle used was ethanol/PEG (3:5 v/v). Single oral doses were administered at two dose levels (0.5 and 100 mg/kg) to groups of male and female rats as follows:

Group	No of animals	Dose	Label	Sampling	Time point (s)
B1	5/sex	0.5 mg/kg	GP-U-^{14}C	Blood	0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, 72, and 96 h
				Urine	8, 24, 48, 72, 96, 120, 144, and 168 h
				Faeces	24, 48, 72, 96, 120, 144, and 168 h
				selected tissues	after 7 days
C1	5/sex	0.5 mg/kg pre-treatment*	GP-U-1^{4}C	Blood	0.25, 0.5, 1, 2, 4, 8, 12, 24 and 48 h
D1	5/sex	100 mg/kg	GP-U-^{14}C	expired air	24, 48 h
				blood	0.25, 0.5, 1, 2, 4, 8, 12, 24 and 48 h
				urine	8, 24, 48, 72, 96, 120, 144, and 168 h
				faeces	24, 48, 72, 96, 120, 144, and 168 h
				selected tissues	after 7 days
D2	5/sex	100 mg/kg	TP-U-^{14}C	expired air	24, 48 h
				blood	0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, 72, and 96 h
				urine	8, 24, 48, 72, 96, 120, 144, and 168 h
				faeces	24, 48, 72, 96, 120, 144, and 168 h
				selected tissues	after 7 days
F1	12 males	0.5 mg/kg	GP-U-^{14}C	selected tissues	at t_{max}, (12h) $t_{max/2}$, (48h) $t_{max/4}$, (66h) $t_{max/8}$, (96h)**
F2	12 males	100 mg/kg	GP-U-^{14}C	selected tissues	at t_{max}, (24h) $t_{max/2}$, (50h) $t_{max/4}$, (72h) $t_{max/8}$, (96h)**
F5	12 females	0.5 mg/kg	GP-U-^{14}C	selected tissues	at t_{max}, (12h) $t_{max/2}$, (23h) $t_{max/4}$, (40h) $t_{max/8}$, (70h)**
F6	12 females	100 mg/kg	GP-U-^{14}C	selected tissues	at t_{max}, (12h) $t_{max/2}$, (44h) $t_{max/4}$, (72h) $t_{max/8}$, (96h)**
G1	6 males	0.5 mg/kg	GP-U-^{14}C	bile	1, 2, 4, 8, 18, 24, 42, 48 h
				urine	24, 48 h
				faeces	24, 48 h
				GI tract, carcass	after 48 h
G2	6 males	100 mg/kg	GP-U-^{14}C	bile	1, 2, 4, 8, 18, 24, 42 h
				urine	24, 42 h
				faeces	24, 42 h
				GI tract, carcass	after 42 h
G3	5 females	0.5 mg/kg	GP-U-^{14}C	bile	1, 2, 4, 8, 18, 24, 42, 48 h
				urine	24, 48 h
				faeces	24, 48 h
				GI tract, carcass	after 48 h
G4	4 females	100 mg/kg	GP-U-^{14}C	bile	1, 2, 4, 8, 18, 24, 42, 48 h
				urine	24, 48 h

				faeces	24, 48 h
				GI tract, carcass	after 48 h

GP-U-^{14}C = [Glyoxyl-Phenyl-U-^{14}C]- trifloxystrobin

TP-U-^{14}C = [Trifluoromethyl-Phenyl-U-^{14}C]- trifloxystrobin

* daily oral doses (0.5 mg/kg) of non-radiolabelled trifloxystrobin for 14 days

** t_{max}, (time at C_{max}) $t_{max/2}$, (time for the depletion to ½ C_{max}) $t_{max/4}$, (time for the depletion to ¼ C_{max}) $t_{max/8}$ (time for the depletion to 1/8 C_{max})**

Radioactivity in liquid samples (e.g. urine, bile, plasma, and cage washes) was measured directly by LSC. Radioactivity in the organs/tissues and faeces was measured either by combustion followed by LSC or LSC after digestion with a tissue solubliser.

Excretion

After administration of [Glyoxyl-Phenyl-U-^{14}C]-trifloxystrobin the majority of the radioactivity was eliminated within 48 hours. 85 – 96% of the radioactivity was excreted independent of the dose level, pretreatment with trifloxystrobin (unlabelled), or the sex of the animals. Seven days after administration the dose was almost completely eliminated.

The route of elimination was influenced by the sex of the animals. Irrespective of pretreatment and dose level, female rats eliminated twice the amount of the radioactivity in the urine than males. Consequently, a greater quantity of radioactivity was excreted via faeces in males.

Table B.6.1: Summary of excretion data

Excretion after oral administration (% of Dose)												
Label	**[Glyoxyl-Phenyl-U-^{14}C]**										**[Trifluoromethyl-Phenyl-U-^{14}C]**	
Group	**B1**		**C1$^{\#}$**		**D1**		**G1**	**G3**	**G2**	**G4**	**D2**	
	Low dose		**Repeat dose**		**High dose**		**Bile duct cannulated rats**				**High dose**	
							Low dose		**High dose**			
Sex	**male**	**female**	**male**	**female**	**male**	**female**	**male**	**female**	**male**	**female**	**male**	**female**
Dose (mg/kg)	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-	13.9	30.0	13.9	36.2	8.4	19.6	6.5	8.6	2.5	5.3	6.2	15.8
24-48h	3.2	3.9	2.9	4.0	2.7	5.8	5.7	6.0	1.6	0.8*	2.4	8.8
48-168h	1.7	1.3	1.6	1.5	1.0	1.2	n.a.	n.a.	n.a.	n.a.	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-	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	41.0	46.5	34.7	19.1**	n.a.	n.a.
Faeces 0-24h	57.1	50.3	54.5	41.0	45.9	32.6	7.6	5.4	8.6	20.8	52.7	25.8
24-48h	19.0	11.5	20.1	11.8	33.2	26.9	19.7	9.2	35.6	8.3*	26.6	29.0
48-72h	2.2	1.0	3.5	2.4	2.3	4.2	n.a.	n.a.	n.a.	n.a.	3.2	10.4
72-169h	1.1	0.5	1.2	0.8	0.7	0.5	n.a.	n.a.	n.a.	n.a.	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	n.a.	n.a.	n.a.	n.a.	<0.01	<0.01	n.a.	n.a.	n.a.	n.a.	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

n.a. - not applicable, * 24-42 hours ** 0-42 hours, $^{\#}$ daily oral doses (0.5 mg/kg) of non-radiolabeled trifloxystrobin for 14 days

Excretion via the bile was the major route of elimination in both sexes. At the low dose, more than half of the dose excreted with the faeces was derived from biliary excretion. Biliary excretion appeared to be independent of sex.

Based on urinary excretion data, there was evidence of enterohepatic circulation at the low dose level, more clearly so in females than males. In bile-duct cannulated female rats (Group G3) the urinary excretion decreased markedly to 15% of the dose within 48 hours compared to non-bile-duct cannulated female rats of

Group B1 (34%). In males 12% of the dose was renally excreted in bile-duct cannulated rats compared to 17% in intact animals.

The enterohepatic circulation was also apparent at the high dose level in both sexes. However, the results, particularly those of the female rats (Group G4), were hampered by the low total excretion. (Note: when this experiment was repeated in females, see. B.6.1.1 b, the apparent low excretion was considered a result of poor recovery of radiolabeled material from the gut).

After administration of the [Trifluoromethyl-Phenyl-U-^{14}C] trifloxystrobin a small amount of the dose, i.e. 0.08% and 0.05% in male and female rats respectively, was exhaled, while with the [Glyoxyl-Phenyl-U-^{14}C] label the expired air contained less than 0.01% of the dose.

Tissue Residues

Depletion kinetics were investigated in male and female rats receiving [Glyoxyl-Phenyl-U-^{14}C] trifloxystrobin at two dose levels, i.e. 0.5 mg/kg (Groups F1, F5) and 100 mg/kg (Groups F2, F6). The tissue residues were measured at four different time points time at C_{max}, time for the depletion to ½ C_{max}, time for the depletion to ¼ C_{max} and time for the depletion to1/8 C_{max}.

In all tissues the highest values were found at the time of maximal blood residues irrespective of the dose level and the sex of the animals. The elimination of the residual radioactivity from tissues and organs was monophasic. The residual radioactivity depleted from tissues and organs with half-life times (assuming first order kinetics) of 13 to 33 hours independent of the dose level and the sex of the animals except of blood and spleen. These tissues showed a slower depletion with half-life times of 30 – 82 hours and 38 – 68 hours.

Table B.6.2: Depletion of residual radioactivity from selected tissues (Half-life time – hours)

Half-life time [h]				
Label	**[Glyoxyl-Phenyl-U-^{14}C] trifloxystrobin**			
Group	**F1**	**F2**	**F5**	**F6**
Sex	**male**	**male**	**female**	**female**
Dose [mg/kg bw]	0.55	101.9	0.54	104.7
Blood	38	40	30	82
Bone	30	26	13	28
Brain	15	31	27	33
Fat (abdominal)	18	18	18	33
Heart	23	26	19	26
Kidneys	23	31	21	30
Liver	21	28	15	23
Lungs	28	28	15	29
Muscle (skeletal)	20	24	18	25
Ovaries	n.a.	n.a.	22	24
Plasma	24	23	14	18
Spleen	39	42	38	68
Testes	26	23	n.a.	n.a.
Uterus	n.a.	n.a	22	22

n.a.: not applicable

Seven days after a single oral dose of [Glyoxyl-Phenyl-U-^{14}C] trifloxystrobin at the low dose level (Group B1, 0.5 mg/kg) the residues were very low not exceeding 0.014 ppm trifloxystrobin equivalents. The pattern of tissue residues in the animals pre-treated with non-radiolabeled trifloxystrobin at a daily dose of 0.5 mg/kg for 14 days (Group C1) was identical to that in the non-pre-treated animals of Group B1.

At the high dose (100 mg/kg, Group D1) the residues were about 126-fold and 108-fold higher than at the low dose in males and females, respectively. The highest residues were found in blood, kidneys and liver. The residues in all tissues were higher in female rats than in males except in liver and plasma.

Seven days after the administration of [Trifluoromethyl-Phenyl-U-^{14}C] trifloxystrobin at a dose level of 100 mg/kg (Group D2) the residual radioactivity in all tissues, except plasma, was higher in female rats than in males particularly in kidneys, liver, and spleen.

Table B.6.3: Tissue residues 7 days after administration of the radiolabeled trifloxystrobin [ppm trifloxystrobin equivalents]

Tissue residues [ppm trifloxystrobin equivalents]								
Label	**[Glyoxyl-Phenyl-U-^{14}C]**						**[Trifluoromethyl-Phenyl-U-^{14}C]**	
Group	**B1**		**C1**		**D1**		**D2**	
Sex	**male**	**female**	**male**	**female**	**male**	**female**	**male**	**female**
Dose (mg/kg bw)	0.48	0.50	0.42	0.48	105.4	101.7	97.5	105.2
Blood	0.007	0.011	0.008	0.011	1.205	1.616	1.278	1.589
Bone	< 0.0009	< 0.0009	< LD*	= LD*	0.054	0.061	0.065	0.067
Brain	< 0.0007	< 0.0007	0.0008	< 0.0008	0.040	0.047	0.042	0.062
Fat (abdom.)	0.001	0.004	0.002	0.002	0.121	0.191	0.139	0.293
Heart	< 0.0010	0.001	0.002	0.002	0.165	0.175	0.167	0.191
Kidneys	0.011	0.013	0.011	0.014	1.388	1.625	1.022	1.936
Liver	0.011	0.009	0.011	0.008	1.612	1.176	1.046	1.949
Lungs	0.002	0.003	0.003	0.003	0.313	0.309	0.241	0.346
Muscle (skelet.)	< 0.0007	0.0007	< 0.0008	< 0.0008	0.055	0.055	0.047	0.055
Ovaries	n.a.	0.006	n.a.	< 0.0042	n.a.	0.171	n.a.	0.198
Plasma	0.003	0.0007	0.002	0.0007	0.223	0.084	0.111	0.091
Spleen	0.002	0.004	0.002	0.006	0.334	0.739	0.367	0.758
Testes	0.0007	n.a.	< 0.0008	n.a.	0.064	n.a.	0.053	n.a.
Uterus	n.a.	< 0.0023	n.a.	< 0.002	n.a.	0.081	n.a.	0.112
Carcass	0.0009	0.0009	0.001	0.001	0.103	0.142	0.123	0.216
Total Residues [% of dose]	0.4	0.4	0.5	0.4	0.3	0.3	0.3	0.4

n.a. - not applicable; *LD - limit of detection (0.0003 ppm trifloxystrobin equivalents)

The radioactivity in the blood was associated predominantly with the blood cells in both sexes, although a sex difference in the extent of binding to blood cells was evident for both labels.

Male and female rats had blood/plasma ratios of 4:1 and 18:1 for the [Glyoxyl-Phenyl-U-^{14}C] label and 11:1 and 17:1 for the [Trifluoromethyl-Phenyl-U-^{14}C] label. Besides the sex difference, male rats showed a clear label difference since the ratio was twice as high for the [Trifluoromethyl-Phenyl-U-^{14}C] label as for the [Glyoxyl-Phenyl-U-^{14}C] label. Concomitant to the residues in blood cells, the residues in the spleen were markedly elevated in females rats compared to males.

Table B.6.4: Residues in plasma and blood cells seven days after administration of the radiolabeled test substances [ppm trifloxystrobin equivalents]

Residues in plasma and blood cells [ppm trifloxystrobin equivalents]				
Group	**D1**		**D2**	
Sex	**Male**	**female**	**male**	**female**
Plasma	0.223	0.084	0.111	0.091
Blood cells	0.982	1.532	1.167	1.498
ratio of blood cells/plasma	4.4	18.2	10.5	16.5

Comparing the tissue residues of the [Trifluoromethyl-Phenyl-U-^{14}C] label (Group D2) with that of the [Glyoxyl-Phenyl-U-^{14}C] label (Group D1), sex and label-related differences were found.

In kidneys, liver, and plasma of male rats the residues were higher after administration of the [Glyoxyl-Phenyl-U-^{14}C] label. In contrast to the males, administration of the [Trifluoromethyl-Phenyl-U-^{14}C] label yielded higher residues in fat and liver of female rats. There were no clear differences between labels in residues in the other tissues.

Absorption and Blood Kinetics

Maximum residues in the blood were reached between 12 and 24 hours after dosing, after an initial small peak, independent of the dose level, sex of the animal, and the site of label. Depletion to half maximal values occurred faster in females than males (see summary table).

After administration of the [Glyoxyl-Phenyl-U-^{14}C] trifloxystrobin at the low dose (0.5 mg/kg, Group B1), the areas under the curve (AUC $_{0-96\ h}$) were in the same range for male and female rats indicating an equal bioavailability. At the high dose level (100 mg/kg, Group D1), the AUC $_{0-48\ h}$ was about one third higher in male rats (335 mg.h/kg) than in females (214 mg.h/kg). However, when considering the huge variation of blood residues in the individual animals, the very similar AUC's of males and females after dosing of the [Trifluoromethyl-Phenyl-U-^{14}C] trifloxystrobin at the high dose (100 mg/kg, Group D2), the presence of a sex difference of bioavailability is not clear. Absorption from the gut at the high dosed appears to be saturated since the AUC $_{0-48\ h}$ was approximately 129-fold higher than that at the low dose while the dose level ratio was 200:1.

The experiment with bile-duct cannulated rats demonstrated that, at the low dose (Groups G1, G3), 56% and 65% of the dose were absorbed by male and female rats, respectively. At the high dose level the biliary excretion decreased leading to a lower extent of absorption of 41% (Group G2) and 27% of the dose (Group G4) in male and female rats, respectively. As previously noted the results, particularly those of the female rats (Group G4), were hampered by poor recoveries of radiolabeled material.

Table B.6.5: Summary of Blood Kinetics

Label	**[Glyoxyl-Phenyl-U-^{14}C]**				**[Trifiuormethyl-Phenyl-U-^{14C}]**	
Group	**B1**		**D1**		**D2**	
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
$t_{c\ max/2}$ [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	n.a.	n.a.	375.1	331.6

n.a. - not applicable

[Study 2] Absorption, distribution amd excretion of [trifluormethyl-phenyl-(U)-14C] and [glyoxyl-phenyl-(U)-14C] CGA 279202 in the rat (extension) (Anonymous, 1998b, M-136744-01-1)

In order to further examine the cleavage between Glyoxyl-Phenyl and Trifluoromethyl-Phenyl moiety an additional study was conducted in 1998. The absorption, excretion and tissue residues after oral administration of [Trifluoromethyl-Phenyl-U-^{14}C] trifloxystrobin was investigated in male and female rats at the low dose level 0.5 mg/kg (Group B2) and the depletion kinetics of the tissue residues at both low and high dose levels (100 mg/kg) (Groups F3, F4, F7, F8).

In addition, the experiments with [Glyoxyl-Phenyl-U-^{14}C] trifloxystrobin in bile duct cannulated female rats administered at the high dose (Group G4) were repeated due to the low recovery of radioactivity in the earlier study.

The vehicle used was ethanol/PEG (3:5 v/v). Single oral doses were administered at two dose levels (0.5 and 100 mg/kg) to groups of male and female rats as follows:

Group	**No of animals**	**Treatment**	**Sampling**	**Time point (s)**
B2	**5/sex**	0.5 mg/kg TP-U-^{14}C	Blood	0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, 72, and 96 h
			Urine	8, 24, 48, 72, 96, 120, 144, and 168 h
			Faeces	24, 48, 72, 96, 120, 144, and 168 h
			selected tissues	after 7 days
F3	**12 males**	0.5 mg/kg TP -U-^{14}C	selected tissues	at t_{max}, (12h) $t_{max/2}$, (48h) $t_{max/4}$, (80h) $t_{max/8}$ (120h)*

Group	No of animals	Treatment	Sampling	Time point (s)
F4	**12 males**	100 mg/kg TP-U-^{14}C	selected tissues	at t_{max}, (24h) $t_{max/2}$, (50h) $t_{max/4}$, (72h) $t_{max/8}$ (96h)*
F7	**12 males**	0.5 mg/kg TP-U-^{14}C	selected tissues	at t_{max}, (12h) $t_{max/2}$, (24h) $t_{max/4}$, (42h) $t_{max/8}$ (72h)*
F8	**12 males**	100 mg/kg GP-U-^{14}C	selected tissues	at t_{max}, (12h) $t_{max/2}$, (44h) $t_{max/4}$, (72h) $t_{max/8}$ (96h)*
G4a	**4 females**	100 mg/kg GP-U-^{14}C	Bile	1, 2, 4, 8, 18, 24, 42, 48 h
			Urine	24, 48 h
			Faeces	24, 48 h
			GI tract, carcass	after 48 h

GP-U-^{14}C = [Glyoxyl-Phenyl-U-^{14}C] trifloxystrobin
TP-U-^{14}C = [Trifluoromethyl-Phenyl-U-^{14}C] trifloxystrobin
* t_{max}, (time at C_{max}) $t_{max/2}$, (time for the depletion to ½ C_{max}) $t_{max/4}$, (time for the depletion to ¼ C_{max}) $t_{max/8}$ (time for the depletion to 1/8 C_{max})**

Radioactivity in liquid samples (e.g. urine, bile, plasma, and cage washes) was measured directly by LSC. Radioactivity in the organs/tissues and faeces was measured either by combustion followed by LSC or LSC after digestion with a tissue solubliser.

Excretion

Excretion after oral administration of [Trifluoromethyl-Phenyl-U-^{14}C] trifloxystrobin at the low dose (Group B2) the radioactivity was moderately rapid, predominantly with the faeces. Within 48 hours 93% of the dose was excreted independent of the sex of the animal. The route of elimination was influenced by the sex of the animals. Approximately double the amount of radioactivity was excreted with the urine in females (33% of the dose) compared to males (16%). The faeces contained 80% and 62% of the dose in male and female rats, respectively.

Table B.6.6: Summary of excretion data [% of dose]

Excretion after oral administration (% of Dose)				
Label	**[Trifluoromethyl-Phenyl-U-^{14}C]**		**[Glyoxyl-Phenyl-U-^{14}C]**	
Group	**D2**		**G4a**	**G4 (Original experiment)**
	Low dose		**Bile duct cannulated rats**	**Bile duct cannulated rats**
Sex	**male**	**Female**	**female**	**female**
Dose (mg/kg)	0.52	0.55	100.2	99.9
Urine 0-	13.1	28.5	1.7	5.3
24-48h	2.0	4.0	1.2	0.8*
48-168h	1.2	1.0	n.a.	n.a.
subtotal	16.3	33.5	2.8	6.1
Faeces 0-	70.7	46.6	3.6	20.8
24-48h	8.0	14.6	15.9	8.3*
48-168h	1.6	1.1	n.a.	n.a
subtotal	80.3	62.3	19.5	29.1
Bile 0-	n.a.	n.a.	8.9	13.3
24-48h	n.a.	n.a.	9.0	5.8*
subtotal	n.a.	n.a.	17.9	19.1 **
Cage Wash	0.1	0.8	0.5	0.4
Total Excretion	96.6	96.6	40.7	54.8
Gastrointestinal tract	n.a.	n.a.	50.0	18.29
Tissues	n.a.	n.a.	1.4	1.4
Recovery	n.a.	n.a.	92.1	74.5

n.a. - not applicable, * 24-42 hours ** 0-42 hours, # daily oral doses (0.5 mg/kg) of non-radiolabeled trifloxystrobin for 14 days

In the repeat experiment with bile-duct cannulated female rats dosed with 100 mg/kg [Glyoxyl-Phenyl-U-^{14}C] trifloxystrobin, similar results were obtained compared to the earlier study. Within 48 hours the bile-duct cannulated female rats excreted about 18%, 3%, and 20% with the bile, urine, and faeces, respectively.

The low recovery determined in the previous experiment was believed to be due to the incorrect determination of the dose remaining in the gastrointestinal tract.

Tissue Residues

Independent of the dose level and the sex of the animals the highest tissue residues were found between 12 and 24 hours after administration. The residual radioactivity depleted from tissues and organs with half-life times of 12 to 34 hours independent of the dose level and the sex of the animals, except for muscle and testes (37 hours) in Group F3, blood (36 – 41 hours) and spleen (38 – 99 hours) in groups F3, F4, and F7.

Table B.6.7: Depletion of residual radioactivity from selected tissues (Half-life time – hours)

Half-life time [h]				
Label	**[Trifluoromethyl-Phenyl-U-^{14}C] trifloxystrobin**			
Group	**F3**	**F4**	**F7**	**F8**
Sex	**male**	**male**	**female**	**female**
Dose [mg/kg bw]	0.48	107.2	0.51	109.98
Time interval [h]	12-120	24-96	12-72	12-96
Blood	36*	38	41	25****
Bone	33	21	16**	19
Brain	30*	32	33	20
Fat (abdominal)	33	20	25***	25
Heart	21*	22	21	16****
Kidneys	21	29	22	27
Liver	34	22	19	12****
Lungs	22*	24	24	22
Muscle (skeletal)	37	26	23	20
Ovaries	n.a.	n.a.	25	20
Plasma	28	16.5	13	14
Spleen	99	38	42	22****
Testes	37	21	n.a.	n.a.
Uterus	n.a	n.a.	19	18

n.a.: not applicable, * Interval 12 - 80 hours, ** Interval 12 - 40 hours,
*** Interval 24 - 72 hours, **** Interval 12 - 72 hours

Highest tissue residues were found between 12 and 24 hours after administration. Seven days after a single oral administration of [Trifluoromethyl-Phenyl-U-^{14}C] trifloxystrobin at the low dose (Group B2) the terminal residues were very low and independent of the sex of the animal. The highest concentrations of radioactivity were found in blood, kidneys and liver. All the other tissue residues did not exceed 0.006 ppm. Compared to the respective data of the first study, obtained with the Glyoxyl-Phenyl label, no significant differences were found.

Table B.6.8 Tissue residues 7 days after administration of [Trifluoromethyl-Phenyl-U-^{14}C] trifloxystrobin [ppm trifloxystrobin equivalents]

Tissue residues [ppm trifloxystrobin equivalents]		
Label	**[Trifluoromethyl-Phenyl-U-^{14}C]**	
Group	**B2**	
Sex	**male**	**female**
Dose [mg/kg bw]	0.52	0.55
Blood	0.014	0.009
Bone	< 0.0011	< 0.0011
Brain	0.001	0.0008
Fat (abdominal)	0.003	0.003
Heart	0.002	0.001
Kidneys	0.010	0.012
Liver	0.012	0.007
Lungs	0.005	0.003
Muscle (skeletal)	0.001	0.0007
Ovaries	n.a.	< 0.004
Plasma	0.002	< 0.0006
Spleen	0.005	0.006
Testes	0.001	n.a.
Uterus	n.a.	0.0015
Carcass	0.001	0.001
Total Residues [% of dose]	0.5	0.3

n.a. - not applicable

[Study 3] The metabolism of [glyoxyl-phenyl-(U)-14C] and [trifluormethyl-phenyl-(U)-14C] CGA 279202 in the rat (Anonymous, 1998c, M-136745-01-1)

In a 1997 investigation of the metabolic pathway of trifloxystrobin in male and female rats, samples from the following experimental parts of the main toxicokinetics study (Miller, T., 1996, see section 5.1.1) and the supplementary toxicokinetics study (Anonymous, 1998b) were analyzed.

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 extracts of specimens were separated and analyzed 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 Bio-Imaging Analyser and quantification by scraping off the radioactive fractions followed by LSC. Non-radioactive fractions on TLC plates were located under UV-light at 254 nm. Pre-purification of urine, bile, and faeces extracts was carried out by solid phase extraction. MS- and NMR-spectroscopy as well as high voltage electrophoresis were used for structure elucidation of metabolites. In addition CGA 347242 and CGA 373463 were characterized as metabolites by comparison with authentic reference substances.

Table B.6.12: Metabolism in the rat - urinary metabolite profile

Label	PERCENT OF DOSE														Assignment
	GP-U-^{14}C						TP-U-^{14}C				GP-U-^{14}C				
Group	B1		C1		D1		B2		D2		G1	G3	G2	G4a	
	Low dose		Repeat dose		High dose		Low dose		High dose		bile-cannulated animals				
											Low dose		High dose		
Sex	M	F	M	F	M	F	M	F	M	F	M	F	M	F	
Dose (mg/kg bw)	0.48	0.5	0.42	0.48	105.4	101.7	0.52	0.55	97.5	105.2	0.46	0.5	113.7	100.2	
Metabolite Fraction U1	1.5	1.2	1.5	1.2	1.1	0.6							0.4	0.1	MET 16U(≡CGA 367619) CGA 373463
U2	2.4	1.4	6.1	1.6							traces			0.1	MET 18U[1]
U3	4	1.6		1.5	1.5	0.9					0.7	0.4	0.5	0.2	CGA 347242
U4					0.5	0.2					1.8	1.1	1.3		
U5					2.1	0.6									
U6							1.4		1	0.6					MET 12U(≡CGA 354870)
U7					0.2	traces							traces		
U8					0.2	traces									
U9	1.5		1.4	traces	0.5	0.4	0.9		0.2	0.4	traces			<0.1	MET 7U
U9a							0.4		0.3	0.2				<0.1	
U10					0.8	traces	0.7		0.4	0.3	traces		0.2		MET 13U[2]
U11	traces		traces		0.2	traces	0.7		0.4	0.3	traces			traces	MET 14U
U11a													0.1		
U12	traces		traces		0.4				traces						
U13	traces	traces	traces	traces	0.3	0.3	0.3		0.2	0.3	traces	traces	traces	0.1	
U14	traces	traces	traces	traces	0.3	0.5	1.6		0.8	0.6	traces	traces	traces		
U15							4.4	1.8	2.3	1.4					MET 11U
U16				traces	0.6	0.7	0.8		0.4	0.8	traces		traces	0.1	
U17		0.8		1.2		0.6				0.8				0.1	MET 10U/19U[3]
U18		6.1		7.2		4.8		6.6		4.5				0.6	MET 6U
U19		0.6		1		0.5			0.3	0.3		2.7		<0.1	MET 8U/20U[4]
U20		1.6		1.4	0.1	0.6			0.2	0.7	traces		0.3	0.2	MET 4U
U21	1	1	1.1	1.5	0.8	1	traces		0.7	0.9				0.1	MET 5U
U22		1.9		2.5		1.9				2		1.1		0.2	MET 3U
U23		4.6		6.3		4.9		5.6		4.2		2		0.2	MET 2U
U24		4.1		5.8		3.9	0.8	6.1		4				0.3	MET 1U
U24a															
U25										0.2					
U26						traces			traces	0.4	traces				
unresolved	6.7	8.9	6.6	9	1.6	3	3.1	12.4	1.5	1.7	9.7	1.2	7.3	0.5	
Total	17.1	33.9	16.8	40.2	11.1	25.3	15.1	32.5	8.7	24.6	12.2	14.6	4.1	2.8	
Actual Recovery (%)	**106**	**106**	**106**	**104**	**95**	**96**	**103**	**105**	**96**	**94**	**122**	**108**	**104**	**98**	

1 tentative assignment from preparative work due to degradation of the genuine metabolite
2 tentatively
3 MET 10U is the major component
4 MET 20U is the major component

Table B.6.13: Metabolism in the rat - faecal metabolite profile

Label	PERCENT OF DOSE														Assignment
	GP-U-^{14}C						TP-U-^{14}C				GP-U-^{14}C				
Group	B1		C1		D1		B2		D2		G1	G3	G2	G4a	
	Low dose		Repeat dose		High dose		Low dose		High dose		bile-cannulated animals				
											Low dose		High dose		
Sex	M	F	M	F	M	F	M	F	M	F	M	F	M	F	
Dose (mg/kg bw)	0.48	0.5	0.42	0.48	105.4	101.7	0.52	0.55	97.5	105.2	0.46	0.5	113.7	100.2	
Metabolite Fraction F1		traces		traces				1.4	1.5	1.1					
F2	4.6	2.0	4.0	1.5	2.0	0.9	3.7	1.5	0.7	0.5					MET 9F
															NOA 417076[1]
F3	3.6	1.6	3.5	1.5	2.3	1.1	3.2	1.4	2.0	1.4					MET 5F
F4	2.6		1.8	traces			1.3	2.1	1.0	1.5					MET 6F
															NOA 414412[1]
F5	3.8	2.7	3.4	2.3	2.1	1.7	2.3		1.2	0.9					
F6	4.1	3.5	4.2	2.8	3.3	3.1	4.3	3.3	2.6	3.1	0.7	0.3	0.4	0.2	MET 4F (≡CGA 321113)
F7	3.9	6.1	4.7	5.3	2.0	1.9	6.5	5.3	1.7	2.4					MET 3F/8F[2]
F8	2.5	3.4	2.8	2.7	1.4	1.5	2.8	2.2	1.5	2.1					MET 7F
F9	7.7	10.1	8.6	7.9	4.0	4.0	11.0	12.5	3.7	4.5	0.4		0.4		MET 2F (≡NOA 405637)
F10	5.6	5.2	5.1	4.3	37.7	31.1	5.8	6.8	46.9	32.6	23.2	12.1	41.6	21.0	PARENT
F11											0.3	0.2	0.7		
unresolved	24.1	17.2	22.3	15.8	16.2	8.9	23.0	14.5	12.7	8.6	2.1	1.6	1.0	0.7	
Sum Extract	62.4	51.7	60.4	44.1	71.0	54.2	64.2	50.9	75.5	58.7	26.7	14.2	44.0	21.9	
Non-extractable	13.6	10.1	14.2	8.7	8.1	5.3	15.5	10.9	7.0	6.5	0.6	0.4	0.2	0.1	
Total	76.0	61.8	74.6	52.8	79.1	59.5	79.7	61.8	82.5	65.2	27.3	14.6	44.2	22.0	
Actual Recovery (%)	106	105	101	106	91	97	107	105	99	98	110	110	101	97	

1 NOA 417076 and NOA 414412 do not correspond to a faeces fraction but are included in the unresolved radioactivity, accounting for about 0.2 and 0.1% of dose, respectively.

2 MET 3F is the major component

Table B.6.14: Metabolism in the rat - biliary metabolite profile

Label	PERCENT OF DOSE				Assignment
	GP-U-^{14}C				
Group	G1	G3	G2	G4a	
	Low dose		High dose		
Sex	M	F	M	F	
Dose (mg/kg bw)	0.46	0.5	113.7	100.2	
Metabolite Fraction G1	traces		0.3		
G2	traces		0.4		
G3	traces		0.6		
G4	1.9		1.5	0.7	
G5	1.0		1.5	0.2	
G6	2.5	2.8	2.6	1.6	MET 5G[1]
G7	traces	2.3	1.2	0.7	
G8	traces	1.9	1.7	1.1	
G9	3.5	4.1	3.0	2.4	MET 6aG/6bG[2]
G10	traces		1.6	1.0	
G11	traces		traces		
G12	1.7	3.4	1.5	1.1	MET 4aG
					MET 4bG[3]
G13	9.9	9.4	5.7	4.3	MET 1G
G14	1.0	2.0	0.7	0.4	MET 7G
G15	5.9	7.5	3.9	1.6	MET 2G (≡ CGA 321113)
G16			0.6		
G17			0.6		MET 8G
unresolved	12.2	13.1	4.2	3.0	
Total	39.6	46.5	31.6	17.9	
Actual Recovery	100%	109%	99%	101%	

1 tentative assignment from preparative work due to degradation of the genuine metabolite

2 the ratio of MET 6aG/MET 6bG is approximately 2:1

3 MET 4bG does not correspond to a bile fraction but is incorporated in the unresolved radioactivity. The ratio of MET 4aG/MET 4bG is approximately 4/1. Therefore, MET 4bG accounts for about 0.3 to 0.9% of dose in the various dosing groups.

The patterns of metabolites seen in urine, faeces, and bile were essentially different from each other.

The urinary patterns were dependent on sex, position of label, partly on dose level but independent of pre-treatment. The faeces patterns were qualitatively independent of sex, dose, pre-treatment, and position of label, with some quantitative variations. Also the bile patterns were qualitatively independent of sex and dose with some quantitative variations.

Ultimately about 35 metabolites were isolated from urine, faeces, and bile of the high dose male and female animals and identified by spectroscopy. In addition CGA 347242, CGA 373463, NOA 414412, and NOA 417076 were characterized as metabolites by co-chromatography with reference substances.

Based on the structures of these metabolites the metabolic pathways of trifloxystrobin were derived (see Figure 5.1). These were:

- hydrolysis of the methyl ester to the corresponding acid (major pathway)
- O-demethylation of the methoxyimino group yielding a hydroxyimino compound (major pathway)
- oxidation of the methyl side chain to a primary alcohol, followed by partial oxidation to the respective carboxylic acid (major pathway)
- hydrolysis of the imino group of the glyoxyl-phenyl moiety to yield a ketone with subsequent chain shortening by oxidative decarboxylation affording ultimately 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 dose. The primary cleavage products are prone to further degradation mainly by the above mentioned processes. For the trifluoromethyl-phenyl part these include 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 affording a ketone followed by oxidation of the methyl group to an intermediary carboxylic acid. This α-keto acid can either be reduced to a α-hydroxy acid or alternatively chain shortening by oxidative decarboxylation to trifluoromethyl-benzoic acid occurs. 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 a α-keto acid and subsequent chain shortening by oxidative decarboxylation ultimately yields phthalic acid.

Glucuronic and, to a lesser extent, sulphuric acid conjugates were generated from metabolites containing a hydroxy group.

The majority of metabolites resulted of more than one of the above mentioned transformations.

About 4 – 7% and 31 – 47% of the low and high dose, respectively, were eliminated in the faeces as unchanged parent.

The oxidation of the methyl side chain to a primary alcohol was more pronounced in female rats resulting in sex specific major metabolites. The degradation resulted in metabolites which 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 sulphuric acid conjugates. After hydrolysis (by the gut microflora) these metabolites were ultimately eliminated via

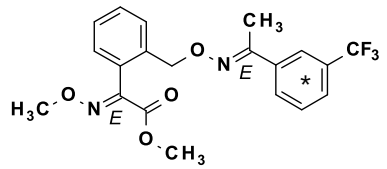
faeces, together with unchanged trifloxystrobin escaping absorption, or via urine after enterohepatic circulation and further transformation.

In conclusion, the extent of trifloxystrobin metabolism was dependent on dose but the degradation of the amount absorbed was almost complete and independent of sex, dose, and pre-treatment. At the low dose level (0.5 mg/kg bw) almost complete degradation took place, whereas at the high dose level (100 mg/kg bw) about 31 – 47% were excreted unchanged. This reflected the different extent of absorption at these dose levels.

The major metabolic pathways postulated were significantly influenced by the sex of the animals but not by the dose level and pre-treatment within the limits of this study. The sex dependent excretion pattern and the sex-related differences in tissue residues observed in earlier toxicokinetic studies indicated quantitative and/or qualitative differences in the metabolism of trifloxystrobin in male and female rats. The gender based difference in elimination routes was considered to be a result of unique metabolism in one sex, and not related to differences in relative abundance and preferred route of elimination of common metabolites.

[Study 4] In vitro metabolism in rats and humans (Solà, 2015, M-473161-02-1)

In a 2015 study (Sola) the comparative metabolism of [trifluoromethyl-phenyl-UL-^{14}C] trifloxystrobin was investigated in animal *in vitro* systems by incubating the test substance with liver microsomes from male Wistar rats and humans of both genders in the presence of NADPH cofactor. Deviations from the study protocol include a lower a buffer concentration of 10 mM rather than 100 mM. The identity of the metabolites has not been stated and only codes reported.



* denotes the ^{14}C label, 99% purity by radio-HPLC

The test substance concentration was 15 µM and the protein concentration 1 mg/mL. The temperature was 37°C and the incubation period 1 hour. This test duration was considered as reasonable because the enzymatic reaction were already obtained with the reference substance testosterone after 10 minutes, i.e. a hydroxylation of testosterone to hydroxy-testosterone. This reaction is a well-known CYP3A microsomal activity which is shared between rats and humans. Sampling of the test system was conducted at beginning and end of the incubation. Samples were radio-assayed and analysed by reversed phase radio-HPLC following protein precipitation with acetonitrile and centrifugation of the proteins. Each incubation test was conducted in triplicate. Radioactivity measurements (radioassaying) were conducted by liquid scintillation counting (LSC). LS counting was stopped after 10 minutes counting time, at maximum. The radioactive recovery of the incubates was determined by comparison of the radioactivity in the supernatants after centrifugation to the whole incubates before centrifugation.

The radioactive components after microsome incubation of ^{14}C-trifloxystrobin were separated by radio-HPLC. The LOQ (limit of quantification) was set on the basis of the following criteria: the coefficient of variation (cv) of the mean peak area <20% and threshold for the remaining linearity levels <15%. Thus, the LOQ was set to 507 dpm (corresponding to 0.002 µg or 5.6 x 10^{-6} µMol ^{14}C-trifloxystrobin). Radiopeaks below LOQ were not considered for calculation of the composition of the metabolites. Radio-HPLC analysed the testosterone incubates.

The 6β-hydroxytestosterone was identified by LC-MS/MS using electrospray ionisation in the positive mode. A set of 6-β-hydroxytestosterone calibration solutions was prepared from the methanolic stock solution by dilution with additional methanol.

The recovery of radioactivity was measured in the microsome incubations at the end of incubation amounting to 91.0 and 95.7% of the applied radioactivity in the systems with rat and human liver microsomes. The metabolic activity of the microsomes was demonstrated by formation of 6-β-hydroxy-testosterone from testosterone by testosterone 6-β-hydroxylase (positive control) despite testosterone 6-β-hydroxylase activity in liver microsomes from both species being lower than usually obtained. The reason is attributed to two factors:

1. The testosterone concentration used in the present study (100 µM) was close to the Km value for testosterone 6-β-hydroxylase activity in HLM and in male RLM.
2. The 10-fold lower molarity of the incubation buffer was not optimal for this CYP3A-catalysed reaction.

For rat liver microsomes, the radio-chromatogram showed 5 very small peaks (Figure 5.1.1-1) at T=0. Two of them exceeded the LOQ and amounted to 1.0% (T19) and 0.4% (T20) of the peak sum (= applied amount of ^{14}C-trifloxystrobin), respectively. They were assessed as impurities of the test substance (see Table 6.14.a1). However, after 1-hour incubation, trifloxystrobin was completely metabolized resulting in 18 radiolabeled metabolites (Figure 5.1.1-1 and Table 6.14.a1). Four metabolites amounted for more than 5% of the applied amount (T05, T08, T09, T11). Four metabolites were tentatively detected below the LOQ (T01, T07, T13, T16) and the remaining 10 metabolites were observed in the range between 0.7 and 4.5% of the applied amount.

At the beginning of the incubation with human liver microsomes the respective radio-chromatogram revealed four very small peaks below the LOQ and one (T19) amounting to 0.5% of the applied amount (Figure 5.1.1-2). These compounds are assessed as impurities of the test substance (Table 6.14.a1). After 1-hour incubation in HLM trifloxystrobin was also completely metabolized resulting in 7 radiolabeled metabolites (T05, T06, T08, T09, T10, T11 and T17) (Table 6.14.a1 and Figure 5.1.1-2). Two of these metabolites accounted for more than 20% each of the applied amount. These were T09 (24.4%) and T11 (73.3%). Four metabolites were below the LOQ (T05, T08, T10 and T17). The remaining metabolite T06 accounted for 2.3% of the applied amount.

Table 6.14.a1: Metabolites due to incubation of ^{14}C-Trifloxystrobin in rat and human liver microsomes

(% of sum of radiopeaks; mean of each 3 replicates; peaks < LOQ are denoted as 0)

Metabolite	Retention (min)	Rat, 0 hour	Rat, 1 hour	Human, 0 hour	Human, 1 hour
T01	4.1	0	0	0	0
T01	5.9	0	3.3	0	0
T03	7.8	0	1.8	0	0
T04	10.6	0	1.4	0	0
T05	10.8	0	5.3	0	0
T06	12.2	0	1.7	0	2.3
T07	12.4	0	0	0	0
T08	12.8	0	17.8	0	0
T09	13.0	0	14.2	0	24.4
T10	13.5	0	0.7	0	0
T11	14.3	0	41.5	0	73.3
T12	15.7	0	4.5	0	0
T13	16.5	0	0	0	0
T14	16.7	0	3.8	0	0

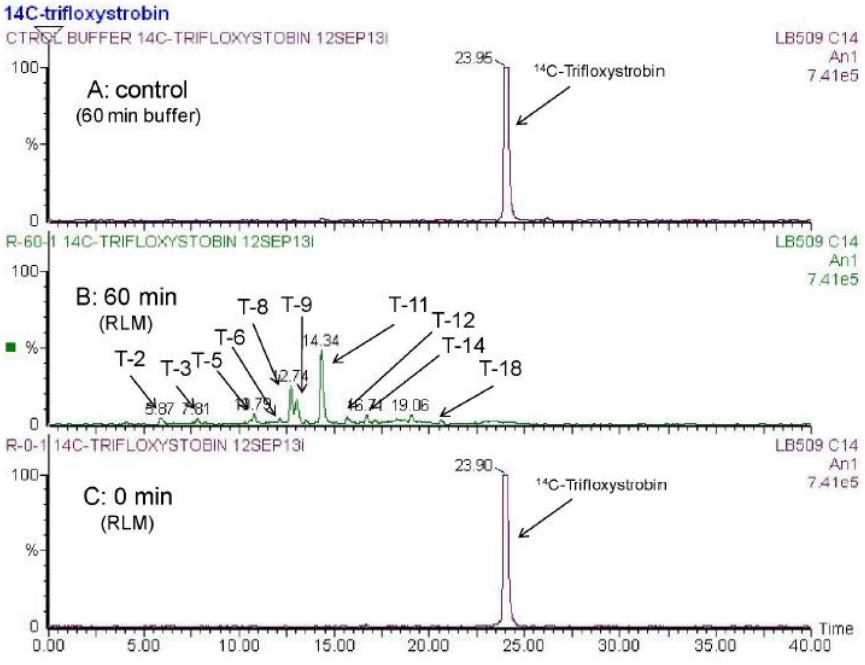
Metabolite	Retention (min)	Rat, 0 hour	Rat, 1 hour	Human, 0 hour	Human, 1 hour
T15	17.2	0	0.8	0	0
T16	17.5	0	0	0	0
T17	19.1	0	2.5	0	0
T18	20.7	0	0.8	0	0
T19	23.8	1.0	0	0.5	0
Trifloxystrobin	24.0	98.6	0	99.5	0
T20	24.5	0.4	0	0	0
	Sum*	100	99.4	100	97.7

* If small peaks below the LOQ are also considered in the peak sum it is 100%.

At first view, the *in vitro* incubation of ^{14}C-trifloxystrobin in rat and human liver microsomes revealed different chromatographic metabolite profiles. However, this observation is only due to the higher number of metabolites formed by rat microsomes than human. All metabolites formed by humans were also formed by rats, including the two largest human metabolites (T09 and T11). The human liver microsomes do not generate any metabolite that is not formed by rat liver microsomes.

Figure 5.1.1-1: Radio-HPLC profile of ^{14}C-Trifloxystrobin incubated in rat liver microsomes

Above: buffer control
Middle: after 1 hour incubation
Below: after 0 hour incubation



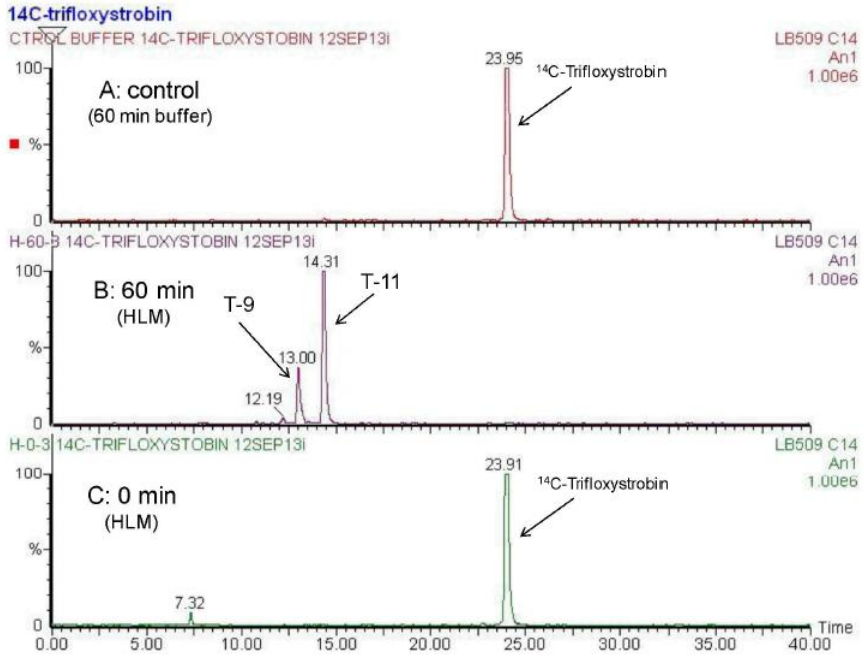
RLM: Rat liver microsomes

Figure 5.1.1-2: Radio-HPLC profile of ^{14}C-Trifloxystrobin incubated in human liver microsomes

Above: buffer control

Middle: after 1 hour incubation

Below: after 0 hour incubation



HLM: Human liver microsomes

After administration of [Glyoxyl-Phenyl-U-^{14}C] trifloxystrobin maximal blood residues were found at 12 to 24 hours. Irrespective of the dose level and the sex of the animals, the residues in all tissues depleted with half-life times of 14 – 40 hours, except blood and spleen of female rats dosed with the high dose (82 and 68 hours respectively).

Seven days after administration of [Glyoxyl-Phenyl-U-^{14}C] trifloxystrobin at the low dose (0.5 mg/kg), the tissue residues were very low (total residues <0.5% of the administered dose). At the high dose residues were about 126 and 108-fold higher than the low dose level, in males and females respectively. Some sex and label-specific differences in tissue residues were observed at the high dose level. Generally tissue residues were higher in females than males. Label-specific differences were noted in the fat, kidneys, liver and plasma.

Within 48 hours, 72 – 96% of the dose was eliminated with the urine and faeces independent of the dose level, pretreatment with non-radiolabeled trifloxystrobin, the site of label, and the sex of the animals. However, the routes of elimination were different in male and female rats. Within seven days, male rats eliminated approximately 15% of the dose via kidneys while females excreted 33% in the urine. Bile-duct cannulated rats demonstrate that the bile (ca. 44% of the dose) is the principal route of elimination in both males and females. There was evidence of the involvement of enterohepatic circulation in the excretion process.

The half-life times of the tissue residues after oral administration trifloxystrobin at a low and high dose, were very similar with either of the two labels demonstrating that the small amount of label-specific metabolites formed do not influence the overall depletion kinetics.

Trifloxystrobin was extensively metabolized at the low dose level (0.5 mg/kg) ca. 5% unchanged parent in faeces, whereas at the high dose level (100 mg/kg bw) 31-47% unchanged parent was found in faeces. The amount of parent found in faeces at the low dose does not correlate well with levels of absorption seen at this dose. This may be an artefact of the relatively low recoveries of radiolabel from the faeces at the low dose.

In most experimental groups the metabolites identified accounted for between ca. 60 – 70% of the administered dose. The extent of characterisation is considered acceptable given the extensive metabolism of trifloxystrobin.

The reactions involved in the major metabolic pathways of trifloxystrobin were (1) hydrolysis of the methyl ester to the corresponding acid, (2) demethylation of the methoxyimino group yielding a hydroxyimino compound, (3) oxidation of the methyl side chain to a primary alcohol, followed by partial oxidation to the respective carboxylic acid, and (4) cleavage between the glyoxyl-phenyl and trifluoromethyl-phenyl moiety.

The major metabolic pathways of trifloxystrobin operative in the rat were not significantly influenced by the dose and pretreatment but were by the sex of the animals resulting in some female specific urinary metabolites. The comparative *in vitro* metabolism study with rat and human microsomes showed that no unique metabolites were produced by human microsomes exposed to trifloxystrobin. The two metabolites produced by the human microsomes (T9 24.4% and T11 73.3%) were also produced by the rat in high quantities (T9 14.2% and T11 41.5%).

The sex dependent excretion pattern and the sex-related differences in tissue residues indicated quantitative and/or qualitative differences in the metabolism of trifloxystrobin in male and female rats. This gender based difference in elimination route was considered to be a result of unique metabolism in females, and not related to differences in relative abundance and preferred route of elimination of common metabolites.

Based on the structures of the urinary, faecal, and biliary metabolites identified by spectroscopic means ('H-NMR and MS) or characterized the metabolic pathways operative in the rat were proposed as summarized in Figure 5.1.

Figure 5.1: Proposed metabolism of Trifloxystrobin in rat, goat and hen

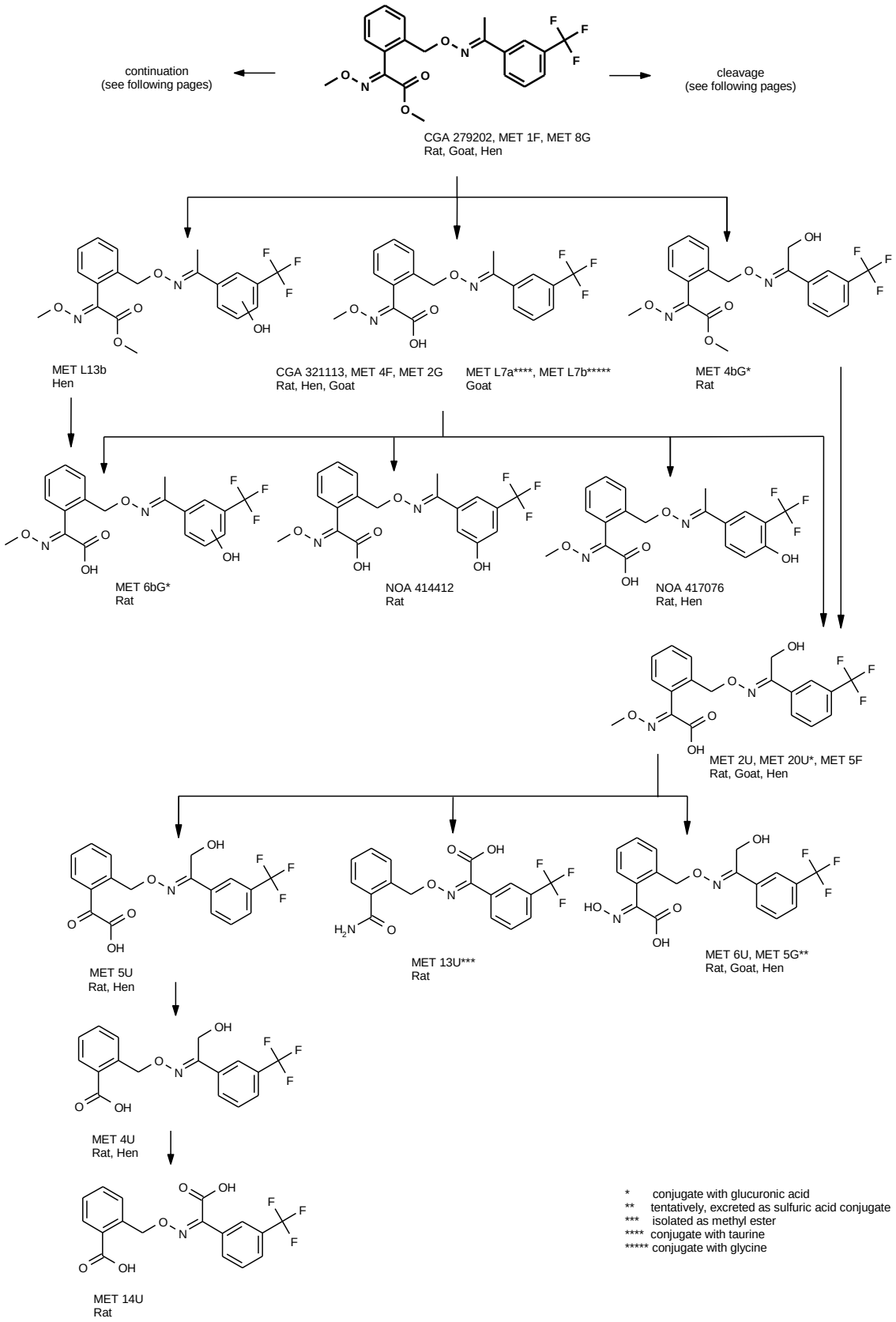


Figure 5.1: continued

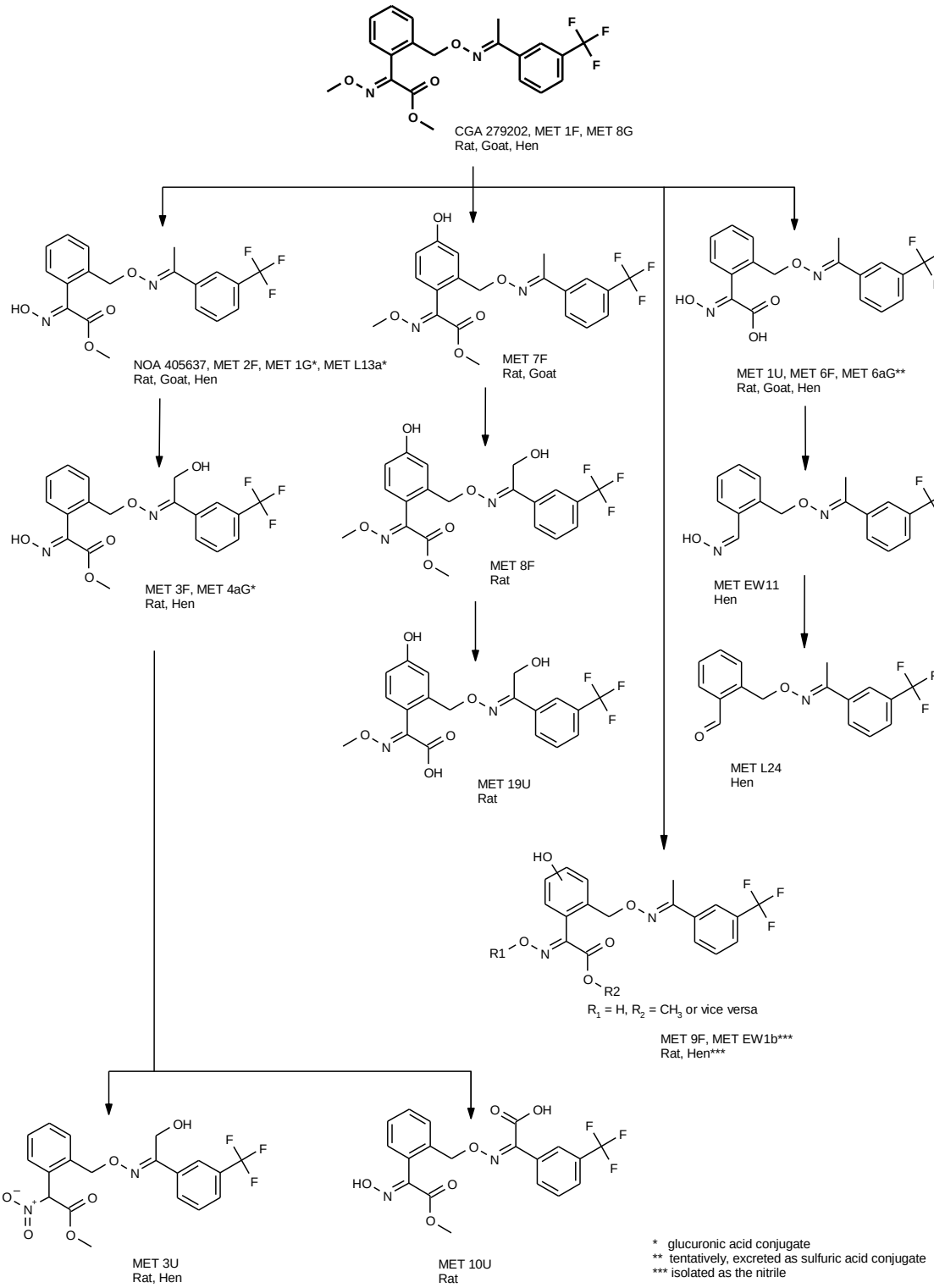
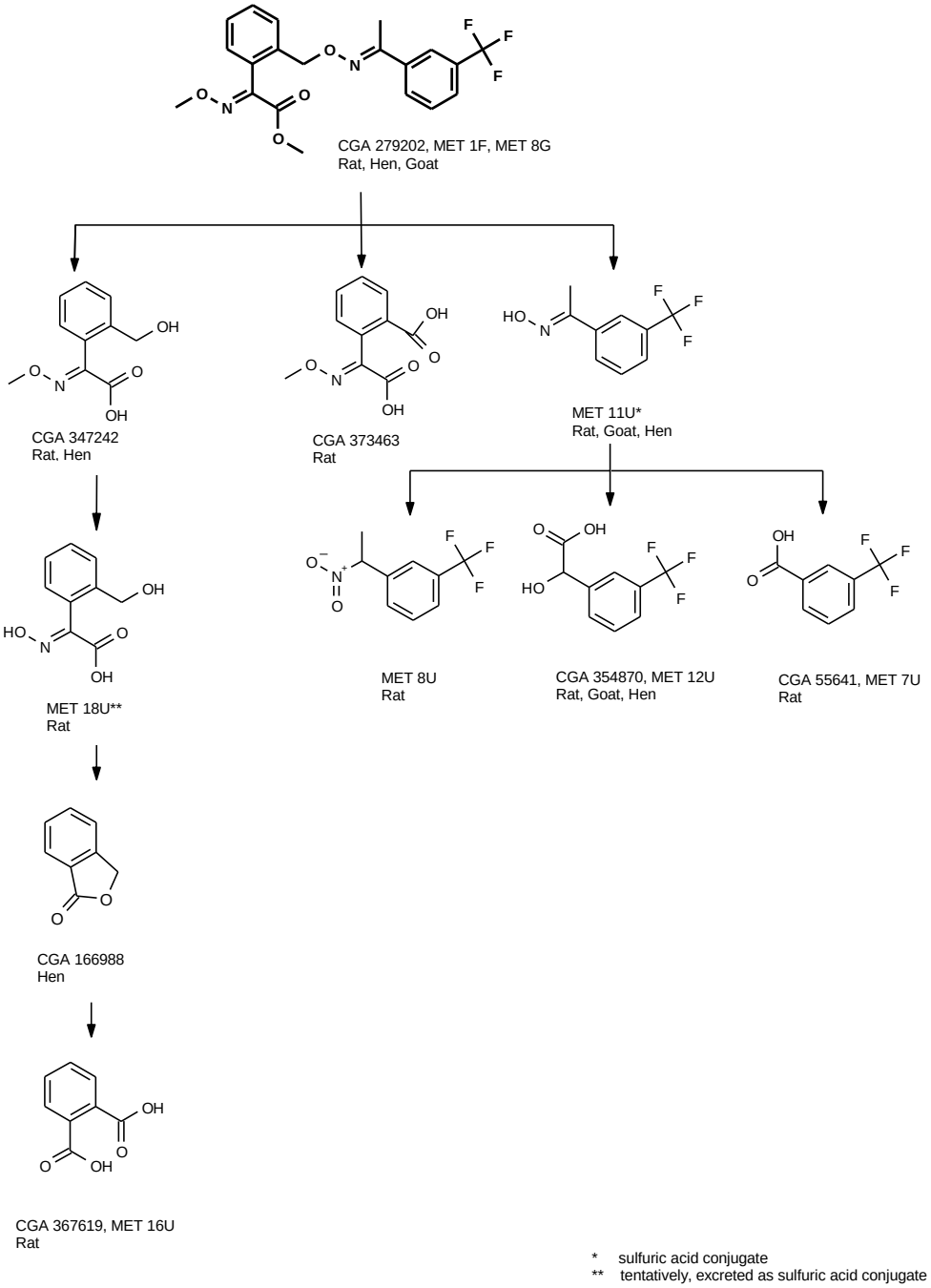


Figure 5.1: continued (cleavage)



2.1.2 Human data

No data are available.

2.1.3 Other data

No data are available.

3 HEALTH HAZARDS

3.1 Acute toxicity – oral route

Not relevant for this proposal.

3.2 Acute toxicity - dermal route

Not relevant for this proposal.

3.3 Acute toxicity - inhalation route

Not relevant for this proposal.

3.4 Skin corrosion/irritation

Not relevant for this proposal.

3.5 Serious eye damage/eye irritation

Not relevant for this proposal.

3.6 Respiratory sensitisation

Not relevant for this proposal.

3.7 Skin sensitisation

Not relevant for this proposal.

3.8 Germ cell mutagenicity

Not relevant for this proposal.

3.9 Carcinogenicity

Not relevant for this proposal.

3.10 Reproductive toxicity

3.10.1 Animal data

3.10.1.1 Adverse effects of sexual function and fertility

[Study 1a] Range-finding study for the multigeneration study in rats (Anonymous, 1995))

Non GLP, a dose-range-finding study is not intended to comply with OECD guidelines

In this range-finding study Sprague Dawley derived rats (15/sex/dose) were fed diets containing trifloxystrobin (96.4 %) at levels of 0, 100, 1000 or 2000 ppm. An overview of the mean doses administered is shown below.

TEST SUBSTANCE MEAN DAILY INTAKE (mg trifloxystrobin/kg bw/day)				
Dose ppm	**Males**	**Females**		
	premating	**premating**	**gestation**	**lactation**
100	6.0	7.9	8.7	16.5
1000	53.5	67.0	81.8	168.6
2000	109.6	140.5	162.0	321.7

The treatment started 2 weeks before mating and continued throughout gestation and lactation until 14 days post-partum. After 2 weeks premating dietary exposure to the test substance, animals were paired 1:1 within each dose group (15 animals per sex and dose) until there was evidence of positive mating or for 14 days, whichever occurred first.
Mortality, clinical signs, body weights, food consumption, mating, gestation and delivery parameters, pup survival and development were recorded. A gross necropsy examination of the main organs of the thoracic and abdominal cavities and in particular the reproductive organs was performed on all animals. For the adult females the number of corpora lutea in each ovary, and the number of implantation sites were recorded.

There were no parental mortalities and no clinical signs related to the treatment.

Body weight gain and food consumption was dose dependently reduced at 1000 and 2000 ppm in both sex.

Food consumption

		Trifloxystrobin (ppm)						
		0	**100**	***(%)[a]***	**1000**	***(%)[a]***	**2000**	***(%)[a]***
Males								
Day 1-8	Premating (g/animal/d)	23.0	24.0	*(+4)*	19.7**	*(-14)*	19.5**	*(-15)*
Day 8-12		26.4	27.4	*(+4)*	25.7	*(-3)*	26.4	*(±0)*
Females								
Day 1-8	Pre-mating (g/animal/d)	14.8	17.1**	*(+16)*	13.0*	*(-12)*	13.0	*(-12)*
Day 8-12		18.5	19.1	*(+3)*	17.7	*(-4)*	18.4	*(-1)*
GD 0-7	Gestation (g)	22.7	23.1	*(+2)*	21.9	*(-4)*	22.1	*(-3)*
GD 7-14		23.9	25.0	*(+5)*	22.9	*(-4)*	22.1**	*(-8)*
GD 14-21		22	24.5**	*(+11)*	21.1	*(-4)*	21.1	*(-4)*
LD 0-7	Lactation (g)	35.2	36.1	*(+3)*	35.1	*(±0)*	34.1	*(-3)*
LD 7-14		62.7	64.1	*(+2)*	60.6	*(-3)*	55.3	*(-12)*

[a] *% difference to control,* GD Gestation day, LD Lactation day

*/** Statistically significant difference from control, $p \leq 0.05$/ $p \leq 0.01$

Body weight (g) and Body weight gain (g)

	Study Phase	**Trifloxystrobin (ppm)**						
		0	**100**	***(%)[a]***	**1000**	***(%)[a]***	**2000**	***(%)[a]***
Males								
Day 1	Pre-mating and mating	418.7	418.7	*(±0)*	418.3	*(±0)*	416.3	*(-1)*
Day 8		443.4	440.9	*(-1)*	431.1	*(-3)*	423.5*	*(-4)*

	Study Phase	Trifloxystrobin (ppm)					
		0	**100**	*(%)[a]*	**1000**	*(%)[a]*	**2000**	*(%)[a]*
Day 12		451.1	447.7	*(-1)*	438.4	*(-3)*	427.0**	*(-5)*
Day 15		446.0	440.9	*(-1)*	429.2	*(-4)*	419.1**	*(-6)*
Day 22		463.7	456.5	*(-2)*	445.7	*(-4)*	439.3**	*(-5)*
Day 29		483.0	475.3	*(-2)*	461.2	*(-5)*	452.1**	*(-6)*
Day 1 – 29		64.2	56,6	*(-13)*	42,9**	*(-33)*	35,9**	*(-44)*
Females								
Day 1	Pre-mating	222.3	222.5	*(±0)*	223.1	*(±0)*	221.0	*(-1)*
Day 8		233.5	236.7	*(+1)*	232.2	*(-1)*	228.3	*(-2)*
Day 12		240.5	241.4	*(±0)*	237.6	*(-1)*	230.7	*(-4)*
Day 15		247.6	246.2	*(-1)*	241.2	*(-3)*	232.5*	*(-6)*
Day 1 – 15		25,3	23,7	*(-6)*	18,2	*(-28)*	11,6**	*(-54)*
GD 0	Gestation	245.7	248.2	*(+1)*	239.9	*(-2)*	229.1**	(-7)
GD 7		276.4	281.9	*(+2)*	272.6	*(-1)*	263.6	(-5)
GD 14		308.8	313.7	*(+2)*	301.6	*(-2)*	292.4*	(-5)
GD 21		397.0	405.3	*(+2)*	383.6	*(-3)*	367.0*	(-8)
GD 0 – 21		151,2	157,2	*(+4)*	143,8	*(-5)*	137,9	(-9)
LD 0	Lactation	289.0	296.6	*(+3)*	281.8	*(-2)*	265.0**	*(-8)*
LD 7		306.7	311.1	*(+1)*	301.7	*(-2)*	285.4**	*(-7)*
LD 14		328.5	334.2	*(+2)*	323.6	*(-1)*	308.0*	*(-6)*
LD 0 – 14		39,5	37,5	*(-5)*	41,7	*(+6)*	43,0	*(+9)*

[a] *% difference to control,* GD Gestation day, LD Lactation day

*/** Statistically significant difference from control, $p \leq 0.05$/ $p \leq 0.01$

There was no compound-related effect on the mating index or fertility index. No treatment-related effect was observed for the gestation length or the number of implantation sites. Gestation and parturition indices were not affected by treatment.

Reproductive data

Parameter	Trifloxystrobin (ppm)			
	0	**100**	**1000**	**2000**
No. pregnant / no. mated	15 / 15	14 / 15	15 / 15	15 / 15
No. of females killed or found dead	0	0	0	0
No. dams with liveborn pups	15	14	15	15
No. dams with stillborn pups	0	2	1	1
Mean no. corpora lutea/dam	18.4	18.9	17.5	18.1
Mean no. implantation sites/dam	15.0	15.2	14.4	14.2
Mating index (%)	100	100	100	100
Fertility index (%)	100	93.3	100	100
Gestation index (%)	100	100	100	100
Parturition Index (%)	100	100	100	100

Parameter	Trifloxystrobin (ppm)			
	0	100	1000	2000
Mean duration of gestation (days)	22.1	22.0	22.1	22.1

The live birth index was not affected by the treatment. A total of 13, 10, 4 and 10 pups died or were missing in the control to high dose group, respectively. Viability and lactation indices were thus not affected by treatment. Several of the pups which died had not suckled, five had subcutaneous haemorrhage, two had wound and crust/scurf, all pups from one control group litter had poor hair growth. One low dose group pup had spina bifida. These findings were not considered treatment-related. Litter weights and litter weight gain were reduced in the high dose group of 2000 ppm in both sexes from day 7 post-partum. There were no treatment-related necropsy observations in offspring.

Litter data

Parameter	Trifloxystrobin (ppm)							
	0	100		1000		2000	
No. of pups delivered	213	198		201		198	
Mean litter size	14.2	14.1		13.4		13.2	
No. of pups delivered	213	198		201		198	
No. of stillborn pups	0	2		1		1	
No. of liveborn pups	213	196		200		197	
Live birth index (%)	100	99.0		99.5		99.5	
No. of pups died / sacrificed moribund	5	5		2		6	
No. of pups missing	8	5		2		4	
Pups surviving day 0 to 4	208	190		199		194	
Viability index (%)	97.7	96.9		99.5		98.5	
Pups surviving day 4 to 14	200	186		196		187	
Lactation index (%)	96.2	97.9		98.5		96.4	
Mean pup weight (g)							
Day 0	5.9	5.8	*(-2)* [a]	6.0	*(+2)* [a]	5.8	*(-2)* [a]
Day 4	8.4	8.6	*(+2)* [a]	9.0	*(+7)* [a]	8.0	*(-5)* [a]
Day 7	12.3	12.6	*(+2)* [a]	12.6	*(+2)* [a]	10.8*	*(-12)* [a]
Day 14	24.0	24.2	*(+1)* [a]	23.4	*(-2)* [a]	20.4**	*(-15)* [a]
Day 0-14	18.1	18.4	*(+2)* [a]	17.5	*(-3)* [a]	14.7**	*(-19)* [a]
Sex ratio (% males)							
Day 0	42.3	55.1		49.5		42.1	
Day 14	41.5	54.8		50.5		42.2	

[a] *% difference to control*
*/** Statistically significant difference from control, $p \leq 0.05$/ $p \leq 0.01$

Based on these findings an appropriate high dose level at 1500 ppm for a two-generation study with trifloxystrobin was recommended.

[Study 1b] Multigeneration study in rats (Anonymous, 2001, M-039264-02-1)

GLP, quality assured and OECD 416 (1983) compliant, EU Guideline (Annex V Two-generation reproduction study 30/5/1988))

In this study two successive generations (F0 and Fl) of Sprague Dawley derived rats (30/sex/dose) were fed diets containing trifloxystrobin (purity 96.4%) at levels of 0, 50, 750 and 1500 ppm. Summary of overall mean doses received is shown below:

TEST SUBSTANCE MEAN DAILY INTAKE (mg trifloxystrobin/kg bw/day)							
Dose ppm	**F0**						
	Males		**Females**				
	1st pre- and post mating	**2nd**	**Premating**	**1st mating**		**2nd mating**	
				gestation	**lactation**	**gestation**	**lactation**
50	3.28	2.28	4.05	3.50	7.60	3.13	7.17
750	48.06	32.85	58.04	53.47	112.77	47.93	107.33
1500	97.32	73.13	123.14	111.80	219.77	98.03	220.03

Dose ppm	**F1**			
	Males	**Females**		
	Pre- and post mating	**premating**	**gestation**	**lactation**
50	3.78	4.36	3.43	8.00
750	58.44	66.99	54.37	119.87
1500	126.66	146.03	114.40	242.00

After 10 weeks premating dietary exposure to the test substance, animals were paired 1:1 within each dose group (30 animals per sex and dose) until there was evidence of positive mating or for 19 days, whichever occurred first. Litters were culled to 4 male and 4 female pups, where possible, on day 4 post partum. After weaning of the F1 pups, the F0 parent animals were remated to produce second litters. The F1 generation was selected from the first litters (i.e. first mating) of the F0 generation.

Clinical signs, bodyweights, food consumption, mating, gestation and delivery parameters, pup survival and development were recorded. A gross necropsy examination was performed on all pups not selected for mating. Parent animals were necropsied after weaning of the second (F0 parents) or first (F1 parents) litters and subjected to macroscopic examination, with histopathological investigation (in all control and high dose animals) of vagina, uterus, ovaries, testes, epididymides, seminal vesicles, prostate, pituitary gland, liver, pancreas and all gross lesions.

F0 Generation

There were no treatment-related clinical signs or treatment-related deaths among the F0 parent animals.

At 1500 ppm, throughout the F0 generation for males and during the premating period for females, bodyweights and overall bodyweight gain were significantly lower than controls. At 750 ppm, for the males, bodyweights were slightly lower than controls throughout the premating period, however the differences from the control value for both bodyweights and bodyweight gain were minimal. Overall weight gain for the females at 750 ppm was significantly lower than controls.

During the two gestation periods, overall weight gain of the females was lower than controls at ≥750 ppm, differences from the control value attaining statistical significance at 1500 ppm at the second mating only.

During the two lactation periods, overall weight gain as a percentage of controls in of the females at 750 and 1500 ppm was increased. Bodyweights during the two lactation periods were reduced at ≥750 ppm, differences from control value attained statistical significance on all occasions at 1500 ppm only.

At 50 ppm, for both sexes, bodyweights and weight gain were similar to that of the control group throughout the F0 generation.

Table B.6.34a: Body weights (g) of F0 generation

Parameter	**Generation**	**Dose (ppm)**					
		0	**50**	*(%)[a]*	**750**	*(%)[a]*	**1500**	*(%)[a]*
Males								
Day 0	F0 - 1st pre- and postmating	185.6	185.6	*(100%)*	186.1	*(100%)*	185.5	*(100%)*
Day 8		240.3	238.6	*(99%)*	240.5	*(100%)*	234.0	*(97%)*
Day 15		289.1	288.2	*(100%)*	287.5	*(99%)*	279.9	*(97%)*
Day 22		325.8	326.7	*(100%)*	324.9	*(100%)*	313.2	*(96%)*
Day 29		353.6	355.1	*(100%)*	350.4	*(99%)*	338.8	*(96%)*
Day 36		381.2	384	*(101%)*	375.7	*(99%)*	**362.3***	***(95%)***
Day 43		402.9	406	*(101%)*	395.2	*(98%)*	**378.5****	***(94%)***
Day 50		421.6	424.6	*(101%)*	413.4	*(98%)*	**393.1****	***(93%)***
Day 57		436.4	437.1	*(100%)*	425.1	*(97%)*	**406.4****	***(93%)***
Day 64		448.2	450.2	*(100%)*	437.1	*(98%)*	**415.3****	***(93%)***
Day 68		453.6	455.1	*(100%)*	442.7	*(98%)*	**418.5****	***(92%)***
Day 71		450.4	453	*(101%)*	440.8	*(98%)*	**412.1****	***(91%)***
Day 78		454.6	456.1	*(101%)*	448.1	*(99%)*	**417.9****	***(93%)***
Day 85		464.4	466.7	*(103%)*	458.2	*(101%)*	**424.1****	***(93%)***
Day 92		476.2	478.7	*(103%)*	469.7	*(101%)*	**433.8****	***(93%)***
Day 99		483	485.7	*(102%)*	474.4	*(100%)*	**439.2****	***(92%)***

Parameter	**Generation**	**Dose (ppm)**					
		0	**50**	*(%)[a]*	**750**	*(%)[a]*	**1500** *(%)[a]*
Day 106		492	494.5	*(102%)*	485.1	*(100%)*	**447.4**** ***(93%)***
Day 113		502.6	504.8	*(100%)*	492.7	*(98%)*	**454.9**** ***(91%)***
Day 120		509.3	511.9	*(101%)*	498.3	*(98%)*	**461.6**** ***(91%)***
Day 127		514.7	517.1	*(100%)*	503.6	*(98%)*	**464.7**** ***(90%)***
Day 134		517.5	521.3	*(101%)*	507.3	*(98%)*	**467.3**** ***(90%)***
Day 141		521.9	524.7	*(101%)*	508.5	*(97%)*	**465.5**** ***(89%)***
Day 148	F0 – 2nd post-mating	521.7	524.3	*(100%)*	511.7	*(98%)*	**469**** ***(90%)***
Day 155		525.2	530.8	*(101%)*	515.8	*(98%)*	**473.1**** ***(90%)***
Day 162		533.6	536.2	*(100%)*	522.5	*(98%)*	**478.6**** ***(90%)***
Day 169		538.7	541.9	*(101%)*	527.4	*(98%)*	**479.5**** ***(89%)***
Day 176		543.9	549.4	*(101%)*	533.7	*(98%)*	**485.8**** ***(89%)***
Day 183		554.9	557.2	*(100%)*	540.3	*(97%)*	**490.7**** ***(88%)***
Day 190		544.4	561.6	*(103%)*	545.9	*(100%)*	**497.6*** ***(91%)***
Females							
Day 0	F0 - 1st pre-mating	147.1	146.7	*(100%)*	147.2	*(100%)*	146.4 *(100%)*
Day 8		173.9	173	*(99%)*	171.1	*(98%)*	170.2 *(98%)*
Day 15		197.9	196.9	*(99%)*	193.0	*(98%)*	191.0 *(97%)*
Day 22		215.0	213.9	*(99%)*	209.0	*(97%)*	**205.3*** ***(95%)***
Day 29		229.6	228.2	*(99%)*	220.9	*(96%)*	**215.3**** ***(94%)***
Day 36		240.3	239.8	*(100%)*	231.3	*(96%)*	**226.2**** ***(94%)***
Day 43		249.3	248.9	*(100%)*	241.0	*(97%)*	**234.6**** ***(94%)***
Day 50		257.3	257.1	*(100%)*	248.2	*(96%)*	**240.8**** ***(94%)***
Day 57		263.5	262.9	*(100%)*	252.0	*(96%)*	**245.5**** ***(93%)***
Day 64		269.9	269.2	*(100%)*	257.3	*(95%)*	**250.2**** ***(93%)***
Day 68		271.9	269.6	*(99%)*	**256.4*** ***(94%)***		**249.4**** ***(92%)***
Day 71		274.3	273.7	*(100%)*	**258.8*** ***(94%)***		**249.0**** ***(91%)***
Gestation day 1	F0 - 1st gestation	273.7	270.8	*(99%)*	**258.2*** ***(94%)***		**247.5**** ***(90%)***
Gestation day 7		292.7	290.2	*(99%)*	**277.7*** ***(95%)***		**269.9**** ***(92%)***

Parameter	Generation	Dose (ppm)					
		0	**50**	*(%)[a]*	**750**	*(%)[a]*	**1500**	*(%)[a]*
Gestation day 14		321.7	318.6	*(99%)*	**305.6***	***(95%)***	**295.0****	***(92%)***
Gestation day 21		408.7	400.7	*(98%)*	**381.8***	***(93%)***	**371.5****	***(91%)***
Lactation day 0	F0 - 1st lactation	302.8	302.2	*(100%)*	291.6	*(96%)*	**277.5****	***(92%)***
Lactation day 7		311.5	310.6	*(100%)*	301.7	*(97%)*	**286.4****	***(92%)***
Lactation day 14		326.2	322.5	*(99%)*	312.4	*(96%)*	**297.9****	***(91%)***
Lactation day 21		316.7	315.7	*(100%)*	310.7	*(98%)*	**294.0****	***(93%)***
Gestation day 1	F0 - 2nd gestation	311.2	303.5	*(98%)*	**286.8****	***(92%)***	**273.1****	***(88%)***
Gestation day 7		329.9	319.6	*(97%)*	**306.1****	***(93%)***	**293.3****	***(89%)***
Gestation day 14		357	348.7	*(98%)*	**330.4****	***(93%)***	**316.5****	***(89%)***
Gestation day 21		451.3	438.6	*(97%)*	**413.4****	***(92%)***	**397.2****	***(88%)***
Lactation day 0	F0 - 2nd lactation	346	340.9	*(99%)*	**322.2****	***(93%)***	**302.1****	***(87%)***
Lactation day 7		347.1	345.4	*(100%)*	334.7	*(96%)*	**314.0****	***(90%)***
Lactation day 14		356.6	352.6	*(99%)*	342.1	*(96%)*	**324.8****	***(91%)***
Lactation day 21		353.4	347.5	*(98%)*	343.7	*(97%)*	**328.4****	***(93%)***

[a] % of control
* statistically significant difference from control p<0.05
** statistically significant difference from control p<0.01
Findings considered related to treatment with trifloxystrobin are written in **bold letters**

Table B.6.34b: Body weight gain (g) of F0 generation

Parameter	Generation	Dose (ppm)					
		0	**50**	*(%)[a]*	**750**	*(%)[a]*	**1500**	*(%)[a]*
Males								
Day 1 – 71	F0 - 1st pre- and post-mating	264.8	267.4	*(101%)*	254.7	*(96%)*	226.6	***(86%)***
Day 1 – 99		297.4	300.1	*(101%)*	288.3	*(97%)*	253.7	***(85%)***
Day 1 – 134		331.9	335.7	*(101%)*	321.2	*(97%)*	**281.8****	***(85%)***
Day 141 – 183	F0 -2nd post-mating	33.0	32.5	*(98%)*	31.8	*(96%)*	**25.2***	***(76%)***
Females								
Day 1 – 68	F0 - 1st pre-	124.8	122.9	*(98%)*	**109.3****	***(88%)***	**103.0****	***(83%)***

Parameter	**Generation**	**Dose (ppm)**						
		0	**50**	*(%)[a]*	**750**	*(%)[a]*	**1500**	*(%)[a]*
	mating							
GD 0 – 7	F0 - 1st gestation	19	19.4	*(102%)*	19.5	*(103%*	22.4	*(118%)*
GD 7 - 14		29	28.4	*(98%)*	27.9	*(96%)*	**25.0***	***(86%)***
GD 14 – 21		87	82.1	*(94%)*	**76.2***	***(88%)***	**76.6****	***(88%)***
GD 0 – 21		135	129.9	*(96%)*	123.6	*(92%)*	124	*(92%)*
LD 0 – 7	F0 - 1st lactation	8.7	7	*(80%)*	10.2	*(117%)*	8.9	*(102%)*
LD 7 - 14		14.7	11.9	*(81%)*	10.7	***(73%)***	11.5	***(78%)***
LD 14 – 21		-9.4	-6.9	- [x]	-1.7	- [x]	-3.8	- [x]
LD 0 – 21		14	12	*(86%)*	19.2	*(137%)*	16.5	*(118%)*
GD 0 – 7	F0 – 2nd gestation	18.7	16.1	*(86%)*	19.3	*(103%)*	20.2	*(108%)*
GD 7 - 14		27.1	29	*(107%)*	24.3	*(90%)*	**23.2****	***(86%)***
GD 14 – 21		94.2	90.1	*(96%)*	83	*(88%)*	**80.8***	***(86%)***
GD 0 – 21		140.1	133.9	*(96%)*	126.6	*(90%)*	**124.1****	***(89%)***
LD 0 – 7	F0 – 2nd lactation	1.2	4.5	*(375%)*	12.5**	*(1042%)*	11.9**	*(992%)*
LD 7 - 14		9.5	7.1	*(75%)*	7.4	*(78%)*	10.9	*(115%)*
LD 14 – 21		-3.3	-5.1	*(155%)*	1.6	- [x]	3.6*	- [x]
LD 0 – 21		7.4	6.6	*(89%)*	21.5**	*(291%)*	26.3**	*(355%)*

[a] % of control

[x] calculation not reasonable due to negative value in control

GD: gestation day

* statistically significant difference from control $p<0.05$

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

Findings considered related to treatment with trifloxystrobin are written in **bold letters**

At 1500 ppm food consumption was slightly reduced from the start of the dosing period (both sexes). At 750 ppm, for males, food consumption was slightly lower than controls from the start of the dosing period. In general, differences from the control value were minimal - the only difference to attain statistical significance was during the first week of the dosing period. For females, during the premating period, food consumption was generally slightly lower than that of the control group. Differences from the control value attained statistical significance on several occasions.

For the females at ≥750 ppm, there was a reduction in food consumption during the gestation and the lactation periods (second mate) and during days 14 and 21 post partum of the first lactation period.

At 50 ppm, for both sexes, food consumption was similar to that of the control group throughout the F0 generation.

Table B.6.34c: Food consumption (g) of F0 generation

Parameter	**Generation**	**Dose (ppm)**					
		0	**50**	*(%)[a]*	**750**	*(%)[a]*	**1500**	*(%)[a]*
Males								
Day 1-8	F0 - 1st pre- and post-mating	20.5	20.8	*(101%)*	**18.7****	***(91%)***	**17.9****	***(87%)***
Day 8-15		25.2	25.7	*(102%)*	24.8	*(98%)*	24.3	*(96%)*
Day 15-22		24.4	24.5	*(100%)*	23.8	*(98%)*	23.6	*(97%)*
Day 22-29		26.2	26.5	*(101%)*	25.6	*(98%)*	25.6	*(98%)*
Day 29-36		24.8	25.3	*(102%)*	23.9	*(96%)*	**23.0****	***(93%)***
Day 36-43		26.9	27.6	*(103%)*	25.7	*(96%)*	**24.9****	***(93%)***
Day 43-50		26.2	26.2	*(100%)*	25.1	*(96%)*	**24.2****	***(92%)***
Day 50-57		24.7	24.6	*(100%)*	24.9	*(101%)*	23.9	*(98%)*
Day 57-64		24.5	24.2	*(99%)*	23.8	*(97%)*	**21.5****	***(98%)***
Day 64-68		24.7	24.5	*(99%)*	24.3	*(98%)*	**23.1***	***(94%)***
Day 92-99		23.9	24.3	*(102%)*	22.8	*(95%)*	**22.1****	***(92%)***
Day 99-106		24	24.6	*(103%)*	24	*(100%)*	23.0	*(96%)*
Day 106-113		23.7	24	*(101%)*	23.4	*(99%)*	22.6	*(95%)*
Day 113-120		23.7	24.1	*(102%)*	23.7	*(100%)*	22.9	*(97%)*
Day 120-127		23.2	23	*(99%)*	23.1	*(100%)*	22.4	*(97%)*
Day 127-134		22.9	23.1	*(101%)*	23.1	*(101%)*	22.4	*(98%)*
Day 162-169	F0 -2nd post-mating	24.6	25.4	*(103%)*	25.6	*(104%)*	23.4	*(95%)*
Day 169-176		23.9	25	*(105%)*	25.1	*(105%)*	23.6	*(99%)*
Day 176-183		25	24.5	*(98%)*	24.5	*(98%)*	23.7	*(95%)*
Day 183-190		23.2	24.6	*(106%)*	24.7	*(106%)*	23.6	*(102%)*
Females								
Day 1-8	F0 - 1st pre-mating	15.3	15.2	*(99%)*	**13.0****	***(85%)***	**13.3****	***(87%)***
Day 8-15		18.7	18.5	*(99%)*	**17.6***	***(94%)***	18.2	*(97%)*
Day 15-22		17.7	17.7	*(100%)*	**16.4****	***(93%)***	17.2	*(97%)*
Day 22-29		19.3	19.2	*(99%)*	**17.7****	***(92%)***	18.4	*(95%)*
Day 29-36		17.4	17.4	*(100%)*	**15.7****	***(90%)***	**16.5***	***(95%)***
Day 36-43		18.5	18.5	*(100%)*	**17.4****	***(94%)***	18.2	*(98%)*

Parameter	**Generation**	**Dose (ppm)**					
		0	**50**	*(%)[a]*	**750**	*(%)[a]*	**1500**	*(%)[a]*
Day 43-50		18.6	18.2	*(98%)*	**17.3****	***(93%)***	18.0	*(97%)*
Day 50-57		17.1	17.2	*(101%)*	16.9	*(101%)*	17.6	*(103%)*
Day 57-64		16.7	16.6	*(99%)*	16.0	*(101%)*	16.3	*(98%)*
Day 64-68		17.5	**16.4***	***(94%)***	**15.9****	***(91%)***	16.8	*(96%)*
Day 1-68		176.8	174.9	*(99%)*	163.9	*(93%)*	170.5	*(96%)*
GD 0 – 7	F0 - 1st gestation	18.4	18.4	*(100%)*	18.1	*(98%)*	18.5	*(101%)*
GD 7 - 14		21.1	21.0	*(100%)*	20.4	*(97%)*	20.4	*(97%)*
GD 14 – 21		22.2	22.3	*(100%)*	21.4	*(96%)*	21.5	*(97%)*
GD 0 – 21		61.7	61.7	*(100%)*	59.9	*(97%)*	60.4	*(98%)*
LD 0 – 7	F0 - 1st lactation	30.6	31.0	*(101%)*	31.2	*(102%)*	29.1	*(95%)*
LD 7 - 14		50.6	49.3	*(97%)*	48.0	*(95%)*	45.8	*(91%)*
LD 14 – 21		62.5	62.3	*(100%)*	58.1	*(93%)*	**52.9****	***(85%)***
LD 0 – 21		143.7	142.6	*(99%)*	137.3	*(96%)*	**127.8**	***(89%)***
GD 0 – 7	F0 – 2nd gestation	17.3	16.8	*(97%)*	16.3	*(94%)*	15.8	*(91%)*
GD 7 - 14		22.2	21.8	*(98%)*	21.2	*(95%)*	**21.0***	***(95%)***
GD 14 – 21		22.9	22.3	*(97%)*	21.7	*(95%)*	**21.0****	***(92%)***
GD 0 – 21		62.4	60.9	*(98%)*	59.2	*(95%)*	57.8	*(93%)*
LD 0 – 7	F0 – 2nd lactation	31.3	32.9	*(105%)*	32.9	*(105%)*	34.7	*(111%)*
LD 7 - 14		52.0	51.7	*(99%)*	49.6	*(95%)*	**47.0****	***(90%)***
LD 14 – 21		68.3	64.2	*(94%)*	**61.0***	***(89%)***	**57.6****	***(84%)***
LD 0 – 21		151.6	148.8	*(98%)*	143.5	*(95%)*	139.3	*(91%)*

[a] % of control

GD: gestation day

* statistically significant difference from control $p<0.05$

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

Findings considered related to treatment with trifloxystrobin are written in **bold letters**

1st and 2nd Mating

Male and female mating and fertility indices, maternal gestation and parturition indices and the duration of gestation were unaffected by treatment at either mating. A total of 30, 30, 29 and 27 females in the control, 50, 750 and 1500 ppm dose groups, respectively, were pregnant and gave birth to live young at the first mating. A total of 28, 26, 29 and 28 females in the control, 50, 750 and 1500 ppm dose groups, respectively, were pregnant and gave birth to live young at the second mating.

Table B.6.34d: Reproductive performance F0 (1st and 2nd mating)

Parameter		Dose (ppm)			
		0	**50**	**750**	**1500**
F0 generation – 1st mating					
Female mating index	%	100	100	96.7	96.7
Female fertility index	%	100	100	100	93.1
Duration of gestation	mean days	22.2	22.3	22.3	22.2
Gestation index	%	100	100	100	100
Parturition index	%	100	100	100	100
Litter size	mean	13.5	12.7	12.0	12.4
Number of pups	total n	406	382	349	336
Number of dead pups	n	1	11**	1	0
Sex ratio	% live males	54.3	53.6	55.0	49.7
Viability index	%	97.5	94.6	99.4	94.6
Lactation index	%	99.6	100	98.2	97.2
F0 generation – 2nd mating					
Female mating index	%	100	96.6	100	100
Female fertility index	%	93.3	92.9	96.7	93.3
Duration of gestation	mean days	22.0	21.9	22.1	22.0
Gestation index	%	100	100	100	100
Parturition index	%	100	100	100	100
Litter size	mean	13.9	13.6	13.2	12.5
Number of pups	total n	390	353	383	351
Number of dead pups	n	2	3	1	2
Sex ratio	% live males	46.4	54.0	46.1	54.4
Viability index	%	98.7	93.1	97.6	96.6
Lactation index	%	100	99.0	99.5	97.7

* statistically significant difference from control p<0.05
** statistically significant difference from control p<0.01
Findings considered related to treatment with trifloxystrobin are written in **bold letters**

F1a Pups

Both the viability index (percentage of pups surviving days 0 to 4 post partum) and the lactation index (percentage of pups surviving days 4 to 21 post partum) were comparable in all groups. The sex ratios of the F1a pups on days 0 and 21 post partum were similar in all groups. In the control, 50, 750 and 1500 ppm dose groups, respectively, 30, 29, 29 and 27 dams successfully reared their litters to weaning on day 21 post partum.

Mean pup weight at birth was similar in all groups. At 1500 ppm, there was a marked reduction in weight gain of pups throughout the lactation period with the result that mean pup bodyweights were significantly lower than controls from day 7 post partum through to weaning on day 21 postpartum (sexes separately and combined). At 750 ppm, there was a retardation of pup weight gain, such that mean pup bodyweights were significantly lower than controls on days 14 and 21 post partum (sexes separately and combined). At 50 ppm, mean pup weights and mean pup weight gain were similar to that of the control group after culling on day 4 post partum through to weaning on day 21 post partum.

Table B.6.35: Bodyweight development in F1a pups (g)

Parameter	**Generation**	**Dose (ppm)**					
		0	**50**	*(%)[a]*	**750**	*(%)[a]*	**1500**	*(%)[a]*
Males & females combined								
LD 0 birth	F1a	6.1	6.1	*(100%)*	6.1	*(100%)*	6.2	*(102%)*
LD 4 prior reduction		9.2	9.4	*(102%)*	9.3	*(101%)*	8.6	*(93%)*
LD 4 after reduction		9.2	9.5	*(103%)*	9.3	*(101%)*	8.6	*(93%)*
LD 7 (week 1)		15	15.2	*(101%)*	14.5	*(97%)*	**12.7****	**(85%)**
LD 14 (week 2)		30.1	30.2	*(100%)*	**28.3***	***(94%)***	**23.7****	**(79%)**
LD 21 (week 3)		49.3	49	*(99%)*	**44.7****	***(91%)***	**35.3****	**(72%)**
Males								
LD 0 birth	F1a	6.2	6.3	*(102%)*	6.3	*(102%)*	6.4	*(103%)*
LD 4 prior reduction		9.4	9.7	*(103%)*	9.5	*(101%)*	8.8	*(94%)*
LD 4 after reduction		9.4	9.7	*(103%)*	9.5	*(101%)*	8.8	*(94%)*
LD 7 (week 1)		15.3	15.6	*(102%)*	15	*(98%)*	**12.9****	***(84%)***
LD 14 (week 2)		30.7	30.8	*(100%)*	**29***	***(94%)***	**24.2****	***(79%)***
LD 21 (week 3)		50.3	50.4	*(100%)*	**46***	***(91%)***	**36.1****	***(72%)***
Females								
LD 0 birth	F1a	5.9	5.9	*(100%)*	6	*(102%)*	6.0	*(102%)*
LD 4 prior reduction		8.9	9.1	*(102%)*	9	*(101%)*	8.3	*(93%)*
LD 4 after reduction		9	9.3	*(103%)*	9.1	*(101%)*	8.3	*(92%)*
LD 7 (week 1)		14.7	15	*(102%)*	14.2	*(97%)*	**12.3****	***(84%)***
LD 14 (week 2)		29.5	29.8	*(101%)*	**27.8***	***(94%)***	**23.2****	***(79%)***
LD 21 (week 3)		48.3	47.7	*(99%)*	**43.9***	***(91%)***	**35****	***(72%)***

[a] % of control

* statistically significant difference from control $p<0.05$

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

Findings considered related to treatment with trifloxystrobin are written in **bold letters**

For the pups at 1500 ppm, mean values for eye opening were delayed by 0.7 days in comparison to the control group. Differences from the control value were statistically significant.

No treatment-related macroscopic findings were noted at necropsy of the F1a pups not chosen as F1 parents.

F1b Pups

At birth, mean litter size did not differ between groups. The sex ratios of the F1b pups on days 0 and 21 post partum were similar in all groups. Both the viability index and the lactation index were comparable in all groups. In the control, 50, 750 and 1500 ppm dose groups, respectively, 28, 25, 29 and 28 dams successfully reared their litters to weaning on day 21 post partum.

Mean pup weight at birth was similar in all groups. At 1500 ppm, there was a marked reduction in weight gain of pups throughout the lactation period with the result that mean pup bodyweights were significantly lower than controls from day 7 post partum through to weaning on day 21 postpartum (sexes separately and combined). At 750 ppm, there was a retardation of pup weight gain after culling on day 4 post partum, such that mean pup bodyweights were significantly lower than controls on days 14 and 21 post partum (sexes separately and combined). At 50 ppm mean pup weights and mean pup weight gain were similar to that of the control group after culling on day 4 post partum through to weaning on day 21 post partum.

Table B.6.36: Bodyweight development in F1b pups (g)

Parameter	**Generation**	**Dose (ppm)**					
		0	**50**	*(%)[a]*	**750**	*(%)[a]*	**1500**	*(%)[a]*
Males & females combined								
LD 0 birth	F1b	5.9	6.2	*(105%)*	6	*(102%)*	6.3	*(107%)*
LD 4 prior reduction		8.7	9.1	*(105%)*	8.7	*(100%)*	8.2	*(94%)*
LD 4 after reduction		8.9	9.3	*(104%)*	8.8	*(99%)*	8.3	*(93%)*
LD 7 (week 1)		14.5	15	*(103%)*	13.7	*(94%)*	**12.2****	***(84%)***
LD 14 (week 2)		30.2	30.6	*(101%)*	**27.4****	***(91%)***	**23.6****	***(78%)***
LD 21 (week 3)		51.8	52	*(100%)*	**46.2****	***(89%)***	**37.7****	***(73%)***
Males								
LD 0 birth	F1b	6.1	6.3	*(103%)*	6.3	*(103%)*	6.4	*(105%)*
LD 4 prior reduction		8.9	9.3	*(104%)*	8.9	*(100%)*	8.4	*(94%)*
LD 4 after reduction		9.1	9.5	*(104%)*	9.1	*(100%)*	8.5	*(93%)*
LD 7 (week 1)		14.7	15.4	*(105%)*	13.9	*(95%)*	**12.5****	***(85%)***
LD 14 (week 2)		30.6	31.3	*(102%)*	**27.7****	***(91%)***	**24.0****	***(78%)***
LD 21 (week 3)		53.1	53.6	*(101%)*	**46.9****	***(88%)***	**38.6****	***(73%)***
Females								
LD 0 birth	F1b	5.7	6.0	*(105%)*	5.8	*(102%)*	6.0	*(105%)*
LD 4 prior reduction		8.5	8.9	*(105%)*	8.4	*(99%)*	8.0	*(94%)*
LD 4 after reduction		8.7	9.1	*(105%)*	8.5	*(98%)*	8.1	*(93%)*
LD 7 (week 1)		14.2	14.7	*(104%)*	13.3	*(94%)*	**12.0****	***(85%)***
LD 14 (week 2)		29.8	29.8	*(100%)*	**27.0****	***(91%)***	**23.1****	***(78%)***
LD 21 (week 3)		50.6	50.2	*(99%)*	**45.2****	***(89%)***	**36.7****	***(73%)***

[a] % of control

* statistically significant difference from control $p<0.05$

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

Findings considered related to treatment with trifloxystrobin are written in **bold letters**

For the pups at 1500 ppm, mean values for eye opening were significantly delayed by 0.6 days in comparison to the control group.

No treatment-related macroscopic findings were noted at necropsy of the F1b pups.

F0 Adult Macroscopic and microscopic Examination

At 1500 ppm, absolute spleen weights (males), adrenal and brain weights (female) were significantly reduced. For both sexes, relative weights of most organs were increased and significantly different from the respective control value, likely as a result of the decreased bodyweights.

At 750 ppm, in females, absolute brain weights were slightly but significantly reduced; and relative liver and ovaries weights slightly but significantly increased.

For males, relative kidneys and liver weights were minimally but significantly increased.

Table B.6.36a: Affected organ weights F0 parents

Affected organ weights F0 parents

	males				females			
Dose level (ppm)	**0**	**50**	**750**	**1500**	**0**	**50**	**750**	**1500**
absolute weights [g]								
body (exsanguinated)	538	543	532	480**	321	315	298**	281**
spleen	0.806	0.782	0.760	0.698**	0.609	0.600	0.567	0.559
liver	19.84	20.39	20.67	20.30	12.78	12.17	12.55	12.47
kidney	3.249	3.324	3.390	3.352	2.234	2.206	2.132	2.150
testes	4.183	4.039	4.130	4.140	---	---	---	---
ovaries	---	---	---	---	0.207	0.207	0.217	0.209
adrenals	0.072	0.070	0.070	0.067	0.088	0.086	0.085	0.081**
brain	2.364	2.392	2.352	2.342	2.246	2.234	2.173*	2.138**
relative to body weight								
liver	3.675	3.743	3.885**	4.222**	3.986	3.866	4.213**	4.430**
kidney	0.605	0.614	0.640*	0.699**	0.698	0.702	0.717	0.766**
testes	0.782	0.750	0.778	0.865**	---	---	---	---
ovaries	---	---	---	---	0.065	0.066	0.073*	0.074**
brain	0.443	0.444	0.445	0.491**	0.703	0.714	0.734	0.762**

Dunnett's t-Test *) P<0.05 **) P<0.01

No treatment-related changes were observed at histopathological examination of the reproductive organs of the control and high dose (1500 ppm) groups.

Microscopic examination of the liver showed an increased incidence of males and females at 1500 ppm with minimal hypertrophy of centrilobular hepatocytes. Microscopic examination of the kidneys and spleen showed an increased incidence of males and females at 1500 ppm and of males at 750 ppm with minimal pigmentation of renal tubules. A decreased incidence of males and females with splenic hemosiderosis was noted at ≥ 750 ppm.

Table B.6.37: Summary of microscopic findings in F0 Parents

		Dose Group (ppm)/Incidence of lesion							
		males				females			
		0	**50**	**750**	**1500**	**0**	**50**	**750**	**1500**
Kidney:	**Tissues Examined**	30	30	30	30	30	30	30	30
	Pigmentation	1	0	4	7	0	0	0	3
Liver:	**Tissues Examined**	30	30	30	30	30	30	30	30
	Hepatocellular hypertrophy	3	1	4	10	1	0	1	5
Spleen:	**Tissues Examined**	30	30	30	30	30	30	30	30
	hemosiderosis	17	20	12	9	23	22	15	8

F1 Generation

The selected F1 animals were representative of the F1a generation in that the 750 ppm and 1500 ppm dose groups had lower bodyweights. Throughout the F1 generation, bodyweights in both groups (both sexes) remained significantly lower than controls, but bodyweight gain was usually similar to that of the control group.

During the gestation period, overall weight gain of the females was significantly lower than controls at ≥ 750 ppm. The resulting lower bodyweights during the gestation period were significantly different from controls on all occasions. This effect was more pronounced in the high dose group than in the mid dose group.

As in the F0 generation, for the females at ≥ 750 ppm, overall weight gain and weight gain between days 14 and 21 of the lactation period were superior to that of the control group.

In the 50 ppm group, bodyweights and bodyweight gain were similar to that of the control group throughout the F1 generation.

Table B.6.38a: Body weights (g) of F1 generation

Parameter	**Generation**	**Dose (ppm)**					
		0	**50**	*(%)[a]*	**750**	*(%)[a]*	**1500**	*(%)[a]*
Males								
Day 1	F1 - pre- and post-mating	160.3	161.6	*(101%)*	**143.2****	***(89%)***	**111.8****	***(70%)***
Day 8		217.2	219.6	*(101%)*	**195.7****	***(90%)***	**159.1****	***(73%)***
Day 15		270.6	271.9	*(100%)*	**247.1****	***(91%)***	**205.1****	***(76%)***
Day 22		318.5	318.4	*(100%)*	**294.2****	***(92%)***	**250.3****	***(79%)***
Day 29		351.2	354.1	*(101%)*	**327.1****	***(93%)***	**287.8****	***(82%)***
Day 36		378.1	381.9	*(101%)*	**261.2****	***(69%)***	**313.3****	***(83%)***
Day 43		403.9	409.9	*(101%)*	**378.1****	***(94%)***	**340.3****	***(84%)***
Day 50		419.5	425.4	*(101%)*	**291.2****	***(69%)***	**355.2****	***(85%)***
Day 57		437.2	443.3	*(101%)*	**406.6****	***(93%)***	**369.6****	***(85%)***
Day 64		449.9	455.3	*(101%)*	**416.6****	***(93%)***	**380.4****	***(85%)***
Day 68		456.7	463.7	*(102%)*	**425.5****	***(93%)***	**388.7****	***(85%)***
Day 71		455.6	461.1	*(101%)*	**420.8****	***(92%)***	**382.6****	***(84%)***
Day 78		461.6	465.3	*(101%)*	**431.3****	***(93%)***	**396.1****	***(86%)***
Day 85		473.9	478.4	*(101%)*	**437.2****	***(92%)***	**404.9****	***(85%)***
Day 92		488	490.2	*(100%)*	**451.4****	***(93%)***	**415.1****	***(85%)***
Day 99		497.8	502.6	*(101%)*	**463.6****	***(93%)***	**425.2****	***(85%)***
Day 106		506.4	512.7	*(101%)*	**470.8****	***(93%)***	**433.5****	***(86%)***

Parameter	**Generation**	**Dose (ppm)**					
		0	**50**	*(%)[a]*	**750**	*(%)[a]*	**1500**	*(%)[a]*
Day 113		516.9	524.6	*(101%)*	**480.5****	***(93%)***	**442.3****	***(86%)***
Day 120		525.1	531.3	*(101%)*	**489.9***	***(93%)***	**448.5****	***(85%)***
Females								
Day 1	F1 - pre-mating	137.7	136.6	*(99%)*	**122.7***	***(89%)***	**97.5****	***(71%)***
Day 8		168.8	166.9	*(99%)*	**152.7****	***(90%)***	**127.8****	***(76%)***
Day 15		194.9	193.0	*(99%)*	**176.0****	***(90%)***	**153.9****	***(79%)***
Day 22		217	214.6	*(99%)*	**197.0****	***(91%)***	**174.5****	***(80%)***
Day 29		233	232.6	*(100%)*	**211.8****	***(91%)***	**191.6****	***(82%)***
Day 36		246.9	245.9	*(100%)*	**224.6****	***(91%)***	**203.4****	***(82%)***
Day 43		257.7	256.5	*(100%)*	**234.9****	***(91%)***	**214.6****	***(83%)***
Day 50		266.8	265.2	*(99%)*	**242.2****	***(91%)***	**222.9****	***(84%)***
Day 57		274.2	271.6	*(99%)*	**248.8****	***(91%)***	**231.2****	***(84%)***
Day 64		279.9	278.1	*(99%)*	**255.3****	***(91%)***	**236.8****	***(85%)***
Day 68		283.3	280.9	*(99%)*	**257.6****	***(91%)***	**241.1****	***(85%)***
Day 71		285.7	283.4	*(99%)*	**258.2****	***(90%)***	**237.6****	***(83%)***
Gestation day 1	F1 - gestation	280.9	283.0	*(101%)*	**257.1****	***(92%)***	**236.5****	***(84%)***
Gestation day 7		303.3	301.6	*(99%)*	**278.0****	***(92%)***	**258.5****	***(85%)***
Gestation day 14		332.7	330.0	*(99%)*	**305.1****	***(92%)***	**282.2****	***(85%)***
Gestation day 21		419.3	412.8	*(98%)*	**384.2****	***(92%)***	**348.0****	***(83%)***
Lactation day 0	F1 - lactation	317.6	313.3	*(99%)*	**289.6****	***(91%)***	**259.6****	***(82%)***
Lactation day 7		325.3	325.3	*(100%)*	**300.3****	***(92%)***	**278.1****	***(85%)***
Lactation day 14		339.8	339.2	*(100%)*	**315.4****	***(93%)***	**290.0****	***(85%)***
Lactation day 21		330.2	331.1	*(100%)*	**313.8****	***(95%)***	**297.2****	***(90%)***

[a] % of control

* statistically significant difference from control $p<0.05$

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

Findings considered related to treatment with trifloxystrobin are written in **bold letters**

Table B.6.38b: Bodyweight gains (g) of F1 generation

Parameter	**Generation**	**Dose (ppm)**					
		0	**50**	*(%)[a]*	**750**	*(%)[a]*	**1500**	*(%)[a]*
Males								
Day 1 – 71	F1 - pre- and post-mating	364.8	369.7	*(101%)*	347.2	*(95%)*	336.7	*(92%)*
Females								
Day 1 – 68	F1 - pre-mating	148	146.8	*(99%)*	**135.5***	***(92%)***	140.1	*(95%)*
GD 0 – 7	F1 - gestation	22.4	18.6	*(83%)*	20.8	*(93%)*	22.0	*(98%)*
GD 7 - 14		29.4	28.3	*(96%)*	27.1	*(92%)*	**23.6****	***(80%)***
GD 14 – 21		86.6	82.9	*(96%)*	79.1	*(91%)*	**65.8****	***(76%)***
GD 0 – 21		138.4	129.8	*(94%)*	**127.0***	***(92%)***	**111.5****	***(81%)***
LD 0 – 7	F1 - lactation	7.7	12	*(156%)*	10.7	*(139%)*	18.6**	*(242%)*
LD 7 - 14		14.5	13.9	*(96%)*	15.2	*(105%)*	11.9	*(82%)*
LD 14 – 21		-9.6	-8.1	*- [x]*	-1.6*	*- [x]*	7.2**	*- [x]*
LD 0 – 21		12.6	17.8	*(141%)*	24.2**	*(192%)*	37.7**	*(299%)*

[a] % of control
[x] calculation not reasonable due to negative value in control
GD gestation day
* statistically significant difference from control p<0.05
** statistically significant difference from control p<0.01
Findings considered related to treatment with trifloxystrobin are written in **bold letters**

At 1500 ppm (both sexes) and at 750 (females), food consumption was reduced and usually significantly different from the control group throughout the F1 generation. At 50 ppm (both sexes) and at 750 ppm (males), food consumption was similar to that of the control group throughout the F1 generation.

Table B.6.38c: Food consumption (g) of F1 generation

Parameter	**Generation**	**Dose (ppm)**					
		0	**50**	*(%)[a]*	**750**	*(%)[a]*	**1500**	*(%)[a]*
Males								
Day 1-8	F1 - pre- and post-mating	23.0	23.8	*(103%)*	21.6	*(94%)*	**17.3****	***(75%)***
Day 8-15		24.4	24.5	*(100%)*	**22.9***	***(94%)***	**19.7****	***(81%)***
Day 15-22		27.7	28.3	*(102%)*	27.0	*(97%)*	**24.3****	***(88%)***

Parameter	**Generation**	**Dose (ppm)**						
		0	**50**	*(%)[a]*	**750**	*(%)[a]*	**1500**	*(%)[a]*
Day 22-29		26.7	27.1	*(101%)*	25.6	*(96%)*	**24.1****	***(90%)***
Day 29-36		26.8	28.2	*(105%)*	26.8	*(100%)*	25.3	*(94%)*
Day 36-43		27.5	28.1	*(102%)*	26.6	*(97%)*	**25.5****	***(93%)***
Day 43-50		26.1	26.4	*(101%)*	24.8	*(95%)*	**23.9****	***(92%)***
Day 50-57		26.2	26.4	*(101%)*	25.0	*(95%)*	24.9	*(91%)*
Day 57-64		27.1	27.8	*(103%)*	26.4	*(97%)*	25.9	*(92%)*
Day 64-68		22.2	23.0	*(104%)*	22.5	*(101%)*	22.7	*(102%)*
Day 92-99		25.4	26.4	*(104%)*	25.5	*(100%)*	24.1	*(95%)*
Day 99-106		27.0	27.7	*(103%)*	26.3	*(97%)*	25.5	*(94%)*
Day 106-113		24.5	25.6	*(104%)*	24.5	*(100%)*	24.0	*(98%)*
Day 113-120		25.6	26.1	*(102%)*	25.1	*(98%)*	24.4	*(95%)*
Females								
Day 1-8	F1 - pre-mating	18.9	18.5	*(98%)*	**17.2****	***(91%)***	**15.0****	***(79%)***
Day 8-15		18.3	17.9	*(98%)*	**16.6****	***(91%)***	**14.7****	***(80%)***
Day 15-22		20.7	20.3	*(98%)*	**19.0****	***(92%)***	**17.7****	***(86%)***
Day 22-29		19.5	19.0	*(97%)*	**17.7****	***(91%)***	**16.9****	***(87%)***
Day 29-36		19.9	19.8	*(99%)*	**18.3****	***(92%)***	18.1	*(91%)*
Day 36-43		19.4	19.4	*(100%)*	**18.3***	***(94%)***	**17.8****	***(92%)***
Day 43-50		18.9	17.9	*(95%)*	**16.7****	***(88%)***	**16.9****	***(89%)***
Day 50-57		18.7	18.1	*(97%)*	**17.4***	***(89%)***	**17.1****	***(91%)***
Day 57-64		19.3	19.0	*(98%)*	**18.1****	***(90%)***	**17.9****	***(93%)***
Day 64-68		16.1	15.9	*(99%)*	15.4	*(96%)*	16.1	*(100%)*
Day 1-68		219.7	185.8	*(85%)*	**174.7**	***(80%)***	**168.2**	***(77%)***
GD 0 – 7	F1 - gestation	19.7	19.2	*(97%)*	18.9	*(96%)*	19.4	*(98%)*
GD 7 - 14		22.4	21.9	*(98%)*	**21.0***	***(94%)***	**20.5****	***(92%)***
GD 14 – 21		21.9	21.7	*(99%)*	**20.6***	***(94%)***	**18.9****	***(86%)***
GD 0 – 21		64.0	62.8	*(98%)*	60.5	*(95%)*	58.8	*(92%)*
LD 0 – 7	F1 - lactation	30.6	33.2	*(108%)*	31.7	*(104%)*	30.7	*(100%)*

Parameter	**Generation**	**Dose (ppm)**					
		0	**50**	*(%)[a]*	**750**	*(%)[a]*	**1500**	*(%)[a]*
LD 7 - 14		51.4	54.0	*(105%)*	50.0	*(97%)*	**46.2***	***(90%)***
LD 14 – 21		68.8	70.1	*(102%)*	64.2	*(93%)*	**58.6****	***(85%)***
LD 0 – 21		150.8	157.3	*(104%)*	145.9	*(97%)*	135.5	***(90%)***

[a] % of control
GD gestation Day
* statistically significant difference from control p<0.05
** statistically significant difference from control p<0.01
Findings considered related to treatment with trifloxystrobin are written in **bold letters**

Mating

Male and female mating and fertility indices, maternal gestation and parturition indices and the duration of gestation were unaffected by treatment. A total of 29, 28, 28 and 29 females in the control, 50, 750 and 1500 ppm dose groups, respectively, were pregnant and gave birth to live young.

Table B.6.38d: Reproductive performance of F1 generation

Parameter		**Dose (ppm)**			
		0	**50**	**750**	**1500**
F1 generation – mating					
Female mating index	%	96.7	96.7	93.3	96.7
Female fertility index	%	100	96.6	100	100
Duration of gestation	mean days	22.0	22.1	22.1	22.0
Gestation index	%	100	100	100	100
Parturition index	%	100	100	100	100
Litter size	mean	12.8	13.0	12.5	12.0
Number of pups	total n	371	363	351	347
Number of dead pups	n	1	0	2	1
Sex ratio	% live males	47.8	49.3	43.3	47.4
Viability index	%	93.0	97.0	99.4**	96.5
Lactation index	%	98.7	99.5	99.1	99.1

* statistically significant difference from control p<0.05
** statistically significant difference from control p<0.01
Findings considered related to treatment with trifloxystrobin are written in **bold letters**

F2 Pups

At birth, mean litter size did not differ between groups. The sex ratios of the F2 pups on days 0 and 21 post partum were similar in all groups. Both the viability index and the lactation index) were comparable in all groups. In the control, 50, 750 and 1500 ppm dose groups, respectively, 28, 28, 28 and 29 dams successfully reared their litters to weaning on day 21 post partum.

Mean pup weight at birth was similar in all groups. There was a retardation in weight gain at 750 and 1500 ppm throughout the lactation period, such that mean bodyweights were significantly lower than controls on days 7, 14 and 21 post partum (both groups) and on day 4 post partum (females, 1500 ppm group). At 50 ppm, mean pup weights and mean pup weight gain were similar to that of the control group after culling on day 4 post partum, through to weaning on day 21 post partum.

Table B.6.39: Bodyweight development in F2 pups (g)

Parameter	**Generation**	**Dose (ppm)**					
		0	**50**	*(%)[a]*	**750**	*(%)[a]*	**1500**	*(%)[a]*
Males & females combined								
LD 0 birth	F2	6.0	6.1	*(102%)*	6.0	*(100%)*	6.1	*(102%)*
LD 4 prior reduction		9.0	9.3	*(103%)*	8.7	*(97%)*	8.5	*(94%)*
LD 4 after reduction		9.1	9.4	*(103%)*	8.8	*(97%)*	8.5	*(93%)*
LD 7 (week 1)		14.9	15.0	*(101%)*	**13.6****	***(91%)***	**12.5****	***(84%)***
LD 14 (week 2)		29.8	30.2	*(101%)*	**26.6****	***(89%)***	**23.3****	***(78%)***
LD 21 (week 3)		51.8	51.8	*(100%)*	**44.5****	***(86%)***	**37.3****	***(72%)***
Males								
LD 0 birth	F2	6.3	6.3	*(100%)*	6.2	*(98%)*	6.3	*(100%)*
LD 4 prior reduction		9.2	9.5	*(103%)*	8.8	*(96%)*	8.6	*(93%)*
LD 4 after reduction		9.3	9.5	*(102%)*	8.9	*(96%)*	8.7	*(94%)*
LD 7 (week 1)		15.1	15.2	*(101%)*	**13.6****	***(90%)***	**12.7****	***(84%)***
LD 14 (week 2)		30.1	30.7	*(102%)*	**26.4****	***(88%)***	**23.7****	***(79%)***
LD 21 (week 3)		53.3	53.1	*(100%)*	**44.4****	***(83%)***	**38.1****	***(71%)***
Females								
LD 0 birth	F2	5.8	5.9	*(102%)*	5.8	*(100%)*	5.9	*(102%)*
LD 4 prior reduction		9.0	9.1	*(101%)*	8.5	*(94%)*	8.3	*(92%)*
LD 4 after reduction		9.1	9.3	*(102%)*	8.6	*(95%)*	**8.3***	***(91%)***
LD 7 (week 1)		14.7	14.8	*(101%)*	**13.5***	***(92%)***	**12.2****	***(83%)***
LD 14 (week 2)		29.3	29.8	*(102%)*	**26.6****	***(91%)***	**22.9****	***(78%)***
LD 21 (week 3)		50.4	50.5	*(100%)*	**44.2****	***(88%)***	**36.5****	***(72%)***

[a] % of control
* statistically significant difference from control $p<0.05$
** statistically significant difference from control $p<0.01$
Findings considered related to treatment with trifloxystrobin are written in **bold letters**

For the pups at 1500 ppm, mean values for eye opening were significantly delayed by 0.7 days in comparison to the control group.

No treatment-related macroscopic findings were noted at necropsy of the F2 pups.

F1 Adult Macroscopic and microscopic Examination

At 1500 ppm, male spleen and brain weights and female brain and kidneys weights were the only absolute organ weights which were significantly lower than the respective control value. At 750 ppm, male brain weights and female kidneys and liver weights were significantly lower than the controls. These findings were considered to be due to the decreased bodyweights and not to be a specific toxic effect on target organs. At 1500 ppm, (for both sexes) and at 750 ppm (females), relative organ weights of most organs were increased and significantly different from the respective control value. These findings were considered to be due to the decreased bodyweights and not to be a specific toxic effect on target organs.

Table B.6.39a: Affected organ weights in F1 generation parents

Affected organ weights F1 parents

	males				females			
Dose level (ppm)	**0**	**50**	**750**	**1500**	**0**	**50**	**750**	**1500**
absolute weights [g]								
body (exsanguinated)	507	512	470**	431**	313	303	283**	261**
spleen	0.825	0.862	0.765	0.730**	0.612	0.592	0.560	0.582
liver	20.89	20.68	19.80	19.86	13.25	12.84	11.82**	12.43
kidney	3.283	3.222	3.090	3.101	2.266	2.180	2.059**	2.060**
testes	4.240	4.309	4.180	4.199	---	---	---	---
ovaries	---	---	---	---	0.212	0.232	0.239	0.205
adrenals	0.068	0.067	0.068	0.069	0.085	0.086	0.105	0.085
thymus	0.488	0.534	0.488	0.459	0.320	0.347	0.360*	0.319
brain	2.382	2.353	2.285**	2.246**	2.188	2.179	2.132	2.112**
relative to body weight								
liver	4.094	4.034	4.216	4.604**	4.226	4.246	4.186	4.767**
kidney	0.648	0.631	0.658	0.721**	0.723	0.723	0.730	0.790**
testes	0.840	0.845	0.891	0.980**	---	---	---	---
ovaries	---	---	---	---	0.068	0.077	0.085*	0.079**
adrenals	0.014	0.013	0.015	0.016**	0.027	0.029	0.037	0.033
thymus	0.097	0.105	0.104	0.107	0.103	0.115*	0.128**	0.123**
brain	0.473	0.462	0.489	0.525**	0.702	0.724	0.759**	0.813**

Dunnett's t-Test *) P<0.05 **) P<0.01

No treatment-related macroscopic changes were observed at terminal necropsy of the F1 parent animals.

No treatment-related microscopic changes were observed at microscopic examination of the reproductive organs of the control and high dose groups.

Microscopic examination of the liver showed an increased incidence of males and females at ≥ 750 ppm with minimal to moderate hypertrophy of centrilobular hepatocytes. Microscopic examination of the spleen showed decreased incidence of males and females with splenic hemosiderosis at ≥ 750 ppm.

Table B.6.40: Summary of microscopic findings in F1 Parents

		Dose Group (ppm)/Incidence of lesion							
		males				females			
		0	**50**	**750**	**1500**	**0**	**50**	**750**	**1500**
Liver:	**Tissues Examined**	30	30	30	30	30	30	30	30
	Hepatocellular hypertrophy	1	1	14	24	0	1	7	9
Spleen:	**Tissues Examined**	30	30	30	30	30	30	30	30
	Decreased hemosiderosis	14	12	5	2	23	22	17	14

In conclusion dose levels of ≥ 750ppm were associated with reduced food consumption and retarded bodyweight gain in the parent F0 and F1 animals. Additionally bodyweight gains of the pups were retarded during the lactation period (F1 a and b, and F2 animals). The marked increases in weight gains noted in parental females at ≥ 750ppm during the lactation periods were thought to be due to the reduced bodyweight development in the pups of these groups. These pups were suckled longer and weaned later than usual by the

dams, so that the normal reduction in bodyweight of the dams as milk reduction reduced prior to day 21 post partum, did not occur.

At 1500 ppm retardation in the eye opening landmark was noted during both lactation periods in the F1 generation and F2 generation was observed, although this was likely to be secondary to the bodyweight changes. There were no effects on reproductive parameters. There was an increased incidence of minimal to moderate hypertrophy of centrilobular hepatocytes in males and females (both generations at 1500 ppm, and F1 generation at 750 ppm). There was also an increased incidence of F0 animals with minimal pigmentation of renal tubules in the 1500 ppm group and in F0 males in the 750 ppm group. Based on these effects the NOAEL is considered to be 50 ppm for adults (equivalent to approx. 2.3 - 7.5 and 3.1 - 10.4 mg/kg bw/day in males and females respectively). In both F1 and F2 pups, bodyweight decreases were significant at 750 ppm and above, thus the NOAEL is 50 ppm (2.3 mg/kg bw/d based on lowest dose in parents). There were no effects on reproductive parameters, therefore the NOAEL is 1500 ppm (73.13 mg/kg bw/d).

3.10.1.2 Developmental toxicity

[Study 1a] Developmental range-finding study in rats Oral route (Anonymous, 1993, M-052919-01-1)

Non GLP, as a dose-range-finding study is not intended to comply with OECD guidelines

In this study trifloxystrobin (purity not reported) was administered by gavage to mated female Sprague Dawley derived rats (7/dose) dissolved in 0.5% aqueous sodium-carboxymethylcellulose at levels of 0, 10, 100 and 1000 mg/kg bw/day from gestation day 6 through gestation day 15. Animals were observed daily for clinical signs of toxicity. Bodyweights and food consumption were measured regularly. All females were sacrificed on gestation day 21 and subjected to a gross necropsy and caesarean section. Fetuses were individually weighed, sexed, and examined for external abnormalities.

There were no deaths or treatment-related clinical signs during the course of the study.

The food consumption was slightly reduced in the highest dose group of 1000 mg/kg bw/day during the treatment period between days 6 and 16 of pregnancy. Body weights of the pregnant animals were not affected by the treatment but slightly reduced body weight gain occurred troughout the treatment period from day 6 to 16.

Feed consumption during gestation

	Trifloxystrobin (mg/kg bw/day)					
	0	**10**		**100**		**1000**	
Mean feed consumption [g/animal/d] *(% difference to control)*						
Day 0-6	18.7	20.3	*(+12)*	18.8	*(+4)*	20.2	*(+12)*
Day 6-11	21.9	22.4	*(+6)*	21.5	*(+2)*	20.0	*(-5)*
Day 11-16	23.8	24.0	*(+1)*	23.6	*(-1)*	20.9	*(-12)*
Day 16-21	24.4	25.6	*(+5)*	26.0	*(+7)*	26.6	*(+9)*

*/** Statistically significant difference from control, $p \leq 0.05$/ $p \leq 0.01$

Maternal body weight and body weight gain

	Trifloxystrobin (mg/kg bw/day)						
	0	**10**		**100**		**1000**	
Body weight [g] *(% difference to control)*							
Day 0	200.8	202.7	*(+1)*	202.8	*(+1)*	202.1	*(+1)*
Day 1	204.6	209.4	*(+2)*	205.9	*(+1)*	209.8	*(+3)*
Day 2	207.9	214.6	*(+3)*	211.3	*(+2)*	213.8	*(+3)*
Day 3	212.9	218.6	*(+3)*	217.4	*(+2)*	219.9	*(+3)*
Day 4	217.2	223.4	*(+3)*	221.5	*(+2)*	224.4	*(+3)*
Day 5	220.1	227.7	*(+3)*	228.3	*(+4)*	227.8	*(+3)*
Day 6	220.0	227.7	*(+3)*	226.9	*(+3)*	228.3	*(+4)*
Day 7	224.9	232.7	*(+3)*	229.7	*(+2)*	232.1	*(+3)*
Day 8	231.4	241.6	*(+4)*	238.2	*(+3)*	238.7	*(+3)*
Day 9	238.0	247.8	*(+4)*	244.7	*(+3)*	243.5	*(+2)*
Day 10	243.6	251.6	*(+3)*	251.6	*(+3)*	249.4	*(+2)*
Day 11	248.6	257.1	*(+3)*	257.9	*(+4)*	252.4	*(+2)*
Day 12	253.7	264.7	*(+4)*	261.4	*(+3)*	257.9	*(+2)*
Day 13	261.5	269.1	*(+3)*	269.2	*(+3)*	264.1	*(+1)*
Day 14	267.5	278.2	*(+4)*	279.9	*(+5)*	270.2	*(+1)*
Day 15	278.6	286.6	*(+3)*	287.3	*(+3)*	276.4	*(-1)*
Day 16	289.6	296.5	*(+2)*	299.5	*(+3)*	286.9	*(-1)*
Day 17	303.1	313.7	*(+3)*	313.3	*(+3)*	303.7	*(±0)*
Day 18	318.5	326.6	*(+3)*	329.5	*(+3)*	317.4	*(±0)*
Day 19	333.7	343.2	*(+3)*	344.5	*(+3)*	334.1	*(±0)*
Day 20	345.2	358.2	*(+4)*	362.8	*(+5)*	349.1	*(+1)*
Day 21	363.6	373.3	*(+3)*	376.4	*(+4)*	365.3	*(±0)*
Day 21[a, #]	259.3	270.2	*(+4)*	265.5	*(+2)*	260.0	*(±0)*
Cumulative body weight gain [g] *(% difference to control)*							
Day 0 – 6	19.2	25.1	*(+31)*	24.1	*(+26)*	26.2	*(+36)*
Day 6 – 11	28.6	29.4	*(+3)*	31.0	*(+8)*	24.1	*(-16)*
Day 11 – 16	41.0	39.5	*(-4)*	41.6	*(+1)*	34.5	*(-16)*
Day 16 – 21	73.9	76.7	*(+4)*	76.9	*(+4)*	78.3	*(+6)*
Day 6 – 15[b]	58.6	58.9	*(+1)*	60.4	*(+3)*	48.1	*(-18)*
Day 6 – 21[c]	143.6	145.6	*(+1)*	149.4	*(+4)*	136.9	*(-5)*
Day 0 – 21	162.8	170.6	*(+5)*	173.6	*(+7)*	163.1	*(±0)*
Day 0 – 21 (corr.[a])[#]	58.5	67.5	*(+15)*	62.7	*(+7)*	57.9	*(-1)*
Gravid uterus weight [g] *(% difference to control)*							
Day 21	104.3	103.1	*(-1)*	110.9	*(+6)*	105.3	*(+1)*

*/** Statistically significant difference from control, $p \leq 0.05$/ $p \leq 0.01$
[#] No statistical analyses were performed.
[a] Corrected for uterus weight
[b] During treatment
[c] Until study termination

There were no treatment-related gross lesions at necropsy in females treated in any group.

All mated animals were pregnant with viable foetuses. Post-implantation losses and foetal body weights were unaffected by the treatment in all dose groups. There were no significant differences regarding the mean gravid uterus and carcass weights at scheduled necropsy observed between the groups.

Caesarean section data

Parameter	Trifloxystrobin (mg/kg bw/day)			
	0	10	100	1000
No. pregnant / no. mated	7 / 7	7 / 7	7 / 7	7 / 7
Mean no. corpora lutea/dam	16.6	16.3	18.0	17.6
Mean no. implantation sites/dam	14.1	14.3	15.3	15.1
No. females with total implant loss	0	0	0	0
Pre-implantation loss (%/dam)	14.2	11.6	12.1	12.7
Post-implantation loss (mean/dam)	0.3	0.6	0.7	1.0
Early resorptions/dam	0.3	0.6	0.7	1.0
Late resorptions/dam	0	0	0	0
No. dams with live foetuses	7	7	7	7
Total no. of live foetuses	97	96	102	99
Dead foetuses	0	0	0	0
Mean litter size	13.9	13.7	14.6	14.1
Mean foetal weight (g)	5.6	5.5	5.6	5.4
Sex ratio (% males)	42.2	46.9	58.8	51.5

*/** Statistically significant difference from control, $p \leq 0.05$/ $p \leq 0.01$

Trifloxystrobin was very slightly toxic to dams at the highest dose of 1000 mg/kg bw/day indicated by the slight reduction in food intake and slightly reduced body weight gain during the treatment. There was no indication of developmental toxicity after treatment with trifloxystrobin.

[Study 1b] Developmental toxicity study in rats (IIA 5.6) Oral route (Anonymous, 1999a, M-039420-02-1)

GLP, quality assured and OECD 414 (1981) compliant. EU Guideline (Annex V Teratogenicity - rodent 30/5/1988).

In this study trifloxystrobin (purity 96.4%) was administered by gavage to mated female Sprague Dawley derived rats (20-23/dose) dissolved in 0.5% aqueous Na-carboxymethylcellulose at levels of 0, 10, 100 and 1000 mg/kg bw/day from gestation day 6 through gestation day 15. Dose levels were based on a range finding study (see above). Animals were observed daily for clinical signs of toxicity. Bodyweights and food consumption were measured regularly. All females were sacrificed on gestation day 20 and subjected to a gross necropsy and caesarean section. Fetuses were individually weighed, sexed, and examined for external, skeletal and visceral abnormalities.

All dams survived until terminal sacrifice. Haemorrhagic discharge in the perineal region was seen in one dam at 100 mg/kg bw/day and 6 top dose group females. This finding was observed for one day only and all these animals had normal pregnancies. Three of these animals had no resorptions and the remainder had 1-4 resorptions.

Reduced bodyweight gains were noted in the top dose group during treatment. There was a dose related and statistically significant reduction in food consumption in the 100 and 1000 mg/kg bw/day dose groups during the treatment period. At 1000 mg/kg bw/day food consumption was 70 and 85% of control values at days 6-11 and 11-16 respectively and at 100 mg/kg bw/day 92% for both periods.

Table B.6.41a: Maternal bodyweight, body weight gain and food consumption

Parameter	**Dose (mg/kg bw/day)**					
	0	**10**	*(%)[a]*	**100**	*(%)[a]*	**1000**	*(%)[a]*
Body weight (g)							
Day 0	202.1	201.5	*(100%)*	201.5	*(100%)*	202.1	*(100%)*
Day 6	231.4	229.4	*(99%)*	230.4	*(100%)*	229.8	*(99%)*
Day 7	235.2	232.7	*(99%)*	233.3	*(99%)*	229.2	*(97%)*
Day 8	239.7	237.6	*(99%)*	237.1	*(99%)*	**230.9***	***(96%)***
Day 9	245.9	243.2	*(99%)*	242.5	*(99%)*	**234.6****	***(95%)***
Day 10	251.9	249.5	*(99%)*	249.3	*(99%)*	**241.9***	***(96%)***
Day 11	259.1	256.6	*(99%)*	256.5	*(99%)*	**247.2***	***(95%)***
Day 12	266.3	262.9	*(99%)*	262.2	*(98%)*	**252.5****	***(95%)***
Day 13	273.2	268.7	*(98%)*	268.3	*(98%)*	**259.6***	***(95%)***
Day 14	280.4	275.8	*(98%)*	276.2	*(99%)*	**264.3****	***(94%)***
Day 15	289.9	284	*(98%)*	285.3	*(98%)*	**275.2***	***(95%)***
Day 16	300.2	294.9	*(98%)*	296.3	*(99%)*	**284.2****	***(95%)***
Day 17	314.6	308.3	*(98%)*	310.4	*(99%)*	301.6	*(96%)*
Day 18	329.8	324.1	*(98%)*	326.5	*(99%)*	317.1	*(96%)*
Day 19	344.9	337.2	*(98%)*	342.4	*(99%)*	332.8	*(96%)*
Day 20	359.8	352.1	*(98%)*	358.8	*(100%)*	347.9	*(97%)*
Day 21	375.6	365.7	*(97%)*	373.3	*(99%)*	362.7	*(97%)*
Food consumption (g/animal/day)							
Day 0 - 6	23.0	22.4	*(97%)*	22.5	*(98%)*	22.1	*(96%)*
Day 6 - 11	25.0	24.4	*(98%)*	**23.1***	***(92%)***	**17.6****	***(70%)***
Day 11 - 16	27.1	26.4	*(97%)*	**25.0***	***(92%)***	**22.9****	***(85%)***
Day 16 - 21	27.7	26.8	*(97%)*	27.5	*(99%)*	28.8	*(104%)*
Body weight gain (g)							

Parameter	**Dose (mg/kg bw/day)**						
	0	**10**	*(%)[a]*	**100**	*(%)[a]*	**1000**	*(%)[a]*
Day 0 - 6	29.3	27.9	*(95%)*	29.0	*(99%)*	27.7	*(95%)*
Day 6 - 11	27.7	27.2	*(98%)*	26.0	*(94%)*	**17.4****	***(63%)***
Day 11 - 16	41.1	38.3	*(93%)*	39.8	*(97%)*	37.0	*(90%)*
Day 16 - 21	75.4	70.8	*(94%)*	77.0	*(102%)*	78.5	*(104%)*
Day 6 - 16	68.8	65.5	*(95%)*	65.9	*(96%)*	**54.4****	***(79%)***
Day 0 - 21	173.5	164.2	*(95%)*	171.8	*(99%)*	160.5	*(93%)*
Uterine / carcass weight							
Gravid uterus (g)	97.7	91.9	*(94%)*	104.6	*(107%)*	101.5	*(104%)*
Carcass (g)	277.9	273.8	*(99%)*	268.6	*(97%)*	**261.2****	***(94%)***
Net weight change							
Day 6 - 21	46.5	44.4	*(95%)*	**38.2***	***(82%)***	**31.4****	***(68%)***

[a] % of control
* statistically significant difference from control $p<0.05$
** statistically significant difference from control $p<0.01$
Findings considered related to treatment with trifloxystrobin are written in **bold letters**

Pregnancy status was not affected by treatment. The number of dams with viable fetuses at scheduled sacrifice was 23/24, 22/24, 20/24 and 22/24 at 0, 10, 100, and 1000 mg/kg bw/day respectively. Necropsy revealed no further macropathological findings in treated animals. Preimplantation losses, number of implantation sites and early and late implantation losses were comparable between groups. No dead or aborted fetuses were noted. Numbers of live fetuses per litter and foetal weights were not affected by treatment. Necropsy of the dams revealed no macroscopically observable pathological changes.

Table B.6.41b: Caesarean section data

Parameter	Dose (mg/kg bw/day)			
	0	10	100	1000
No. pregnant / no. mated	23 / 24	22 / 24	20 / 24	22 / 24
Mean no. corpora lutea / dam	16.5	16.4	16.6	16.9
Mean no. implantation sites / dam	14.4	13.9	15.6	15.0
Pre-implantation loss (% of corpora lutea)	13.8	13.4	6.0	10.3
Post-implantation loss (mean no. / dam)	1.1	1.1	1.1	1.3
early resorptions	1.1	1.1	1.1	1.3
late resorptions	0.0	0.0	0.0	0.0
Post-implantation loss (% of impl. per animal)	8.2	7.9	7.0	9.1
No. of fetuses	306	281	290	302
No. dead fetuses	0	0	0	0
Mean no. live fetuses/dam	13.3	12.8	14.5	13.7
Sex ratio (% males)	45.1	49.8	46.9	54.0
Mean fetal weight, both sexes (g)	5.4	5.3	5.3	5.3
Mean fetal weight of males (g)	5.6	5.5	5.5	5.5
Mean fetal weight of females (g)	5.2	5.2	5.2	5.1

Historical control data (rats) – caesarean section data

Historical control data	Mean	±	SD	Range of group means	
				Minimum	Maximum
Corpora lutea	16.9	±	2.5	14.3	18.1
Implantation sites	14.8	±	2.7	13.3	15.7
Pre-implantation loss [mean %]	12.0	±	14.0	3.4	19.6
Post-implantation loss [mean %]	5.5	±	10.3	2.4	9.9
Resorptions [N]	0.8	±	1.3	0.3	1.4
Live litter size [N]	14.0	±	3.0	12.6	14.9
Fetal weights [g]	5.5			2.8 [a]	7.2 [a]
Males	5.6			2.8 [a]	7.2 [a]
Females	5.3			2.9 [a]	6.9 [a]
Sex ratio	Male 50%			-	-

[a] Range of litter means
22 studies performed at the test facility between 1988–1994 with Tif:RAI f (SPF) rats ([redacted])
(735 pregnant dams; 729 litters with 10234 viable fetuses)

Fetal external examination

External examination revealed no treatment related abnormalities.

Summary of total malformations, anomalies and variations – rat study

Dose (mg/kg bw/d)		**0**	**10**	**100**	**1000**
Litters evaluated	[N]	23	22	20	22
Fetuses evaluated	[N]	306	281	290	302
Live	[N]	306	281	290	302
Dead	[N]	0	0	0	0
Total malformations					
Fetal incidence	No. (%)	1 (0.3)	1 (0.4)	0 (0.0)	0 (0.0)
Litter incidence	No. (%)	1 (4.3)	1 (4.5)	0 (0.0)	0 (0.0)
Affected fetuses / litter	%	1.09 ± 5.21	0.35 ± 1.64	0.00 ± 0.00	0.00 ± 0.00
Total anomalies[a]					
Fetal incidence	No. (%)	17 (5.6)	22 (7.8)	13 (4.5)	13 (4.3)
Litter incidence	No. (%)	11 (47.8)	12 (54.5)	10 (50.0)	10 (45.5)
Affected fetuses / litter	%	5.20 ± 6.65	7.48 ± 9.42	4.54 ± 5.13	5.04 ± 6.77
Total variations					
Fetal incidence	No. (%)	160 (52.3)	151 (53.7)	155 (53.4)	169 (56.0)
Litter incidence	No. (%)	23 (100)	22 (100)	20 (100)	22 (100)
Affected fetuses / litter	%	52.28 ± 3.04	53.29 ± 6.48	53.42 ± 3.42	56.36 ± 7.13

Statistical analysis: Litter incidence: Chi-square + Fisher's Exact test; affected fetuses/litter: Kruskal-Wallis + Mann-Whitney U-test; * $p \leq 0.05$, ** $p \leq 0.01$

[a] Note: according to the study report Anomaly is defined as: rare, slight to moderate, permanent or reversible structural change that is not considered to impair fetal survival, development or function.

Fetal visceral examination

Table B.6.42: Foetal visceral observations (fetal % incidence/litter % incidence)

Finding	**Dose level (mg/kg bw/day) fetal % incidence (litter % incidence)**			
	0	**10**	**100**	**1000**
Total fetuses examined (litters examined)	149/23	135/22	139/20	146/22
Umbilical hernia	0.7 (4.3)			
Enlarged thymus	2.0 (13.0)	2.2 (4.5)	2.2 (15.0)	7.5* (31.8)
Pulmonary hyperplasia	-	2.2 (4.5)	-	-
Accessory lobulets	0.7 (4.3)	1.5 (9.1)	0.7 (5.0)	1.4 (9.1)
Renal pelvic dilatation	2.0 (13.0)	0.7 (4.5)	-	0.7 (4.5)
Total visceral observations	5.4 (34.8)	5.2 (18.2)	2.9 (20.0)	9.6 (45.5)

* Statistically significant ($p<0.05$)

The only apparently treatment related finding was an enlarged thymus (considered a variation) seen in 11 fetuses in the top dose group. This incidence was statistically significant and outside the historical control range, historical database of 4793 fetuses and 725 litters (range of fetal % incidence/litter % incidence: 0.0-6.0%/0.0-29.2%).

Fetal skeletal examination

Table B.6.43: Foetal skeletal observations (fetal % incidence/litter % incidence)

Finding	**Dose level (mg/kg bw/day) fetal % incidence (litter % incidence)**			
	0	**10**	**50**	**250**
Total fetuses examined (litters examined)	157/23	146/22	151/20	156/22
TOTAL SKELETAL MALFORMATIONS	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
SKELETAL ANOMALIES[#]				
Asymmetrically shaped sternebra 1	-	3.4* (9.1)	0.7 (5.0)	0.6 (4.5)
TOTAL SKELETAL ANOMALIES	8.9 (39.1)	13.7 (50.0)	8.6 (50.0)	7.7 (40.9)
TOTAL SKELETAL VARIATIONS	99.4 (100)	100 (100)	100 (100)	100 (94.1)

* Statistically significant ($p<0.05$)

[#] Only anomalies with an apparent treatment related increase in foetal incidence/litter incidence given in table.

Summary of skeletal malformations, anomalies and variations – rat study

Dose (mg/kg bw/d)		**0**	**10**	**100**	**1000**
Litters evaluated	[N]	23	22	20	22
Fetuses evaluated	[N]	157	146	151	156
Live	[N]	157	146	151	156
Dead	[N]	0	0	0	0
Total skeletal malformations					
Fetal incidence	No. (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Litter incidence	No. (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Affected fetuses / litter	%	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Total skeletal anomalies[a]					
Fetal incidence	No. (%)	14 (8.9)	20 (13.7)	13 (8.6)	12 (7.7)
Litter incidence	No. (%)	9 (39.1)	11 (50.0)	10 (50.0)	9 (40.9)
Affected fetuses / litter	%	8.05 ± 11.55	13.30 ± 17.59	8.70 ± 9.76	9.21 ± 13.39
Total skeletal variations					
Fetal incidence	No. (%)	156 (99.4)	146 (100)	151 (100)	156 (100)
Litter incidence	No. (%)	23 (100)	22 (100)	20 (100)	22 (100)
Affected fetuses / litter	%	99.46 ± 2.61	100 ± 0.00	100 ± 0.00	100 ± 0.00

Statistical analysis: Litter incidence: Chi-square + Fisher's Exact test; affected fetuses/litter: Kruskal-Wallis + Mann-Whitney U-test; * $p \leq 0.05$, ** $p \leq 0.01$

[a] Note: according to the study report Anomaly is defined as: rare, slight to moderate, permanent or reversible structural change that is not considered to impair fetal survival, development or function.

Fetuses with fused/partially fused sternebrae

Parameter		**Dose (mg/kg bw/d)**				
		0	**10**	**100**	**1000**	**HCD**
No. fetuses evaluated		157	146	151	156	5426
No. litters evaluated		23	22	20	22	728
Sternebra(e)						
Sternebra 1, fused/ partially fused 1 and 2	Fetuses affected [N]	6	7	4	4	-
	Fetal incidence [%]	**3.8**	**4.8**	2.6	2.6	0.0 – 2.9
	Litter incidence [%]	**17.4**	**22.7**	**15.0**	**13.3**	0.0 – 13.0
Sternebra 2, fused/ partially fused 2 and 3	Fetuses affected [N]	0	0	0	0	-
	Fetal incidence [%]	0.0	0.0	0.0	0.0	-
	Litter incidence [%]	0.0	0.0	0.0	0.0	-
Sternebra 3, fused/ partially fused 3 and 4	Fetuses affected [N]	0	0	0	0	-
	Fetal incidence [%]	0.0	0.0	0.0	0.0	0.0 – 0.6
	Litter incidence [%]	0.0	0.0	0.0	0.0	0.0 – 4.2
Sternebra 4, fused/ partially fused 4 and 5	Fetuses affected [N]	0	0	0	0	-
	Fetal incidence [%]	0.0	0.0	0.0	0.0	0.0 – 0.6
	Litter incidence [%]	0.0	0.0	0.0	0.0	0.0 – 4.5

Statistical analysis: Chi-square + Fisher´s Exact test; * $p \leq 0.05$

Values exceeding HCD are written in **bold letters**

HCD: 22 studies performed at the test facility (1988–1994) with Tif:RAI f (SPF) rats ([redacted]) (278 litters with 5426 viable fetuses examined)

Fetuses with asymmetrically shaped sternebrae

Parameter		Dose (mg/kg bw/d)				
		0	**10**	**100**	**1000**	**HCD**
No. fetuses evaluated		157	146	151	156	5426
No. litters evaluated		23	22	20	22	728
Sternebra(e)						
Sternebra 1, asymmetrically shaped	Fetuses affected [N]	0	5*	1	1	-
	Fetal incidence [%]	0.0	**3.4**	0.7	0.6	0.0 – 0.7
	Litter incidence [%]	0.0	**9.1**	**5.0**	4.5	0.0 – 4.8
Sternebra 2, asymmetrically shaped	Fetuses affected [N]	2	0	0	1	-
	Fetal incidence [%]	**1.3**	0.0	0.0	0.6	0.0 – 0.7
	Litter incidence [%]	**8.7**	0.0	0.0	4.5	0.0 – 4.8
Sternebra 3, asymmetrically shaped	Fetuses affected [N]	0	0	0	0	-
	Fetal incidence [%]	0.0	0.0	0.0	0.0	0.0 – 0.7
	Litter incidence [%]	0.0	0.0	0.0	0.0	0.0 – 4.8
Sternebra 4, asymmetrically shaped	Fetuses affected [N]	2	0	0	0	-
	Fetal incidence [%]	1.3	0.0	0.0	0.0	0.0 – 1.9
	Litter incidence [%]	8.7	0.0	0.0	0.0	0.0 – 13.6
Sternebra 5, asymmetrically shaped	Fetuses affected [N]	7	2	2	3	-
	Fetal incidence [%]	**4.5**	1.4	1.3	1.9	0.0 – 2.2
	Litter incidence [%]	**30.4**	9.1	10.0	13.6	0.0 – 16.7
Sternebra 6, asymmetrically shaped	Fetuses affected [N]	3	2	3	2	-
	Fetal incidence [%]	**1.9**	1.4	**2.0**	1.3	0.0 – 1.6
	Litter incidence [%]	8.7	9.1	**15.0**	9.1	0.0 – 12.5

Statistical analysis: Chi-square + Fisher´s Exact test; * $p \leq 0.05$
Values exceeding HCD are written in **bold letters**
HCD: 22 studies performed at the test facility (1988–1994) with Tif:RAI f (SPF) rats ([redacted]) (278 litters with 5426 viable fetuses examined)

No skeletal malformations were observed in this study. The skeletal anomalies observed consisted of fused or asymmetric sternebrae, irregular ossification of the cranial bones, poor ossification of metacarpal-, additional cervical vertebral arches and bipartite thoracic vertebral centers. Although the incidence of asymmetrically shaped sternebrae-1 was significantly increased and outside historical controls in the low dose group the lack of any dose response indicates this finding was incidental (historical database of 5426 fetuses and 728 litters (fetal % incidence/litter % incidence ranges: 0.0-0.7%/0.0-4.8%)).

In conclusion maternal toxicity was evident at 1000 mg/kg bw/day based on effects on bodyweight and food consumption. Although food consumption was marginally affect at 100 mg/kg bw/day in the absence of other findings this dose level is considered the NOAEL for maternal toxicity. The NOAEL for fetotoxicity was 100 mg/kg bw/day based on the increased incidence of enlarged thymus found in the 1000 mg/kg group. There was no evidence of a teratogenic potential.

After discussion at the PRAPeR TC 144, the maternal NOAEL was reduced to 10 mg/kg bw/d based on the decreased body weight gain and food consumption observed at higher doses. The developmental NOAEL remained at 100 mg/kg bw/d.

[Study 2a] Developmental range-finding study in rabbits (Anonymous, 1994a, M-053339-01-1)

Non GLP, a dose-range-finding study is not intended to comply with OECD guidelines

In this study groups of 5 artifically inseminated Russian (Chbb:HM) rabbits were administered trifloxystrobin (purity 97.1%) by gavage in 0.5% aqueous sodium carboxymethylcellulose at doses of 0, 20, 100, 500 and 1000 mg/kg bw/day from days 7 to 19 of gestation. Animals were observed daily for clinical signs of toxicity.

Bodyweights and food consumption were measured regularly. All females were sacrificed on gestation day 29 and subjected to a gross necropsy and caesarean section. Fetuses were individually weighed, sexed, and examined for external abnormalities.

There were no deaths at any dose level. Treatment-related clinical signs such as reduced locomotor activity (from day 11 onwards) and haemorrhagic discharge in the perineal area (several occations between day 15 and 29) occurred at the high dose of 1000 mg/kg bw/day.

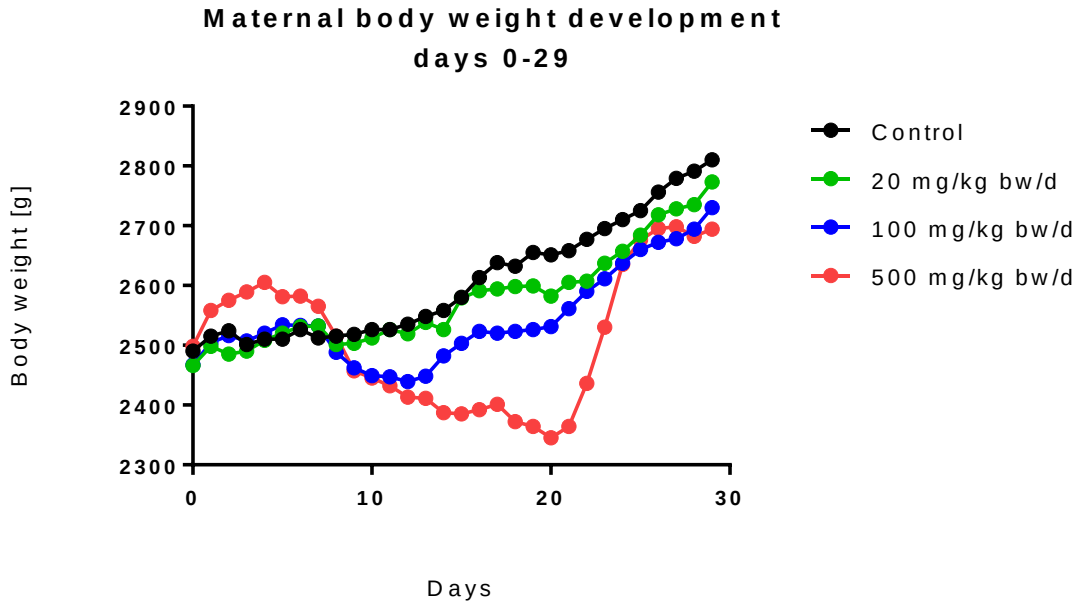
The treatment with trifloxystrobin had no effect on the weight gains and food consumption of the pregnant animals in the low dose group. Dose dependend effects on food consumption and body weight were reported for the dose groups ≥ 100 mg/kg bw/day. Food consumption was consistently decreased during the treatment period in the 100 mg/kg bw/day group by up to 57% and at 500 mg/kg bw/day by up to 77% with an effect on body weight and body weight gain. Losses of body weight were observed during treatment.

Food consumption during gestation

	Trifloxystrobin (mg/kg bw/day)						
	0	**20**		**100**		**500**		**1000**
Mean food consumption [g/animal/d] *(% difference to control)*							
Day 0-4	93.0	117.8	*(+27)*	128.5	*(+38)*	114.1	*(+23)*	-
Day 4-7	101.7	115.9	*(+14)*	119.7*	*(+18)*	106.7	*(+5)*	-
Day 7-12	102.8	94.6	*(-8)*	43.9*	*(-57)*	23.5*	*(-77)*	-
Day 12-16	129.9	94.6	*(-27)*	84.4	*(-35)*	32.9**	*(-75)*	-
Day 16-20	134.4	98.2	*(-27)*	92.6	*(-31)*	47.6*	*(-65)*	-
Day 20-24	116.7	109.8	*(-6)*	132.3	*(+13)*	107.0	*(-8)*	-
Day 24-29	98.6	109.8	*(+11)*	126.1	*(+28)*	162.2*	*(+65)*	-

*/** Statistically significant difference from control, $p \leq 0.05$/ $p \leq 0.01$

\- Food consumption is given only for dams with viable foetuses: At 1000 mg/kg bw/day, all pregnant animals had total resorptions.



Maternal body weight and body weight gain

	Trifloxystrobin (mg/kg bw/day)							
	0	**20**		**100**		**500**		**1000**
Body weight [g] *(% difference to control)*								
Day 0	2490	2466	*(-1)*	2467	*-19*	2498	*(±0)*	-
Day 1	2515	2498	*(-1)*	2504	*(±0)*	2558	*(+2)*	-
Day 2	2524	2485	*(-2)*	2516	*(±0)*	2575	*(+2)*	-
Day 3	2501	2490	*(±0)*	2507	*(±0)*	2589	*(+4)*	-
Day 4	2510	2508	*(±0)*	2520	*(±0)*	2605	*(+4)*	-
Day 5	2510	2520	*(±0)*	2534	*(+1)*	2581	*(+3)*	-
Day 6	2526	2531	*(±0)*	2533	*(±0)*	2582	*(+2)*	-
Day 7	2512	2532	*(+1)*	2532	*(+1)*	2565	*(+2)*	-
Day 8	2515	2501	*(-1)*	2488	*(-1)*	2515	*(±0)*	-
Day 9	2518	2503	*(-1)*	2462	*(-2)*	2457	*(-2)*	-
Day 10	2526	2512	*(-1)*	2449	*(-3)*	2445	*(-3)*	-
Day 11	2526	2526	*(±0)*	2447	*(-3)*	2432	*(-4)*	-
Day 12	2535	2519	*(-1)*	2439	*(-4)*	2413	*(-5)*	-
Day 13	2548	2538	*(±0)*	2448	*(-4)*	2411	*(-5)*	-
Day 14	2558	2526	*(-1)*	2482	*(-3)*	2387	*(-7)*	-
Day 15	2580	2579	*(±0)*	2503	*(-3)*	2385	*(-8)*	-
Day 16	2613	2591	*(-1)*	2523	*(-3)*	2392	*(-8)*	-
Day 17	2638	2594	*(-2)*	2520	*(-4)*	2401	*(-9)*	-
Day 18	2632	2598	*(-1)*	2523	*(-4)*	2372*	*(-10)*	-
Day 19	2655	2599	*(-2)*	2526	*(-5)*	2364*	*(-11)*	-
Day 20	2651	2582	*(-3)*	2531	*(-5)*	2345*	*(-12)*	-
Day 21	2658	2605	*(-2)*	2561	*(-4)*	2364*	*(-11)*	-
Day 22	2577	2607	*(+1)*	2590	*(+1)*	2436	*(-5)*	-
Day 23	2695	2637	*(-2)*	2611	*(-3)*	2530	*(-6)*	-
Day 24	2710	2657	*(-2)*	2637	*(-3)*	2635	*(-3)*	-
Day 25	2725	2684	*(-2)*	2660	*(-2)*	2676	*(-2)*	-
Day 26	2756	2718	*(-1)*	2672	*(-3)*	2695	*(-2)*	-
Day 27	2779	2728	*(-2)*	2678	*(-4)*	2698	*(-3)*	-
Day 28	2791	2735	*(-2)*	2694	*(-2)*	2682	*(-4)*	-
Day 29	2810	2773	*(-1)*	2730	*(-1)*	2694	*(±0)*	-
Day 29 [a]	2471	2409	*(-1)*	2448	*(±0)*	2424	*(+2)*	-
Cumulative body weight gain [g] *(% difference to control) [#]*								
Day 0 – 4	21	41	*(+95)*	52	*(+148)*	107	*(+410)*	-
Day 4 – 7	2	25	*(+1150)*	12	*(+500)*	-40	*(-2100)*	-
Day 7 – 12	23	-13	*(-157)*	-93	*(-504)*	-151**	*(-757)*	-
Day 12 – 16	78	72	*(-8)*	83	*(+6)*	-22**	*(-128)*	-
Day 16 – 20	38	-9	*(-124)*	9	*(-76)*	-46	*(-221)*	-
Day 20 – 24	59	75	*(+27)*	106	*(+80)*	289**	*(+390)*	-
Day 24 – 29	100	116	*(+16)*	93	*(-7)*	59	*(-41)*	-
Day 7 – 19 [b#]	143	67	*(-53)*	-6	*(-104)*	-201	*(-241)*	-
Day 7 – 29 [c#]	298	241	*(-19)*	198	*(-34)*	129	*(-57)*	-
Day 0 – 29 [#]	320	307	*(-4)*	263	*(-18)*	196	*(-39)*	-
Day 0 – 29 [#] (corrected [a])	-19	-57	*(+200)*	-19	*(±0)*	-74	*(+289)*	-
Gravid uterus weight [g] *(% difference to control)*								
Day 29	339	364	*(+7)*	282	*(-17)*	270	*(-20)*	-

*/** Statistically significant difference from control, $p \leq 0.05$/ $p \leq 0.01$

[#] No statistical analyses were performed.

- Body weights only for dams with viable foetuses: at 1000 mg/kg bw/day, all dams had total resorptions.

[a] Corrected for uterus weight

[b] During treatment

[c] Until study termination

There were no treatment-related gross lesions at necropsy in females treated at any dose group. However, dilatation of gall bladder was seen in one animal of the high dose group and mottled lung in a high-mid dose animal. Both observations were not considered treatment-related.

Pregnancy status was not affected by treatment. The number of corpora lutea, preimplantation losses, and numbers of implantation sites were comparable between groups. One dam at 500 mg/kg bw/day and all pregnant dams at 1000 mg/kg bw/day had total resorptions. The mean gravid uterus weight was reduced in the 500 mg/kg bw/day group. There were no dead or aborted fetuses in any group. The numbers of live fetuses per litter and fetal weights were decreased by treatment of 500 mg/kg bw/day. Abnormalities were not observed on foetal external examination.

Caesarean section data

Parameter	Trifloxystrobin (mg/kg bw/day)				
	0	**20**	**100**	**500**	**1000**
No. pregnant / no. mated	5 / 5	5 / 5	5 / 5	4 / 5	4 / 5
No. females with resorptions only	0	0	0	1	4
Mean no. corpora lutea/dam	9.0	8.6	7.8	7.8	6.0
Mean no. implantation sites/dam	7.6	7.6	6.0	6.3	6.0
Pre-implantation loss (%/dam)	15.2	10.9	22.9	18.9	0.0
Post-implantation loss (mean/dam)	0.8	0.0	0.4	2.0	6.0
Early resorptions/dam	0.6	0.0	0.0	2.0	6.0
Late resorptions/dam	0.2	0.0	0.4	0.0	0.0
No. dams with live foetuses	5	5	5	3	0
Total no. of live foetuses	34	38	28	17	0
Dead foetuses	0	0	0	0	0
Mean litter size	6.8	7.6	5.6	4.3	0.0**
Mean foetal weight (g)	36.7	35.6	36.6	34.8	-
Sex ratio (% males)	55.9	42.1	50.0	64.7	-

*/** Statistically significant difference from control, $p \leq 0.05$/ $p \leq 0.01$

In this developmental range finding study in rabbits, trifloxystrobin was toxic to dams at doses ≥ 100 mg/kg bw/day and developmentally toxic at doses ≥ 500 mg/kg bw/day. Based on these results, 500 mg/kg bw/day was selected as high dose level for the main developmental toxicity study with trifloxystrobin.

[Study 2b] Developmental toxicity study in rabbits (Anonymous, 1999b, M-039377-03-1)

GLP, quality assured and OECD 414 (1981) compliant. Oral Study EU Guideline (Annex V Teratogenicity - non-rodent 30/5/1988).

In this study groups of 17-19 presumed-pregnant Russian (Chbb:HM) rabbits were administered trifloxystrobin (purity 96.4%) by gavage in 0.5% aqueous Na-carboxymethylcellulose at doses of 0, 10, 50, 250 and 500 mg/kg bw/day from days 7 to 19 of gestation. Dose levels were based on a range finding study (see above). Measurements of bodyweight, food consumption and an assessment of clinical signs were made regularly. The animals were sacrificed on day 29 of gestation, macroscopic pathological changes in maternal organs noted, and the ovaries and uteri examined. Fetuses were weighed and examined for visceral and skeletal abnormalities.

No treatment related mortality or clinical signs occurred. One dam of the 50 mg/kg bw/day dose group died spontaneously without having exhibited any clinical signs before death. At necropsy haemorrhagic contents of uterus was found with this animal.

At ≥ 250mg/kg bw/day there was a dose related reduction in bodyweight gain and a significant bodyweight loss during the treatment period. Reduced food consumption was associated with these findings.

Table B.6.44a: Maternal body weight, body weight gain and food consumption

Parameter	**Dose (mg/kg bw/day)**								
	0	**10**	*(%)[a]*	**50**	*(%)[a]*	**250**	*(%)[a]*	**500**	*(%)[a]*
Body weight (g)									
Day 0	2715	2707	*(100%)*	2737	*(100%)*	2703	*(100%)*	2724	*(100%)*
Day 4	2739	2732	*(100%)*	2780	*(101%)*	2733	*(100%)*	2760	*(101%)*
Day 7	2747	2724	*(99%)*	2772	*(101%)*	2720	*(99%)*	2752	*(100%)*
Day 8	2752	2721	*(99%)*	2761	*(100%)*	2681	*(97%)*	2710	*(98%)*
Day 9	2743	2735	*(100%)*	2750	*(100%)*	2643	*(96%)*	2655	*(97%)*
Day 10	2744	2736	*(100%)*	2750	*(100%)*	2627	*(96%)*	2634	*(96%)*
Day 11	2750	2733	*(99%)*	2754	*(100%)*	2614	*(95%)*	2611	*(95%)*
Day 12	2749	2734	*(99%)*	2750	*(100%)*	2600	*(95%)*	2601	*(95%)*
Day 13	2762	2743	*(99%)*	2765	*(100%)*	2607	*(94%)*	**2603***	***(94%)***
Day 14	2772	2757	*(99%)*	2764	*(100%)*	**2611***	***(94%)***	**2609***	***(94%)***
Day 15	2799	2775	*(99%)*	2783	*(99%)*	**2633***	***(94%)***	**2615***	***(93%)***
Day 16	2809	2788	*(99%)*	2804	*(100%)*	2655	*(95%)*	**2622****	***(93%)***
Day 17	2814	2793	*(99%)*	2808	*(100%)*	**2652***	***(94%)***	**2630****	***(93%)***
Day 18	2821	2791	*(99%)*	2813	*(100%)*	**2650***	***(94%)***	**2626****	***(93%)***
Day 19	2820	2784	*(99%)*	2811	*(100%)*	**2637***	***(94%)***	**2613****	***(93%)***
Day 20	2811	2783	*(99%)*	2807	*(100%)*	**2637***	***(94%)***	**2600****	***(92%)***
Day 21	2815	2785	*(99%)*	2814	*(100%)*	2674	*(95%)*	**2638***	***(94%)***
Day 22	2820	2791	*(99%)*	2825	*(100%)*	2709	*(96%)*	2672	*(95%)*
Day 23	2827	2799	*(99%)*	2835	*(100%)*	2739	*(97%)*	2710	*(96%)*
Day 24	2836	2814	*(99%)*	2842	*(100%)*	2765	*(97%)*	2746	*(97%)*
Day 25	2858	2842	*(99%)*	2863	*(100%)*	2781	*(97%)*	2769	*(97%)*
Day 26	2876	2862	*(100%)*	2871	*(100%)*	2790	*(97%)*	2779	*(97%)*
Day 27	2893	2876	*(99%)*	2887	*(100%)*	2798	*(97%)*	2793	*(97%)*

Parameter	**Dose (mg/kg bw/day)**							
	0	**10**	*(%)[a]*	**50**	*(%)[a]*	**250**	*(%)[a]*	**500**	*(%)[a]*
Day 28	2910	2887	*(99%)*	2899	*(100%)*	2797	*(96%)*	2810	*(97%)*
Day 29	2919	2911	*(100%)*	2918	*(100%)*	2834	*(97%)*	2820	*(97%)*
Food consumption (g/animal/day)									
Day 0 - 4	127.0	122.6	*(97%)*	130.8	*(103%)*	123.9	*(98%)*	136.0	*(107%)*
Day 4 - 7	121.8	109.3	*(90%)*	123.4	*(101%)*	112.0	*(92%)*	116.6	*(96%)*
Day 7 - 12	109.5	103.9	*(95%)*	93.7	*(86%)*	**37.8****	***(35%)***	**37.6****	***(34%)***
Day 12 - 16	106.7	83.5	*(85%)*	91.9	*(93%)*	**56.3****	***(57%)***	**52.0****	***(53%)***
Day 16 - 20	98.8	91.4	*(98%)*	102.3	*(110%)*	**64.4****	***(69%)***	**58.7****	***(63%)***
Day 20 - 24	92.8	93.6	*(103%)*	107.2	*(118%)*	122.4	*(135%)*	109.4	*(121%)*
Day 24 - 29	90.6	90.6	*(91%)*	98.5	*(99%)*	107.4	*(108%)*	118.1**	*(118%)*
Body weight gain (g)									
Day 0 - 4	24	25		43		30		36	
Day 4 - 7	8	-9		-8		-13		-8	
Day 7 - 12	2	10		-22		**-120****		**-150****	
Day 12 - 16	59	55		54		55		**21****	
Day 16 - 20	2	-5		2		-18		-23	
Day 20 - 24	25	31		36		**128****		**146****	
Day 24 - 29	83	97		76		68		75	
Day 7 - 20	64	59	*(92%)*	34	*(53%)*	**-83****	***(-130%)***	**-152****	***(-238%)***
Uterine / carcass weight (g)									
Gravid uterus	340	367	*(108%)*	307	*(90%)*	301	*(89%*	295	*(87%)*
Carcass	2579	2544	*(99%)*	2611	*(101%)*	2533	*(98%*	2525	*(98%)*
Net weight change (g)									
Day 6 - 21	-167	-180		-162		-187		-226	

[a] % of control

* statistically significant difference from control $p<0.05$

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

Findings considered related to treatment with trifloxystrobin are written in **bold letters**

Pregnancy status was not affected by treatment. The number of dams with viable fetuses at scheduled sacrifice was 19/19, 18/19, 16/19, 17/19 and 18/19, at 0, 10, 50, 250 and 500 mg/kg bw/day respectively. Necropsy

revealed no further macropathological findings in treated animals. The number of corpora lutea, preimplantation losses, numbers of implantation sites, and postimplantation losses were comparable between groups. There were no dead or aborted fetuses in any group. The numbers of live fetuses per litter and fetal weights were unaffected by treatment.

Table B.6.44b: Caesarean section data

Parameter	Dose (mg/kg bw/day)				
	0	**10**	**50**	**250**	**500**
No. pregnant / no. mated	19 / 19	18 / 19	17 / 19	18 / 19	19 / 19
No. of dams pregnant, used for calculation	19	18	16	18	19
No. dams with resorptions only	0	0	0	1	1
Mean no. corpora lutea / dam	7.9	8.6	7.8	7.9	7.5
Mean no. implantation sites / dam	6.4	7.6	6.3	6.1	5.7
Pre-implantation loss (% of corpora lutea)	18.18	10.2	21.1	24.4	27.6
Post-implantation loss (mean no. / dam)	0.3	0.4	0.6	0.7	0.6
early resorptions	0.3	0.4	0.6	0.7	0.5
late resorptions	0.1	0.0	0.0	0.0	0.1
Post-implantation loss (% of impl. per animal)	6.1	5.4	12.6	14.7	10.0
No. of fetuses	116	130	90	97	97
No. dead fetuses	0	0	0	0	0
Mean no. live fetuses/dam	6.1	7.2	5.6	5.4	5.1
Sex ratio (% males)	45.7	37.7	35.6	46.4	46.4
Mean fetal weight, both sexes (g)	40.0	37.3	40.2	38.2	39.3
Mean fetal weight of males (g)	39.9	37.2	38.4	36.5	39.6
Mean fetal weight of females (g)	39.9	37.2	40.5	37.7	39.0

Fetal External Examination

Table B.6.45: Foetal external observations (fetal % incidence/litter % incidence)

Finding	Dose level (mg/kg bw/day) fetal % incidence (litter % incidence)				
	0	**10**	**50**	**250**	**500**
Total fetuses examined (litters examined)	116(19)	130(18)	90(16)	97(17)	97(18)
Craniocele		0.8 (5.6)			
Gastrochisis				1.0 (5.9)	
Acromicria, forelimb				1.0 (5.9)	
Ectodactyly, forelimb				1.0 (5.9)	
Position anomaly, forelimb		2.3 (11.1)	2.2 (12.5)	3.1 (11.8)	2.1 (5.6)
Total external observations	0.0 (0.0)	3.1 (16.7)	2.2 (12.5)	4.1 (17.6)	2.1 (5.6)

At external fetal examination two fetuses with malformations were seen; a single fetus of the low dose group showed craniocele and at 250 mg/kg bw/day a fetus (No 59/3) exhibited the following malformations; gastrochisis, acromicria and ectodactyly of the left forelimb. Since all these findings occurred in single individuals only, without any dose dependency or statistical significance, they were not considered treatment related.

Forelimb position anomaly (unilateral) was evenly distributed among all treated groups. Although there was no dose relationship the incidence was outside historical range in some groups, historical database of 2564 fetuses and 456 litters (fetal % incidence/litter % incidence ranges: 0.0-2.5%/0.0-13.3%). The study authors considered that this anomaly was thought to be most likely due to restriction of movement in the uterus.

Fetal Visceral Examination

Table B.6.46: Foetal visceral observations (fetal % incidence/litter % incidence)

Finding	**Dose level (mg/kg bw/day) fetal % incidence (litter % incidence)**				
	0	**10**	**50**	**250**	**500**
Total fetuses examined (litters examined)	116(19)	130(18)	90(16)	97(17)	97(18)
Small gall bladder		1.5 (5.6)	2.2 (12.5)	1.0 (5.9)	1.0 (5.6)
Aplasia of gall bladder			1.1 (6.3)		2.1 (11.1)
Total visceral observations	0.0 (0.0)	1.5 (5.6)	1.0 (5.9)	1.0 (5.9)	3.1 (16.7)

A visceral malformation, aplasia of the gall bladder occurred in one low-mid and in two high dose fetuses. Give the low incidence and lack of treatment relationship this finding was considered to be incidental (no historical control data supplied). The Applicant comments that- 'Aplasia of the gall bladder' belongs to a group of findings associated with the gall bladder, which spontaneously occur in rabbits quite frequently. The finding is mostly reported as 'small gall bladder' or 'missing gall bladder. Other related findings are 'enlarged gall bladder' or 'dilatation of the gall bladder'. Although 'aplasia of the gall bladder' does not specifically appear in the lists of historical control data, it's relevance can be assessed together with the other gall bladder findings, as they are all considered to be of the same ontogenic origin. All of them are considered to be developmental variations, mostly spontaneous in nature and of little or no toxicological concern.

One or two small gall bladders were found in all treated groups. However the incidence was well within the historical range and demonstrates no dose relationship, historical database of 2562 fetuses and 456 litters (fetal % incidence/litter % incidence ranges: 0.0-2.4%/0.0-11.1%).

Fetal Skeletal Examination

Table B.6.47: Foetal skeletal observations (fetal % incidence/litter % incidence)

Finding	**Dose level (mg/kg bw/day) fetal % incidence (litter % incidence)**				
	0	**10**	**50**	**250**	**500**
Total fetuses examined (litters examined)	116(19)	130(18)	90(16)	97(17)	97(18)
SKELETAL MALFORMATIONS					
Reduced interparietal bone		0.8 (5.6)	1.1 (6.3)		
Reduced parietal bone		0.8 (5.6)			
Reduced frontal bone		0.8 (5.6)			
Reduced nasal bone		0.8 (5.6)			
Fore limb - absent ossification ulna				0.8 (5.9)	
Fore paw - adactyly				0.8 (5.6)	
Pelvic girdle - absent ossification pubis					1.0 (5.6)
Total skeletal malformations	*0.0 (0.0)*	*0.8 (5.6)*	*1.1 (6.3)*	*1.0 (5.9)*	*1.0 (5.6)*
SKELETAL ANOMALIES[#] Historical control data from 2562 fetuses/455 litters with Russian Chbb:HM rabbits (20 studies, 1989-1995)##					
Asymmetrically shaped sternebra 1 0.0-2.3 (0.0-13.3)##		0.8 (5.6)	1.1 (6.3)	2.1 (5.9)	3.1 (5.6)
Fused sternebra 2 and 3 0.0-5.7 (0.0-20.0)##	0.9 (5.3)	0.8 (5.6)	1.1 (6.3)	4.1 (23.5)	4.1 (22.2)
Asymmetrically shaped sternebra 2 0.0-4.1 (0.0-13.3)		0.8 (5.6)	1.1 (6.3)	2.1 (11.8)	4.1 (16.7)
Fused sternebra 3 and 4 0.0-9.2 (0.0-33.3)##	1.7 (10.5)	1.5 (5.6)	1.1 (6.3)	5.2 (23.5)	10.3* (33.3)
Asymmetrically shaped sternebra 3 0.0-2.7 (0.0-10.5)##		0.8 (5.6)		2.1 (11.8)	3.1 (16.7)
Fused sternebra 4 and 5 0.0-8.0 (0.0-29.4)##	3.4 (21.1)	1.5 (11.1)	4.4 (25.0)	7.2 (35.3)	8.2 (33.3)
Asymmetrically shaped sternebra 4 0.0-3.2 (0.0-17.6)		0.8 (5.6)		4.1 (23.5)	2.1 (11.1)

Total skeletal anomalies	*10.3 (42.1)*	*6.9 (38.9)*	*7.8 (37.5)*	*21.6 (70.6)*	*21.6 (50)*
SKELETAL VARIATIONS **Historical control data from 2562 fetuses/455 litters with Russian Chbb:HM rabbits (20 studies, 1989-1995)##**					
1st sternebra, poor ossification 0.0-1.6 (0.0-11.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	1.0 (5.9)	0.0 (0.0)
5th sternebra, poor ossification 36.0-62.9 (64.7-100)##	62.9 (94.7)	58.5 (100.0)	51.1 (81.3)	55.7 (94.1)	48.5 (94.4)
5th sternebra, absent ossification 0.0-18.4 (0.0-53.3)##	5.2 (26.3)	12.3 (38.9)	7.8 (37.5)	11.3 (35.3)	13.4 (33.3)
6th sternebra, poor ossification 0.0-2.3 (0.0-13.3)##	0.0 (0.0)	0.8 (5.6)	2.2 (12.5)	2.1 (11.8)	1.0 (5.6)
Sutural Bone(s) 0.0-7.5 (0.0-36.8)	0.0 (0.0)	0.8 (5.6)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Slot in parietal bone 0.0-5.0 (0.0- 27.8)##	4.3 (26.3)	7.7 (38.9)	4.4 (25.0)	3.1 (11.8)	8.2 (38.9)
Small hole in parietal bone 0.0-5.3 (0.0-29.4)	0.0 (0.0)	2.3 (16.7)	1.1 (6.3)	1.0 (5.9)	3.1 (16.7)
1st metacarpal, absent ossification 0.0- 0.9 (0.0-5.9)	0.0 (0.0)	0.0 (0.0)	1.1 (6.3)	0.0 (0.0)	1.0 (5.6)
Caudal vertebral center, absent ossification 5.5- 36.0 (20.0- 78.6)##	16.4 (47.4)	26.9 (66.7)	22.2 (43.8)	27.8 (76.5)	28.9 (61.1)
Caudal vertebral center, poor ossification 3.2- 27.7 (17.6-76.5)	6.9 (21.1)	3.8 (16.7)	18.9* (56.3)	10.3 (47.1)	13.4 (50.0)
Additional caudal vertebral center 0.0- 27.5 (0.0-68.4)##	25.9 (68.4)	10.8** (50.0)	17.8 (50.0)	6.2** (29.4)	8.2** (27.8)
Additional (13th) rib(s) 0.0- 7.5 (0.0- 27.8)##	0.9 (5.3)	0.0 (0.0)	4.4 (18.8)	2.1 (11.8)	3.1 (11.1)
Medial phalanx, anterior digit-5 poor ossification 0.0- 63.8 (0.0-94.7)##	1.7 (5.3)	0.8 (5.6)	1.1 (6.3)	3.1 (17.6)	4.1 (22.2)
Total skeletal variations	*84.5 (100)*	*82.3 (100)*	*82.2 (100)*	*82.5 (94.1)*	*79.4 (94.4)*

Only anomalies with an apparent treatment related increase in foetal incidence/litter incidence given in table.
Corrected historical control values based on revised HCD.

Skeletal malformations were observed in a low dose fetus (reduced interparietal, parietal, frontal and nasal bones), a 50 mg/kg bw/day fetus (reduced interparietal bone), a 250 mg/kg bw/day fetus (No. 59/3 – see above, absent ossification of ulna), and a high dose fetus (absent ossification of pubis). All these malformations were considered to be spontaneous and not related to dosing with trifloxystrobin.

Skeletal anomalies observed in the fetuses consisted mainly of fused, fragmented or asymmetric sternebrae, irregular ossification of scapula, and displaced cervical and caudal vertebral centers. The incidence of fused sternebrae and asymmetrically shaped sternebrae was slightly increased in the two higher dose groups. For some anomalies the incidence was outside the control range. Statistical significance was reached only for the occurrence of fused sternebrae 3 and 4 in the high dose group, the incidence of this finding was also outside the historical control range; historical database of 2562 fetuses and 455 litters (fetal % incidence/litter % incidence ranges: 0.0-9.2%/0.0-33.3%).

The fetal incidence of fused sternebrae-3 and -4 was slightly higher in the 500 mg/kg group than the controls and was regarded as treatment-related.

Skeletal variations occurred in about two thirds of fetuses from almost all litters in all dose groups. They consisted mainly of poor or absent ossification of sternebra-l, -5, -6, cranial findings (sutural bones, slot or hole in parietal bone), absent ossification of metacarpal-l, tail bone variations (poor or absent ossification of or additional caudal vertebral centers), additional ribs, and poor ossification of the medial phalanx of anterior digit-5. Poor ossification of the caudal vertebral centers showed statistically significant higher values for the low-mid dose group when compared to controls. However, since there was no dose-relationship and since this value was within the historical control values historical database of 2562 fetuses and 455 litters (fetal % incidence/litter % incidence ranges: 3.2-27.7%/17.6-76.5%), therefore it was considered not to be treatment-related.

In conclusion, maternal toxicity was evident at ≥ 250 mg/kg bw/day based on effects on bodyweight and food consumption. There was apparent increased incidence of skeletal anomalies at ≥ 250 mg/kg bw/day although statistical significance was only achieved at the top dose. Based on these effects the NOAEL for maternal and developmental toxicity was 50 mg/kg bw/day. There was no evidence of a teratogenic potential.

3.10.2 Human data

No data are available

3.10.3 Other data (e.g. studies on mechanism of action)

Studies on lactating ruminants:

[Study 1] The metabolism of [trifluormethyl-phenyl(U)-^{14}C] CGA 279202 after multiple oral administration to lactating goats (Anonymous, 1997a, M-034501-01-1)

Report:	KCA 6.2.3/01; M-034501-01-1
Title:	The metabolism of [trifluormethyl-phenyl(U)-^{14}C] CGA 279202 after multiple oral administration to lactating goats
Report No.:	09/97
Document No.:	M-034501-01-1
Guideline(s):	Residue Chemistry Test Guidelines, OPPTS 860.1300, Nature of the Residue - Plants, Livestock EPA, Washington, August 1996 Commission of the European Communities Working Document 7030/VI/95 - Rev.2, 6/1/1997 Appendix F: Metabolism and Distribution in Domestic Animals
Guideline deviation(s):	none
GLP/GEP:	**yes**

Summary

The metabolism of [trifluoromethyl-phenyl-UL-^{14}C]trifloxystrobin in two lactating goats was investigated after oral administration of the radiolabelled test substance at a dose rate 4.24 mg/kg bw/day for four consecutive days (one dose per day). This dose rate corresponded to 103.8 mg/kg in the diet. Milk was collected twice daily and excreta were collected on daily basis. The goats were slaughtered 6 hours after the last dose (78 hours after the first dose) and leg muscle, tenderloin, omental and perirenal fat, kidney and liver as well as blood, bile and the gastrointestinal tract were dissected. Pooled milk samples and pooled tissues were analysed for the radioactive residues.

During the whole test period 0.077, 44.46 and 17.45% of the dose totally applied were eliminated via milk, faeces and urine, respectively. These values were mean data of the respective samples from both goats. In total less than 1% of the administered dose was recovered in the milk and tissues. Summing up all radioactive residues in milk, excreta, organs and tissues and the gastro-intestinal tract, the sum resulted in a total recovery of 88.14% of the dose. The rest is assumed to be in the carcass that was not analysed.

The highest total radioactive residues (TRR) in milk were measured after an interval of 48-72 h amounting to 0.121 mg eq/kg; however, since the last milk sample was collected after an interval of 78 hours, it is not possible to determine definitively whether or not this was the plateau.

TRR in tissues amounted to 4.815 mg eq/kg in liver, 1.830 mg eq/kg in kidneys, 0.058 mg eq/kg in muscle and 0.191 mg eq/kg in fat. The average residues in milk were 0.085 mg eq/kg (pooled over the whole study period 0 – 78 hours).

The extractability of the milk and tissue samples by conventional extraction at room temperature was high (≥ 85% of the respective TRR) with the exception of liver (66.5% of TRR). Microwave assisted extraction at elevated temperatures (≤ 180°C) released additional radioactive residues and thus increasing the extractability to 100%.

Identification of extracted trifloxystrobin residues was conducted by one- and two-dimensional radio-TLC and radio-HPLC with reference compounds co-chromatographed, as well as by additional spectroscopic methods (MS and NMR).

The parent substance trifloxystrobin was found as the main residue component in fat (79% of TRR) and pooled milk (51.6% of TRR).

CGA 321113 (M5) formed by ester hydrolysis of trifloxystrobin was the main residue component in muscle (57.2% of TRR) and kidney (54.3% of TRR). The taurine and glycine conjugates of CGA 321113 were also major metabolites in liver (27.8% and 10.7% of TRR, respectively) and the taurine conjugate was major in milk (13.0% of TRR in the milk pool). Eight other identified metabolites were minor and did not exceed 5% of TRR in edible matrices.

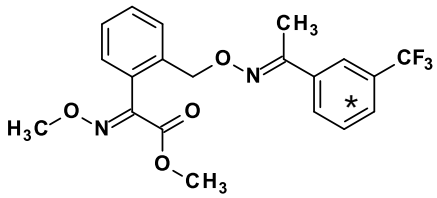
Based on the structures identified the metabolism of trifloxystrobin in lactating goats proceeded predominantly via hydrolysis, demethylation and hydroxylation reactions followed by conjugation with taurine and glycine:

- Deposition of intact trifloxystrobin as the major residue component in fat and milk.
- Hydrolysis of the methyl ester group trifloxystrobin to CGA 321113 as the predominant residue component in muscle and kidney and as major in metabolite urine and faeces.
- Conjugation of CGA 321113 with taurine to metabolite L7a as the major metabolite in liver and milk or conjugation with glycine to the minor metabolite L7b.
- Conjugation of metabolite NOA 405637 (2F, M27) with glucuronic acid to metabolite L5 (Met 1G).
- Hydroxylation at the aminooxymethyl group of metabolite CGA 321113 (M5) to metabolite NOA 443152 (2U, M10) and subsequent demethylation to metabolite 6U (M23).
- Hydroxylation of the glyoxyl-phenyl ring in position 4 yielding trace amounts of metabolite 7F.
- Demethylation of the methoxyimino group of trifloxystrobin to metabolites NOA 405637 (2F, M27), NOA 412443 (1U) and 6U (M23).
- As a minor reaction breakdown of the molecule between the two phenyl rings to Met U8 (M58) and sulfate conjugation to metabolite 11U (M59) or further oxidations to metabolite CGA 354870 (12U, M60).
- Comparison of metabolites in goats and rat indicated as similar metabolite pattern in both species. All major metabolites identified in goat were also described in rat and the general pathways in goat were similar to those in the rat.
- The parent substance trifloxystrobin and its hydrolyzed metabolite CGA 321113 (M5) can serve as analytical targets for a residue method in food of animal origin since these two components contributed mainly to the total residues.

I. Materials and Methods

A. Materials

1. Test Material

IUPAC Name	Methyl (2E)-(methoxyimino)[2-({[(1E)-{1-[3-(trifluoromethyl) phenyl]ethylidene}amino]oxy}methyl)phenyl]acetate (accord. to software ACD, ver.12.2) Methoxyimino-{2-[l-(3-trifluoromethyl-phenyl)-ethylidene-aminooxymethyl]-phenyl}-acetic acid methyl ester
Code name	CGA 279202
Common name	Trifloxystrobin
Empirical formula	$C_{20}H_{19}F_3N_2O_4$
CAS Number	141517-21-7
Molar mass, non-labelled	408.4 g/mol
Chemical structure	 * position of the radiolabel
Radiolabelled test material	[trifluoromethyl-phenyl-UL-^{14}C] trifloxystrobin
Batch number	Mo-65.3B-1
Specific radioactivity used for administration (after radiodilution)	577 kBq/mg = 15.6 µCi/mg
Original radiochemical purity	98.7% (stock solution applied to the gelatine capsules) (radio-TLC)
Dose level	4.24 mg/kg bw equivalent to 103.8 mg/kg in the diet 1 capsule per day to each goat for four consecutive days;
Vehicle	gelatine capsules containing D-(+)-lactose monohydrate
Stability of the test compound	The radiochemical purity of the stock solution and in an acetonitrile/water extract of the dosing capsules was 98.9% at the start of the dosing and 97.8% after the last administration. Thus the test material remained stable on the carrier during the administration phase.

2. Test Animals

Species	Goat
Strain	*Gemsfarbige Gebirgsziege*
Breeding facility	[redacted]
Sex and numbers involved	2 female animals
Age	2 years and 4 months; 1 year and 3 months
Body weight	51.68 and 35.06 kg (at the first day of treatment), 49.00 and 34.10 kg (at the test end)
Acclimatisation	7 days before first treatment in their respective cages.
Identification	Ear tag
Housing	Metabolic cages with artificial light and 12/12 hours day/night cycle Temperature and relative humidity were measured throughout the study (temperature: 18-24°C; relative humidity: 55-97%)
Feed and water	Controlled daily diet containing 600 g concentrate (UFA Nr. 867), 1000 g maize cubes and 100 g hay which provided in two portions (850 g per portion). The amount of refused feed was weighed and recorded in order to calculate the daily feed intake. Tap water was provided *ad libitum* in automatic drinkers.

B. Study Design

Dosing

The radiolabelled test compound with an original specific radioactivity of 2.19 MBq/mg (59.2 µCi/mg) was diluted with the non-labelled test compound in acetonitrile solution to achieve a specific radioactivity of 577 kBq/mg for the metabolism study. Aliquots the resulting stock solution containing 170 mg trifloxystrobin each were applied to gelatine capsules which contained D-(+)-lactose monohydrate. After evaporation of the solvent the capsules were sealed.

Two lactating goats were orally dosed with one gelatine capsule per goat and day for four consecutive days. The resulting dose rate was 4.24 mg a.s./kg bw/day, at mean, corresponding to a daily mean dose of 103.8 mg/kg feed. The actual dose rates amounted to 3.48 and 5.00 mg/kg bw/day due to the different body weights and daily feed consumptions.

The purity and stability of the test substance were determined by TLC in the stock solution and in an acetonitrile/water extract of remaining capsules at the first and after the last administration.

Sample collection

Sampling of urine, faeces, cage wash and milk

Samples of urine, faeces and cage washes (ethanol/water, 1:1, v/v) were collected daily in the morning for analysis from the day before the first administration until slaughter in 24 hour intervals and stored frozen.

Each goat was milked twice daily (morning and evening). Milk samples were collected from day 7 of the adaptation period onwards and stored at 4°C. The last milking took place just before slaughter. Morning and evening milk were kept separately.

Sacrifice and dissection of organs and tissues

The animals were sacrificed approximately 6 hours after the last dose (= 78 hours after the first dose). The goats were stunned with a stunning bolt and immediately exsanguinated by severing the major neck vessels. Blood was collected under addition of heparin and was stored refrigerated until shipment to the analytical laboratory on the next day.

Tissue samples dissected were leg muscle, tenderloin, omental and perirenal fat, kidney and liver. Bile and the gastrointestinal tract were also sampled. Leg muscle, tenderloin, omental and perirenal fat as well as contents of the gastrointestinal tract were homogenized and stored refrigerated until shipment to the analysing laboratory the same day. The other specimens (kidney, liver, bile) were stored intact and refrigerated until shipment to the analysing laboratory on the same day.

Sample preparation

Faeces and rumen content

Faeces were homogenized by mixing in the collection box using a rod. An aliquot was weighed and homogenized in a Cutter Cut-o-Mat with a few strokes at reduced speed in the beginning and thorough homogenization afterwards. Variable amounts of dry ice were added to obtain a “dry” homogenate. In the laboratory samples of frozen faeces were homogenized again using a Moulinette SE cutter. Three aliquots were analyzed by combustion and radioassaying.

The content of the gastrointestinal tract was thoroughly homogenised by using a rod that was agitated manually. An aliquot was weighed into a box and kept refrigerated. In the laboratory samples of rumen content were homogenized in a Moulinette SE cutter. Three aliquots were analyzed by combustion and radioassaying. Aliquots of faeces and urine from both animals were combined for each interval prior to extraction. The aliquots were based on equal percentages of the total weight of the samples.

Milk

Aliquots of milk from both animals were combined for each interval prior to extraction. The aliquots were based on equal percentages of the total weight of the samples.

Organ and tissue samples
Muscle and fat samples were cut into pieces. Aliquots of leg muscle homogenates representing the total sample together with the entire homogenates of tenderloin, omental fat and perirenal fat were stored refrigerated until transport to the analytical laboratory on the same day.

In the analytical laboratory samples of tissues were cut into smaller pieces with a knife and frozen in liquid nitrogen and homogenized in a Moulinette SE cutter. Three aliquots of each sample were analyzed for the residue content by radioassaying after digestion with tissue solubilizer via LSC. A pool of muscle samples was prepared by mixing corresponding aliquots of forequarter, hindquarter and tenderloin. Aliquots of omental and perirenal fat were pooled prior to extraction. The aliquots were based on equal percentages of the total weight of the samples.

Extraction and extract analysis
Individual extraction procedures for the various sample materials are described below. Analysis of the obtained extracts was performed by one- and two-dimensional (2D-)radio-TLC on silica gel or RP18 plates and radio-RP18-HPLC. References for co-chromatography were either obtained from metabolites which were isolated from the liver and which were identified by LC-MS and NMR or from identified metabolites in other metabolism studies with ^{14}C-trifloxystrobin.

Milk (Table B.7.2.3-3)
Milk was homogenized with acetonitrile by shaking. The mixture was decanted and the precipitate was extracted twice with acetonitrile/water (4:1, v/v). The volume of the combined extracts was reduced before partitioning with hexane (4x). The combined hexane phase was reduced in volume and purified by preparative TLC. The resulting fractions were analyzed and quantified by 2D-TLC or HPLC.

The polar acetonitrile phase obtained after hexane partitioning and re-extraction with acetonitrile was cleaned up using RP-18 SPE and eluted with water, methanol and hexane. The methanolic fraction was analyzed by 2D-TLC and HPLC.

Tissues (Table B.7.2.3-4 - Table B.7.2.3-7)
Muscle
The homogenized sample of muscle was extracted with acetonitrile by stirring at room temperature. After filtration the residues were extracted three times with acetonitrile/water (4:1, v/v) followed by extraction with methanol/water (4:1, v/v). The extracts were combined and the volume was reduced, followed by partitioning with hexane (4 times). The hexane phases were individually re-extracted with acetonitrile.

The polar fraction was purified by RP18-SPE, eluted with water, methanol and hexane. The methanolic fraction was analyzed by HPLC and 2D-TLC. The hexane fraction was purified by preparative TLC and the resulting four radioactive zones were eluted with methanol and analyzed by 2D-TLC and/or HPLC.

Fat
Fat was dissolved in n-hexane by slightly heating in a water bath. The solution was decanted and partitioned with acetonitrile. The acetonitrile phases were combined and concentrated. An aliquot of this fraction was fractionated using RP-18 SPE and eluted with water, methanol and n-hexane.

The water fraction was analyzed by 2D-TLC. The methanolic fraction was purified by preparative Si60-TLC. The resulting three radioactive zones were eluted with methanol. Two fractions were analyzed further by HPLC and one of them additionally by 2D-TLC. The hexane fraction from the RP-18-SPE fractionation was also separated by preparative TLC into three zones which were eluted with methanol and analysed by 2D-TLC and HPLC.

Liver
The homogenized sample of liver was extracted conventionally with acetonitrile at room temperature. After centrifugation the remaining solids were extracted with acetonitrile/water (4:1, v/v, 4x) followed by an extraction with methanol/water (4:1, v/v, 1x). The combined acetonitrile fraction was reduced in volume and partitioned four times with n-hexane. The hexane phases were individually re-extracted with acetonitrile.

The polar acetonitrile phase was concentrated and fractionated by RP18-SPE. The RP18 cartridge was eluted stepwise with water, methanol and hexane. The methanol fraction was analyzed by HPLC and 2D-TLC.

The n-hexane phase obtained after partitioning and re-extraction with acetonitrile was separated by preparative TLC into three radioactive zones. These zones were eluted with methanol and analyzed by 2D-TLC and/or HPLC.

Non-extractable residues after conventional extraction of liver were exhaustively extracted with 2-propanol/H_2O (4:1, v/v) by microwave assistance under nitrogen. The temperature was increased in three steps from 100 to 150°C. The solution was decanted from the remaining residues and reduced in volume followed by RP18 SPE clean-up. The RP18 cartridge was stepwise eluted with mater and methanol. The methanolic phase fraction was analyzed by 2D-TLC.

Another aliquot of conventionally extracted liver homogenate was also extracted with 2-propanol/ H_2O (4:1, v/v) by microwave assistance at temperatures up to 180°C. The extract was purified on a R18-SPE cartridge, which was eluted with water, methanol and methylene chloride. The methanolic fraction was analyzed by HPLC.

For preparation of radioactive liver metabolites used as reference substances a larger amount of liver was extracted with acetonitrile and acetonitrile/water (4/1, v/v). The extract was concentrated in a rotary evaporator and diluted with 0.1N sodium phosphate buffer (pH 7). The resulting solution was applied to C18-flash chromatography. The C18 column that was stepwise eluted with 0.02N phosphate buffer (pH 7), and various solutions of phosphate buffer/methanol (9/1, 7/3 and 1/1). The lastly eluted fraction was separated on a semi-preparative C18 column with a water/methanol gradient. One fraction was further purified on the same column with a water/methanol gradient adjusted to pH 3 with formic acid. Two resulting fractions (L7a and L7b) were purified by HPLC (operated by an acetonitrile/water gradient) and identified by MS and NMR.

Metabolite L7a was identified as taurine conjugate of CGA 321113 (M5). The carboxylic OH group is replaced by –NH-CH_2-CH_2-SO_3H. Metabolite L7b was identified as glycine conjugate of CGA 321113. The carboxylic OH group is replaced here by –NH-CH_2-COOH.

Kidney

The homogenized sample of kidneys was extracted with acetonitrile at room temperature. After centrifugation the sample was additionally extracted four times with acetonitrile/water (4:1, v/v) followed by an extraction with methanol/water (4:1, v/v). The combined acetonitrile extract was reduced and partitioned with n-hexane. The hexane phase was re-extracted with acetonitrile.

The polar acetonitrile phase was reduced in volume and an aliquot was fractionated using a RP18-SPE cartridge which was eluted with water, methanol and hexane. The methanolic fraction was analyzed by HPLC and 2-D TLC.

The hexane phase obtained after partitioning and re-extraction with acetonitrile was separated by preparative TLC into five radioactive zones which were eluted with methanol. The fractions were analyzed by 2D-TLC and/or HPLC.

Excreta

Faeces: The pooled faeces sample was extracted with methanol followed by extraction with methanol/water (4:1, v/v, 2x) at room temperature. After each extraction step the homogenate was centrifuged. The supernatant solutions were combined and the volume was reduced. The extract was analyzed directly by 2D-TLC and HPLC.

Urine: The pooled urine sample was directly analyzed by 2D-TLC and HPLC.

Analytical methods

The radioactivity of all samples and fractions were measured by radioassaying via LSC with automatic quench correction. Liquid samples were directly measured. Solid samples were combusted and the formed $^{14}CO_2$ collected in a basic liquid scintillator before LSC measurement. The LOQ of LSC measurement depended on the different matrices. It ranged from 0.0004 (milk) to 0.0028 (kidney) mg eq/kg.

Radio-HPLC was performed using RP18 columns (250 x 4.6 mm, particle size 5 µm) that were operated with various gradient systems of pH 7-buffer/methanol. The HPLC system was equipped with UV detector and a radiomonitor with a 400 µL scintillator cell.

Radio-TLC was performed using precoated silica gel 60 F_{254} and RP18 $F_{254}S$ plates. The plates were developed with several different solvent mixtures (for silica gel plates) or pH 7-buffer/acetonitrile (RP18 plates) after chamber saturation. Two-dimensional silica gel TLC plates were developed with two different solvent mixtures in rectangular directions. Radioactive spots were detected by radioluminiscence (bioimaging) analysing, i.e. by exposure of the TLC plates to phosphor imaging plates and scanning of the imaging plates thereafter. For quantitative radioactivity determination the respective spots/zones were scraped off, added to methanol and a scintillation cocktail and radioassayed by LSC. Non-radioactive reference compounds were visualized by fluorescence quenching under UV light (254 nm). Due to the different R_f values of the reference compounds in the different TLC systems a clear identification of the radiopeaks via co-chromatography was possible.

MS identification of metabolites was performed by combination with HPLC. Ionisation was conducted by atmospheric pressure chemical ionization (APCI) in the positive mode. For LC-MS a RP18 column (10 cm x 2.0 mm i.d., particle size 3 µm) was operated with a gradient mixture of water and acetonitrile. A radiomonitor with a 50 µL measuring cell was also involved.

NMR spectra for structure elucidation were conducted using a 500 MHz NMR spectrometer in deuterated acetonitrile.

Storage stability of samples

During the study, all homogenized samples were stored at -20°C prior to extraction and analysis. The storage stability of residues during the analytical period was investigated in milk and liver (chosen as representative matrices).

The storage stability of residues in liver was determined by comparison of metabolite pattern of fresh liver extract with that of the same extract stored at -20°C for about 7 months and with that obtained from liver stored at -20°C and extracted after about 4 months and after about 23 months (at the end of the analytical phase). There was no difference in extractability and the composition of the metabolite patterns did not change significantly during the storage of whole liver or during storage of liver extracts.

To determine the storage stability of residues in milk, the metabolite pattern of the unpolar phase and the polar phase of fresh milk extract was compared with that of milk stored at -20°C and extracted after about 4 months and of milk stored and extracted at the end of experimental phase. Comparison of the overall patterns showed no significant change of the extractability or the metabolite composition during storage of milk or milk extracts.

Therefore, the results of the study are considered not to be affected by the length and conditions of storage of the samples analyzed.

II. Results and Discussion

Test design and animal health

Two lactating goats received a daily dose of 170.41 mg [trifluoromethyl-phenyl-UL-^{14}C] trifloxystrobin for four consecutive days. Based on their body weight and the daily feeding rate this dose represented a mean intake of 4.24 mg/kg bw/day or 103.8 mg/kg feed/day. The animals were sacrificed 6 hours after the last dose. No unusual appearance or behaviour of the animals was observed.

Elimination and recovery of radioactivity (Table B.7.2.3-1)

During this test period, 0.077% of the total dose was eliminated via milk and 44.46% via faeces and 17.45% via urine. In total, less than 1% of the dose was detected in edible organs and tissues. These values were mean data of the respective samples from both goats. Summing up all radioactive residues in milk, excreta,

organs and tissues and the gastro-intestinal tract, the sum resulted in a total recovery of 88.14% of the dose. The rest is assumed to be in the carcass that was not analysed.

Total radioactive residues in milk, tissues and organs and excreta

The total radioactive residues (TRR) recovered in milk, excreta and tissues were measured individually for each goat, however presented in Table B.7.2.3-1 as mean values together with the standard deviation.

After sacrifice, TRR amounted to 4.815 mg eq/kg in liver, 1.830 mg eq/kg in kidney, 0.058 mg eq/kg in muscle and 0.191 mg eq/kg in fat (Table B.7.2.3-1). All TRR values are the mean levels of sample pools from both goats and weighted based on the individual body weight. In relation to the total dose administered, the TRR values in liver and kidney corresponded to 0.539% and 0.031%, respectively, and in muscle and fat to 0.038% and 0.079%, respectively.

Table B.7.2.3-1: Distribution of residues in milk, excreta and tissues of two lactating goats following oral administration of [trifluoromethyl-phenyl-UL-^{14}C]trifloxystrobin at a mean dose rate of 4.24 mg/kg bw/day for four consecutive days.

Matrix (Pooled sample from both goats)	**Sampling time [h]**	**% of total dose administered**	**TRR of sample pool [mg eq/kg]****
		Mean*	**Mean***
Total Milk	**0-78**	**0.077**	**0.085**
Total Faeces	**0-78**	**44.457**	-
Total Urine	**0-78**	**17.449**	-
Cage Wash	0-78	0.466	-
Cage Debris	0-78	0.005	-
Total Eliminated	**0-78**	**62.455**	-
Leg Muscle	78	0.036	0.058
Tenderloin	78	0.002	0.059
Total Muscle	*78*	*0.038*	*0.058*
Omental Fat	78	0.050	0.182
Perirenal Fat	78	0.029	0.209
Total Fat	*78*	*0.079*	*0.191*
Kidneys	78	0.031	1.830
Liver	78	0.539	4.815
Total Tissue	**78**	**0.687**	-
Blood	78	0.063	0.248
Bile	78	0.170	71.315
GIT/Rumen	78	24.762	-
Total Recovery*	**0-78**	**88.138**	-

* weighted mean value of the pooled samples from both animals based on their proportion to the body weight
** [mg eq/kg] = mg/kg expressed as parent equivalents

Time course of total radioactive residues in milk

The highest residues were found in milk 48 – 72 h after first administration with maximum TRR of 0.121 mg eq/kg (Table B.7.2.3-2); however, since the last milk sample was collected after an interval of 78 hours, it is not possible to determine definitively whether or not this was the plateau. In general, radioactive residues were excreted rapidly into milk. The TRR in milk peaked out 6-7 h after each administration.

Table B.7.2.3-2: Time course of TRR in milk following oral administration [trifluoromethyl-phenyl-UL-^{14}C]trifloxystrobin to two goats at a mean daily dose rate of 4.24 mg/kg bw for four consecutive days

Interval [h]	% of total dose administered Mean*	TRR [mg eq/kg]** Mean*
0-7	0.007	0.069
7-24	0.012	0.063
0-24	*0.018*	*0.065*
24-31	0.009	0.087
31-48	0.016	0.086
24-48	*0.025*	*0.087*
48-55	0.012	**0.121**
55-72	0.016	0.087
48-72	*0.027*	*0.099*
72-78	0.007	0.101
0-78	**0.077**	**0.085**

* weighted mean value of the pooled samples from both animals based on the amount of individual samples
** [mg eq/kg] = mg/kg expressed as parent equivalents

Extraction efficiency of residues

Samples of milk and tissues (muscle, kidney, liver) were extracted with acetonitrile and with acetonitrile/water (4:1, v/v) (2-4 times). With exception of milk, an additional extraction followed with methanol/water (4:1, v/v). Fat was extracted with hexane, faeces with methanol followed by methanol/water (4:1, v/v) (2 times). Details of extraction and fractionation of radioactive residues are given in the extraction schemes for milk (Table B.7.2.3-3), for muscle (Table B.7.2.3-4) and for fat (Table B.7.2.3-5), liver (Table B.7.2.3-6) and kidney (Table B.7.2.3-7).

The extractability of muscles, fat and kidneys after conventional extraction amounted to 90.3 – 93.8% of TRR, to 95.5% of TRR for milk and to 89.5% of the TRR for faeces. Conventional extraction of liver with acetonitrile and acetonitrile/water (4:1, v/v, 4x) and methanol/water (4:1, v/v) liberated 66.5% of TRR. However, microwave assisted extraction at temperatures up to 150 – 180°C released additional 28.5 – 33.5% of TRR and consequently released the radioactive residues completely.

Non-extractable residues amounted to 0.0038 mg eq/kg (4.5% of TRR) for milk, 0.0056 mg eq/kg (9.7%) for total muscle, 0.0126 mg eq/kg (6.6%) for total fat, 0.2408 mg eq/kg (5.0%) for liver and 0.1135 mg eq/kg (6.2%) for kidney and 10.5% for faeces (Table B.7.2.3-8 – Table B.7.2.3-10).

Distribution of parent compound and metabolites in organs and tissues, milk and excreta

The distribution of trifloxystrobin residues in urine, faeces, milk, muscle, fat, kidney, and liver is summarized in Table B.7.2.3-8 to Table B.7.2.3-10. Chemical names and structural formulae of the identified metabolites can be found in the "List of compounds identified" at the end of this study summary.

Metabolites in milk (Table B.7.2.3-9)
Overall 95.5% of TRR in milk (TRR: 0.085 mg eq/kg in the 0 – 78 hours pool) were extracted conventionally with acetonitrile and water.

The parent compound trifloxystrobin was the main residue component and accounted for 51.6% of TRR (0.0439 mg eq/kg). In addition, the taurine conjugate of metabolite CGA 321113 (Met L7a) was identified as major metabolite representing 13.0% of TRR (corresponding to 0.011 mg eq/kg).

Minor metabolites were identified as CGA 321113 (M5), CGA 354870 (M60, Met 12U), Met 6U (M23), NOA 443152 (M10, Met 2U), and NOA 412443 (M29, Met 1U): They accounted for 0.9 – 4.0% of the residues in milk (<0.01 mg eq/kg). Two unknown metabolites were also detected at trace levels (≤ 0.01 mg eq/kg).

Metabolites in muscle (leg and tenderloin) (Table B.7.2.3-9)
Overall 90.3% of the TRR (TRR: 0.058 mg eq/kg) were extracted by conventional extraction using acetonitrile followed by acetonitrile/water (4:1, v/v, 3x) and by methanol/water (4:1, v/v, 1x).

The major residue components in muscle were identified as acid metabolite CGA 321113 (M5), accounting for 57.2% of TRR (0.0332 mg eq/kg) and parent compound accounting for 20.6% of TRR (0.012 mg/kg).

Minor metabolites were identified as NOA 443152 (M10, Met 2U), CGA 354870 (M60, Met 12U) and NOA 412443 (M29, Met 1U) amounting to 0.3 – 2.0% of TRR (< 0.01 mg eq/kg). In addition, two conjugates of CGA 321113, i.e. the taurine conjugate (L7a) and the glycine conjugate (L7b) were both present at 1.2% (0.0007 mg eq/kg). Met U8 (M58) was found at a trace level of 0.3% of TRR (0.0002 mg eq/kg). Three unknown metabolites were detected also a trace amounts (≤ 1.7% of TRR, ≤ 0.001 mg eq/kg).

Metabolites in fat (omental and perineal) (Table B.7.2.3-9)
Overall 93.4% of TRR in fat (TRR: 0.191 mg eq/kg) was extracted with hexane. The primary extract was partitioned with acetonitrile. 92.0% of TRR partitioned into acetonitrile.

Trifloxystrobin was the predominant residue component in fat and accounted for 79.0% of TRR (0.1509 mg eq/kg). Besides parent compound acid metabolite CGA 321113 (M5) was identified as a major metabolite accounting for 10.4% of TRR (0.0198 mg eq/kg).

Three additional minor metabolites were identified as CGA 354870 (M60, Met 12U), NOA 443152 (M10, Met 2U) and NOA 412443 (M29, Met 1U). They accounted for 0.4 – 0.7% of TRR (0.0008 – 0.0013 mg eq/kg).

Metabolites in kidney (Table B.7.2.3-10)
Overall 93.8% of TRR in kidney (TRR: 1.830 mg eq/kg) was extracted by conventional extraction with acetonitrile followed by acetonitrile/water (4:1, v/v, 2x) and finally 0.7% of TRR with methanol/water (4:1, v/v, 1x).

The parent substance trifloxystrobin contributed only 1.8% of TRR (0.0337 mg/kg). The main component was identified as acid metabolite CGA 321113 (M5) accounting for 54.3% of TRR (0.9930 mg eq/kg). The taurine conjugate of CGA 321113 (Met L7a) was identifies as a major metabolite which accounted for 12.7% of TRR (0.2328 mg eq/kg); the corresponding glycine conjugate (Met L7b) was found at 5.2% TRR (0.0950 mg eq/kg).

Several minor metabolites were identified as NOA 443152 (M10, Met 2U, NOA 412443 (M29, Met 1U), CGA 300624 (M59, Met 11U), Met U8 (M58) accounting for 0.3 – 3.1% of TRR (0.0054 – 0.0565 mg eq/kg).

Metabolites in liver (Table B.7.2.3-10)
Conventional extraction with acetonitrile and acetonitrile/water (4:1, v/v, 4x) released 65.0% of TRR from liver (TRR: 4.815 mg eq/kg). An additional extraction step with methanol/water liberated only minor amounts (1.5% of the TRR) of the remaining radioactivity.

Subsequent exhaustive extraction with 2-propanol/water (4/1) using microwave assistance released additionally 28.5% of TRR at 150°C or 33.5% of TRR at 180°C, resulting in total extractable residues of 95.0 – 100% of TRR.

The parent substance trifloxystrobin contributed only 2.8% of TRR (0.1359 mg/kg). The major components identified were acid metabolite CGA 321113 (M5) amounting to 13.0% of TRR (0.6260 mg eq/kg) and its two conjugates, the taurine conjugate (Met L7a) and glycine conjugate (Met L7b) accounting for 27.8 and 10.7% of TRR (1.3366 and 0.5152 mg eq/kg), respectively.

Minor metabolites were identified as M23 (Met 6U, 4.0% of TRR, 0.1928 mg eq/kg) CGA 354870 (M60, Met 12U, 0.0004 mg eq/kg), Met U8 (M58, 2.5% of TRR, 0.1180 mg eq/kg), and Met L5 (glucuronide conjugate of metabolite NOA 405637, M27, 4.6% of TRR, 0.2223 mg eq/kg).

CGA 357276 (M42) accounted for 7.0% (0.3368 mg eq/kg), but was discussed as a probable degradation product generated during microwave assisted extraction.

Metabolite stability upon exhaustive extraction

To investigate the stability of relevant metabolites several control samples (urine, CGA 321113 isolated from urine, the taurine and glycine conjugate of CGA 321113 L7a and L7b and trifloxystrobin) were incubated in aqueous propanol in the microwave oven at 100 – 150°C. TLC and HPLC showed that Met L7a and L7b and parent trifloxystrobin were completely stable under these conditions. The acid metabolite CGA 321113 (M 5) was quantitatively converted to CGA 357276 (M42) by thermal decarboxylation. Therefore, CGA 357276 (M 42) found in the microwave extract of liver was attributed to CGA 321113 (M 5).

In addition, the C18 purified fraction from the first liver extract was also incubated in aqueous propanol under microwave conditions. This experiment demonstrated also the conversion of CGA 321113 (M5) to CGA 357276 (M42) and the stability of Met L7a and Met L7b.

Metabolites in urine (Table B.7.2.3-8)

93.9% of the radioactive residues in urine were identified by HPLC and TLC co-chromatography. The main metabolite in urine was the acid metabolite CGA 321113 (M5) amounting to 70.4% of the renally excreted residues. Metabolites NOA 412443 (M29, Met) CGA 354870 (M60, Met 12U), CGA 300624 (M59, Met 11U), Met U8 (M58), NOA 443152 (M10) were identified in urine accounting for 3.2 – 7.2% of renal residues. Parent compound trifloxystrobin was not present in urine.

Metabolites in faeces (Table B.7.2.3-8)

Overall 89.5% of the total ^{14}C-residue in faeces was extracted with methanol and water extraction. All extracted residue components were identified. The parent compound trifloxystrobin accounted for 21.7% of the identified residue. The acid metabolites CGA 321113 (M5) and NOA 405637 (M27, Met 2F) were detected as major metabolites accounting for 35.5% and 10.2%, respectively. Additional metabolites identified were M58 (Met), NOA 443152 (M10, Met 2U), NOA 412443 (M29, Met 1U), the glycine conjugate of CGA 321113 (Met L7b) and Met 7F (trifloxystrobin hydroxylated glyoxyl-phenyl ring in position 4); these accounted for 2.9 – 8.0%.

Table B.7.2.3-3: Extraction and sample preparation scheme for milk

	Milk
TRR [mg eq/kg]*	**0.085**
% of TRR	**100**
1x Acetonitrile followed by 2x Acetonitrile/Water (4:1, v/v)	95.5
Hexane phase	47.2
Separation by prep. TLC:	
Fraction 1	**0.2**
Fraction 2	**46.2**
Fraction 3	0.8
Acetonitrile phase	48.3
Fractionation (C_{18} column):	
H_2O	2.3
Methanol	**45.5**
Hexane	0.5
Total extracted	95.5
PES	4.5
Accountability	100

* [mg eq/kg] = mg/kg expressed as parent equivalents
PES: post extraction solids = non-extractable radioactivity
Accountability: sum of extracts and PES
Extract/fraction **in bold**: extract used for quantitative analysis.

Table B.7.2.3-4: Extraction and sample preparation scheme for muscle (leg and tenderloin)

	Total muscle
TRR [mg eq/kg]*	**0.058**
% of TRR	**100**
1x Acetonitrile followed by 3x Acetonitrile/Water (4:1, v/v) followed by Methanol/Water (4:1, v/v)	90.3
Hexane phase	74.0
Separation by prep. TLC:	
Fraction 1	**11.3**
Fraction 2	**41.8**
Fraction 3	**20.2**
Fraction 4	0.7
Acetonitrile phase	16.3
Fractionation (C_{18} column):	
H_2O	1.0
Methanol	**15.0**
Hexane	0.3
Total extracted	90.3
PES	9.7
Accountability	100

* [mg eq/kg] = mg/kg expressed as parent equivalents
PES: post extraction solids = non-extractable radioactivity.
Accountability: sum of extracts and PES
Extract/fraction **in bold**: extract used for quantitative analysis.

Table B.7.2.3-5: Extraction and sample preparation scheme for fat (omental and perineal)

	Total fat
TRR [mg eq/kg]*	**0.191**
% of TRR	**100**
1x Hexane extraction	93.4
Partitioning with acetonitrile	
Hexane phase	1.4
Acetonitrile phase	92.0
Fractionation (C_{18} column):	
H_2O	**4.3**
Methanol	78.7
Separation by prep. TLC:	
Fraction 1	**4.4**
Fraction 2	**73.5**
Fraction 3	0.8
Hexane phase	9.0
Separation by prep. TLC:	
Fraction 1	0.6
Fraction 2	7.9
Fraction 3	0.5
Total extracted	93.4
PES	6.6
Accountability	100

* [mg eq/kg] = mg/kg expressed as parent equivalents
PES: post extraction solids = non-extractable radioactivity.
Accountability: sum of extracts and PES
Extract/fraction **in bold**: extract used for quantitative analysis.

Table B.7.2.3-6: Extraction and sample preparation scheme for liver

	Liver
TRR [mg eq/kg]*	**4.815**
% of TRR	**100**
1x Acetonitrile followed by 4x Acetonitrile/Water (4:1, v/v)	65.0
Hexane phase	17.9
Separation by prep. TLC:	
Fraction 1	**3.0**
Fraction 2	**12.0**
Fraction 3	**2.9**
Acetonitrile phase	47.1
Fractionation (C_{18} column):	
H_2O	0.5
Methanol	**46.5**
Hexane	0.1
1x Methanol/Water (4:1, v/v)	1.5
PES (after conventional extraction)	33.5
Microwave extraction 1 (up to 150°C) (2-propanol/H_2O, 4:1, v/v)	28.5
Fractionation (C_{18} column):	
H_2O	2.2
Methanol	**26.3**
PES (after microwave extraction 1)	5.0
Microwave extraction 2 (up to 180°C) (2-propanol/H_2O, 4:1, v/v)	33.5
Fractionation (C_{18} column):	
H_2O	2.8
Methanol	28.3
Dichloromethane	2.4
PES (after microwave extraction 2)	0.0
Total extracted (conventional)	66.5
Total extracted (conventional followed by exhaustive)	95-100
Accountability	100

* [mg eq/kg] = mg/kg expressed as parent equivalents
PES: post extraction solids = non-extractable radioactivity
Accountability: sum of extracts and PES
Extract/fraction **in bold**: extract used for quantitative analysis.

Table B.7.2.3-7: Extraction and sample preparation scheme for kidney

	Kidney
TRR [mg eq/kg]*	**1.830**
% of TRR	**100**
1x Acetonitrile followed by 2x Acetonitrile/Water (4:1, v/v)	93.1
Hexane phase	5.3
Separation by prep. TLC:	
Fraction 1	**1.4**
Fraction 2	**1.6**
Fraction 3	**0.5**
Fraction 4	0.4
Fraction 5	**1.4**
Acetonitrile phase	87.8
Fractionation (C_{18} column):	
H_2O	2.5
Methanol	**85.2**
Hexane	0.1
Methanol/H_2O (4:1, v/v)	0.7
PES	6.2
Total extracted (conventional)	93.8
Accountability	100

* [mg eq/kg] = mg/kg expressed as parent equivalents
PES: post extraction solids = non-extractable radioactivity.
Accountability: sum of extracts and PES
Extract/fraction **in bold**: extract used for quantitative analysis.

Table B.7.2.3-8: Radioactive residues in pooled urine and faeces of lactating goats following oral administration of [trifluoromethyl-phenyl-UL-^{14}C]trifloxystrobin at a mean daily dose rate of 4.24 mg/kg bw for four consecutive days

	Urine	Faeces
Compound	**% of renally excreted**	**% of faecally excreted**
Parent compound		
Trifloxystrobin (CGA 279202)	-	**21.7**
Free metabolites		
CGA 321113 (M 5)	**70.4**	**35.5**
NOA 443152 (Met 2U, M 10)	3.2	4.1
Met 7F	-	8.0
Met 6U	-	-
NOA 405637 (Met 2F, M 27)	-	**10.2**
NOA 412443 (Met 1U, M 29)	7.2	3.0
Met U8 (M58)*	4.9	3.9
CGA 300624 (Met 11U, M 59)	4.5	-
CGA 354870 (Met 12U, M 60)	3.7	-
CGA 357276 (M42)**	-	-
Conjugated metabolites		
Taurine conjugate of CGA 321113 (Met L7a)	-	-
Glycine conjugate of CGA 321113 (Met L7b)	-	2.9
L5***	-	-
Total identified	93.9	89.3
Characterized metabolites		
N11	-	-
LR5	-	-
MU2	-	-
MU3	-	-
MU4	-	-
Not analyzed (polar)	**6.1**	**-**
Not analyzed (unpolar)	**-**	**-**
Total not analyzed	**6.1**	**-**
Total extracted	**100.0**	**89.5**
PES	-	**10.5**
Accountability	**100.0**	**100.0**

* Metabolite U8 (M58) was identified in the parallel goat metabolism study with the [GP-^{14}C] label (Anonymous 1997b, M-034517-01-1) and used here as reference substance for co-chromatography.

** CGA 357276 was not an incurred metabolite, but generated from CGA 321113 by thermal degradation during microwave assisted high-temperature extraction.

*** The unknown metabolite L5 was not identified within this study, but in the parallel study with the [GP-^{14}C] label (Anonymous 1997b, M-034517-01-1) as metabolite 1G, i.e. the glucuronide of metabolite NOA 405637 (M27, Met 2F).

Table B.7.2.3- 9: Radioactive residues in pooled milk, muscle and fat of lactating goats following oral administration of [trifluoromethyl-phenyl-UL-^{14}C]trifloxystrobin at a mean daily dose rate of 4.24 mg/kg bw for four consecutive days

	Milk		Total muscle		Total fat	
TRR [mg eq/kg]	**0.085**		**0.058**		**0.191**	
Compound	**% TRR**	**mg eq/kg**	**% TRR**	**mg eq/kg**	**% TRR**	**mg eq/kg**
Parent compound						
Trifloxystrobin (CGA 279202)	**51.6**	**0.0439**	**20.6**	**0.012**	**79.0**	**0.1509**
Free metabolites						
CGA 321113 (M5)	3.6	0.0031	**57.2**	**0.0332**	**10.4**	**0.0198**
NOA 443152 (Met 2U, M 10)	1.9	0.0016	2.0	0.0012	0.4	0.0008
Met 7F	-	-	-	-	-	-
Met 6U (M 23)	4.0	0.0034	-	-	-	-
NOA 405637 (Met 2F, M 27)	-	-	-	-	-	-
NOA 412443 (Met 1U, M 29)	0.9	0.0008	1.3	0.0007	0.5	0.0009
Met U8 (M 58)*	-	-	0.3	0.0002	-	-
CGA 300624 (Met 11U, M 59)	-	-	-	-	-	-
CGA 354870 (Met 12U, M 60)	3.1	0.0026	1.7	0.001	0.7	0.0013
CGA 357276 (M 42)**	-	-	-	-	-	-
Conjugated metabolites						
Taurine conjugate of CGA 321113 (Met L7a)	**13.0**	**0.011**	1.2	0.0007	-	-
Glycine conjugate of CGA 321113 (Met L7b)	-	-	1.2	0.0007	-	-
L5***	-	-	-	-	-	-
Characterized metabolites						
N11	-	-	-	-	-	-
LR5	-	-	-	-	-	-
MU2	11.8	0.0100	1.7	0.001	-	-
MU3	-	-	0.6	0.0003	-	-
MU4	2.1	0.0017	0.6	0.0004	-	-
Total extracted	**95.5**	**0.0746**	**90.3**	**0.052**	**93.4**	**0.1784**
Not analyzed (polar)	**2.3**	**0.0020**	**1.3**	**0.0008**	**0.3**	**0.0006**
Not analyzed (unpolar)	**1.3**	**0.0011**	**0.7**	**0.0004**	**2.2**	**0.0042**
Total not analyzed	**3.6**	**0.0031**	**2.0**	**0.0012**	**2.5**	**0.0048**
PES	**4.5**	**0.0038**	**9.7**	**0.0056**	**6.6**	**0.0126**
Accountability	**100.0**	**0.085**	**100.0**	**0.058**	**100.0**	**0.191**

* Metabolite U8 (M58) was identified in the parallel goat metabolism study with the [GP-^{14}C] label Anonymous 1997b, M-034517-01-1) and used here as reference substance for co-chromatography.

** CGA 357276 was not an incurred metabolite, but generated from CGA 321113 by thermal degradation during microwave assisted high-temperature extraction.

*** The unknown metabolite L5 was not identified within this study, but in the parallel study with the [GP-^{14}C] label (Anonymous 1997b, M-034517-01-1) as metabolite 1G, i.e. the glucuronide of metabolite NOA 405637 (M27, Met 2F).

Table B.7.2.3-10: Radioactive residues in pooled liver and kidney of lactating goats following oral administration of [trifluoromethyl-phenyl-UL-^{14}C]trifloxystrobin at a mean daily dose rate of 4.24 mg/kg bw for four consecutive days

	Liver[#]		Liver[##]		Kidney	
TRR [mg eq/kg]	**4.815**				**1.830**	
Compound	**% TRR**	**mg eq/kg**	**% TRR**	**mg eq/kg**	**% TRR**	**mg eq/kg**
Parent compound						
Trifloxystrobin (CGA 279202)	**2.8**	**0.1359**	**2.8**	**0.1359**	**1.8**	**0.0337**
Free metabolites						
CGA 321113 (M5)	**13.0**	**0.6260**	**13.0**	**0.6260**	**54.3**	**0.9930**
NOA 443152 (Met 2U, M 10)	-	-	-	-	3.1	0.0565
Met 7F	-	-	-	-	-	-
Met 6U (M 23)	4.0	0.1928	4.4	0.2118	-	-
NOA 405637 (Met 2F, M 27)	-	-	-	-	-	-
NOA 412443 (Met 1U, M 29)	-	-	-	-	1.9	0.0348
Met U8 (M 58)*	2.5	0.1180	3.6	0.1725	2.7	0.049
CGA 300624 (Met 11U, M 59)	-	-	-	-	0.3	0.0054
CGA 354870 (Met 12U, M 60)	0.0	0.0004	0.0	0.0004	-	-
CGA 357276 (M 42)**	-	-	7.0	0.3368	-	-
Conjugated metabolites						
Taurine conjugate of CGA 321113 (Met L7a)	27.8	1.3366	27.8	1.3366	12.7	0.2328
Glycine conjugate of CGA 321113 (Met L7b)	9.0	0.4354	10.7	0.5152	**5.2**	**0.0950**
L5***	4.6	0.2223	4.6	0.2223	-	-
Characterized metabolites						
N11	-	-	-	-	0.8	0.0144
LR5	-	-	4.2	0.2013	-	-
MU2	-	-	-	-	-	-
MU3	-	-	-	-	-	-
MU4	-	-	-	-	-	-
Total extracted	**66.5**	**3.202**	**95.0**	**4.574**	**93.8**	**1.7165**
Not analyzed (polar)	**2.1**	**0.1011**	**16.2**	**0.7820**	**10.6**	**0.1946**
Not analyzed (unpolar)	**0.7**	**0.0335**	**0.7**	**0.0335**	**0.4**	**0.0073**
Total not analyzed	**2.8**	**0.1346**	**16.9**	**0.8155**	**11.0**	**0.2019**
PES	**33.5**	**1.613**	**5.0**	**0.2408**	**6.2**	**0.1135**
Accountability	**100.0**	**4.815**	**100.0**	**4.815**	**100.0**	**1.830**

* Metabolite U8 (M58) was identified in the parallel goat metabolism study with the [GP-^{14}C] label (Anonymous 1997b, M-034517-01-1) and used here as reference substance for co-chromatography.

** CGA 357276 was not an incurred metabolite, but generated from CGA 321113 by thermal degradation during microwave assisted high-temperature extraction.

*** The unknown metabolite L5 was not identified within this study, but in the parallel study with the [GP-^{14}C] label (Anonymous 1997b, M-034517-01-1) as metabolite 1G, i.e. the glucuronide of metabolite NOA 405637 (M27, Met 2F).

[#] results after conventional extraction.

[##] results after an additional exhaustive extraction with microwave assistance.

III. Conclusion

The metabolism of [trifluoromethyl-phenyl-UL-^{14}C]trifloxystrobin in two lactating goats was investigated after oral administration of the radiolabelled test substance at a dose rate of 4.24 mg/kg bw/day for four consecutive days (one dose per day). This dose rate corresponded to 103.8 mg/kg in the diet. Milk was collected twice daily and excreta were collected on daily basis. The goats were slaughtered 6 hours after the

last dose (78 hours after the first dose) and leg muscle, tenderloin, omental and perirenal fat, kidney and liver as well as blood, bile and the gastrointestinal tract were dissected. Pooled milk samples and pooled tissues were analysed for the radioactive residues.

TRR levels

During the whole test period 0.077, 44.46 and 17.45% of the dose totally applied were eliminated via milk, faeces and urine, respectively. These values were mean data of the respective samples from both goats. In total, less than 1% of the administered dose was recovered in the milk and tissues. Summing up all radioactive residues in milk, excreta, organs and tissues and the gastro-intestinal tract, the sum resulted in a total recovery of 88.14% of the dose. The rest is assumed to be in the carcass that was not analysed.

The highest total radioactive residues (TRR) in milk were measured after an interval of 48 – 72 hours after start of administration amounting to 0.121 mg eq/kg; however, since the last milk sample was collected after an interval of 78 hours, it is not possible to determine definitively whether or not this was the plateau.

TRR in organs and tissues amounted to 4.815 mg eq/kg in liver, 1.830 mg eq/kg in kidneys, 0.058 mg eq/kg in muscle and 0.191 mg eq/kg in fat. The average residues in milk were 0.085 mg eq/kg (pooled over the whole study period 0 – 78 hours).

Extractability

The extractability of the milk and tissue samples by conventional extraction at room temperature was high (≥ 85% of the respective TRR) with the exception of liver (66.5% TRR). Microwave assisted extraction at elevated temperatures (≤ 180°C) released additional radioactive residues and thus increasing the extractability to 100%.

Major residue components

- Trifloxystrobin was found as the major compound in fat (79.0%, 0.151 mg/kg) and in pooled milk (51.6%, 0.0439 mg/kg) and to a smaller extent in muscle (20.6%, 0.012 mg/kg), liver (2.8%, 0.191 mg/kg) and kidneys (1.8%, 0.0337 mg/kg). Trifloxystrobin was also a major residue component in faeces (21.7%), but not detectable in urine.
- CGA 321113 (M5) formed by ester hydrolysis of trifloxystrobin was the main residue component in muscle (57.2% of TRR, 0.0332 mg eq/kg) and kidney (54.3% of TRR, 0.9930 mg eq/kg). It was also a major metabolite in liver (13.0% of TRR, 0.626 mg eq/kg), fat (10.4% of TRR, 0.0198 mg eq/kg) and was also found in milk (3.6% of TRR, 0.0031 mg eq/kg). CGA 321113 was the predominant component in urine (70.4% of renally excreted radioactivity) and was also a major metabolite in faeces (35.5% of faecally excreted radioactivity).
- The taurine conjugate of CGA 321113, i.e. metabolite L7a was a major metabolite in liver (27.8% of TRR, 1.3366 mg eq/kg), as well as in kidney (12.7% of TRR, 0.2328 mg eq/kg), and milk (13.0% of TRR, 0.011 mg eq/kg in a 0 – 72 hours pool). In muscle it was present to a lower extent (1.2% of TRR, 0.0007 mg eq/kg).
- The glycine conjugate of CGA 321113, i.e. metabolite L7b was also a major metabolite in liver (up to 10.7% or TRR, 0.5152 mg eq/kg after exhaustive extraction) and a main metabolite in kidney (5.2% of TRR, 0.0950 mg eq/kg). It was present also in muscle (1.2% of TRR, 0.0007 mg eq/kg), and in faeces (2.9% of faecally excreted radioactivity).
- The minor metabolites CGA 354870 (M60, Met 12U), NOA 412443 (M29, Met 1U), NOA 443152 (M10, Met 2U), M23 (Met 6U) and M58 (Met 8U) were detected at a level generally below 5% of TRR in all matrices and < 0.01 mg eq/kg in milk, muscle and fat. They slightly exceeded a level of 0.1 mg eq/kg in liver and 0.01 mg eq/kg in kidneys due the higher TRR values in liver and kidneys. Additional very minor unknown metabolites were detected in milk and muscle (all ≤ 0.01 mg eq/kg) and at something higher levels in liver and kidney (0.201 mg eq/kg; 4.2% TRR and 0.014 mg eq/kg; 0.8% TRR).

Metabolic reactions

Based on the structures identified the metabolism of trifloxystrobin in lactating goats proceeded predominantly via hydrolysis, demethylation and hydroxylation reactions followed by conjugation with taurine and glycine:

- Deposition of trifloxystrobin as the major residue component in fat and milk.
- Hydrolysis of the methyl ester group trifloxystrobin to CGA 321113 (M 5) as the predominant residue component in muscle and kidney and as major metabolite in urine and faeces.
- Conjugation of CGA 321113 with taurine to metabolite L7a as the major metabolite in liver and milk or conjugation with glycine to the minor metabolite L7b.
- Hydroxylation at the aminooxymethyl group of metabolite CGA 321113 (M5) led to metabolites NOA 443152 (2U, M10) and subsequent demethylation to metabolite 6U (M 23).
- Demethylation of the methoxyimino group of trifloxystrobin to metabolites NOA 405637 (2F, M27), NOA 412443 (1U) and 6U (M23).
- Hydroxylation of the glyoxyl-phenyl ring in position 4 yielding trace amounts of metabolite 7F.
- As a minor reaction breakdown of the molecule between the two phenyl rings to Met U8 (M58) and sulfate conjugation to metabolite CGA 300624 (11U, M59) or further oxidations to metabolite CGA 354870 (12U, M60).

In summary, major metabolites identified in lactating goat were also found in rat and hen and the general metabolic pathways were similar to those in rat and hen. A cleavage of the molecule between the two phenyl rings was observed for the [trifluoromethyl-phenyl-UL-^{14}C] label as a minor reaction in the pathways leading to three minor label specific metabolites.

The parent substance trifloxystrobin and its hydrolyzed metabolite CGA 321113 (M5) can serve as analytical targets for a residue method in food of animal origin since these two components contributed mainly to the total residues.

Based on these results the metabolic pathway of [trifluoromethyl-phenyl-UL-^{14}C]trifloxystrobin in lactating goats is proposed as shown in Figure B.7.2.3-1.

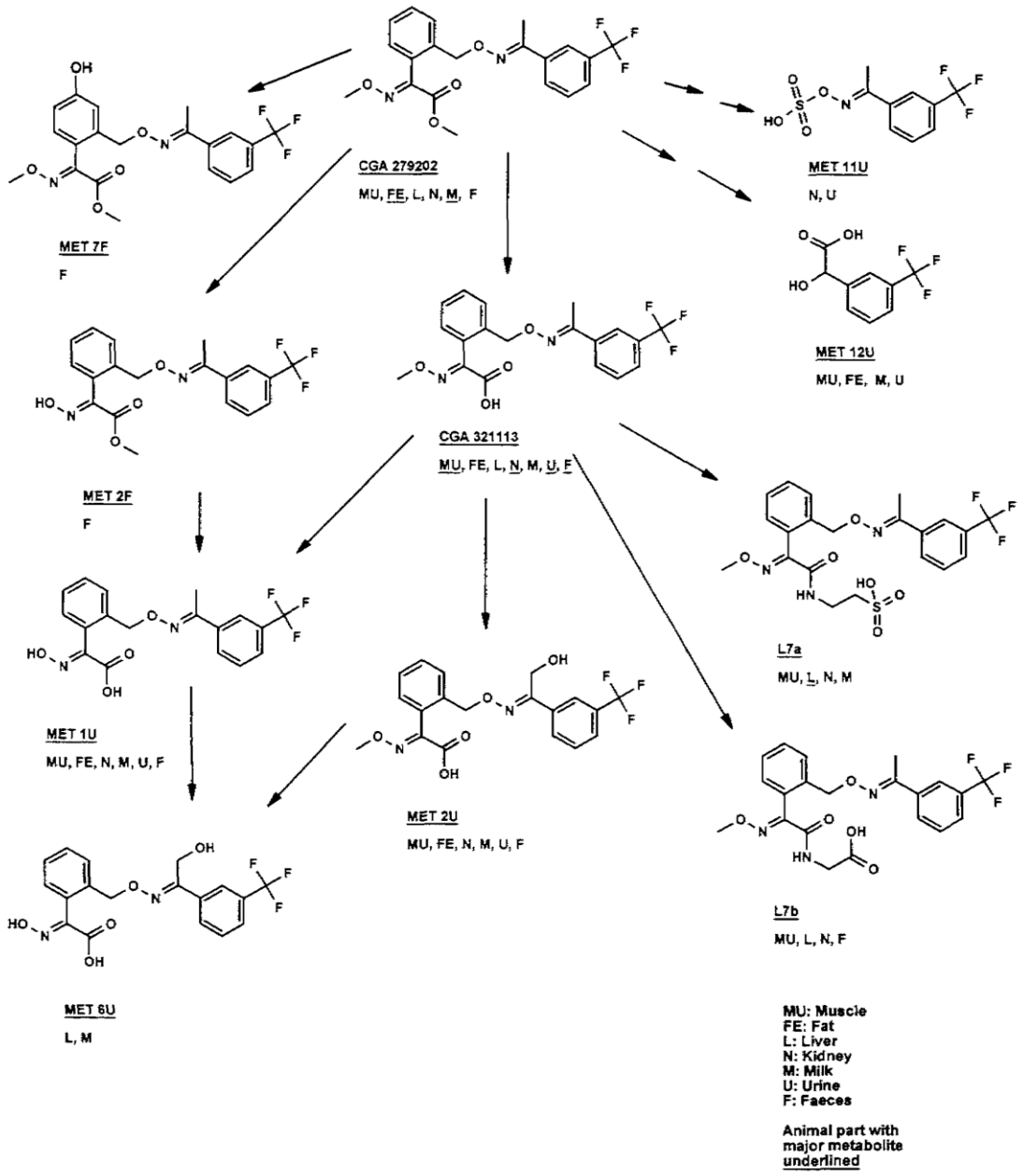
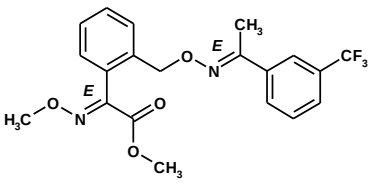
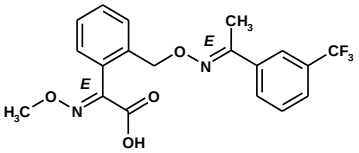
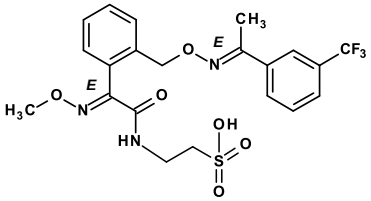
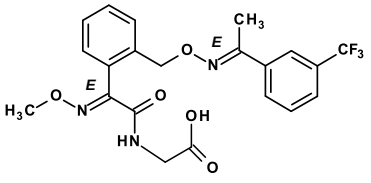
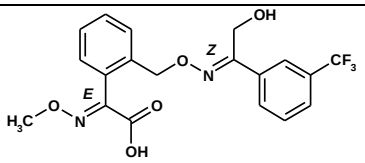
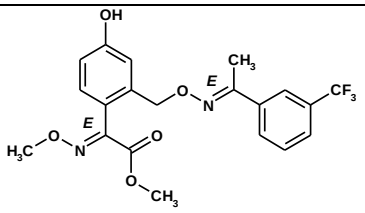
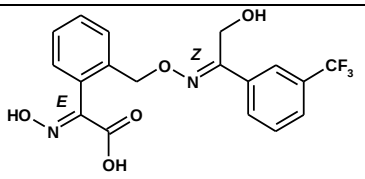
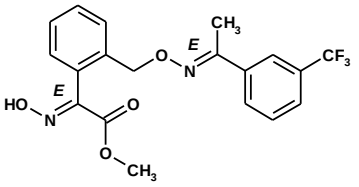
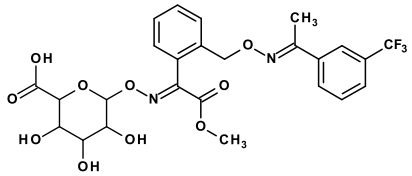
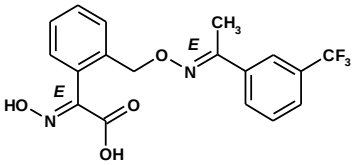
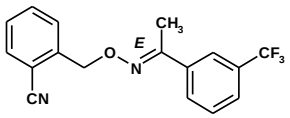
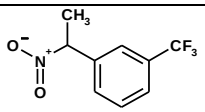
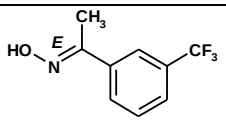
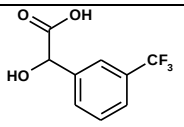


Figure B.7.2.3-1: Proposed metabolic pathway of [trifluoromethyl-phenyl-UL-^{14}C]trifloxystrobin in the lactating goat

List of compounds identified in this metabolism study

No	Common Name/Code	Chemical Name	Chemical structure
1	Trifloxystrobin/ CGA 279202 (*EE*-isomer)	methyl (2E)-(methoxyimino) (2-{[({(1E)-1-[3-(trifluoromethyl) phenyl]ethylidene}amino)oxy] methyl}phenyl)acetate (*E.E*)-methoxyimino-{2-[1-(3-trifluoro methyl-phenyl)-ethylideneaminooxymethyl]-phenyl}-acetic acid methyl ester	
5	CGA 321113 (*EE*-isomer)	(2*E*)-(methoxyimino)(2-{[({(1*E*)-1-[3-(trifluoromethyl)phenyl]ethylidene}amino)oxy]methyl}phenyl) acetic acid *(E,E)*-methoxyimino-{2-[1-(3-trifluoro methyl-phenyl)-ethylideneaminooxy-methyl]-phenyl}-acetic acid	
5a	Taurine conjugate of CGA 321113/ Met L7a (*EE*-isomer)	Taurine conjugate of (2*E*)-(methoxyimino)(2-{[({(1*E*)-1-[3-(trifluoromethyl)phenyl]ethylidene}amino)oxy]methyl}phenyl) acetic acid Taurine conjugate of *(E,E)*-methoxyimino-{2-[l-(3-trifluorornethyl-phenyl)-ethylidene-aminooxymethyl]-phenyl}-acetic acid	
5b	Glycine conjugate of CGA 321113/ Met L7b (*EE*-isomer)	Glycine conjugate of (2*E*)-(methoxyimino)(2-{[({(1*E*)-1-[3-(trifluoromethyl)phenyl]ethylidene}amino)oxy]methyl}phenyl) acetic acid Glycine conjugate of *(E,E)*-methoxyimino-{2-[l-(3-trifluoromethyl-phenyl)-ethylidene-aminooxymethyl]-phenyl}-acetic acid	

No	Common Name/Code	Chemical Name	Chemical structure
10	NOA 443152/ Met 2U	(2*E*)-(2-{[({(1*Z*)-2-hydroxy-1-[3-(trifluoromethyl)phenyl]ethylidene } amino)oxy]methyl}phenyl) (methoxyimino)acetic acid 2-[2-hydroxy-1-(3-trifluoromethyl-phenyl)-ethylidene-aminooxymethyl]-phenyl}-methoxyimino-acetic acid	
19	Met 7F (*EE*-isomer)	methyl (2*E*)-(4-hydroxy-2-{[({(1*E*)-1-[3-(trifluoromethyl)phenyl] ethylidene}amino)oxy]methyl} phenyl)(methoxyimino)acetate {4-hydroxy-2-[1-(3-trifluoromethyl-phenyl)-ethylideneaminooxymethyl]-phenyl}-methoxyimino-acetic acid methyl ester	
23	Met 6U	(2*E*)-(hydroxyimino)(2-{[({(1*Z*)-2-hydroxy-1-[3-(trifluoromethyl)phenyl]ethylidene } amino)oxy]methyl}phenyl)acetic acid hydroxyimino-{2-[2-hydroxy-1-(3-trifluoro methyl-phenyl)-ethylidene aminooxymethyl]-phenyl}-acetic acid	
27	NOA 405637 (*E*,*E*-isomer)/ 2F	methyl (2*E*)-(4-hydroxy-2-{[({(1*E*)-1-[3-(trifluoromethyl)phenyl] ethylidene}amino)oxy]methyl} phenyl)(methoxyimino)acetate hydroxyimino-{2-[1-(3-trifluoromethyl-phenyl)-ethylideneaminooxymethyl]-phenyl}-acetic acid methyl ester	

No	Common Name/Code	Chemical Name	Chemical structure
27a	Met 1G (L5)*	glucuronic acid conjugate of methyl (2*E*)-(hydroxyimino)(2-{[({(1*E*)-1-[3-(trifluoromethyl) phenyl]ethylidene}amino)oxy] methyl}phenyl)acetate glucuronic acid conjugate of hydroxyimino-{2-[l-(3-trifluoromethyl-phenyl)-ethylideneaminooxymethyl]-phenyl}-acetic acid methyl ester	
29	NOA 412443 (*EE*-isomer)/ Met 1U	(2*E*)-(hydroxyimino)(2-{[({(1*E*)-1-[3-(trifluoromethyl)phenyl] ethylidene}amino)oxy]methyl} phenyl)acetic acid hydroxyimino-{2-[1-(3-trifluoro-methyl-phenyl)-ethylidene-amino-oxymethyl]-phenyl}-acetic acid	
42	CGA 357276** (*E*-isomer)	2-{[({(1*E*)-1-[3-(trifluoromethyl) phenyl]ethylidene}amino)oxy] methyl}benzonitrile 2-[1-(3-trifluoromethyl-phenyl)-ethylideneaminooxymethyl]-benzonitrile	
58	Met 8U	l-(l-nitro-ethyl)-3-trifluoromethyl-benzene	
59	CGA 300624 (*E*-isomer) Met 11U	(1*E*)-N-hydroxy-1-[3-(trifluoromethyl) phenyl]ethanimine 1-(3-trifluoromethyl-phenyl)-ethanone oxime	
60	CGA 354870/ Met 12U	hydroxy[3-(trifluoromethyl)phenyl] acetic acid hydroxy-(3-trifluoromethyl-phenyl)-acetic acid	

* Met 27a (L5) was not identified within this study, but in the parallel study with the [GP-^{14}C] label (Anonymous, 1997b, M-034517-01-1) as metabolite 1G, i.e. the glucuronide of metabolite NOA 405637 (M27, Met 2F).

** CGA 357276 (M42) was not an incurred metabolite, but generated from CGA 321113 by thermal degradation during microwave assisted high-temperature extraction.

[Study 2] The metabolism of [glyoxyl-phenyl-(U)-^{14}C] CGA 279202 after multiple oral administration to lactating goats (Anonymous, 1997b, M-034517-01-1)

Goats dosed with [^{14}C-GP] Trifloxystrobin

Report:	KCA 6.2.3/02; M-034517-01-1
Title:	The metabolism of [glyoxyl-phenyl-(U)-^{14}C] CGA 279202 after multiple oral administration to lactating goats
Report No.:	14/97
Document No.:	M-034517-01-1
Guideline(s):	Residue Chemistry Test Guidelines, OPPTS 860.1300, Nature of the Residue - Plants, Livestock EPA, Washington, August 1996 Commission of the European Communities Working Document 7030/VI/95 - Rev.2, 6/1/1997 Appendix F: Metabolism and Distribution in Domestic Animals
Guideline deviation(s):	none
GLP/GEP:	**yes**

Summary

The metabolism of [glyoxyl-phenyl-UL-^{14}C]trifloxystrobin in two lactating goats was investigated after oral administration of the radiolabelled test substance at a dose rate of 4.13 mg/kg bw/day for four consecutive days (one dose per day). This dose rate corresponded to 100.4 mg/kg in the diet.
Milk was collected twice daily and excreta were collected on daily basis. The goats were slaughtered 6 hours after the last dose (78 hours after the first dose) and leg muscle, tenderloin, omental and perirenal fat, kidney and liver as well as blood, bile and the gastrointestinal tract were dissected. Pooled milk samples and pooled tissues were analysed for the radioactive residues.

During the whole test period 0.06%, 36.04% and 18.91% of the dose totally applied were eliminated via milk, faeces and urine, respectively. These values were mean data of the respective samples from both goats. In total, less than 1% of the administered dose was recovered in the milk and tissues. Summing up all radioactive residues in milk, excreta, organs and tissues and the gastro-intestinal tract, the sum resulted in a total recovery of 80.88% of the dose. The rest is assumed to be in the carcass that was not analysed.

The highest TRR in milk were measured in the third-day-interval (48 – 72 hours from begin) amounting to approx. 0.105 mg eq/kg. The TRR peak in the milk was measured in the morning milk amounting to 0.116 mg eq/kg; however, since the last milk sample was collected after an interval of 78 hours, it is not possible to determine definitively whether or not this was the plateau.

TRR in tissues amounted to 3.913 mg eq/kg in liver, 2.331 mg eq/kg in kidney, 0.077 mg eq/kg in muscle and 0.356 mg eq/kg in fat. The average residues in milk were 0.089 mg eq/kg (pooled over the whole study period 0 – 78 hours).

The extractability of the milk and tissue samples by conventional extraction at room temperature was high (≥ 86% of the respective TRR) with the exception of liver (68.7% of TRR). Microwave assisted extraction at elevated temperatures (≤ 180°C) released additional radioactive residues and thus increasing the extractability to 99.5% of TRR.

Identification of extracted trifloxystrobin residues was conducted by one- and two-dimensional radio-TLC and radio-HPLC with reference compounds co-chromatographed.

The parent substance trifloxystrobin was found as the main residue component in fat (82% of TRR) and milk (73.8% of TRR). CGA 321113 (M5) was the major metabolite in muscle (51.1% of TRR), kidney (73.5% of TRR) and liver (39.6% of TRR). It was also detected in fat (11.3% of TRR) and milk (4.8% of TRR). Other

identified minor metabolites did not exceed 5% of the TRR in edible matrices, with the exception of the taurine and glycine conjugates of CGA 321113 which were present in liver at 5.2% and 11.8% of TRR, respectively.

Based on the structures identified the metabolism of trifloxystrobin in lactating goat proceeded predominantly via hydrolysis, demethylation and hydroxylation reactions followed by taurine and glycine conjugation:

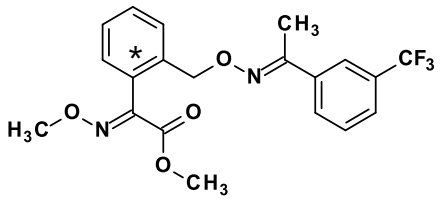
- Deposition of intact trifloxystrobin as the major residue component in fat and milk.
- Hydrolysis of the methyl ester group of trifloxystrobin to CGA 321113 (M5) as the predominant metabolite in muscle, liver and kidneys and as major metabolite in urine and faeces.
- Conjugation of CGA 321113 with glycine to metabolite L7b being a major metabolite in liver and conjugation with taurine to the minor metabolite L7a.
- Demethylation of the methoxyimino group of trifloxystrobin to metabolites NOA 405637 (2F, M27), NOA 412443 (1U) and 6U (M 23).
- Conjugation of metabolite NOA 405637 (2F, M 27) with glucuronic acid to metabolite L5 (Met 1G).
- Hydroxylation at the aminooxymethyl group to metabolites NOA 443152 (2U, M 10)3F (M 22) and 6U (M 23).
- Hydroxylation of the glyoxyl-phenyl ring in position 4 yielding trace amounts of the metabolite 7F.
- No label-specific metabolites (resulting from cleavage of the aminooxymethyl bridge between the phenyl rings) were detected in this study.
- Comparison of metabolites in the excreta of goats and rat indicated a similar metabolite pattern in both species. All major metabolites identified in goat were also described in rat and the general pathways in goat were similar to those in the rat.

The parent substance trifloxystrobin and its hydrolyzed metabolite CGA 321113 (M5) can serve as analytical targets for a residue method on trifloxystrobin residues in food of animal origin since these two components contributed mainly to the total residues.

I. Materials and Methods

A. Materials

1. Test Material

IUPAC Name	Methyl (2E)-(methoxyimino)[2-({[(1E)-{1-[3-(trifluoromethyl) phenyl]ethylidene}amino]oxy}methyl)phenyl]acetate (accord. to software ACD, ver.12.2) Methoxyimino-{2-[l-(3-trifluoromethyl-phenyl)-ethylidene-aminooxymethyl]-phenyl}-acetic acid methyl ester
Code name	CGA 279202
Common name	Trifloxystrobin
Empirical formula	$C_{20}H_{19}F_3N_2O_4$
Molar mass, non-labelled	408.4 g/mol
Chemical structure	 * position of the radiolabel
Radiolabelled test material	[glyoxyl-phenyl-UL-^{14}C] trifloxystrobin
Batch number	TYP-V-26

Specific radioactivity used for the test (after radiodilution)	610 kBq/mg = 16.5 µCi/mg
Original radiochemical purity	99.8% (stock solution applied to the gelatine capsules) (radio TLC)
Dose level	4.13 mg/kg bw/day equivalent to 100.4 mg/kg in the diet 1 capsule per day to each goat for four consecutive days;
Vehicle	gelatine capsules containing D-(+)-lactose monohydrate
Stability of the test material	The radiochemical purity of the test substance in the stock solution and in an acetonitrile/water extract of the dosing capsules was 96.5% at the start of the dosing and 97.1% after the last administration. Thus the test material remained stable on the carrier during the administration phase.

2. Test Animals

Species	Goat
Strain	*Gemsfarbige Gebirgsziege*
Breeding facility	Pierre Schlunegger, CH-1606, Lavaux, Switzerland, Forel, Lavaux, Switzerland
Sex and numbers involved	2 female animals
Age	11 and 13 months
Body weight	40.1 and 47.5 kg (at the first day of treatment), 37.81 and 45.71 kg (at the test end)
Acclimatisation	7 days before first treatment in their respective cages
Identification	Ear tag
Housing	Metabolic cages with artificial light and 12/12 day/night cycle Temperature: 18 - 20°C; relative air humidity: 45 - 60%
Feed and water	Controlled daily diet containing 600 g concentrate (UFA Nr. 867), 1000 g maize cubes and 100 g hay which were provided in two portions (850 g per portion). During the 5th day of acclimatisation both goats received a double ration on hay to enable them to regain their normal appetite. On the last day of treatment the animals received the morning portion only. The amount of refused feed was weighed back and recorded in order to calculate the daily feed intake. Tap water was provided *ad libitum* in automatic drinkers.

B. Study Design

Dosing

The radiolabelled test compound with an original specific radioactivity of 0.896 MBq/mg (24.2 µCi/mg) was diluted with the non-labelled test compound in acetonitrile solution to achieve a specific radioactivity of 610 kBq/mg for the metabolism study. Aliquots of the resulting stock solution containing 171 mg trifloxystrobin each were applied to gelatine capsules which contained D-(+)-lactose monohydrate. After evaporation of the solvent the capsules were sealed.

Two lactating goats were orally dosed with one gelatine capsule per goat and day for four consecutive days. The resulting dose rate was 4.13 mg a.s./kg bw/day corresponding to a daily mean dose of 100.4 mg/kg feed. The actual dose rates for the two animals were 4.52 and 3.74 mg/kg bw/day due to the different body weights and daily feed consumptions.

The purity and stability of the test substance were determined by TLC in the stock solution and in an acetonitrile/water extract of remaining capsules at the first and after the last administration.

Sample collection

Sampling of urine, faeces, cage wash, and milk

Samples of urine, faeces and cage washes (ethanol/water, 1:1, v/v) were collected daily in the morning from the day before the first administration until slaughter in 24 hour intervals and stored frozen.

Each goat was milked twice daily (morning and evening). Milk samples were collected from day 7 of the adaptation period onwards and stored at 4°C. The last milking took place just before slaughter. Morning and

evening milk were kept separately.

Sacrifice and dissection of organs and tissues
The animals were sacrificed approximately 6 hours after the last dose (= 78 hours after the first dose). The goats were stunned with a stunning bolt and immediately exsanguinated by severing the major neck vessels. Blood was collected under addition of heparin and was stored refrigerated until shipment to the analytical laboratory on the next day.

Tissue samples dissected were leg muscle, tenderloin, omental and perirenal fat, kidney and liver. Bile and the gastrointestinal tract were also sampled. Leg muscle, tenderloin, omental and perirenal fat as well as contents of the gastrointestinal tract were homogenized and stored refrigerated until shipment to the analysing laboratory the same day. The other organs (kidney, liver, bile) were stored intact and refrigerated until shipment to the analysing laboratory the same day.

Sample preparation

Faeces and rumen content
Faeces were homogenized by mixing in the collection box using a rod. An aliquot was weighed and roughly homogenized in a Cutter Cut-o-Mat with a few strokes at reduced speed in the beginning and thoroughly homogenized afterwards. Variable amounts of dry ice were added to obtain a "dry" homogenate. In the laboratory samples of frozen faeces were homogenized again using a Moulinette SE cutter. Three aliquots were analyzed by combustion and radioassaying.

The content of the gastrointestinal tract was thoroughly homogenised by using a rod that was agitated manually. An aliquot was weighed into a box and kept refrigerated. In the laboratory samples of rumen content were homogenized in a Moulinette SE cutter. Three aliquots were analyzed by combustion and radioassaying. Aliquots of faeces and urine from both animals were combined for each interval prior to extraction. The aliquots were based on equal percentages of the total weight of the samples.

Milk
Aliquots of milk from both animals of each interval were combined for each interval prior to extraction. The aliquots were based on equal percentages of the total weight of the samples.

Organ and tissue samples
Muscle and fat samples were cut into pieces. Aliquots of leg muscle homogenates representing the total sample together with the entire homogenates of tenderloin, omental fat and perirenal fat were stored refrigerated until transport to the laboratory on the same day.

In the analysing laboratory samples of tissues were cut into smaller pieces with a knife, frozen in liquid nitrogen and homogenized in a Moulinette SE cutter. Three aliquots of each sample were analyzed for the residue content by radioassaying after digestion with tissue solubilizer via LSC. A pool of muscle samples was prepared by mixing corresponding aliquots of leg muscle and tenderloin. Aliquots of omental and perirenal fat were pooled prior to extraction. The aliquots were based on equal percentages of the total weight of the samples.

Extraction and extract analysis

Individual extraction procedures for the various sample materials are described below. The extracts were analysed by one- and two-dimensional (2D-) radio-TLC on silica gel or RP18 plates and by radio-RP18-HPLC. References for co-chromatography were either obtained from metabolites which were isolated from the liver and which were identified by LC-MS and NMR or from identified metabolites in other metabolism studies with ^{14}C-trifloxystrobin.

Milk (Table B.7.2.3-13)
Milk was homogenized with acetonitrile by mechanical shaking. After centrifugation the precipitate was extracted twice with acetonitrile/water (4:1, v/v). The extracts were combined and the volume was reduced. The resulting solution was partitioned three times with hexane. The three hexane phases were individually

re-extracted with acetonitrile. The combined polar (acetonitrile) fraction was analyzed by 2D-TLC and HPLC.

Tissues

Muscle (leg and tenderloin) (Table B.7.2.3-14)
The homogenized sample of muscle was extracted with acetonitrile by stirring at room temperature. After filtration the residues were extracted three times with acetonitrile/water (4:1, v/v). All extracts were combined and the volume was reduced. The resulting aqueous raw extract was partitioned with hexane (3 times). The three hexane phases were individually re-extracted with acetonitrile.

An aliquot of the combined polar (aqueous) extract was cleaned-up by RP-18 SPE. The RP18 cartridge was eluted with water and methanol/water (1:9, v/v) (extract 1), followed by methanol/water (4:1, v/v) and methanol (extract 2) and methanol (extract 3).

The three extracts were further analyzed as follows:

- Extract 1 was purified by preparative TLC. The resulting three radioactive zones were scraped out, eluted with methanol and analyzed by 2D-TLC.
- Extract 2 was analyzed by HPLC and 2D-TLC. An aliquot of the extract was further cleaned up using an ENV^+-cartridge that was eluted twice with water (resulting in fraction 1 and 2), methanol/water (1:9, v/v), methanol/water (1:4, v/v), methanol (fraction 3) and methanol (fraction 4). Fractions 2 and 3 from elution of the ENV cartridge were analyzed by 2D-TLC.
- Extract 3 of the primary RP18 clean-up was analyzed by 2D-TLC. The radioactive zone was eluted with methanol and re-chromatographed on TLC.

Fat (omental and perirenal) (Table B.7.2.3-15)
The homogenized sample of fat was extracted with acetonitrile using a mechanical shaker, followed by ultrasonication at 40°C for 4 h and additional extraction in a mechanical shaker. The remaining solids were additionally extracted with acetonitrile/water (4:1, v/v, 2x). The combined extracts were evaporated and redissolved in acetonitrile followed by partitioning with hexane (3 times). The hexane phases were individually re-extracted with acetonitrile. The acetonitrile extracts were combined and an aliquot was analyzed by 2D-TLC.

Another aliquot of the combined acetonitrile extract was purified by silica gel SPE that was eluted with hexane and dichloromethane/hexane (1:9, v/v) (fraction 1), dichloromethane/hexane (4:1, v/v), dichloromethane and methanol (fraction 2), followed by methanol and water (fraction 3). An aliquot of fraction 2 containing the predominant portion of radioactivity was purified by semi-preparative TLC and also analyzed by 2D-TLC on Si-60 TLC plates. The resulting two radioactive zones from the semi-preparative TLC were eluted with methanol. One of them was analyzed by HPLC and 2D-TLC.

Liver (Table B.7.2.3-16)
The homogenized liver sample was extracted with acetonitrile at room temperature. After centrifugation the remaining solids were extracted with acetonitrile/water (4:1, v/v, 3x). The combined extracts were evaporated to dryness, redissolved in acetonitrile and partitioned three times with hexane. The three hexane phases were individually re-extracted with acetonitrile. The acetonitrile extracts were combined and analyzed by 2D-TLC.

Another aliquot of the combined acetonitrile extract was purified by RP-18 SPE. The RP18 column was eluted with water (fraction 1), methanol/water mixtures (1/4, 2/3, 3/2 and 4/1, v/v) (fraction 2) and finally by methanol (fraction 3). Fraction 2 was analyzed by HPLC.

Non-extractable residues after conventional extraction of liver were exhaustively extracted with 2-propanol/H2O (4:1, v/v) and microwave assistance. The temperature was increased in three steps from 100° to 150° and finally to 180°C. The extracts were concentrated and added to RP18 material that was applied to the top of a RP18 column. This column was stepwise eluted with water, water/methanol solutions (1/9 and 1/4, v/v), methanol and dichloromethane. The medium polar water/methanol eluates were analysed by 2D-TLC and HPLC.

Kidney (Table B.7.2.3-17)
The homogenized kidney sample was extracted with acetonitrile at room temperature. After centrifugation the residue was additionally extracted with acetonitrile/water 4:1, v/v, 2x). The combined extract was evaporated to dryness, redissolved in acetonitrile/water (1:1, v/v) and extracted with n-hexane. After re-extraction of the hexane phase with acetonitrile, the combined polar phase was analyzed by HPLC and 2D-TLC.

Excreta

Faeces
The pooled faeces sample was extracted with methanol followed by extraction with methanol/water (4:1, v/v, 2x) at room temperature. After each extraction step the homogenate was centrifuged. The supernatant solutions were combined and the volume reduced. The resulting extract was analysed directly by 2D-TLC and HPLC.

Urine
The pooled urine sample was directly analyzed by 2D-TLC and HPLC.

Analytical methods

The radioactivity of all samples and fractions were measusured by radioassaying via LSC with automatic quench correction. Liquid samples were directly measured. Solid samples were combusted and the formed $^{14}CO_2$ collected in a basic liquid scintillator before LSC measurement. The LOQ of radioassaying depended on the matrix. It ranged from 0.0003 mg eq/kg (milk) to 0.0033 mg eq/kg (liver).

Radio-HPLC was performed using RP18 columns (250 x 4.0 mm, particle size 5 µm) that were operated with various gradient systems of pH 7-buffer/methanol. The HPLC system was equipped with an UV detector (operated at 254 nm) and a radiomonitor with a 400 µL scintillator cell.

Radio-TLC was performed using precoated silica gel 60 F_{254} and RP18 F_{254} plates. The plates were developed with several different solvent mixtures for both, silica gel plates and RP18 plates after chamber saturation. Two-dimensional (2D) TLC on silica gel plates were developed with different solvent mixtures in rectangular direction. Radioactive spots were detected by a spark chamber radiochromatogram camera. For quantitative radioactivity determination the respective spots/zones were scraped off, added to methanol and a scintillation cocktail and radioassayed by LSC. Nonradioactive reference compounds were visualized by fluorescence quenching under UV light (254 nm). Due to the different Rf values of the reference compounds in the different TLC systems a clear identification of the radiopeaks via cochromatography was possible.

Storage stability of samples
During the study, all homogenized samples were stored at -20 °C prior to extraction and analysis. The storage stability of residues during the analytical period was investigated in milk and liver (chosen as representative matrices).

The storage stability of residues in liver and milk was determined by comparison of metabolite pattern of fresh liver and fresh milk extract with that of the same extract stored at -20°C for about 10 months, until the end of the analytical phase, and with the metabolite pattern in a new extract from liver and milk stored at -20°C for ca. 10 months. For liver, no difference in the extractability and no significant change of the metabolites' composition was recognized during storage of whole liver or the liver extracts.

Milk extracts were analyzed without further purification; therefore, the unpolar metabolites were not well resolved on TLC. However, comparison of the overall pattern of metabolites did not show a significant change during storage of milk or milk extracts.

Therefore, the results of the study are considered not to be affected by the length and conditions of storage of the samples analyzed.

II. Results and discussion

Test design and animal health

Two lactating goats received a daily dose of 170.75 mg [glyoxyl-phenyl-UL-^{14}C]trifloxystrobin for four consecutive days. Based on their body weight and the daily feeding rate this dose represented an intake of 4.13 mg/kg bw/day and a feed concentration of 100.4 mg/kg feed/day as means for both animals. The animals were sacrificed 6 hours after the last dose. No unusual appearance or behaviour of the animals was observed.

Elimination and recovery of radioactivity (Table B.7.2.3-11)

During the whole test period, 0.061% of the total dose was eliminated via milk, 36.04% was excreted with the faeces and 18.91% with the urine. In total, less than 1% of the administered dose was detected in milk, edible organs and tissues. These values were mean data of the respective samples from both goats. Summing up all radioactive residues in milk, excreta, organs and tissues and the gastro-intestinal tract, the sum resulted in a total recovery of 80.88% of the dose. The rest is assumed to be in the carcass that was not analysed.

Total radioactive residues in milk, tissues and organs and excreta

The total radioactive residues (TRR) recovered in milk, excreta and tissues were measured individually for each goat, however presented in Table B.7.2.3-11 as mean values together with the standard deviation.

After sacrifice, TRR amounted to 3.913 mg eq/kg in liver, 2.331 mg eq/kg in kidney, 0.077 mg eq/kg in muscle and 0.356 mg eq/kg in fat (Table B.7.2.3-11). All TRR values are the mean values of sample pools from both goats. In relation to the total dose administered, the TRR values in liver and kidney corresponded to 0.408% and 0.042%, respectively, and in muscle and fat to 0.098% and 0.084%, respectively.

Table B.7.2.3-11: Distribution of residues in milk, excreta and tissues of two lactating goats following oral administration of [glyoxyl-phenyl-UL-^{14}C]trifloxystrobin at a mean daily dose rate of 4.13 mg/kg body weight for four consecutive days

Matrix * (Pooled sample from both goats)	**Sampling time [h]**	**% of total dose administered** **Mean***	**TRR of sample pool [mg eq/kg]**** **Mean***
Total Milk	**0-78**	**0.061**	0.089
Total Faeces	**0-78**	**36.041**	-
Total Urine	**0-78**	**18.912**	-
Cage Wash	0-78	3.469	-
Cage Debris	0-78	0.140	-
Total Eliminated	**0-78**	**58.624**	-
Leg Muscle	78	0.095	0.077
Tenderloin	78	0.003	0.074
Total Muscle	*78*	*0.098*	*0.077*
Omental Fat	78	0.052	0.364
Perirenal Fat	78	0.032	0.343
Total Fat	*78*	*0.084*	*0.356*
Kidneys	78	0.042	2.331
Liver	78	0.408	3.913
Total Tissue	**78**	**0.632**	-
Blood	78	0.097	0.330
Bile	78	0.080	40.813
GIT/Rumen	78	21.442	-
Total Recovery	**0-78**	**80.875**	-

* weighted mean value of the pooled samples from both animals based on their proportion to the body weight
** [mg eq/kg] = mg/kg expressed as parent equivalents

Time course of total radioactive residues in milk

The radioactive residues in milk seems to approach a plateau level of about 0.105 mg eq/kg in the third day interval (48 – 72 h) (Table B.7.2.3-12); however, since the last milk sample was collected after an interval of 78 hours, it is not possible to determine definitively whether or not this was the plateau. In general, radioactive residues were secreted rapidly into milk: TRR in milk was higher in the morning interval 6 – 7 hours after each administration than during the rest of the day 7 – 24 hours after administration. The residue peak in milk was measured in the morning milk amounting to 0.116 mg eq/kg.

Table B.7.2.3-12: Time course of TRR in milk following oral administration [glyoxyl-phenyl-UL-^{14}C] trifloxystrobin at a mean daily dose rate of 4.13 mg/kg bw for four consecutive days

Interval [h]	% of total dose administered Mean*	TRR [mg eq/kg]** Mean*
0-7	0.002	0.025
7-24	0.011	0.076
0-24	*0.013*	*0.059*
24-31	0.009	0.116
31-48	0.012	0.091
24-48	*0.021*	*0.100*
48-55	0.009	0.116
55-72	0.013	0.099
48-72	*0.022*	*0.105*
72-78	0.005	0.112
0-78	**0.061**	**0.089**

* according to total weight and radioactivity of samples collected from both animals
** [mg eq/kg] = mg/kg expressed as parent equivalents

Extraction efficiency of residues

Samples of milk and tissues (muscle, kidney, liver and fat) were extracted with acetonitrile and with acetonitrile/water (4:1, v/v) (2-3 times). Faeces was extracted with methanol followed by methanol/water (4:1, v/v) (2 times). Details of extraction and fractionation of radioactive residues are given in the extraction schemes for milk (Table B.7.2.3-13), for muscle (Table B.7.2.3-14) and for fat (Table B.7.2.3-15), liver (Table B.7.2.3-16) and kidneys (Table B.7.2.3-17).

The extractability of residues in muscles, fat and kidneys after conventional extraction amounted to 88.9 – 98.3% of TRR, 97.2% of TRR for milk and 86.0% of the faecally excreted residues for faeces. Conventional extraction of liver with acetonitrile and acetonitrile/water (4:1, v/v, 3x) liberated 68.7% of the TRR. However, microwave assisted extraction at temperatures up to 180°C released completely the radioactive residues (99.5%).

Non-extractable residues amounted to 0.0025 mg eq/kg (2.8% of TRR) for milk, 0.0085 mg eq/kg (11% of TRR) for total muscle, 0.0061 mg eq/kg (2.1% of TRR) for total fat, 0.0822 mg eq/kg (2.1% of TRR) for liver and 0.1212 mg eq/kg (5.2% of TRR) for kidney.

Distribution of parent compound and metabolites in organs and tissues, milk and excreta

The distribution of trifloxystrobin residues in urine, faeces, milk, muscle, fat, kidney, and liver are summarized in Table B.7.2.3-18 to Table B.7.2.3-20.

Chemical names and structural formulae of the identified metabolites can be found in the “List of compounds identified” at the end of this study summary.

Metabolites in milk (Table B.7.2.3-19)
Overall 97.2% of TRR in pooled milk (TRR: 0.089 mg eq/kg in the 0 – 78 hours pool) were extracted conventionally with acetonitrile and water.

The parent compound trifloxystrobin was the main residue component and accounted for 73.8% of TRR (0.0657 mg/kg).

Several minor metabolites were identified as CGA 321113 (M 5), Met U8 (M58), Met 6U (M23), NOA 443152 (M10, Met), the taurine and glycine conjugate of CGA 321113 (Met L7a and L7b), Met 3F (M22) and Met 2F (M27) accounting for 1.0 – 4.8% TRR (0.0009 – 0.0043 mg eq/kg).

Metabolites in muscle (leg and tenderloin) (Table B.7.2.3-19)
Overall 88.9% of TRR in muscle (TRR: 0.077 mg eq/kg) was extracted conventionally using acetonitrile and water.

The major components in muscle were identified as acid metabolite CGA 321113 (M 5), accounting for 51.1% of TRR (0.0393 mg eq/kg) and parent compound accounting for 26.5% of TRR (0.0204 mg eq/kg).

Minor metabolites were identified as NOA 443152 (M10, Met 2U) and NOA 412443 (M 9, Met 1U) amounting to 1.8% of TRR (0.00014 mg eq/kg) and 2.8% of TRR (0.0022 mg eq/kg), respectively. In addition, the taurine and the glycine conjugates of CGA 321113, (Met L7a) (Met L7b) were detected at a level of 0.3% of TRR (0.0002 mg eq/kg) and 2.0% of TRR (0.0016 mg eq/kg), respectively.

Metabolites in fat (omental and perireneal) (Table B.7.2.3-19)
Overall 98.3% of TRR in fat (TRR: 0.356 mg eq/kg) was extractable with acetonitrile and acetonitrile/water (4:1, v/v, 2x).

Parent trifloxystrobin was the predominant residue component and accounted for 82% of TRR (0.2921 mg/kg). Besides parent compound only one metabolite, i.e. CGA 321113 (M 5) was present, which accounted for 11.3% (0.0401 mg eq/kg).

Metabolites in kidney (Table B.7.2.3-20)
Overall 94.8% of TRR in kidney (TRR: 2.331 mg eq/kg) was extractable by conventional extraction with acetonitrile followed by 2x acetonitrile/water (4:1, v/v).

The predominant residue component was identified as metabolite CGA 321113 (M 5) accounting for 73.5% of TRR (1.7142 mg eq/kg).

Several minor metabolites were identified as taurine and glycine conjugate of CGA 321113 (Met L7a and L7b), Met U8 (M58), Met 1G (glucuronide conjugate of NOA 405637, M27, Met 2F), NOA 443152 (M10, Met 2U) and NOA 412443 (M29, Met 1U). These metabolites accounted for 1.4 – 4.9% of TRR (0.0323 – 0.1144 mg eq/kg).

Metabolites in liver (Table B.7.2.3-20)
After conventional extraction with acetonitrile followed by acetonitrile/water (4:1, v/v, 3x) 68.7% of TRR in liver was extractable at room temperature (TRR: 3.913 mg eq/kg). Exhaustive extraction with 2-propanol/H2O, (4:1, v/v) and microwave assistance at temperatures up to 180°C released additionally 30.8% of TRR, resulting in total extractable residues of 97.9% of TRR.

Conventional and exhaustive extraction revealed the acid metabolite CGA 321113 (M 5) as main residue component, accounting for 39.6% of TRR (1.5494 mg eq/kg). One major metabolite was identified as the glycine conjugate of CGA 321113 (Met L7b) amounting to 11.8% of TRR (0.4614 mg eq/kg). The parent compound was only a minor residue component in the liver amounting to 2.5% of TRR, (0.0966 mg/kg).

Minor metabolites were detected as Met U8 (M58), Met 6U (M23), Met 1G (glucuronide conjugate of NOA 405637, M27, Met 2F), NOA 443152 (M10, Met 2U), the taurine conjugate of CGA 321113 (Met L7a), Met 3F (M22) and Met 2F (M27). They accounted for 0.9 – 5.2% of TRR (0.0337 – 0.2054 mg eq/kg).

CGA 166988 (M 52) accounting for 11.9% of TRR (0.4645 mg eq/kg) was discussed as a probable breakdown product generated during microwave assisted high-temperature extraction.

Metabolites in urine (Table B.7.2.3-18)
84.5% of the renally excreted compounds were identified by HPLC and TLC co-chromatography with reference standards.

The predominant metabolite in urine was identified as the acid metabolite CGA 321113 (M5) amounting to 71.7% of the renal radioactivity. Minor metabolites were identified as U8 (M58), NOA 443152 (M10), NOA 412443 (M29, Met), the taurine and glycine conjugates of CGA 321113 (L7a and L7b) accounting for 1.6 – 7.3% of the renal radioactivity. The parent compound trifloxystrobin was not excreted with the urine.

Metabolites in faeces (Table B.7.2.3-18)
Overall 86.0% of the faecally excreted radioactivity was extracted with methanol followed by 2x methanol/water (4:1, v/v). 81.3% of the faecally excreted residues were identified by HPLC and TLC co-chromatography.

The parent compound trifloxystrobin (CGA 279202) accounted for 48.2%.The acid metabolite CGA 321113 (M 5) was present as major metabolite accounting for 13.3%.

Minor metabolites were identified as Met U8 (M58), NOA 443152 (M10, Met 2U), NOA 412443 (M29, Met 1U), the taurine and glycine conjugates of CGA 321113 (Met L7a and L7b), Met 7F (trifloxystrobin hydroxylated in position 4 of the glyoxyl-phenyl ring) and NOA 405637 (M27, Met 2F). These metabolites accounted for 0.3 – 7.4% of the faecal radioactivity.

Metabolite stability upon exhaustive extraction
In a control experiment the liver extract was incubated in the microwave oven under the same conditions (180°C) as the non-extractable residues. Analysis of the extract before and after incubation showed that taurine and the glycine conjugates of CGA 321113 L7a and L7b remained unchanged. However, the aglycon CGA 321113 (M 5) was quantitatively transformed to CGA 357276 (M 42).

Table B.7.2.3-13: Extraction and sample preparation scheme for milk
(milk pool from both goats and the whole collection period)

	Milk
TRR [mg eq/kg]*	**0.089**
% of TRR	**100**
Acetonitrile followed by 3x Acetonitrile/Water (4:1, v/v)	97.2
Partitioning	
Hexane	2.5
Acetonitrile	**94.7**
Total extracted	97.2
PES	2.8
Accountability	100

* [mg eq/kg] = mg/kg expressed as parent equivalents
PES: post extraction solids = non-extractable radioactivity
Accountability: sum of extracts and PES
Extract/fraction **in bold**: extract used for quantitative analysis.

Table B.7.2.3-14: Extraction and sample preparation scheme for muscle
(pool of leg and tenderloin muscle from both goats)

	Total muscle
TRR [mg eq/kg]*	**0.077**
% of TRR	**100**
Acetonitrile followed by 3x Acetonitrile/Water (4:1, v/v)	88.9
Hexane phase	1.5
Acetonitrile	87.4
Fractionation (C_{18} column)	
H_2O/Methanol (4:1, v/v) followed by Methanol	**83.1**
Methanol	1.1
H_2O followed by H_2O/Methanol (9:1, v/v)	3.2
Separation on prep. TLC (with Methanol)	
Fraction 1	0.5
Fraction 2	2.3
Fractionation (C_{18} column)	
H_2O	0.3
H_2O	0.3
H_2O/Methanol (9:1, v/v) followed by H_2O/Methanol (4:1, v/v) followed by Methanol	**1.6**
Methanol	0.1
Fraction 3	**0.4**
Total extracted	88.9
PES	11.1
Accountability	100

* [mg eq/kg] = mg/kg expressed as parent equivalents
PES: post extraction solids = non-extractable radioactivity
Accountability: sum of extracts and PES
Extract/fraction **in bold**: extract used for quantitative analysis.

Table B.7.2.3-15: Extraction and sample preparation scheme for fat
(pool of omental and perirenal fat from both goats)

	Total fat
TRR [mg eq/kg]*	**0.356**
% of TRR	**100**
Acetonitrile followed by 2x Acetonitrile/Water (4:1, v/v)	98.3
Hexane phase	4.0
Acetonitrile	94.3
Fractionation (Si-60 cartridge column)	
Hexane followed by dichloromethane/hexane (1:9, v/v)	0.0
Dichloromethane/hexane (4:1, v/v) followed by Dichloromethane followed by methanol	94.1
Separation by prep. TLC (solvent system AJ7, Hexane and Methanol)	
Fraction 1	0.8
Fraction 2	**93.3**
Methanol followed by H_2O	0.2
Total extracted	98.3
PES	1.7
Accountability	100

* [mg eq/kg] = mg/kg expressed as parent equivalents
PES: post extraction solids = non-extractable radioactivity
Accountability: sum of extracts and PES
Extract/fraction **in bold**: extract used for quantitative analysis.

Table B.7.2.3-16: Extraction and sample preparation scheme for liver
(liver pool from both goats)

	Liver
TRR [mg eq/kg]*	**3.913**
% of TRR	**100**
Acetonitrile followed by 3x Acetonitrile/Water (4:1, v/v)	68.7
Hexane phase	1.5
Acetonitrile	67.2
Fractionation (C_{18} column)	
H_2O	2.7
H_2O/Methanol (4:1-1:4, v/v)	**62.9**
Methanol	1.6
PES (after conventional extraction)	31.3
Microwave extraction (up to 180°C) (2-Propanol/H_2O, 4:1, v/v)	30.8
Fractionation (C_{18} column)	
H_2O followed by H_2O/Methanol (1:9, v/v)	1.1
H_2O/Methanol (1:4, v/v) followed by Methanol	**26.8**
Methanol followed by Dichloromethane	1.3
Residues	1.6
PES final	0.5
Total extracted (conventional)	68.7
Total extracted (conventional followed by exhaustive)	99.5
Accountability	100

* [mg eq/kg] = mg/kg expressed as parent equivalents
PES: post extraction solids = non-extractable radioactivity
Accountability: sum of extracts and PES
Extract/fraction **in bold**: extract used for quantitative analysis.

Table B.7.2.3-17: Extraction and sample preparation scheme for kidney
(kidney pool from both goats)

	Kidney
TRR [mg eq/kg]*	**2.331**
% of TRR	**100**
Acetonitrile followed by 2x Acetonitrile/Water (4:1, v/v)	94.8
Hexane phase	1.3
Acetonitrile	**93.5**
PES	5.2
Total extracted (conventional)	94.8
Accountability	100

* [mg eq/kg] = mg/kg expressed as parent equivalents
PES: post extraction solids = non-extractable radioactivity
Accountability: sum of extracts and PES
Extract/fraction in **bold**: extract used for quantitative analysis.

Table B.7.2.3-18: Radioactive residues in pooled urine and faeces of lactating goats following oral administration of [glyoxyl-phenyl-UL-^{14}C]trifloxystrobin at a mean daily dose rate of 4.13 mg/kg bw for four consecutive days

	Urine	**Faeces**
Compound	**% of renally excrected residues**	**% of faecally excreted residues**
Parent compound		
Trifloxystrobin (CGA 279202)	-	**48.2**
Free metabolites	-	
CGA 321113 (M5)	**71.7**	**13.3**
NOA 443152 (Met 2U, M 10)	2.7	1.2
Met 7F	-	2.8
Met 3F	-	-
Met 6U	-	-
NOA 405637 (Met 2F, M 27)	-	7.4
NOA 405637, (M27, Met 1G, Met 2F)	-	-
NOA 412443 (Met 1U, M 29)	6.7	2.0
Met U8 (M 58)	1.8	0.8
CGA 166988 (M 52)*	-	-
Conjugated metabolites		
Taurine conjugate of CGA 321113 (Met L7a)	1.6	**0.3**
Glycine conjugate of CGA 321113 (Me L7b)	7.3	**5.3**
Glucuronide of NOA 405637 (2F, Met 1G)	-	-
Total extracted	**100.0**	**86.0**
Not analyzed (polar)	8.1	1.9
Not analyzed (apolar)	-	2.8
Total not analyzed	8.1	4.7
PES (non-extractable)	**-**	**14.0**
Accountability	**100.0**	**100.0**

* artificially generated by degradation during microwave assisted high-temperature extraction.

Table B.7.2.3-19: Radioactive residues in pooled muscle, fat and kidney of lactating goats following oral administration of [glyoxyl-phenyl-UL-^{14}C]trifloxystrobin at a mean daily dose rate of 4.13 mg/kg bw for four consecutive days

	Milk		**Total muscle**		**Total fat**	
TRR [mg eq/kg]	**0.089**		**0.077**		**0.356**	
Compound	**% TRR**	**mg eq/kg**	**% TRR**	**mg eq/kg**	**%TRR**	**mg eq/kg**
Parent compound						
Trifloxystrobin (CGA 279202)	**73.8**	**0.0657**	**26.5**	**0.0204**	**82.0**	**0.2921**
Free metabolites						
CGA 321113 (M5)	4.8	0.0043	**51.1**	**0.0393**	**11.3**	**0.0401**
NOA 443152 (Met 2U, M10)	1.6	0.0015	1.8	0.0014	-	-
Met 7F	-	-	-	-	-	-
Met 3F	2.6	0.0023	-	-	-	-
Met 6U (M23)	4.7	0.0041	-	-	-	-
NOA 405637 (Met 2F, M 27)	1.7	0.0016	-	-	-	-
NOA 412443 (Met 1U, M 29)	-	-	2.8	0.0022	-	-
Met U8 (M58)	1.2	0.001	-	-	-	-
CGA 166988 (M52)*	-	-	-	-	-	-
Conjugated metabolites						
Taurine conjugate of CGA 321113 (Met L7a)	3.3	0.0029	0.3	0.0002	-	-
Glycine conjugate of CGA 321113 (Met L7b)	1.0	0.0009	2.0	0.0016	-	-
Glucuronide of NOA 405637 (2F, Met 1G)	-	-	-	-	-	-
Total extracted	**97.2**	**0.0865**	**88.9**	**0.0685**	**98.3**	**0.3499**
Not analyzed (polar)	-	-	1.7	0.0013	1.0	0.0036
Not analyzed (apolar)	2.5	0.0022	2.7	0.0021	4.0	0.0142
Total not analyzed	2.5	0.0022	4.4	0.0034	5.0	0.0178
PES (non-extractable)	**2.8**	**0.0025**	**11.0**	**0.0085**	**1.7**	**0.0061**
Accountability	**100.0**	**0.089**	**100.0**	**0.077**	**100.0**	**0.356**

* artificially generated by degradation during microwave assisted high-temperature extraction.

Table B.7.2.3-20: Radioactive residues in pooled liver and milk of lactating goats following oral administration of [glyoxyl-phenyl-UL-^{14}C]trifloxystrobin at a mean daily dose rate of 4.13 mg/kg bw for four consecutive days

	Liver		Liver#		Kidney	
TRR [mg eq/kg]	**3.913**		**3.913**		**2.331**	
Compound	**% TRR**	**mg eq/kg**	**% TRR**	**mg eq/kg**	**% TRR**	**mg eq/kg**
Parent compound						
Trifloxystrobin (CGA 279202)	**1.0**	**0.0398**	**2.5**	**0.0966**	**1.8**	**0.0423**
Free metabolites						
CGA 321113 (M5)	**38.1**	**1.4927**	**39.6**	**1.5494**	**73.5**	**1.7142**
NOA 443152 (Met 2U, M10)	1.6	0.0644	1.6	0.0644	1.6	0.0373
Met 7F	-	-	-	-	-	-
Met 3F	0.9	0.0337	0.9	0.0337	-	-
Met 6U (M 23)	2.7	0.1073	2.7	0.1073	-	-
NOA 405637 (Met 2F, M27)	0.9	0.0368	1.9	0.0736	-	-
NOA 412443 (Met 1U, M29)	-	-	-	-	3.8	0.0896
Met U8 (M58)	2.5	0.0981	3.6	0.1425	3.4	0.0796
CGA 166988 (M52)*	-	-	11.9	0.4645	-	-
Conjugated metabolites						
Taurine conjugate of CGA 321113 (Met L7a)	5.2	0.2054	5.2	0.2054	1.4	0.0323
Glycine conjugate of CGA 321113 (Met L7b)	7.9	0.3096	**11.8**	**0.4614**	4.9	0.1144
Glucuronide of NOA 405637 (2F, Met 1G)	1.9	0.0736	1.9	0.0736	3.0	0.0697
Total extracted	**68.7**	**2.6882**	**97.9**	**3.8308**	**94.8**	**2.2098**
Not analyzed (polar)	2.7	0.1057	9.9	0.3863	-	-
Not analyzed (apolar)	3.1	0.1213	4.4	0.1722	1.3	0.0303
Total not analyzed	5.8	0.2270	14.3	0.5585	1.3	0.0303
PES (non-extractable)	**31.3**	**1.2248**	**2.1**	**0.0822**	**5.2**	**0.1212**
Accountability	**100.0**	**3.913**	**100.0**	**3.913**	**100.0**	**2.331**

* artificially generated by degradation during microwave assisted high-temperature extraction.
# after microwave assisted high-temperature extraction

III. Conclusion

The metabolism of [glyoxyl-phenyl-UL-^{14}C]trifloxystrobin in two lactating goats was investigated after oral administration of the radiolabelled test substance at a dose rate of 4.13 mg/kg bw/day for four consecutive days (one dose per day). This dose rate corresponded to 100.4 mg/kg in the diet. Milk was collected twice daily and excreta were collected on daily basis. The goats were slaughtered 6 hours after the last dose (78 hours after the first dose) and leg muscle, tenderloin, omental and perirenal fat, kidney and liver as well as blood, bile and the gastrointestinal tract were dissected. Pooled milk samples and pooled tissues were analysed for the radioactive residues.

TRR levels

During the whole test period 0.06%, 36.04% and 18.91% of the dose totally applied were eliminated via milk, faeces and urine, respectively. These values were mean data of the respective samples from both goats. In total, less than 1% of the administered dose was recovered in the milk and tissues. Summing up all radioactive residues in milk, excreta, organs and tissues and the gastro-intestinal tract, the sum resulted in a total recovery of 80.88% of the dose. The rest is assumed to be in the carcass that was not analysed.

The highest TRR in milk was measured after in the third-day-interval (48 – 72 hours from start of dosing) amounting to approx. 0.105 mg eq/kg; however, since the last milk sample was collected after an interval of

78 hours, it is not possible to determine definitively whether or not this was the plateau. The TRR peak was measured in the morning milk amounting to 0.116 mg eq/kg.

TRR in tissues amounted to 3.913 mg eq/kg in liver, 2.331 mg eq/kg in kidney, 0.077 mg eq/kg in muscle and 0.356 mg eq/kg in fat. The average residues in milk were 0.089 mg eq/kg (pooled over the whole study period 0 – 78 hours).

Extractability

The extractability of the milk and tissue samples by conventional extraction at room temperature was high (≥ 86% of the respective TRR), with the exception of liver (68.7% of TRR). Microwave assisted extraction at elevated temperatures (≤ 180°C) released additional radioactive residues from the liver and thus increasing the extractability to 99.5% of TRR.

Major residue components

- Trifloxystrobin was found as the major compound in fat (82.0% of TRR, 0.292 mg/kg) and in milk (73.8% of TRR, 0.0657 mg/kg) and to a smaller extent in muscle (26.5% of TRR, 0.0204 mg/kg), liver (2.5% of TRR, 0.0398 mg/kg) and kidneys (1.8% of TRR, 0.0423 mg/kg). Trifloxystrobin was also the major residue eliminated via faeces (48.2% of the faecal residues).
- CGA 321113 (M5) formed by ester hydrolysis of trifloxystrobin was the main residue component in muscle (51.1% of TRR, 0.0393 mg eq/kg), kidneys (73.5% of TRR, 1.714 mg eq/kg) and liver (39.6% of TRR, 1.549 mg eq/kg). It was also present in fat (11.3% of TRR, 0.0401 mg eq/kg) and in milk (4.8% of TRR, 0.0043 mg eq/kg). CGA 321113 was the main residue component in urine (71.7% of renal residues) and was detected in faeces (13.3% of the faecal residues).
- Minor metabolites were identified as a taurine conjugate of CGA 321113 (Met L7a), found in liver (5.2%, 0.2054 mg eq/kg), muscle (0.3%, 0.0002 mg eq/kg), kidneys (1.4%, 0.0323 mg eq/kg), milk (3.3%, 0.0029 mg eq/kg), urine (1.6%) and faeces (0.3%).
- The glycine conjugate of CGA 321113 (Met L7b), found in muscle (2.0%, 0.0016 mg eq/kg), liver (11.8%, 0.4614 mg eq/kg), kidneys (4.9%, 0.1144 mg eq/kg), milk (1.0%, 0.0009 mg eq/kg), urine (7.3%) and faeces (5.3%).
- Further minor metabolites were identified as NOA 443152 (M10), Met 3F, M23 (Met 6U), NOA 405637 (M27, Met 2F) and its glucuronide conjugate, NOA 412443 (M29, Met 1U), and M58 (Met U8). They were detected generally at a low level < 5% of TRR and < 0.01 mg eq/kg in milk, muscle and fat. They slightly exceeded a level of 0.01 mg eq/kg in kidneys and a level of 0.1 mg eq/kg in liver due to the higher TRR values in liver and kidneys.

Metabolic reactions

Based on the structures identified the metabolism of trifloxystrobin in lactating goat proceeded predominantly via hydrolysis, demethylation and hydroxylation reactions followed by conjugation with taurine and glycine:

- Deposition of trifloxystrobin as the major residue component in fat and milk.
- Hydrolysis of the methyl ester group of trifloxystrobin to CGA 321113 (M5) as the predominant metabolite in muscle, liver and kidneys and as major metabolite in urine and faeces.
- Conjugation of CGA 321113 with glycine to metabolite L7b as the major metabolite in liver and conjugation with taurine as the minor metabolite L7a.
- Demethylation of the methoxyimino group to metabolites NOA 405637 (2F, M27), NOA 412443 (1U, M29) and 6U (M23).
- Conjugation of metabolite NOA 405637 (2F, M27) with glucuronic acid to metabolite L5 (Met 1G).
- Hydroxylation at the aminooxymethyl group to metabolites NOA 443152 (2U, M10), 3F (M22) and 6U (M23).
- Hydroxylation of the glyoxyl-phenyl ring in position 4 yielding trace amounts of metabolite 7F.
- No label-specific metabolites (resulting from cleavage of the bridge between the phenyl rings) were detected in this study.

In summary, major metabolites identified in lactating goat were also found in rat and hen and the general metabolic pathways were similar to those in rat and goat.

The parent substance trifloxystrobin and its hydrolyzed metabolite CGA 321113 (M5) can serve as analytical targets for a residue method in food of animal origin since these two components contributed mainly to the total residues.

Based on these results the metabolic pathway of [glyoxyl-phenyl-UL-^{14}C]trifloxystrobin in lactating goats is proposed as shown in Figure B.7.2.3-2.

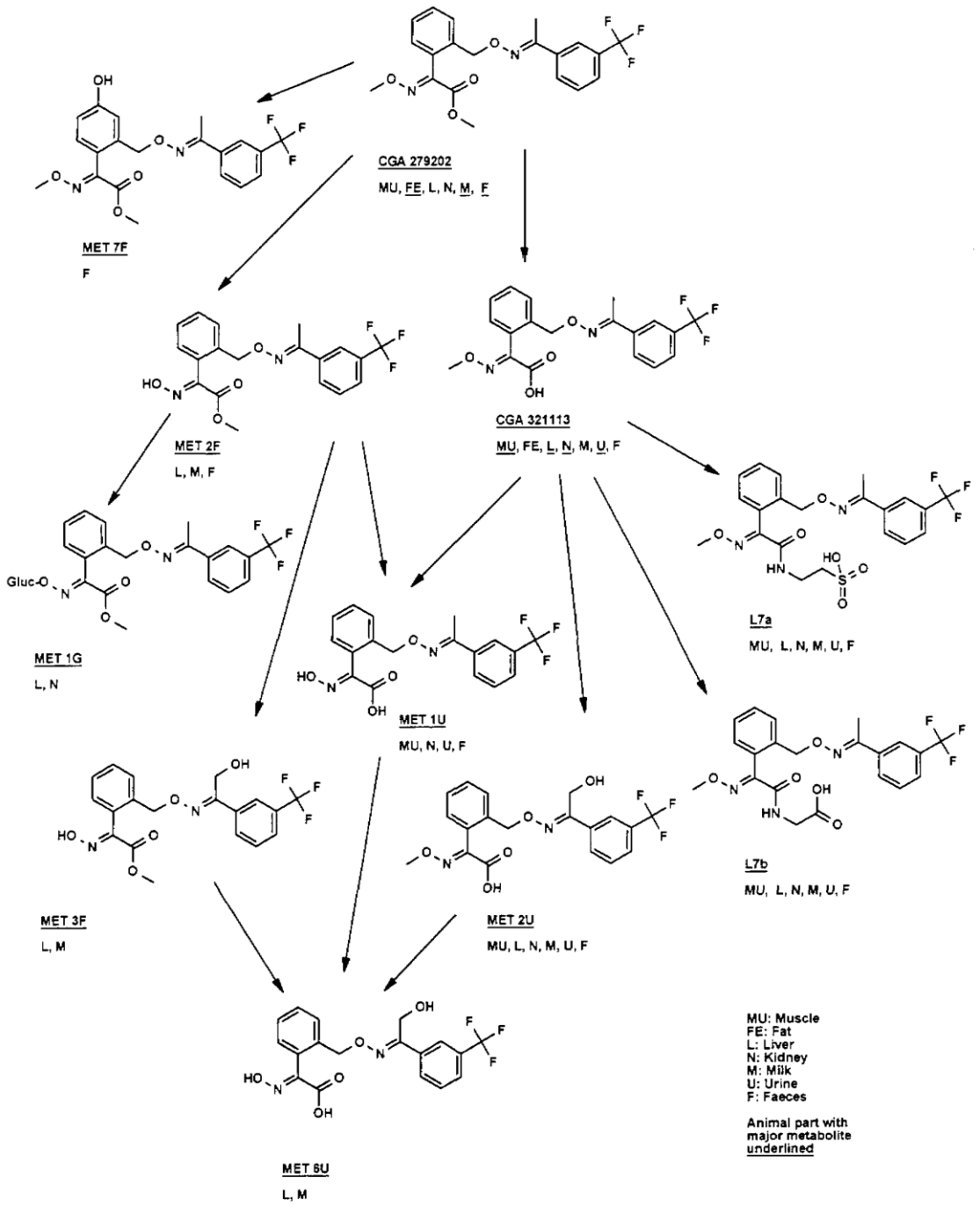
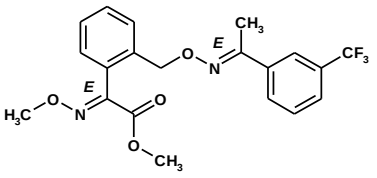
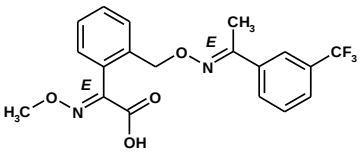
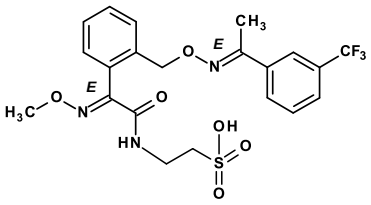
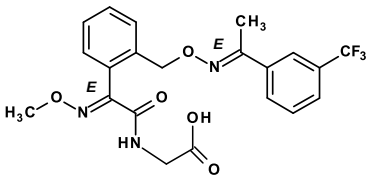
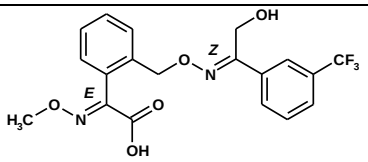
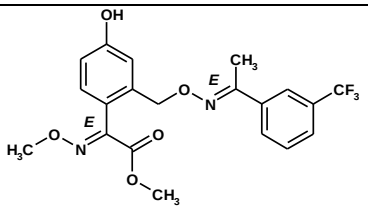
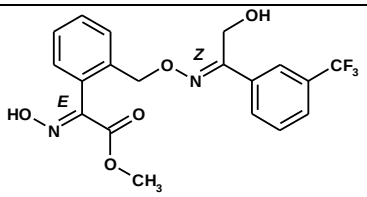
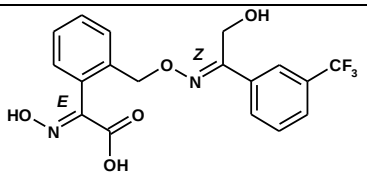
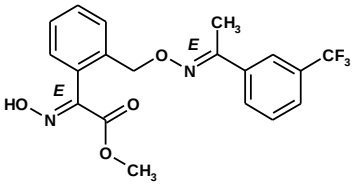
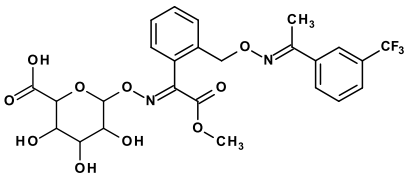
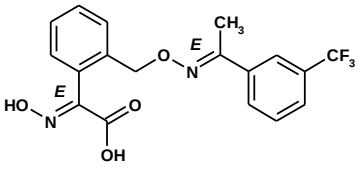
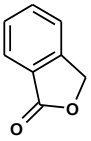
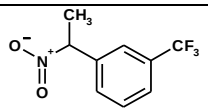


Figure B.7.2.3-2: Proposed metabolic pathway of [glyoxyl-phenyl-UL-^{14}C]trifloxystrobin in the lactating goat

List of compounds identified in this metabolism study

No	Common Name/Code	Chemical Name	Chemical structure
1	Trifloxystrobin/ CGA 279202 *(EE*-isomer)	methyl (2E)-(methoxyimino) (2-{[({(1E)-1-[3-(trifluoromethyl) phenyl]ethylidene}amino)oxy] methyl}phenyl)acetate (*E,E*)-methoxyimino-{2-[1-(3-trifluoro methyl-phenyl)-ethylideneaminooxymethyl]-phenyl}-acetic acid methyl ester	
5	CGA 321113 (*EE*-isomer)	(2*E*)-(methoxyimino)(2-{[({(1*E*)-1-[3-(trifluoromethyl)phenyl]ethylidene} amino)oxy]methyl}phenyl) acetic acid *(E,E)*-methoxyimino-{2-[1-(3-trifluoro methyl-phenyl)-ethylideneaminooxy-methyl]-phenyl}-acetic acid	
5a	Taurine conjugate of CGA 321113/ Met L7a (*EE*-isomer)	Taurine conjugate of (2*E*)-(methoxyimino)(2-{[({(1*E*)-1-[3-(trifluoromethyl)phenyl]ethylidene} amino)oxy]methyl}phenyl) acetic acid Taurine conjugate of *(E,E)*-methoxyimino-{2-[l-(3-trifluorornethyl-phenyl)-ethylidene-aminooxymethyl]-phenyl}-acetic acid	
5b	Glycine conjugate of CGA 321113/ Met L7b (*EE*-isomer)	Glycine conjugate of (2*E*)-(methoxyimino)(2-{[({(1*E*)-1-[3-(trifluoromethyl)phenyl]ethylidene} amino)oxy]methyl}phenyl) acetic acid Glycine conjugate of *(E,E)*-methoxyimino-{2-[l-(3-trifluoromethyl-phenyl)-ethylidene-aminooxymethyl]-phenyl}-acetic acid	
10	NOA 443152/ Met 2U	(2*E*)-(2-{[({(1*Z*)-2-hydroxy-1-[3-(trifluoromethyl)phenyl]ethylidene} amino)oxy]methyl}phenyl) (methoxyimino)acetic acid 2-[2-hydroxy-1-(3-trifluoromethyl-phenyl)-ethylidene-aminooxymethyl]-phenyl}-methoxyimino-acetic acid	
19	Met 7F (*EE*-isomer)	methyl (2*E*)-(4-hydroxy-2-{[({(1*E*)-1-[3-(trifluoromethyl)phenyl] ethylidene}amino)oxy]methyl} phenyl)(methoxyimino)acetate {4-hydroxy-2-[1-(3-trifluoromethyl-phenyl)-	

No	Common Name/Code	Chemical Name	Chemical structure
		ethylideneaminooxymethyl]-phenyl}-methoxyimino-acetic acid methyl ester	
22	Met 3F (*ZE*-isomer)	methyl (2*E*)-(hydroxyimino)(2-{[({(1*Z*)-2-hydroxy-1-[3-(trifluoromethyl)phenyl]ethylidene} amino)oxy]methyl}phenyl)acetate hydroxyimino-{2-[2-hydroxy-1-(3-trifluoro methyl-phenyl)-ethylideneaminooxymethyl]-phenyl}-acetic acid methyl ester	
23	Met 6U	(2*E*)-(hydroxyimino)(2-{[({(1*Z*)-2-hydroxy-1-[3-(trifluoromethyl) phenyl]ethylidene}amino)oxy]methyl} phenyl)acetic acid hydroxyimino-{2-[2-hydroxy-1-(3-trifluoro methyl-phenyl)-ethylidene aminooxymethyl]-phenyl}-acetic acid	
27	NOA 405637 (*EE*-isomer)/ 2F	methyl (2*E*)-(4-hydroxy-2-{[({(1*E*)-1-[3-(trifluoromethyl)phenyl] ethylidene}amino)oxy]methyl} phenyl)(methoxyimino)acetate hydroxyimino-{2-[1-(3-trifluoromethyl-phenyl)-ethylideneaminooxymethyl]-phenyl}-acetic acid methyl ester	
27a	Met 1G (=L5)	glucuronic acid conjugate of methyl (2*E*)-(hydroxyimino)(2-{[({(1*E*)-1-[3-(trifluoromethyl) phenyl]ethylidene}amino)oxy] methyl}phenyl)acetate glucuronic acid conjugate of hydroxyimino-{2-[l-(3-trifluoromethyl-phenyl)-ethylideneaminooxymethyl]-phenyl}-acetic acid methyl ester	
29	NOA 412443 (*EE*-isomer)/ Met 1U	(2*E*)-(hydroxyimino)(2-{[({(1*E*)-1-[3-(trifluoromethyl)phenyl] ethylidene}amino)oxy]methyl} phenyl)acetic acid hydroxyimino-{2-[1-(3-trifluoro-methyl-phenyl)-ethylidene-amino-oxymethyl]-phenyl}-acetic acid	
52*	CGA 166988	2-benzofuran-1(3H)-one 3-*H*-isobenzofuranon-1-one	

No	Common Name/Code	Chemical Name	Chemical structure
58	Met U8	l-(l-nitro-ethyl)-3-trifluoromethyl-benzene	

* artificially generated by degradation during microwave assisted high-temperature extraction.

3.10.3.1 Summary of ruminant metabolism studies in the DAR

Data on ruminant metabolism studies were reviewed in the framework of the peer review under Directive 91/414/EEC.

In summary, goats were dosed daily with [^{14}C-TP] trifloxstrobin at 103.8 mg/kg diet (a mean of 4.24 mg/kg bw/day; 130N) and [^{14}C-GP] trifloxystrobin at 100.4 mg/kg diet (a mean of 4.13 mg/kg bw/day; 127N) for four days. It is noted that in both studies, the goats were dosed for 4 consecutive days rather than 5 as recommended in OECD 503. This is not considered to have had a significant impact on the outcome of the studies.

Up to 18.9 % of the dose was excreted in the urine and 44.5 % in the faeces. Highest residue levels in milk were 0.121 and 0.153 mg/kg. Trifloxystrobin was the major residue.

After sacrifice the highest amount of residue was found in liver and kidney (up to 4.8 and 2.3 mg/kg, respectively). Highest residues in muscle and fat were 0.08 and 0.35 mg/kg, respectively. Trifloxystrobin and the metabolite CGA 321113 were dominant in muscle, fat, kidney and liver. The taurine and glycine conjugates of CGA 321113 (L7a (CGA 321113 taurine conjugate) and L7b (CGA 321113 glycine conjugate)) were also major metabolites in liver. Other identified metabolites did not individually exceed 5 % TRR. Identified metabolites comprised up to 93.5 % TRR (milk), 84.5 % (muscle), 93 % (fat), 90 % (kidney) and 60 % (liver). There was some evidence of cleavage of the molecule between the two phenyl rings with the formation of metabolites 11U and 12 U (CGA 354870). The goat metabolites were all identified in the rat metabolism studies.

Based on the log Kow, trifloxystrobin can be considered to be fat soluble.

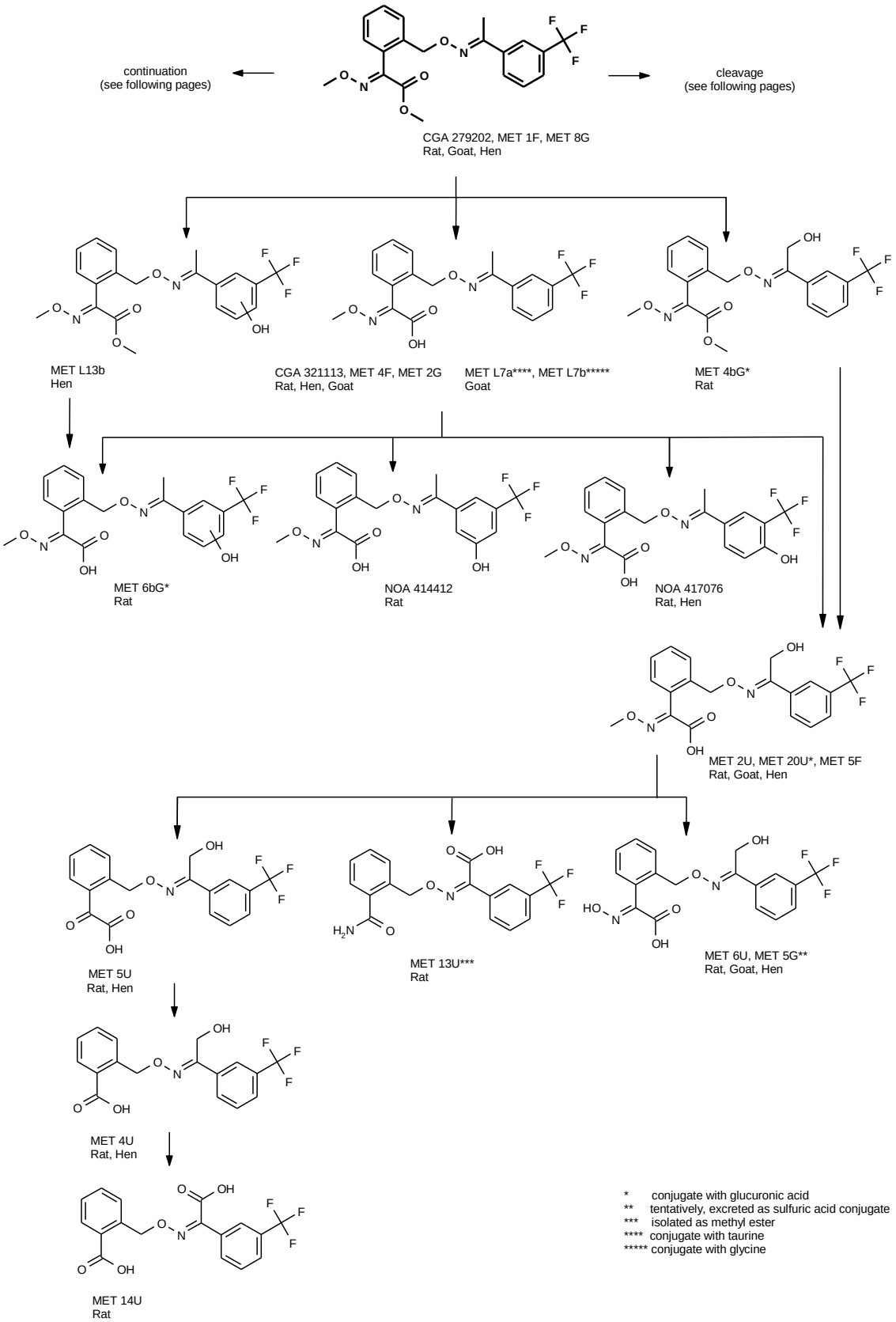


Figure B.7.2.6-1: Proposed metabolism of trifloxystrobin in rat, goat and hen

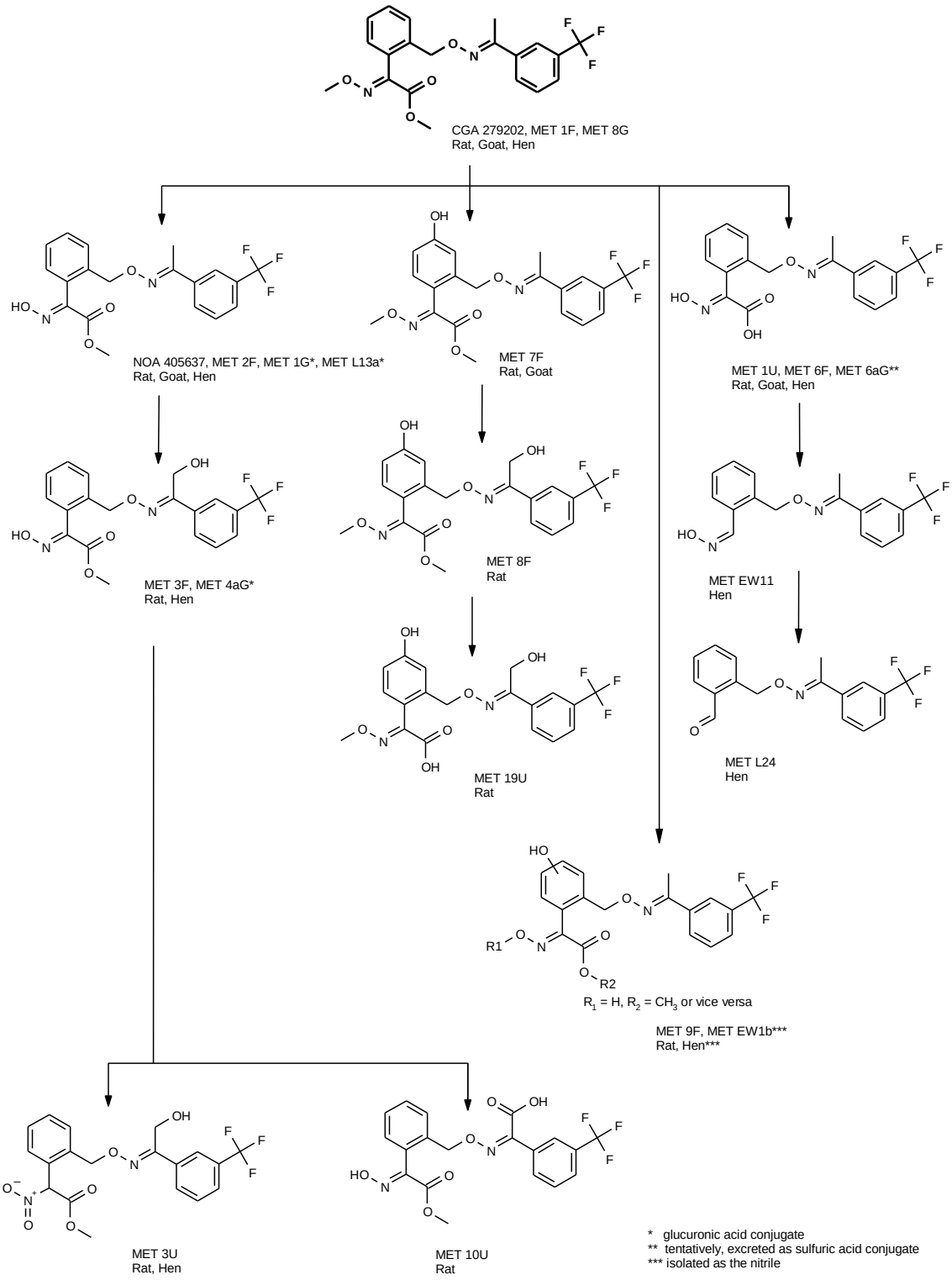


Figure B.7.2.6-1 cont - Continuation: Proposed metabolism of trifloxystrobin in rat, goat and hen

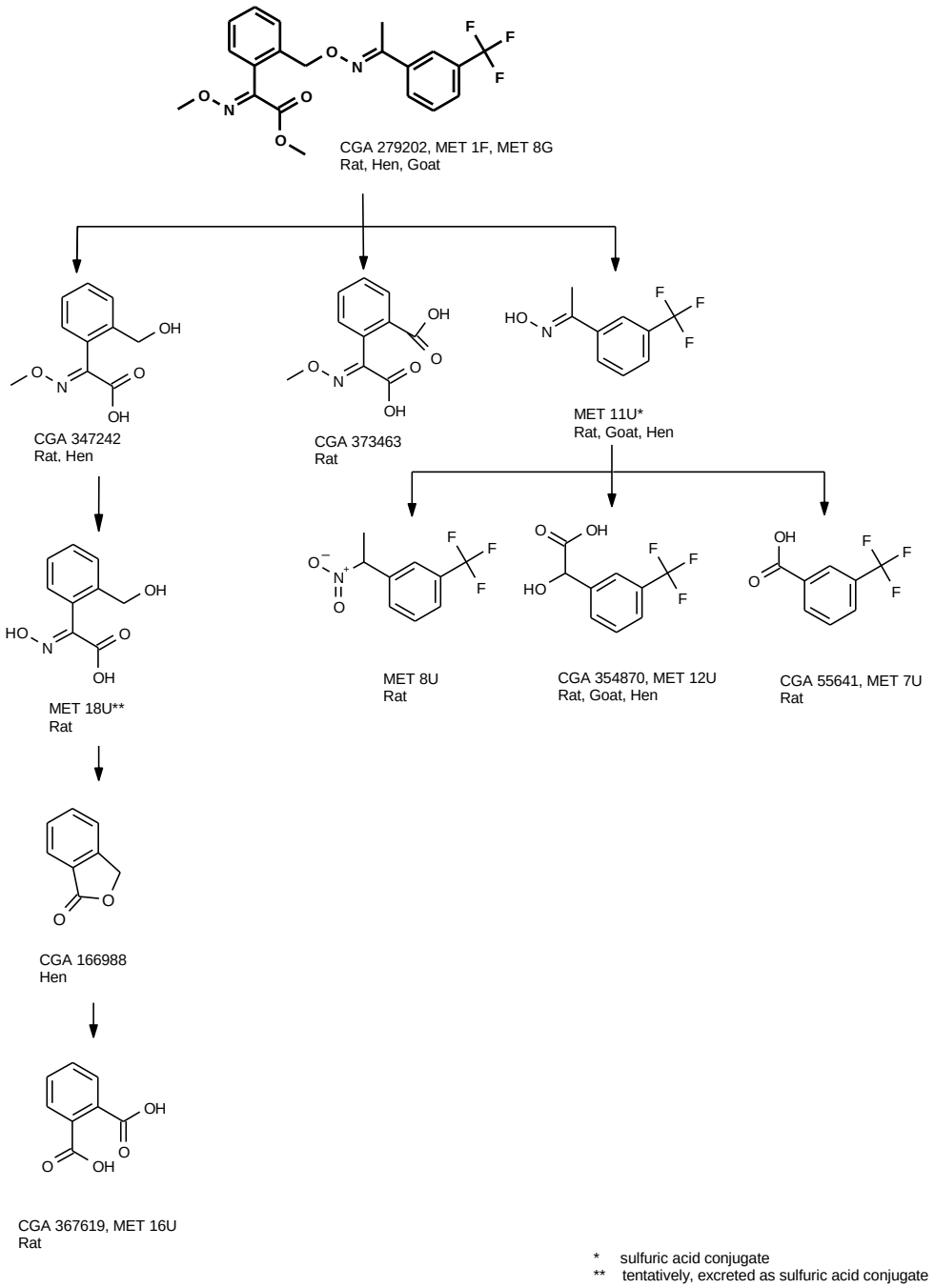


Figure B.7.2.6-1 cont - Cleavage: Proposed metabolism of trifloxystrobin in rat, goat and hen

[Study 3] Magnitude of the residues in meat and milk resulting from the feeding of three levels to dairy cattle (Anonymous, 1997c, M-038221-01-1)

Study:	CGA-279202 – Magnitude of the residues in meat and milk resulting from the feeding of three levels to dairy cattle
Document No.:	M-038221-01-1
Guideline(s):	OPPTS 860.1480 Residue Chemistry Test Guidelines – Meat/Milk/Poultry/Eggs
Guideline deviation(s):	--
GLP/GEP:	**yes**

The ruminant feeding study was conducted according to EPA guideline OPPTS 860.1480. The study parameters comply with OECD guideline 505 (3 dose levels; capsule application for 28 days; 3 animals per dose level; sampling and analysis of meat, 2 fat types, liver, kidney, milk).

Summary

Trifloxystrobin was administered orally (via capsule) to lactating Holstein cow for 28 – 30 consecutive days at average dose rates of 2.0, 5.9 and 21 mg/kg feed dry matter corresponding to 0.065, 0.193 and 0.635 mg/kg bw/day, respectively.

During the course of the study, no residues of trifloxystrobin (CGA 279202) and its acid metabolite CGA 321113 were determined above the LOQ of 0.01 mg/kg in the milk from any sampling event and of any dose group.

No residues above the LOQ of 0.02 mg/kg of both parent compound trifloxystrobin and CGA 321113 were found in samples of round muscle and tenderloin muscle of the 21 mg/kg-dose.

No residues of trifloxystrobin were found in liver and kidney samples at any dose rate. However, its metabolite CGA 321113 was detected in liver (up to 0.09 mg/kg) and kidney (up to 0.02 mg/kg) from cows at the highest dose rate, but not at lower dose rates.

Trifloxystrobin was detected in perirenal fat (up to 0.06 mg/kg) and omental fat (up to 0.05 mg/kg), from cows at the highest dose rate. No residues of its metabolite CGA 321113 were present in perirenal and omental fat from cows of the highest dose rate.

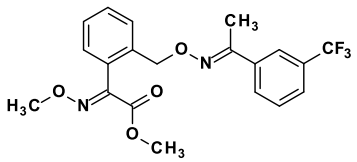
Based on these results it can be concluded that residues of trifloxystrobin or CGA 321113 are not anticipated to be present in food of animal origin when livestock is exposed at the calculated maximum dietary burden.

The ruminant feeding study was conducted according to EPA guideline OPPTS 860.1480. The study parameters comply with OECD guideline 505 (3 dose levels; capsule application for 28 days; 3 animals per dose level; sampling and analysis of meat, 2 fat types, liver, kidney, milk).

I. Materials and Methods

A. Materials

1. Test Material

Chemical Name	Methyl (2E)-(methoxyimino)[2-({[(E)-{1-[3-(trifluoromethyl) phenyl]ethylidene}amino]oxy}methyl)phenyl]acetate (accord. to ACD, ver.12.2) Methoxyimino-{2-[l-(3-trifluoromethyl-phenyl)-ethylidene-aminooxymethyl]-phenyl}-acetic acid methyl ester
Code name	CGA 279202
Common name	Trifloxystrobin
Empirical formula	$C_{20}H_{19}F_3N_2O_4$
CAS Number	141517-21-7
Molar mass	408.4 g/mol
Chemical structure	
Batch number	FL-941274
Purity	96.0%
Stability of the test compound	Additional dosing capsules were analysed to confirm dosing levels and stability during the 30 day dosing period. Dose capsules taken at 0 days and day 29 indicated that the nominal dosages were achieved and that no significant degradation occurred during the dosing period.

2. Test Animals

Species	*Bos taurus*
Strain (breed)	Holstein
Breeding facility	[redacted]
Sex and numbers involved	10 female animals
Age	3-9 years
Body weight	Average at initial dose and at sacrifice (kg):

Animal	1	2	3	Average
Control	662			662
Group 1	631	654	578	621
Group 2	593	587	689	623
Group 3	629	661	600	626

Acclimatisation	The cows were acclimated in treatment groups for twelve days prior to experiment initiation
Feed and water	Cows were fed by treatment group, 9 kg of commercially available dairy concentrate per cow following each milking; hay and water offered *ad libitum*
Housing	The treatment groups (3 animals per group) and one control animal were housed in individual pastures
Husbandry	Temperature: 10-31°C, relative humidity: 29-90%

B. Study Design

1. Dosing regime

Amount of dose (oral)	Group 1 1.97 mg/kg feed (dry matter) (average); 0.061-0.069 mg/kg bw/day (average: 0.064 mg/kg bw/day)

	Group 2 5.88 mg/kg feed (dry matter) (average); 0.174-0.204 mg/kg bw/day (average: 0.193 mg/kg bw/day) Group 3 21.16 mg/kg feed (dry matter) (average); 0.605-0.667 mg/kg bw/day (average: 0.635 mg/kg bw/day)
Food consumption	Average values (without acclimatization period): Control 20.8 kg (dry matter)/cow/day Group 1 20.3 kg (dry matter)/cow/day Group 2 20.4 kg (dry matter)/cow/day Group 3 18.9 kg (dry matter)/cow/day
Vehicle	Gelatine capsule
Timing	Once daily, immediately following the milking in the afternoon
Duration	28-30 days

2. Sample collection

Milk collection	Milk samples were collected on Days 0 (pre-dose), day 1, 3, 7, 14, 21, and 26
Interval from last dose to sacrifice	The animals were sacrificed approximately 20 to 24 hours after receiving the last dose
Tissues collected and analysed	Blood and tissue samples (liver, kidney, perirenal fat, omental fat, round muscle, and tenderloin muscle) were collected on day 28, 29 and 30

3. Storage of samples

All samples were frozen after collection and stored for a maximum of six months for all matrices, except perirenal fat which was stored for 12 months. The storage stability of trifloxystrobin and CGA 321113 was investigated in Study Report ABR97119 (M-038213-02-2) where stability of residues of trifloxystrobin and CGA 321113 was shown in animal meat for at least 12 months under deep-freezer storage conditions and in liver, milk and eggs for approximately 6 months.

4. Extraction and characterization

The method AG-659 (M-038841-01-1) involved extraction of the residue by homogenization twice with acetonitrile/water (4:1, v/v). Milk was extracted once by shaking for 15 minutes in acetonitrile/water (4:1, v/v). After filtration, an aliquot was taken and a 3-layer liquid-liquid partition is performed by adding water saturated with sodium chloride, toluene and hexane. The middle layer is collected, partitioned a second time with hexane, and evaporated. The sample is reconstituted in 0.085% aqueous phosphoric acid/acetone (95:5, v/v) and subjected to a C_{18} solid-phase extraction cleanup. After elution with 0.085% aqueous phosphoric acid/acetone (3:7, v/v), the sample is evaporated to aqueous remainder and partitioned into methyl-tert butyl ether/hexane (1:1, v/v). The methyl-tert butyl ether:hexane is evaporated to dryness and the sample is reconstituted in 0.1% polyethylene glycol in acetone (v/v) for analysis by gas-chromatography using a nitrogen-phosphorus detection (GC-NPD).

The limit of quantification (LOQ) of this method for trifloxystrobin and its metabolite CGA 321113 is 0.01 mg/kg for milk and 0.02 mg/kg for organs and tissues.

The magnitude of the residue of trifloxystrobin and its metabolite CGA 321113 has been studied in lactating dairy cows. Ten lactating Holstein dairy cows (*Bos taurus*; three cows/treatment group, one control cow) were dosed orally, *via* capsule, for 28 – 30 consecutive days with technical CGA 279202. The animals were fed at dose rates (based on feed dry weight) of either 0 mg/kg feed/day (control, 0X), 2.0 mg/kg feed/day ("1X" dose group), 5.9 mg/kg feed/day ("3X" dose group), or 21 mg/kg feed/day ("10X" dose group). In absolute figures the doses were approximately 41.7 mg, 125 mg and 416.7 mg per animal for the treated groups corresponding to daily dose rates of 0.061 – 0.069 mg/kg bw, 0.174 – 0.204 mg/kg bw and 0.605 – 0.667 mg/kg bw.

Milk was collected twice daily. Samples of milk were collected on the day of the first administration of the test item (day 0). Further samples were collected 1, 3, 7, 14, 21, and 26 days following the administration of the first dose. Milk samples were not pooled within treatment groups. Milk samples belonging to the 10X dose group were analysed for trifloxystrobin and CGA 321113.

On day 28 after the administration of the first dose, one animal from the control group and each dose group were sacrificed. On day 29 the second cow from each dose group and on day 30 the third cow from each dose group was sacrificed. Samples of round muscle, tenderloin muscle, omental fat, perirenal fat, liver and kidney were collected at each sampling event for analysis.

II. Findings

Trifloxystrobin was administered orally (via capsule) to lactating Holstein cows for 28 – 30 consecutive days at nominal average dose rates of 2.0, 5.9 and 21 mg/kg feed DM corresponding to 0.065, 0.193 and 0.635 mg/kg bw/day, respectively.

Within the dose period the feed consumption was fairly steady within treatment groups. The 10X group consumed less feed than the other three groups due to one cow in that group being a low milk producer. Milk production was mostly steady except for the low producing cow in the 10X group.

Concurrent recovery experiments were performed along with the analysis of samples. The concurrent recovery levels for matrices of round and tenderloin muscle, omental and perirenal fat, liver and kidney) were at 0.02 mg/kg (LOQ level) and between 0.05 and 1.0 mg/kg for trifloxystrobin and CGA 321113. For milk the levels were between 0.01 mg/kg (LOQ level) and 0.1 mg/kg for trifloxystrobin and CGA 321113. The sample materials chosen served to represent all relevant sample materials collected in this study.

The overall mean values of the concurrent recovery rates per compound and matrix were generally in the range of 70 – 120%, with relative standard deviations less than 20% with exceptions. Details are outlined in Table B.7.4.2-4.

Each analytical set consisted of a control sample, at least one procedural recovery sample, and samples from the dosed cows. The residue results were corrected upward for procedural recovery, where the recovery was less than 100%. Any residues found in the control samples were subtracted from the recoveries, since procedural recoveries are derived from the control samples. The residue results from treated samples were not corrected for any control values observed.

In tissues from cattle belonging to the high dose group (actual 21 mg/kg feed, target 10X dose rate), no residues above the LOQs of 0.02 mg/kg for trifloxystrobin and its acid metabolite CGA 321113 were found in muscle (round and tenderloin) at sacrifice.

In samples of omental fat residues of trifloxystrobin were in the range of 0.03 to 0.05 mg/kg (mean 0.04 mg/kg) and between <0.02 and 0.03 mg/kg (mean 0.02 mg/kg) in perirenal fat. No residues of CGA 321113 (<0.02 mg/kg) were found in samples of omental fat and perirenal fat.

In samples of liver and kidney no residues of trifloxystrobin (<0.02 mg/kg) were found, while residues of CGA 321113 ranged from 0.04 to 0.09 mg/kg (mean 0.06 mg/kg) in liver and from <0.02 to 0.02 mg/kg (mean 0.02 mg/kg) in kidney.

In milk taken throughout the study duration, no residues of trifloxystrobin and its acid metabolite above the LOQ of 0.01 mg/kg were determined in any milk sample.

In the group belonging to the low and middle dose group (actual 2.0 mg/kg feed, target 1X dose rate, and actual 5.9 mg/kg feed, target 3X dose rate) no residues were determined in any tissue sample, except one single sample of perirenal fat belonging to the middle dose group where residues of trifloxystrobin were found at 0.03 mg/kg and CGA 321113 at 0.02 mg/kg. These results were suspect, since the other two animals dosed at this level did not contain detectable residues plus the three animals dosed at the 10x level contained residues of the same magnitude of parent (0.03 to 0.06 mg/kg) and no detectable residues of CGA 321113. Therefore a repeated analysis of these samples was done and showed residues of trifloxystrobin and CGA 321113 below the LOQ (0.02 mg/kg).

Samples of milk belonging to the low and middle dose group were not analysed as residues in samples belonging to the high dose group were below the LOQ for both analytes.

The residues found in the milk during dosing and in tissues and organs collected at the end of the dosing period, are summarized in Table B.7.4.2-5 and Table B.7.4.2-3.

III. Conclusion

A feeding study was conducted with technical trifloxystrobin on dairy cattle in order to elucidate the levels of relevant residues in milk, tissues and organs.

The cows were divided into three dose groups (three animals per group) which are 2.0 mg/kg feed DM (0.061 – 0.069 mg/kg bw) for the low, 5.9 mg/kg feed DM (0.174 – 0.204 mg/kg bw) for the mid and 21.16 mg/kg feed DM (0.605 – 0.667 mg/kg bw) for the high dose group.

During the course of the study, no residues of trifloxystrobin and its metabolite CGA 321113 were determined above the LOQ of 0.01 mg/kg in the milk from any sampling event and of any dose group. No residues above the LOQ of 0.02 mg/kg of both parent compound trifloxystrobin and CGA 321113 were found in any samples of round muscle and tenderloin muscle of the 21 mg/kg-dose sampled on day 28, 29 or 30. Therefore, lower dose groups were not analysed.

No residues of trifloxystrobin were found in liver and kidney samples at any dose rate and any sampling event. However, its metabolite CGA 321113 was detected in liver (up to 0.09 mg/kg, day 29) and kidney (up to 0.02 mg/kg, day 28 and 29) from cows at the highest dose rate, but not at lower dose rates.

Trifloxystrobin was detected in perirenal fat (up to 0.06 mg/kg, day 28) and omental fat (up to 0.05 mg/kg, day 28), from cows at the highest dose rate. A single perirenal fat sample at the intermediate dose rate contained CGA 321113 at 0.02 mg/kg (day 30). It is suggested that this was probably an anomalous result, which was confirmed by the repeated analysis of these samples where residues of trifloxystrobin and CGA 321113 were found below the LOQ (0.02 mg/kg).

It is noted that the conjugates of CGA 321113 were not tested for in the feeding studies (nor does the analytical method used (AG-659) contain a hydrolysis step), yet these metabolites are proposed to be included in the residue definition for risk assessment for all ruminant matrices. Given the very low level of residues expected in animal matrices as a result of the representative uses, it is considered that the lack of data on the CGA 321113 conjugates is not of concern.

Table B.7.4.2-4: Concurrent recovery data for the relevant residues of trifloxystrobin in bovine matrices

Matrix	a.s./ metabolite	n	Spike Level (mg/kg)	Recovery (%)				
				Individual recoveries	Min	Max	Mean	RSD
Milk	Trifloxystrobin	7	**0.01**	107, 82, 87, 83, 66, 115, 110	66	115	93	19.4
		2	0.05	107, 80	80	107	94	-
		2	0.10	109, 87	87	109	98	-
		2	0.50	105, 96	96	105	101	-
		1	1.0	98	98	98	-	-
		14	*Overall*		66	115	95	15.2
	CGA 321113	7	**0.01**	88, 67, 76, 81, 80, 77, 104	67	104	82	14.2
		2	0.05	89, 69	69	89	79	-
		2	0.10	95, 72	72	95	84	-
		2	0.50	74, 78	74	78	76	-
		1	1.0	85	85	85	-	-
		14	*Overall*		67	104	81	12.7
Round muscle	Trifloxystrobin	1	**0.02**	102	102	102	-	-
		1	0.05	92	92	92	-	-
		2	*Overall*		92	102	97	-
	CGA 321113	1	**0.02**	91	91	91	-	-
		1	0.05	87	87	87	-	-
		2	*Overall*		87	91	89	-
Tenderloin muscle	Trifloxystrobin	1	**0.02**	88	88	88	-	-
		1	0.10	91	91	91	-	-
		2	*Overall*		88	91	89	-
	CGA 321113	1	**0.02**	83	83	83	-	-
		1	0.10	79	79	79	-	-
		2	*Overall*		79	83	90	-
Omental fat	Trifloxystrobin	3	**0.02**	106, 133, 88	88	133	109	20.8[1]
		1	0.20	110	110	110	-	-
		1	0.50	80	80	80	-	-
		5	*Overall*		80	133	103	20.0
	CGA 321113	3	**0.02**	91, 82, 61	61	91	78	19.7
		1	0.20	76	76	76	-	-
		1	0.50	65	65	65	-	-
		5	*Overall*		61	91	75	16.4
Perirenal fat	Trifloxystrobin	4	**0.02**	103, 113, 110, 95	95	113	105	7.6
		2	0.05	88, 97	88	97	93	-
		1	0.20	83	83	83	-	-
		1	1.0	88	88	88	-	-
		8	*Overall*		83	113	97	11.2
	CGA 321113	4	**0.02**	117, 94, 101, 74	74	117	97	18.5
		2	0.05	109, 74	74	109	92	-
		1	0.20	56	56	56	-	-
		1	1.0	74	74	74	-	-
		8	*Overall*		56	109	87	24.1[2]

Table B.7.4.2-1 contd: Concurrent recovery data for the relevant residues of trifloxystrobin in bovine matrices

Matrix	a.s./ metabolite	n	Spike Level (mg/kg)	Recovery (%)				
				Individual recoveries	Min	Max	Mean	RSD
Liver	Trifloxystrobin	3	**0.02**	99, 95, 111	95	111	102	8.2
		1	0.05	118	118	118	-	-
		1	0.10	115	115	115	-	-
		1	1.0	100	100	100	-	-
		6	*Overall*		95	119	106	9.0
	CGA 321113	3	**0.02**	97, 83, 78	78	97	86	11.5
		1	0.05	80	80	80	-	-
		1	0.10	77	77	77	-	-
		1	1.0	116	116	116	-	-
		6	*Overall*		77	116	89	17.3
Kidney	Trifloxystrobin	3	**0.02**	135, 121, 147	121	147	134	9.7
		1	0.05	123	123	123	-	-
		1	0.20	127	127	127	-	-
		1	0.50	131	131	131	-	-
		6	*Overall*		121	147	131[3]	7.3
	CGA 321113	3	**0.02**	127, 64, 105	64	127	99	32.4[4]
		1	0.05	73	73	73	-	-
		1	0.20	77	77	77	-	-
		1	0.50	77	77	77	-	-
		6	*Overall*		64	127	87	27.4

LOQ (practical limit of quantification) given **in bold**; RSD: Relative Standard Deviation; n: Number of single values

[1] acceptable since the overall mean RSD was 20% and residues of CGA 279202 were always <0.02 mg/kg in omental fat.

[2] acceptable since the mean RSD at 0.02 mg/kg was <20% and residues of CGA 321113 were always <0.02 mg/kg in perirenal fat.

[3] recoveries of CGA 279202 were >120% at any level (121 to 147%). However, this represents a worst-case in context of residues and doesn´t affect the results of the study as residues of CGA 279202 were always <0.02 mg/kg.

[4] the mean RSD and overall mean RSD were >20% for CGA 321113 in kidney. Residues of CGA 321113 in kidney were determined in a range of <0.02 to 0.02 mg/kg at the highest dose group.

Table B.7.4.2-5: Residue data from ruminant feeding study with trifloxystrobin

Animal No	Matrix	Collection time (day)	Feeding level (actual) (mg/kg)	Residues (mg/kg)	
				Trifloxystrobin	CGA 321113*
4A	Milk	0, 1, 3, 7, 14, 21, 26	21	<0.01	<0.01
4B		0, 1, 3, 7, 14, 21, 26		<0.01	<0.01
4C		0, 1, 3, 7, 14, 21, 26		<0.01	<0.01
4A	Round muscle	28	21	<0.02	<0.02
4B		29		<0.02	<0.02
4C		30		<0.02	<0.02
4A	Tenderloin muscle	28	21	<0.02	<0.02
4B		29		<0.02	<0.02
4C		30		<0.02	<0.02
1A	Omental fat	28	2.0	<0.02	<0.02
1B		29		<0.02	<0.02
1C		30		<0.02	<0.02
3A	Omental fat	28	5.9	<0.02	<0.02
3B		29		<0.02	<0.02
3C		30		<0.02	<0.02
4A		28	21	0.05	<0.02
4B		29		0.03	<0.02
4C		30		0.04	<0.02
1A	Perirenal fat	28	2.0	<0.02	<0.02
1B		29		<0.02	<0.02
1C		30		<0.02	<0.02
3A		28	5.9	<0.02	<0.02
3B		29		<0.02	<0.02
3C		30		0.03	0.02
3A		28	5.9 (repeat)	<0.02	<0.02
3B		29		<0.02	<0.02
3C		30		<0.02	<0.02
4A		28	21	0.06	<0.02
4B		29		0.03	<0.02
4C		30		0.04	<0.02

Animal No	Matrix	Collection time (day)	Feeding level (actual) (mg/kg)	Residues (mg/kg)	
				Trifloxystrobin	CGA 321113*
1A	Liver	28	2.0	<0.02	<0.02
1B		29		<0.02	<0.02
1C		30		<0.02	<0.02
3A		28	5.9	<0.02	<0.02
3B		29		<0.02	<0.02
3C		30		<0.02	<0.02
4A		28	21	<0.02	0.04
4B		29		<0.02	0.09
4C		30		<0.02	0.05
1A	Kidney	28	2.0	<0.02	<0.02
1B		29		<0.02	<0.02
1C		30		<0.02	<0.02
3A		28	5.9	<0.02	<0.02
3B		29		<0.02	<0.02
3C		30		<0.02	<0.02
4A		28	21	<0.02	0.02
4B		29		<0.02	0.02
4C		30		<0.02	<0.02

* residue results of CGA 321113 calculated and expressed as CGA 321113.

Table B.7.4.2-6: Summary of residue data from ruminant feeding study with trifloxystrobin

Matrix	Feeding level (mg/kg)	Residue levels (mg/kg)				
		n	Min.	Max.	Median	Mean
Trifloxystrobin						
Milk	21	21	<0.01	<0.01	<0.01	<0.01
Round muscle	21	3	<0.02	<0.02	<0.02	<0.02
Tenderloin muscle	21	3	<0.02	<0.02	<0.02	<0.02
Omental fat	2	3	<0.02	<0.02	<0.02	<0.02
	5.9	3	<0.02	<0.02	<0.02	<0.02
	21	3	0.03	0.05	0.04	0.04
Perirenal fat	2	3	<0.02	<0.02	<0.02	<0.02
	5.9	6	<0.02	<0.02*	<0.02	<0.02
	21	3	0.03	0.06	0.04	0.04
Liver	2	3	<0.02	<0.02	<0.02	<0.02

Matrix	Feeding level (mg/kg)	Residue levels (mg/kg)				
		n	Min.	Max.	Median	Mean
Trifloxystrobin						
	5.9	3	<0.02	<0.02	<0.02	<0.02
	21	3	<0.02	<0.02	<0.02	<0.02
Kidney	2	3	<0.02	<0.02	<0.02	<0.02
	5.9	3	<0.02	<0.02	<0.02	<0.02
	21	3	<0.02	<0.02	<0.02	<0.02
CGA 321113						
Milk	21	21	<0.01	<0.01	<0.01	<0.01
Round muscle	21	3	<0.02	<0.02	<0.02	<0.02
Tenderloin muscle	21	3	<0.02	<0.02	<0.02	<0.02
Omental fat	2	3	<0.02	<0.02	<0.02	<0.02
	5.9	3	<0.02	<0.02	<0.02	<0.02
	21	3	<0.02	<0.02	<0.02	<0.02
Perirenal fat	2	3	<0.02	<0.02	<0.02	<0.02
	5.9	6	<0.02	<0.02*	<0.02	<0.02
	21	3	<0.02	<0.02	<0.02	<0.02
Liver	2	3	<0.02	<0.02	<0.02	<0.02
	5.9	3	<0.02	<0.02	<0.02	<0.02
	21	3	0.04	0.09	0.05	0.06
Kidney	2	3	<0.02	<0.02	<0.02	<0.02
	5.9	3	<0.02	<0.02	<0.02	<0.02
	21	3	<0.02	0.02	0.02	0.02

Please note:
Residue levels for milk samples refer to results of sampling at day 0, 1, 3, 7, 14, 21, 26.
Residue levels for organ and tissue samples refer to results of sampling at day 28, 29 and 30.
Residue results of CGA 321113 calculated and expressed as CGA 321113.
* Results of repeated analysis showed residues of trifloxystrobin and CGA 321113 below the LOQ (0.02 mg/kg).

[Study 4] Dietary burden calculations and consideration of transfer into animal tissues

Based on the uses for which the applicant seeks approval in the RAR, only apples (in the form of wet pomace) are fed to livestock.

Table B 7.4.5-1 summarizes the inputs used in the dietary burden calculation (using the EFSA animal model 2017, which is based on the OECD feedstuff tables and OECD guidance document on residues in livestock, No. 73):

Table B 7.4.5-1: Input values for dietary burden calculation

Commodity	Median dietary burden		Max. dietary burden	
	Input value (mg/kg)	Comment	Input value (mg/kg)	Comment

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Commodity	Median dietary burden		Max. dietary burden	
	Input value (mg/kg)	Comment	Input value (mg/kg)	Comment
Risk assessment residue definition (animal products): **Ruminants:** Sum of trifloxystrobin and CGA 321113 (free and conjugated), expressed as trifloxystrobin~~, except for ruminant liver and kidney, where it is proposed as: sum of trifloxystrobin and CGA 321113 and its conjugates, expressed as trifloxystrobin.~~ **Poultry:** Sum of trifloxystrobin and CGA 321113, expressed as trifloxystrobin				
Apple, pomace	1.42 ~~0.583~~	Median residue (0.12 mg/kg; defined using the risk assessment residue definition for processed commodities as trifloxystrobin + CGA 321113, expressed as trifloxystrobin see Table B.7.3.3-1b) x PF (11.8 as the median processing factor for apple pomace from the list of end points). ~~Median residue (trifloxystrobin + CGA321113, expressed as trifloxystrobin) x PF~~	N/A – only STMR considered for this processed commodity	

~~(a) a processing factor of 4.86 was calculated for apple pomace (see Section B.7.5 below).~~

The result of this calculation is provided in Table B.7.4.5-2, below.

Table B.7.4.5-2: OECD animal intake and feeding 2017

Animal burden calculation — Trifloxystrobin

According to: "OECD Guidance Document, Series on testing and assessment No 64 and Series on pesticides No 32" and "OECD Guidance Document on Residues in livestock, Series on Pesticides No 73"

Maximum Intake	Cattle						Sheep					
	Beef	500 kg / 12 kg		Dairy	650 kg / 25 kg		Ram/Ewe	75 kg / 2.5 kg		Lamb	40 kg / 1.7 kg	
(mg/kg bw/d)	0.017	mg/kg bw/d	%	0.014	mg/kg bw/d	%	0.012	mg/kg bw/d	%	0.015	mg/kg bw/d	%
Contributor 1	Apple	pomace, wet	20	Apple	pomace, wet	10	Apple	pomace, wet	10	Apple	pomace, wet	10
Contributor 2												
Contributor 3												
Contributor 4												
Median intake	0.0170	mg/kg bw/d		0.0136	mg/kg bw/d		0.0118	mg/kg bw/d		0.0150	mg/kg bw/d	

Intakes >0.004 mg/kg bw/d are highlighted

Maximum Intake	Swine					
	Breeding	260 kg / 6 kg		Finishing	100 kg / 3 kg	
(mg/kg bw/d)		mg/kg bw/d	%		mg/kg bw/d	%
Contributor 1						
Contributor 2						
Contributor 3						
Contributor 4						
Median intake		mg/kg bw/d			mg/kg bw/d	

Maximum Intake	Poultry								
	Broiler	1.7 kg / 0.12 kg		Layer	1.9 kg / 0.13 kg		Turkey	7 kg / 0.5 kg	
(mg/kg bw/d)		mg/kg bw/d	%		mg/kg bw/d	%		mg/kg bw/d	%
Contributor 1									
Contributor 2									
Contributor 3									
Contributor 4									
Median intake		mg/kg bw			mg/kg bw			mg/kg bw	

Intakes expressed on the dry mater basis (mg/kg DM)

mg/kg DM	Cattle		Sheep		Swine	
	Beef	Dairy	Ram/Ewe	Lamb	Breeding	Finishing
Maximum	0.71	0.35	0.4	0.35		
Median	0.71	0.35	0.35	0.35		

	Poultry		
	Broiler	Layer	Turkey
Maximum			
Median			

Intake >0.1 mg/kg DM in red characters

New data requirements — Regulation (EU) No 283/2013)

Relevant groups	Dietary burden expressed in				Most critical diet (a)	Most critical commodity (b)		Trigger exceeded (Yes/No)
	mg/kg bw per day		mg/kg DM					0.004
	Median	Maximum	Median	Maximum				mg/kg bw
Cattle (all diets)	0.017	0.017	0.71	0.71	Beef cattle	Apple	pomace, wet	Yes
Cattle (dairy only)	0.014	0.014	0.35	0.35	Dairy cattle	Apple	pomace, wet	Yes
Sheep (all diets)	0.015	0.015	0.35	0.35	Lamb	Apple	pomace, wet	Yes
Sheep (ewe only)	0.012	0.012	0.35	0.35	Ram/Ewe	Apple	pomace, wet	Yes
Swine (all diets)								No
Poultry (all diets)								No
Poultry (layer only)								No

(a): When several diets are relevant (e.g. cattle, sheep and poultry "all diets"), the most critical diet is identified from the maximum dietary burdens expressed as "mg/kg bw per day"

(b): The most critical commodity is the major contributor identified from the maximum dietary burden expressed as "mg/kg bw per day".

Intakes for dairy and beef cattle and ram/ewes and lambs exceed the 0.004 mg/kg bw/day threshold for requiring feeding studies. Pigs and poultry are not fed apple pomace and so they have no expected dietary burden and there is no requirement for feeding studies in these species.

Comparison of the dietary burden calculated above with the feeding study dose levels considered in the DAR and for renewal (Table B.7.4.5-3) indicates that residues above the current EU MRL in products of animal origin are not expected.

Table B.7.4.5-3: Overview of residues of trifloxystrobin in livestock following dietary exposure of trifloxystrobin (updated following the Pesticides Peer Review Teleconference 146 held on 5 July 2017)

Animal commodity	Residues at the closet feeding level (mg/kg)		Estimated value at 1N level		MRL proposal (mg/kg)	CF	STMR (mg/kg)	HR (mg/kg)
	Mean	Highest	$STMR_{Mo}$ (mg/kg)	HR_{Mo} (mg/kg)				
Cattle (all diets)								
Closest feeding level[a]:	0.065	mg/kg bw	3.8 N Beef cattle (highest diet)					
Muscle	0.04	0.04	0.04	0.04	**0.04**	n.c.	0.04	0.04
Fat	0.04	0.04	0.04	0.04	**0.04**	n.c.	0.04	0.04
Liver	0.04	0.04	0.04	0.04	**0.04**	n.c.	0.04	0.04
Kidney	0.04	0.04	0.04	0.04	**0.04**	n.c.	0.04	0.04
Cattle (dairy only)								
Closest feeding level[a]:	0.065	mg/kg bw	4.8 N Dairy cattle					
Milk[b]	0.02	0.02	0.02	0.02	**0.02**	n.c.	0.02	0.02
Sheep (all diets)								
Closest feeding level[a]:	0.065	mg/kg bw	4.3 N Lamb (highest diet)					
Muscle	0.04	0.04	0.04	0.04	**0.04**	n.c.	0.04	0.04
Fat	0.04	0.04	0.04	0.04	**0.04**	n.c.	0.04	0.04
Liver	0.04	0.04	0.04	0.04	**0.04**	n.c.	0.04	0.04
Kidney	0.04	0.04	0.04	0.04	**0.04**	n.c.	0.04	0.04
Sheep (dairy only)								
Closest feeding level[a]:	0.065	mg/kg bw	5.5 N Ewe					
Milk[b]	0.02	0.02	0.02	0.02	**0.02**	n.c.	0.02	0.02

(a): Closest feeding level and N dose rate related to the maximum dietary burden.

(b): Highest residue level from day D1 to day D2 (daily mean of X cows).
(c): Highest residue level from day D1 to day D2 (daily mean of Y laying hens).

It is noted that the conjugates of CGA 321113 were not tested for in the feeding studies, yet these metabolites are proposed to be included in the residue definition for risk assessment for all ruminant matrices. Given the very low level of residues expected in animal matrices as a result of the representative uses, it is considered that the lack of data on the CGA 321113 conjugates has no appreciable effect on the outcome of the risk assessment.

Summary of reproductive toxicity studies

Effects on reproduction were investigated in a 2-generation feeding study in rats. The teratogenic potential of trifloxystrobin was investigated in two studies, one in rats and one in rabbits, both by gavage dosing. All these studies met the essential requirements of their respective guidelines.

No toxic effects on reproduction were found in a rat 2-generation feeding study with trifloxystrobin at any dose level tested. The highest dose in parental animals of both sexes and the intermediate dose in female parents resulted in reduced food consumption as well as in a retarded bodyweight development. Both dose levels caused a reduced body weight gain in pups of both sexes. Target organs were liver and kidney.

There was no evidence of a teratogenic effect in either rats or rabbits.

Treatment of pregnant female rats with trifloxystrobin resulted in reduced bodyweight gain and lowered food consumption of the dams at the top dose. Reproductive parameters were not affected. Enlarged thymus was found in fetuses of the high dose group. In rabbits, maternal body weight gain and food consumption were reduced after oral treatment trifloxystrobin at the two higher dose levels. No effects on reproduction parameters were seen. There was a slightly increased incidence of fused sternebrae in fetuses which obtained statistically significance at the top dose.

Following discussion of the findings at the PRAPeR TC 144, classification of Trifloxystrobin for effects via lactation (H362 may cause harm to breast fed children) was proposed due to the decreased body weight in pups occurring during the lactation period (predominantly LD4 onwards). In addition, classification for reproductive toxicity (Cat 2, H361) was proposed based on the skeletal anomalies in the rabbit pups.

3.11 Specific target organ toxicity – single exposure

Not relevant for this proposal.

3.12 Specific target organ toxicity – repeated exposure

3.12.1 Animal data

[Study 1] 28 Range-finding study in rats (non-guideline) (Anonymous 1994b, M-040074-01-1)

Detailed study summary and results:

In this study Sprague-Dawley derived rats (5/sex/dose) were administered trifloxystrobin (purity 96.2%) at concentrations of 0, 200, 1000, 4000 and 12000 ppm in the diet for 28 days. A summary of overall mean doses received is shown below;

Dietary inclusion level (ppm)	Overall mean dose received (mg trifloxystrobin/kg bw/day)	
	males	females
200	16.51	16.37
1000	84.35	84.06
4000	337.2	327.0
12000	1074	1005

The study was conducted in accordance with GLP principles at a certified laboratory, but not audited by the Quality Assurance Unit and no on-site inspections were undertaken. Since the study was a range-finder no microscopic evaluations were conducted.

Mortality was checked twice per day and clinical signs daily; bodyweight, food and water consumption were recorded weekly. Haematological and clinical chemical evaluations, also urinalysis were conducted at end of the treatment. Following termination the liver, testes, thymus, ovaries, spleen, adrenal glands, brain, heart, kidneys, thyroids and body (exsanguinated) weights were measured and gross findings noted.

No mortality occurred in any dose group. Soft faeces and diarrhoea were noted at ≥ 4000 ppm.

Bodyweight gain was reduced in male groups at 1000 ppm and above, and top dose group females.

Table B.6.15: Bodyweight changes in 28 day study in the rat

Sex	**males**				**females**			
Dose level (ppm)	**200**	**1000**	**4000**	**12000**	**200**	**1000**	**4000**	**12000**
Mean bodyweight gain (% of control)	104	87	78	66	118	108	100	73

A minimal reduction in overall food consumption (4 – 6% lower than controls) was noted in males at ≥ 1000 ppm male groups and in top dose group females. Food consumption ratios were slightly reduced in week 1 in the high dose group (both sexes). No dose related effect on mean water consumption was noted.

No treatment related changes of the haematological profile were observed. Slightly higher plasma albumin and cholesterol levels were recorded in both sexes at 4000 ppm and above. Slightly increased values were also observed for glucose and urea at the highest dose in both sexes, also for glucose in the 4000 ppm male group and for urea in the 4000 ppm female group.

Relative liver weights were increased in males at 4000 and 12000 ppm (13% and 31% higher than controls, respectively) and also top dose group females (15% higher than controls). At the highest dose level increases were noted for relative kidney weights in both sexes and for adrenals in males.

Based on this limited range finding study the NOAELs in males and females were 200 and 1000 ppm, respectively (equivalent to approximately 17 and 84 mg/kg bw/day, respectively) based on clinical signs, reduced bodyweight gains and organ weight changes.

[Study 2] 90 Day study in the rat EU Guideline (Annex V 90-day repeated oral dosing in a rodent species 30/5/1988) (Anonymous 1997d, M-040135-01-1)

In a 1993-4 study Sprague-Dawley derived rats (15 or 25/sex/dose) were administered trifloxystrobin (purity 96.2%) at concentrations of 0, 100, 500, 2000 (both sexes) and 8000 ppm (females only) in the diet for 13 weeks. The control and top dose groups each included an additional 10 rats/sex (i.e. total group size 25/sex) of which were kept for a 4 weeks recovery period after treatment.

In addition the requirements of the EU Guideline this study included neurotoxicological investigations and histopathological examinations were extended to tissues of the central and peripheral nervous system.

A summary of overall mean doses received is shown below;

Dietary inclusion level (ppm)	**Overall mean dose received (mg trifloxystrobin/kg bw/day)**	
	males	**females**
100	6.4	6.8
500	30.6	32.8
2000	127	133
8000	-	618

Mortality was checked twice per day and clinical signs daily; bodyweight, food and water consumption were recorded weekly. At pretest, day 91 and after recovery all animals from control and top dose groups were subjected to ophthalmology examinations. At the end of the treatment and at the end of the recovery period animals were subjected to haematological, clinical chemical and urinalysis.

A Functional observational battery (FOB) was conducted at week -1, 4, 9, 13 and after the recovery period. The neurological examinations including tests for sensorimotor functions (approach, touch, vision, audition, pain, vestibular), autonomic functions (pupillary reflex, body temperature) and sensorimotor coordination (grip strength, landing foot splay) were conducted at week -1, 4, 9, 13 and after the recovery period.

Following termination the weight of the adrenal glands, body (exsanguinated), brain, heart, kidneys, liver, ovaries, spleen, testes, thymus and thyroid/parathyroid were measured and gross findings noted. Microscopic evaluations were conducted on a range of tissues, and in addition a range of organs/tissues were specifically prepared for neuropathological investigations (glutaraldehyde perfusion/fixation), although histological examination was restricted to control and high dose animals.

One male at 2000 ppm was sacrificed on study day 35 due to moribund condition and one female at this dose level was found dead on study day 16. At 8000 ppm one female was found dead on study day 28 and four further top dose females were sacrificed in moribund condition on study days 30-34. One control male was sacrificed in moribund conditions on study day 69 and on female was found dead study day 43. All the deaths in the 2000 and 8000 ppm dose groups were considered by the study authors to be treatment related.

Transient piloerection and soft faeces at week one were noted with all females of the 8000 ppm group. Hunched posture or hypoactivity were observed with moribund animals. Ophthalmological examination revealed no changes between control and high dose group animals.

Single animals dosed at 2000 and 8000 ppm lost bodyweight (these animals were among those which died prematurely/were sacrificed in moribund condition). The mean terminal bodyweight gain was reduced in males dosed at ≥ 500 ppm and females at ≥ 2000 ppm (see Table B.6.17). During the recovery phase, bodyweight gains above the control values were noted in high dose groups, but terminal weights were still below controls.

Table B.6.16a: Mean body weight (g) during the study

Dose (ppm)	**0**	**100**	**500**	**2000**	**0**	**100**	**500**	**2000**	**8000**
week	**males**				**females**				
-1	192.3	191.9	188.9	191.0	161.0	161.8	165.0	162.6	159.0
1	244.3	243.0	238.1	232. 8*-	190.6	187.5	193.7	185.8	172.2*-
2	293.2	294.3	284.4	275.9*-	213.3	210.2	220.3	207.6	188.2*-
3	325.1	327.2	314.5	302.7*-	231.7	231.6	240.3	225.6	192.0*-
4	356.0	358.9	342.1	329.7*-	243.6	242.0	255.6	232.3	194.9*-
5	375.8	379.0	360.3	338.6*-	253.5	249.9	263.0	241.4	202.3*-
6	400,3	404.0	380.9	360.9*-	267.1	263.1	274.4	252.5	221.1*-
7	412.2	422.9	394.3	375.0*-	272.6	273.6	279.5	256.5	228.1*-
8	433.1	441.2	409.9	389.2*-	278.3	278.8	289.5	261.0	231.9*-
9	442.2	454.7	421.4	398.7*-	288.0	286.8	294.5	270.4	232.4*-
10	450.3	463.9	432.2	404.0*-	294.9	289.5	304.8	273.4*	239.6*-
11	470.0	478.3	440.6	415.1*-	299.3	289.9	306,2	272.1*	241.9*-
12	482.9	492.0	450.0*	422,2*-	301.4	293.6	309.4	276.3*	242.2*-
13	490.3	501.3	459.7	428.9*-	303.2	298.5	313.5	281.3	243.7*-
recovery									
14	488.6			424.2	294.3				255.1*
15	504.5			447.1	302.0				263.5*-
16	522.4			466.7	307.5				272.7*-

17	535.2			481.4	311.7				282.9

Statistical tests and flags used
LEPAGE: * if p_L < 0.01
JONCKHEERE: +- if p_J < 0.01

Food intake was reduced in males dose at ≥ 500 ppm and females at ≥ 2000 ppm (6-10% lower than their respective controls). During recovery, food intake of the high dose animals was higher than control animals. Generally the food consumption ratio was higher than controls in females of the high dose group.

Water consumption was slightly reduced in the top dose group males during the first 4 weeks of the study. In the female high dose group the overall mean water consumption (week 1-13) was 11% lower than controls, but over the recovery period it was comparable.

Slightly higher values for red blood cell parameters (erythrocyte counts, haemoglobin and hematocrit) and a tendency to eosinophilia were noted in females of the top dose group. These relatively minor alterations appeared reversible within the recovery period.

At the end of treatment slightly reduced plasma globulin and total protein levels were observed with high dose males and in females of the 2000 and 8000 ppm groups. In addition, males treated at 2000 ppm had minimally increased cholesterol levels and in females treated at 8000 ppm glucose, urea and potassium were increased. These changes were partly reversible during recovery. The urine excreted by females of the 8000 ppm group was slightly acid.

Statistically significant increases in relative liver and kidney weights were noted in males at 500 (liver only) and 2000 ppm and females at 8000 ppm. Although absolute values for these organs were also mostly higher than control values there was no clear treatment relationship. Liver and kidney weight changes were partly reversible after recovery.

Table B.6.16b: Mean absolute organ weights

Dose (ppm)	**0**	**100**	**500**	**2000**	**0**	**100**	**500**	**2000**	**8000**
	males				**females**				
1st sacrifice (week 14)									
Body w (g)	458.1	476.6	428.5	428.5	283.5	282.9	290.5	268.7	221.2*-
Brain (g)	2.334	2.378	2.315	2.315	2.289	2.222	2.225	2.203	2.135*-
Heart (g)	1.440	1.480	1.303	1.303	0.969	0.977	0.950*	0.979	0.944
Liver (g)	16.42	17.50	17.32	17.32	10.65	9.970	10.42	11.39	11.56
Kidneys (g)	2.912	3.063	2.957	2.957	2.079	1.996	2.009	1.971	1. 853
Adrenals (mg)	62.40	68.48	64.11	64.11	83.17	78.52	91.00	82.10	71.11
Thymus (mg)	408.4	385.0	381.6	381.6	301.6	314.5	330.6	309.8	268.4
Testis (g)	3.631	3.862	3.767	3.767					
Ovary (both)					168.9	171.6	167.5	157.9	158.8
Spleen(g)	0.762	0.788	0.715	0.715	0.617	0.604	0.563	0.619	0.565
Thyroid (mg)	22.97	25.01	25.05	25.05	20.59	24.21	23.79	22.40	22.65
2nd sacrifice (week 18, recovery)									
Body w (g)	502.8			450.0	290.4				259.6
Brain (g)	2.381			2.385	2.275				2.217
Heart (g)	1.457			1.461	0.991				1.062
Liver (g)	18.85			17.15	10.68				10.69
Kidney (g)	3.121			3.004	2.096				1.961

Adrenal mg)	79.11			68.76	89.93				95.04
Thymus (mg)	380.8			349.5	267.6				278.5
Testis (g)	3.781			4.034					
Ovary (both)					164.4				182.1
Spleen (g)	0.764			0.716	0.532				0.528
Thyroid (mg)	25.92			22.52	22.72				24.01

Statistical tests and flags used
LEPAGE: *if p_L < 0.01
JONCKHEERE: +- if p_J < 0.01

Table B.6.16c: Mean organ to bodyweight ratios

Dose (ppm)	0	100	500	2000	0	100	500	2000	8000
	males				females				
1st sacrifice (week 14)									
Brain (0/00)	5.177	5.022	5.410*	5.855 +	8.109	7.890	7.706	8. 237	9.652*
Heart (0/00)	3.158	3.123	3.046	3.369	3.428	3.454	3.290	3. 657	4.271*+
Liver (0/00)	35.86	36.70	40.41*+	43.70*+	37.47	35.21	35.85	42.30	52.24*+
Kidney (both) (0/00)	6.402	6.426	6.913	7.153 +	7.339	7.078	6.945	7. 340	8.378 +
Adrenal (both) (0/00)	0.136	0.144	0.150	0. 147	0.293	0.280	0.314	0.304	0.321
Thymus (0/00)	0.899	0.803	0.886	0.879	1.069	1.113	1.133	1.157	1.214
Testis (both) (0/00)	8.069	8.154	8.814	9. 190					
Ovary (both) (0/00)					0.599	0.608	0.579	0.588	0.717
Spleen (0/00)	1.668	1.657	1.667	1.775	2.177	2.136	1.932	2.299	2.553 +
Thyroid gland (0/00)	0.050	0.053	0.058	0.058	0.073	0.086	0.083	0.083	0.102 +
2nd sacrifice (week 18, recovery)									
Brain (0/00)	4.793			5.343	7.857				8.577
Heart (0/00)	2.909			3.268	3.424				4.098*
Liver (0/00)	37.32			38.16	36.75				41. 07
Kidney (both) (0/00)	6.225			6.714	7.237				7.580
Adrenal (both) (0/00)	0. 158			0.153	0.312				0. 368
Thymus (0/00)	0.764			0.786	0.913				1.067
Testis (both) (0/00)	7.587			9.038					
Ovary (both) (0/00)					0.569				0.702
Spleen (0/00)	1.519			1.605	1.835				2.023
Thyroid gland (0/00)	0.052			0.050	0.079				0.092

Statistical tests and flags used
LEPAGE: * if p_L < 0.01

JONCKHEERE: +- if p_J < 0.01

In the 8000 ppm group (females) gross pathology revealed a small thymus in 3/13 animals at the end of treatment and 1/8 after recovery.

Histopathology of the prematurely dead or moribund sacrificed females revealed minimal, perilobular hepatocellular hypertrophy and acute tubular lesions in kidney (minimal to moderate). In males and females numerous organs with atrophy of the parenchyma were found including the endocrine pancreas, spleen, bone marrow, lymph nodes, salivary gland, mucosa of small intestine, uterus, ovaries, adenohypophysis and thymus.

Histopathology of scheduled sacrificed animals after treatment revealed changes in the liver, pancreas and salivary glands. A minimal hepatocellular hypertrophy in top dose group animals was observed. A minimal to moderate atrophy of the pancreas was seen in most top dose group females and a minimal atrophy in one female dosed at 2000 ppm. A minimal atrophy of the pancreas was observed in two top dose group males. One female treated with 8000 ppm revealed a minimal atrophy of the salivary gland.

After recovery examination of high dose group animals revealed minimal to moderate atrophy of the pancreas in males (2/10) and in female minimal atrophy of the thymus (1/8) and the uterus (1/8).

Table B.6.17: Summary of bodyweight effects, haematology, organ weight changes and histopathological finding in 90 day study in the rat

			DOSE GROUP (PPM)						
BODYWEIGHT GAIN (% of control)			**males**			**females**			
			100	**500**	**2000**	**100**	**500**	**2000**	**8000**
			104	91	80	96	104	83	60
Haematology	**Control**		**males**			**females**			
	males	**females**	**100**	**500**	**2000**	**100**	**500**	**2000**	**8000**
RBCs (T/l)	8.669	8.131	8.502	8.844	8.819	7.972	8.041	8.229	8.459
Hb (mmol/l)	9.432	9.321	9.330	9.290	9.403	9.235	9.250	9.456	9.600
Hct (1)	0.433	0.438	0.429	0.432	0.436	0.428	0.535	0.440	0.452
Eosinophils (G/l)	0.016	0.114	0.014	0.014	0.012	0.144	0.124	0.119	0.070*
Organ weight (% of control)			**males**			**females**			
			100	**500**	**2000**	**100**	**500**	**2000**	**8000**
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*
Histopathology (number of animals affected)									
			males			**females**			
	males	**females**	**100**	**500**	**2000**	**100**	**500**	**2000**	**8000**
No animals examined									
Main group	10	10	10	10	10	10	10	9	8
Recovery group	9	9			10				8
MS + FD	1	1			1			1	5
Total	20	20	10	10	21	10	10	10	21
Kidney: Acute tubular lesion									
MS+FD									5
Total									5
Liver hepatocyte hypertrophy									
Main group					5				7
MS + FD									4
Total					5				11
Pancreas atrophy									

Main group					2			1	7
Recovery group					2				
MS + FD					1			1	4
Total					5			2	12
Salivary gland atrophy									
Main group									1
MS + FD					1			1	5
Total					1			1	6
Uterus atrophy									
Recovery group									1
MS + FD								1	3
Total								1	4
Thymus atrophy									
Recovery group									1
MS + FD					1			1	5
Total					1			1	6

* statistically significant (p< 0.01),MS + FD: moribund sacrificed + found dead

The functional observational battery (FOB) revealed no indications for a potential neurologic or behavioural effect of trifloxystrobin. No changes of toxicological relevance were observed in any of the parameters associated with motor activity. Neuropathological examination of tissues of the central and peripheral nervous system did not reveal any treatment-related neuropathic changes.

The top dose levels used in this study (males- 2000 ppm/females- 8000 ppm) clearly exceeded the maximum tolerated dose. Based on the bodyweight effects and effects seen on the liver and pancreas the original NOAELs were 100 and 500 ppm in males and females respectively (equivalent to approximately 6.4 and 32.8 mg/kg bw/day). Following PRAPeR TC 144, the NOAELs were concluded to be 500 ppm for both males and females (30.6 and 32.8 mg/kg bw/d respectively) based on reduced body weight gain, food consumption and increased organ weight (liver and kidney).

3.12.2 Human data

No data are available.

3.12.3 Other data

No data are available.

3.13 Aspiration hazard

Not relevant for this proposal.

4 ENVIRONMENTAL HAZARDS

4.1 Degradation

4.1.1 Ready biodegradability (screening studies)

4.1.2 BOD_5/COD

4.1.3 Aquatic simulation tests

4.1.4 Other degradability studies

4.2 Bioaccumulation

Partition coefficient

[Study 1] Report on octanol / water partition coefficient - CGA 279202 (Stulz, 1997, M-041647-01-1)

The partion coefficient 1-octanol/water of trifloxystrobin was determined in pH 7.51 (average pH of aqueous phase) according to OECD Guideline 107 and GLP. The test substance was pure active ingredient, batch: AMS 759/101 with a purity of 99.7%.
Six amounts of the test substance between 35.2 and 69.6 mg trifloxystrobin dissolved at room temperature in three different volume ratios of octanol and water (20:20; 40:20; 10:20) in duplicates. After shaking for approximately 24 hours, the amount of trifloxystrobin in water and octanol was analysed by HPLC.
The results show a P_{OW} of 32000 ± 680 and a corresponding log P_{OW} of 4.5 ± 0.0094 at 25 °C.

4.2.1 Bioaccumulation test on fish

[Study 1] Phenyl(A)-U-14C]-CGA-279202 - Flow-through bioconcentration and metabolism study with bluegill sunfish (*Lepomis macrochirus*) (Anonymous, 1997e, M-032004-01-1)

Detailed study summary and results:

Objective:
The overall goal of this study was to determine the bioconcentration factor (BCF) of CGA 279202 for bluegill sunfish *(Lepomis macrochirus)* and to identify the metabolites formed in the fish tissues during a continuous exposure in a flow-through system.

Materials and methods:
Test item: [Phenyl(A)-U-14C]-CGA-279202, reference no.: TYP-V-39-1, radiochemical purity: 98.4%, specific activity: 82.0 µCi/mg, molecular weight: 408.38 g/mol.
Bluegill sunfish were commercially supplied ([redacted]) and held in well water (17-18°C, pH 6.9-7.1, 76-89% oxygen saturation) for 14 d prior testing. They were fed a commercially prepared pelleted food ([redacted] Salmon Starter), *ad libitum*, during the acclimation period and during testing (except for 1 day prior test start and test end). The mean body length was 44 mm and the mean body weight was 1.5 g. A daily photoperiod of 16 hours light was maintained.

The exposure of bluegill to [phenyl(A)-U-14C]-CGA-279202 at nominal concentrations of 0.16 and 1.6 µg/L

was continuous throughout the establishment of a steady state tissue residue concentration and maintained for 28 days. The test material was dispensed via syringe pump into dilution water flowing to each test vessel at the rate of approximately 6 volume turnovers per day (initial loading *ca.* 0.6 g/L/day). Afterwards, fish were transferred to the depuration aquaria into which dilution water was introduced at a rate equal to the flow rate maintained during the last 18 days exposure period (630 mL/min).

During the study, 5 fish were removed from each group for total 14C measurement in the edible and viscera tissues at days 1, 3, 7, 10, 14, 16, 21 and 28 of exposure, and at 1, 3, 7, 10 and 14 days after the depuration phase was initiated. Five fish were collected from the metabolism aquarium on days 21 and 28. These fish were dissected into three portions, edible, viscera and carcass. On day 28, the remaining 238 fish from the metabolism aquarium were harvested for metabolite identification.

Daily observations were made on the appearance and behaviour of the fish and the physical appearance of the test solution.

Hardness and alkalinity were measured at test initiation. Temperature, pH, and dissolved oxygen concentration were measured daily in each test aquarium. Temperature was also continuously monitored in the solvent control aquarium.

Aquaria water samples were collected from the bioconcentration and metabolism exposure aquaria on Days 0, 1, 3, 7, 10, 14, 21 and 28 of exposure. Additional water samples were collected on Days 9, 15 and 16 from the 1.6 µg/L bioconcentration and metabolism exposures. Similarly, triplicate water samples were collected from both bioconcentration aquaria on Days 1, 3, 7, 10 and 14 of depuration. Water samples were collected from the solvent control aquarium on Days 0, 14, 21, and 28 of exposure and on Day 14 of depuration.
Chemical analysis of [phenyl(A)-U-14C]-CGA-279202 was performed by HPLC analysis with radiometric detection.

Validity criteria:

Validity criteria (according to OECD 305, 2012)	**Obtained in this study**
Water temperature variation is less than ±2°C	Water temperature was between 17-18°C
The concentration of dissolved oxygen does no fall below 60% saturation	Remained above 60% saturation during the exposure and depuration periods
The concentration of the test substance is maintained within ±20% of the mean of the measured values during uptake phase	During exposure, mean measured concentrations represented 97.4 and 82.0% of the nominal concentrations, respectively At the lower concentration, a single measurement on day 1 was below 80% of the mean, but the average of two concentrations was > 80%. This concentration showed a BCF (lipid normalised, whole body) of 370. At the higher concentration tested (1.31 µg/L mm, 1.6 nom), which yielded the lower BCF, four measurements were not within 80-120% of the mean. Thus, although the formal validity criteria are not fully met, no impact on the overall study result is expected. A repeat of a vertebrate study on that basis is not justified.
The concentration of the test substance is below its limit of solubility in water	A solvent (0.1 mL acetone/L water) was used in the study
Mortality / adverse effects / disease in all fish are less than 10% at the end of the test	Mortality within the 10% range, no diseases or stress behaviour observed

Results:
During the exposure and the depuration phase, water parameters were as follows: hardness (as CaCO3) 32-36 mg/L, pH 6.8-7.1, temperature 17-18°C and dissolved oxygen saturation of 75-76%.

Analytical Results:
The mean measured concentration of [14C]-residues in the exposure water for the 0.16 and 1.6 µg/L CGA 279202 exposures over the 28-day period ranged from 0.130 to 0.195 µg/L and 0.728 to 1.63 µg/L, respectively, with overall means (± standard deviations) of 0.156 (± 0.028) µg/L (n=27), and 1.31 (± 0.27) µg/L (n=33), respectively. Results of water analyses indicate that mean measured concentrations represented 97.4 and 82.0% of the nominal [Phenyl (A)-U-14C]-CGA 279202 concentration (0.00016 and 0.0016 mg/L trifloxystrobin, respectively).
The concentrations of [14C]-residues in the solvent control during exposure and depuration periods were generally found to be below the minimum detection limit of 0.00500 µg/L at each sampling interval.

Biological Results:
On Days 4 and 12, one mortality in the 1.6 µg/L metabolism and 0.16 µg/L bioconcentration aquarium, respectively, was observed due to the fish getting caught in the drainage tube.

On Days 13 and 26, one mortality was observed in the 0.16 µg/L bioconcentration and 1.6 µg/L metabolism aquarium, respectively. The fish appeared healthy and exhibited normal behaviour throughout the study.
Bioconcentration factors (BCF) for each tissue type were calculated using the mean measured steady state exposure water concentration of CGA-279202 and the mean measured steady state tissue concentrations (based on total [14C]-residues). The mean (± standard deviation) water concentrations of [14C]-residues measured in the water were 0.156 (± 0.028) µg/L (n=27, where n is the total number of measurements used for determination of mean and standard deviation values), and 1.31 (± 0.27) µg/L (n=33) for the 0.16 and 1.6 µg/L CGA-279202 exposures, respectively.

The lipid content of bluegill sunfish was determined to be 5.83% (the procedure is described in Section 4.11). BCF values and BCF values based on lipid content (whole body) are presented in Tables 14 and 15 for the 0.16 and 1.6 µg/L treatment levels, respectively.

For exposure at 0.16 µg/L CGA-279202, tissue steady state was considered to have been reached by Day 1 of exposure for edible tissue, and Day 3 for the nonedible and whole body tissues. Mean (± standard deviation) measured steady state tissue concentrations were calculated, using the results from Day 1 to 28, to be 20.5 (± 6.89) µg/kg, n=40, for edible tissue. Using the results from Day 3 to 28, mean (± standard deviation) measured steady state tissue concentrations were calculated to be 130 (± 36.4) and 67.2 (±13.1) µg/kg, n=35, for nonedible and whole body tissues, respectively. From these data, bioconcentration factors (BCF) of 131, 835 and 431 were calculated for edible, nonedible and whole body tissues, respectively. The whole body BCF based on the lipid content of the fish was calculated to be 7390.

Steady state in the tissues exposed to a nominal CGA-279202 concentration of 1.6 µg/L was considered to have been reached by Day 14 of exposure for edible, nonedible and whole body tissues. Mean (± standard deviation) measured steady state tissue concentrations were calculated, using the results from Day 14 to 28, to be 118 (± 60.2), n=20, 695 (± 187), n=19, and 367 (± 104) µg/kg, n=19, for edible, nonedible and whole body tissues, respectively. From these data, bioconcentration factors (BCF) of 90, 530 and 280 were calculated for edible, nonedible and whole body tissues, respectively. The whole body BCF based on lipid content was calculated to be 4800.
The bioconcentration factors and average tissue concentrations are summarised in Table B.9.2.21.

Table B.9.2.8.1. Bioconcentration factors (BCFs) and mean tissue concentrations of 14C-trifloxystrobin over 28-day exposure period

Tissue type	0.00016 mg/L			0.0016 mg/L		
	Mean measured tissue content [µg/kg wwt (±SD)]*	BCF[d]	BCF based on lipid content[e]	Mean measured tissue content [µg/kg wwt (±SD)]*	BCF[d]	BCF based on lipid content[e]

Edible	20.5 (±6.89) [a]	131	na	118 (±60.2) [c]	90	na
Non-edible	130 (±36.4) [b]	835	na	650 (±187) [c]	530	Na
Whole body	67.2 (±13.1) [b]	431	7393	367 (±104) [c]	280	4800

* Mean measured values calculated as [Phenyl(A)-U-14C]-CGA 279202 equivalents

[a] steady state reached at Day 1- mean (±standard deviation) value calculated for Day 1 to Day 28

[b] steady state reached at Day 3- mean (±standard deviation) value calculated for Day 3 to Day 28

[c] steady state reached at Day 14- mean (±standard deviation) value calculated for Day 14 to Day 28

[d] based on mean measured steady state exposure and tissue concentrations.

[e] based on mean lipid content of 5.83%

At both test concentrations, residues of (14C) trifloxystrobin accumulated within the exposed fish. Equilibrium levels were reached within 3 days in the 0.00016 mg/L group and within 14 days in the 0.0016 mg/L concentration. Within 24 h of being placed in clean water, levels of (14C) in fish had fallen to 69 and 73.4% of final 28 day exposure levels for the 0.0016 and 0.00016 mg/L groups respectively. At the end of the 14-day depuration period, greater than 98% of the accumulated radioactive residue was eliminated from the fish tissue. The respective times for 50 and 90% depuration were given as 0.5 to 2.4 days and 1.5 to 7.8 days. The following metabolites were detected after 28 days exposure to 0.0016 mg/L trifloxystrobin:

Table B.9.2.8.2. Concentration of CGA 279202 and metabolites in fish tissues after 28 days continuous exposure

Compound	Concentration in viscera tissue (mg/kg wwt)	Concentration in edible tissue (mg/kg wwt)
CGA 279202 (trifloxystrobin)	0.537	0.082
CGA 321113	0.179	0.012
CGA 357276	0.059	n.d.
CGA 331409	0.047	0.005
CGA 373466	0.014	n.d.
metabolite A	0.025	0.004
metabolite B	0.503	0.004
metabolite C	0.190	n.d.

n.d. not detected

metabolite A was not identified

metabolite B was characterised as the cystine conjugate of CGA 321113

metabolite C was identified as the glucuronic acid conjugate of CGA 279202

The study was stated to have been conducted in accordance with US EPA Guideline 165-4, which is comparable to OECD No. 305 guideline (May 1989), and in compliance with GLP.

The BCF for whole body tissue is 431 L kg^{-1}. If normalised to 5% lipid, the BCF is 7393 L kg^{-1}

4.2.2 Bioaccumulation test with other organisms

4.3 Acute toxicity

4.3.1 Short-term toxicity to fish

[Study 1] Acute toxicity test of CGA 279202 to rainbow trout (*Oncorhynchus mykiss*) in the flow-through system (Anonymous, 1997f, M-032048-01-1)

Detailed study summary and results:

Objective:
The aim of the study was to assess the acute toxicity of CGA-279202 to rainbow trout (*Oncorhynchus mykiss*), expressed as 96h-LC_{50} for mortality, in a flow-through system.

Materials and methods:
Test item: CGA 279202, purity: 96.4%, Batch No.: P.405009, solubility in water: 0.6 mg/L at 20°C. The test was conducted over a period of 96 hours with *Oncorhynchus mykiss* in dechlorinated tap water. Oxygen content, pH and temperature were measured daily, total hardness was measured at the beginning and at the end of the test. A daily photoperiod of 16 hours light and a transition period of 30 min were maintained.

Juvenile fish were commercially supplied ([redacted]) and held for 42 days under test conditions prior to testing. They were fed a commercially prepared trout food diet supplemented by living, frozen or dried organisms, e.g. Daphnia and larvae of mosquitoes, until 24 h prior to the test. The mean body length was 45 mm and the mean body weight was 0.71 g, corresponding to a fish biomass to solution ratio of 0.15 g/L per 24 h. Fish were not fed during the 96 h exposure period.
Two replicates of ten fish per concentration and control (blank control and vehicle control with 0.096 mL dimethylformamide/L (91.6 mg/L)) were exposed under flow-through conditions in one 20 L-glass aquarium (with 15 L water) per replicate to nominal test concentrations of 0.004, 0.0072, 0.013, 0.023 and 0.042 mg test item/L.

Samples of each test concentration were taken during the starting phase, immediately before the exposure and then in daily intervals in both replicates until the end of the test. They were analysed using HPLC-UV.
At 2, 24, 48, 72, 96-hours, observations of mortality, and sublethal symptoms, such as abnormal behavioural activity and stress were made (swimming behaviour, loss of equilibrium, respiratory function, exophthalmus, pigmentation and other observations). Dead fish, if any, were removed from the test solutions at least at the above mentioned intervals.
The LC_{50} values for 24-72h exposure were calculated according to the maximum likelihood method, using the probit procedure. The LC_{50} value for 96h exposure was calculated according to the method of estimating the bioassay with quantal response, based on the logistic function.

Validity criteria:

Validity criteria (according to OECD 203, 2009)	Obtained in this study
Mortality in the controls does not exceed 10% (or one fish if less than 10 are used) at the end of the test	0%
Dissolved oxygen concentration in the control and test vessels is ≥ 60% throughout the test	89-101%
Measured concentration of the test substance is maintained ± 20% of the nominal concentration, or results are based on mean measured	Results are based on measured concentration, as in two samples the measured concentration was <80% and in two samples it was >120%

concentrations	

Results:

Analytical Results:

Constant conditions were maintained throughout the test, as indicated by the water parameter measurements. Temperature was slightly below the OECD recommendations for this species (12.8-13.5°C measured vs. 13-17°C recommended) but was constant within a range of 1°C. This had no impact on control performance. Test water had an oxygen content of 89-101% of the saturation value, pH 8.4-8.5, a temperature of 12.8-13.5°C and a total hardness of 197-199 mg $CaCO_3$/L.

The test substance appeared homogeneously distributed in the test vessels at all test times and test concentrations. Measured test concentrations of CGA 279202 determined daily ranged from 74 to 121% of the nominal concentrations over the period of the test (two samples < 80% and two samples > 120%).

Table B.9.2.1.1. Summary of the measured concentrations

Conc. nominal	-7.5h		Measured concentrations: initial (0h)		24h		48h		72h		end (96h)		mean 0-96h	
			Mean of repl. A&B		Repl. B		Repl. A		Repl. B		Mean of repl. A&B			
mg/L	mg/L	%	mg/L	%	mg/L	%	mg/L	%	mg/L	%	mg/L	%	mg/L	%
Blank			< 0.0004		< 0.0004		< 0.0004		< 0.0004		< 0.0004		< 0.0004	
Vehicle			< 0.0004		< 0.0004		< 0.0004		< 0.0004		< 0.0004		< 0.0004	
0.004	0.0051	128	0.0037	93	0.0046	115	0.0044	110	0.0035	88	0.0040	100	0.0040	101
0.0072			0.0065	90	0.0087	121	0.0075	104	0.0063	88	0.0070	97	0.0072	100
0.013	0.0156	120	0.0106	82	0.0153	118	0.0121	93	0.0099	76	0.0129	99	0.0122	94
0.023			0.0171	74	0.0261	113	0.0212	92	0.0199	87	0.0221	96	0.0213	93
0.042	0.0493	117	0.0373	89	0.0490	117	0.0387	92	0.0384	91	0.0417	99	0.0410	98
mean		122		85		117		98		86		98		97
min		117		74		113		92		76		96		93
max		128		93		121		110		91		100		101

Repl.: Replicate

Biological Results:

Sublethal effects were observed at concentrations > 0.0072 mg/L, such as loss of equilibrium, change in the swimming behaviour, in the pigmentation and in the respiratory function, hence, the highest concentration with no sublethal and lethal effects was 0.0072 mg/L.

The LC_{50} (96h) of CGA 279202 was determined to be 0.015 mg/L based on actual mean concentrations. This value remained constant in the period 24 to 96 h.

Table B.9.2.1.2 Summary of the mortality over the study period

Conc. actual mean	Cumulative Mortality Number of dead fish					%
mg/L	2-4 h	24 h	48 h	72 h	96 h	96h
Blank	0	0	0	0	0	0
Vehicle	0	0	0	0	0	0
0.0040	0	0	0	0	0	0
0.0072	0	0	0	0	0	0
0.012	0	3	4	4	4	20
0.021	0	18	19	19	20	100
0.041	19	20	20	20	20	100

Conclusions:

The LC_{50} (96h) of CGA 279202 was determined to be 0.015 mg a.s./L based on actual mean concentrations. The highest concentration with no sublethal and lethal effects was 0.0072 mg a.s./L.

[Study 2] Acute toxicity of CGA 279202 to bluegill (*Lepomis macrochirus*) under flow-through conditions (Anonymous, 1997g, M-032068-01-1)

Objective:
The aim of the study was to assess the acute toxicity of CGA-279202 to bluegill (*Lepomis macrochirus*), expressed as 96h-LC_{50} for mortality, in a flow-through system.

Materials and methods:
Test item: CGA 279202, purity: 96.4%, Batch No.: P.405009, solubility in water: 0.6 mg/L at 20°C.
The test was conducted over a period of 96 hours with *Lepomis macrochirus* in dechlorinated tap water. Oxygen content, pH and temperature were measured daily, total hardness was measured at the beginning and at the end of the test. A daily photoperiod of 16 hours light and a transition period of 30 min were maintained.
Juvenile fish were commercially supplied ([redacted]) and held for 25 d under test conditions prior testing. They were fed larvae of mosquitoes supplemented by commercially prepared discus fish food, until 24 h prior to the test. The mean body length was 36 mm and the mean body weight was 0.51 g, corresponding to a fish biomass to solution ratio of 0.06 g/L per 24 h. Fish were not fed during the 96 h exposure period.
Two replicates, each containing ten fish per concentration and control (blank control and vehicle control with 87 mg (91.6 µL) dimethylformamide/L) were exposed under flow-through conditions in one 20 L- glass aquarium (with 15 L water) per replicate to nominal test concentrations of 0.017, 0.031, 0.056, 0.10, 0.18 mg test item/L.
Samples of each test concentration were taken immediately before the exposure and then in daily intervals in both replicates until the end of the test. They were analysed using HPLC-UV.
At 2, 24, 48, 72 and 96 h, observations of mortality and sublethal symptoms, such as abnormal behavioural activity and stress were made (swimming behaviour, loss of equilibrium, respiratory function, exophthalmus, pigmentation and other observations). Dead fish, if any, were removed from the test solutions at least at the above mentioned intervals.

Validity criteria:

Validity criteria (according to OECD 203, 2009)	Obtained in this study
Mortality in the controls does not exceed 10% (or one fish if less than 10 are used) at the end of the test	0%
Dissolved oxygen concentration in the control and test vessels is ≥ 60% throughout the test	65-113%
Measured concentration of the test substance is maintained ± 20% of the nominal concentration, or results are based on mean measured concentrations	Results are based on mean measured concentrations as measured concentrations were in a range of 71-101%

Constant conditions were maintained as indicated by the water parameter measurements.
The mean body length of the fish was slightly above the recommended total length (20 ± 10 mm recommended) as indicated in OECD Guideline 2003. However, this had no impact on control performance.

Results:

Analytical Results:
The temperature was maintained within a range of 22.2-23.9°C. The dissolved oxygen content was between 65-113% of the saturation value, pH was 8.0-8.4 and the total hardness at the start of the exposure was 181 mg $CaCO_3$/L, respectively.
Measured test concentrations of CGA 279202 determined daily ranged from 71-101% of the nominal concentrations over the period of the test. The actual mean concentrations were 0.015, 0.028, 0.046, 0.076; 0.15 mg test substance/L. Small particles appeared at the surface of the test solution after 72 h of exposure at concentrations 0.031 and 0.056 mg a.s./L.

Table B.9.2.1.3 Summary of the measured concentrations

Conc. nominal	Measured concentrations: initial (0h) Mean of repl. A&B		24h Mean of repl. A&B		48h Repl. B		72h Repl. A		end (96h) Mean of repl. A&B		mean 0-96h		Standard Error
mg/L	mg/L	%	mg/L	%	mg/L	%	mg/L	%	mg/L	%	mg/L	%	s
Blank	< 0.01		< 0.01		< 0.01		< 0.01		< 0.01		< 0.01		
Vehicle	< 0.01		< 0.01		< 0.01		< 0.01		< 0.01		< 0.01		
0.017	0.01519	89	0.01512	89	0.01463	86	0.01466	86	0.01691	99	0.01530	90	0.0009
0.031	0.02662	86	0.02882	93	0.02600	84	0.02797	90	0.03122	101	0.02812	91	0.0023
0.056	0.04334	77	0.04828	86	0.04084	73	0.04528	81	0.05210	93	0.04597	82	0.0044
0.10	0.07079	71	0.08049	80	n.d.		n.d.		n.d.		0.07564	76	
0.18	0.12987	72	0.17161	95	n.d.		n.d.		n.d.		0.15074	84	
mean		79		89		81		86		98		84	
min		71		80		73		81		93		76	
max		89		95		86		90		101		91	

n.d.: not determined
Repl.: Replicate

Biological Results:
Sublethal effects were observed after 2-4 h of exposure at concentration 0.076 mg/L, such as a loss of equilibrium and change in the swimming behaviour, hence, the highest concentration with no sublethal and lethal effects was 0.028 mg/L.
After 96 h exposure, mortality occurred at concentrations of 0.046, 0.076 and 0.15 mg test item/L with 5, 100 and 100%, respectively.
The LC_{50} values were calculated according to a Bayesian approach, probit analysis.The LC_{50} (96 h) of CGA 279202 was determined to be 0.054 mg a.s./L based on mean measured concentrations. This value remained constant in the period 24 to 96 h.

Table B.9.2.1.4 Summary of the mortality over 96 hours

Conc. actual mean	Cumulative Mortality Number of dead fish					%
mg/L	2-4 h	24 h	48 h	72 h	96 h	96h
Blank	0	0	0	0	0	0
Vehicle	0	0	0	0	0	0
0.015	0	0	0	0	0	0
0.028	0	0	0	0	0	0
0.046	0	1	1	1	1	5
0.076	11	20	20	20	20	100
0.15	20	20	20	20	20	100

Conclusions:
The LC_{50} (96h) of CGA 279202 was determined to be 0.054 mg a.s./L based on actual mean concentrations.

The highest concentration with no sublethal and lethal effects was 0.028 mg a.s./L.

[Study 3] Acute toxicity of CGA 279202 to the sheepshead minnow, *Cyprinodon variegatus* (Anonymous, 1996a, M-032072-01-1)

Objective:
The aim of the study was to assess the acute toxicity of CGA-279202 to the sheepshead minnow (*Cyprinodon variegatus*), expressed as 96h-LC_{50} for mortality, in a flow-through system.

Materials and methods:
Test item: CGA 279202, Technical ID No.: FL-941274, purity: 96.0% (reanalysed 95.5%), Batch Code: P.405009.
The test was conducted over a period of 96 hours with *Cyprinodon variegatus* in natural seawater ([redacted]), adjusted to a salinity of 16 - 17 ppt with deionized water, aerated and recirculated through particle filters, activated carbon and ultraviolet steriliser. Oxygen content, pH, salinity and temperature were measured daily. A daily photoperiod of 16 hours light and a transition period of 15 min were maintained.
Juvenile fish were commercially supplied ([redacted]) and acclimatized for more than 14 d to test conditions. They were fed a commercial flake food (Tetra Min®) daily during acclimation except for the 48 h immediately preceding the test initiation. At the conclusion of the test the control fish had a mean total length of 22 mm and a mean wet weight of 0.16 g (loading rate was 0.11 g/L at any time and 0.018 g/L per 24 h). Fish were not fed during the 96 h exposure period.
2 x 10 fish per concentration and control (control and solvent control with 0.1 mL dimethylformamide/L) were exposed under flow-through conditions in one 20 L-glass aquarium per replicate to nominal test concentrations of 0.039, 0.066, 0.11, 0.18, and 0.30 mg test item/L.
Analytical determination of test substance concentration (active ingredient) was performed with samples collected from each replicate test vessel after 0 and 96 hours. They were analysed using HPLC-UV.
At 0, 24, 48, 72 and 96 h, observations of mortality, and sublethal symptoms such as lethargy and loss of equilibrium were made.

Validity criteria:

Validity criteria (according to OECD 203, 2009)	Obtained in this study
Mortality in the controls does not exceed 10% (or one fish if less than 10 are used) at the end of the test	0% in both control replicates
Dissolved oxygen concentration in the control and test vessels is ≥ 60% throughout the test	6.5-7.6 mg/L (71-84%)
Measured concentration of the test substance is maintained ± 20% of the nominal concentration, or results are based on mean measured concentrations	Results are based on mean measured concentrations as one value was below 80% of the nominal concentration after 96 h

Constant conditions were maintained throughout the test, as indicated by the water parameter measurements.

Results:

Analytical Results:
The salinity ranged from 16 to 17 ppt, the pH ranged from 8.1 to 8.3, temperature ranged from 21.4 to 22.1°C, and the dissolved oxygen concentration ranged from 6.5 to 7.6 mg/L. Insoluble material was not observed at any time during the test. Mean measured concentrations were 83 to 92% of nominal values and were stable throughout the test. Mean measured concentrations were used for all calculations.

Table B.9.2.1.5 Summary of the measured concentrations in the test system

Nominal concentration [mg/L]	Measured concentration of CGA 279202					
	0 h		96 h		Mean	
0 (control)	nd		nd		Nd	
0 (solvent	nd		nd		Nd	
0.039	0.0356	91%	0.0289	74%	0.0323	83%
0.066	0.0601	91%	0.0582	88%	0.0592	90%
0.11	0.0943	86%	0.103	94%	0.0987	90%
0.18	0.153	85%	0.178	99%	0.166	92%
0.30	0.243	81%	0.275	92%	0.259	86%

nd = not detected

Biological Results:

One hundred percent survival occurred in the control and 95% survival occurred in the solvent control. No sublethal effects were noted in the controls during the exposure period. Sublethal effects were observed in test vessels containing 0.11, 0.18, and 0.30 mg CGA 279202/L during the test.

Table B.9.2.1.6 Summary of the toxicity over 96 hours

Mean Measured Concentration of CGA-279,202 (mg/L)	Rep.	Number Alive 0 hr	24 hr	48 hr	72 hr	96 hr	Number Affected 0 hr	24 hr	48 hr	72 hr	96 hr
ND[1] (control)	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0
ND (solvent control)	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	9	9	9	0	0	0	0	0
0.0323	1	10	10	10	10	10	0	0	0	0	0
	2	10	10	10	10	10	0	0	0	0	0
0.0592	1	10	8	8	6	6	0	0	0	0	0
	2	10	9	9	9	9	0	0	0	0	0
0.0987	1	10	9	6	3	3	0	0	0	3[3]	3[3]
	2	10	9	5	3	3	0	0	0	3[3]	3[3]
0.166	1	10	3	0	0	0	5[2]	3[2]	--	--	--
	2	10	2	0	0	0	5[2]	2[2]	--	--	--
0.259	1	10	0	0	0	0	10[2]	--	--	--	--
	2	10	0	0	0	0	10[2]	--	--	--	--

[1] ND = none detected at or above the limit of quantitation of 0.0143 mg/L.
[2] Affected fish exhibited lethargy and loss of equilibrium.
[3] Affected fish exhibited lethargy.

Conclusions:
Exposure of fish to the test substance resulted in a 96 h-LC_{50} of 0.0780 mg a.s./L, based on mean measured concentrations. The 96 h-NOEC is 0.0323 mg a.s./L.

4.3.2 Short-term toxicity to aquatic invertebrates

[Study 1] Acute toxicity of CGA 279202 to the cladoceran *Daphnia magna* Straus under flow-through conditions (Neumann, C., 1997 , M-032085-01-1)

Detailed study summary and results:

Objective:
The aim of the study was to assess the 48 h-acute toxicity of CGA 279202 to the daphnid, *Daphnia magna*, expressed as LC/EC_{50} for immobilization, under flow-through conditions.

Materials and methods:
Test item: CGA 279202, purity: 96.4%, Batch No.: P.405009.
The test was conducted over a period of 48 hours with *Daphnia magna* STRAUS/Clone 5 in Elendt M4 medium. Oxygen content, pH and temperature were measured at test start and after 48 h, hardness and conductivity were determined from freshly prepared M4 medium. The test was conducted at a 16 h light and 8 h dark photoperiod with a transition period of 30 minutes.
Daphnids from an in-house culture kept under test conditions were fed on workdays with a suspension of green algae (1.0 mL/L of a *Scenedesmus subspicatus* suspension with a density of approximately 108 cells/mL). Juvenile daphnids, less than 24 h old, were used for the test. They were not fed during the 48 hours exposure period.
Two replicates with 10 daphnids each were applied per concentration and control (blank control and solvent control: 89 mg dimethylformamide/L) and were exposed under flow-through conditions in 400 mL glass vessels (with 250 mL solution renewed every hour by intermittent flow) to nominal test concentrations of 0.0075, 0.015, 0.03, 0.06 and 0.12 mg/L.
Water samples of each concentration were taken at hour 0, 24 and 48 and were analyzed using HPLC with UV detection.

Immobilization or other behavioral changes of the daphnids were recorded after 24 and 48 hours of exposure. Other sublethal effects (e.g. lethargic swimming) were also recorded.

Validity criteria:

Validity criteria (according to OECD 202, 2004)	Obtained in this study
Immobilization in the control and solvent control does not exceed 10%	0% in control, 0% in solvent control
Dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels	At 0.12 mg/L, the oxygen saturation at test end was 45-46% (4.0-4.2 mg/L). This had no impact on the outcome of the study. [A]

[A] 100% immobilization were already reached at the lower test concentration of 0.06 mg/L. Oxygen saturation in the control and solvent control was ≥90%.

The conductivity was 677 µS/cm and water hardness was 269 mg $CaCO_3$/L. The pH ranged from 8.1 to 8.5, temperature was constant at 20.0°C, and the oxygen saturation ranged from 96-84% at test start and from 94-81% after 48 h for the controls and nominal test concentrations of 0.0075-0.03 mg/L. At 0.06 and 0.12 mg/L, the oxygen saturation at test end was 68 and 45-46%, respectively.

Results:

Analytical Results:
The test substance appeared homogeneously distributed at nominal test concentrations of 0.0075 to 0.06 mg/L of CGA 279202 throughout the test. A slight turbidity was observed at 0.12 mg a.s./L throughout the test. Presence of test substance on the water surface was recorded for all concentrations. Measured contents

were low for 0.0075 and 0.015 mg a.s./L, therefore, the determination of effect values was based on mean measured concentrations.

Table B.9.2.4.5. Summary of the measured concentrations in the test system

Samples: Nominal concentrations of CGA 279202 (mg/L)	Concentration determined (mg/L)	Values corrected for recovery rate (mg/L)	% of nominal concentration
0 hour, flask 1			
Blank	< 0.0006	< 0.0006	-
Vehicle	< 0.0006	< 0.0006	-
0.0075	0.0048	0.0046	61.3
0.015	0.0098	0.0093	62.0
0.03	0.0213	0.0209	69.7
0.06	0.0554	0.0543	90.5
0.12	0.104	0.102	85.0
48 hours, flask 1			
Blank	< 0.0006	< 0.0006	-
Vehicle	< 0.0006	< 0.0006	-
0.0075	0.0053	0.0050	66.7
0.015	0.0106	0.0101	67.3
0.03	0,0259	0,0254	84.7
0.06	0.0639	0.0626	104.3
0.12	0.140	0.137	114.2
Stock solution 2.5005 mg/g	2.7661 mg/g	2.7118 mg/g	108.5

Biological Results:
After 48 h of exposure, rates of 5, 20, 70, 100 and 100% immobilization were observed at mean measured concentrations of 0.0048, 0.010, 0.023, 0.06 and 0.12 mg/L, respectively. The estimation of effect values was based on mean measured concentrations according to the Probit-model.

Table B.9.2.4.5.Summary of the toxicity over 48 hours

Mean measured Concentration [mg/L]	**Number of immobilised daphnids (48 h)**			
	Replicate: 1	Replicate: 2	Total	%
Control	0	0	0	0
Solvent Control	0	0	0	0
0.0048	0	1	1	5
0.010	1	3	4	20
0.023	8	6	14	70
0.06	10	10	20	100
0.12	10	10	20	100

EC_{50} (48 h)	**0.016 mg/L**
95% confidence limit	0.012 - 0.021 mg/L
Slope	1.61
Goodness of fit X_2	0.9, df = 3, p = 0.83
NOEC (48 h)	<0.0048 mg/L
EC_{10} (48 h)	0.007 mg/L
EC_{50} (24 h)	0.047 mg/L
95% confidence limit	0.037 - 0.059 mg/L
Slope	2.1
Goodness of fit X_2	0.9, df = 3, p = 0.83

Conclusions:
Exposure of daphnids to the test substance resulted in a 48 hour-EC_{50} of 0.016 mg a.s./L, with a 95% confidence interval of 0.012-0.021 mg a.s./L, based on mean measured concentrations.
The 48 hour NOEC is <0.0048 mg a.s./L.

[Study 2] Boeri, R. L.; Magazu, J. P.; Ward, T. J.; 1997; Acute toxicity of CGA 279202 to the daphnid, *Daphnia magna*; M-032084-01-1)

Objective:
The aim of the study was to assess the 48 h-acute toxicity of CGA 279202 to the daphnid, *Daphnia magna*, expressed as LC/EC_{50} for immobilization, under flow-through conditions.

Materials and methods:
Test item: CGA 279202, Technical ID: FL-941274, ARS 31748, purity: 96.0% (reanalysed 96.3%), Batch No.: P.405009.
The test was conducted over a period of 48 hours with *Daphnia magna* in deionized water (carbon filtered, adjusted to hardness of 160-180 mg/L, aerated and recirculated through particle filters, activated carbon and UV sterilizer). Oxygen content, pH, conductivity and temperature were measured daily. The test was conducted at a 16 h light and 8 h dark photoperiod with a transition period of 15 minutes.
Daphnids were commercially supplied (Aquatic Research Organisms, Hampton, New Hampshire) and held for 20 d under test conditions (19.2-20.3°C, dissolved oxygen concentration ≥ 8.4 mg/L, feeding: yeast/trout chow suspension and *Selenastrum capricornutum* daily) until juveniles produced from the cultured adults were collected. Juvenile daphnids, less than 24 h old, were used for the test. They were not fed during the 48 hours exposure period. At the end of the test, control daphnids averaged 0.47 mg and the loading rate during the definitive toxicity test was approximately 0.00031 g/L at any time and 0.000051 g/L/24 hours.
Two replicates with 10 daphnids each were applied per concentration and control (blank control and solvent control: 0.1 mL dimethylformamide/L) and were exposed under flow-through conditions in 20 L-glass aquaria (with 15 L water, without aeration, and intermittent flow resulting in 6.1 volume additions per 24 h) to nominal test concentrations of 6.5, 12, 18, 31, and 50 µg/L. Daphnids were exposed in glass cylinders with Nitex® screen attached to the bottom with silicone adhesive. These exposure chambers were suspended within each test vessel.
Analytical determination of test substance concentration was performed with samples collected from each replicate test vessel after 0 and 48 hours. Samples collected from replicate 1 test vessels at initiation and replicate 2 test vessels at termination were analyzed using HPLC with UV detection.
The numbers of surviving organisms, the occurrence of sublethal effects, and observations of insolubility were determined visually and recorded after 24 and 48 hours. Dead organisms were removed every 24 hours or when first observed.

Validity criteria:

Validity criteria (according to OECD 202, 2004)	Obtained in this study
Immobilization in the control and solvent control does not exceed 10%	0% in control, 5% in solvent control
Dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels	8.6 to 8.8 mg/L (95-98%)

Results:
The conductivity ranged from 540 to 600 µmhos/cm, the pH ranged from 8.5 to 8.6, temperature ranged from 20.0 to 20.9°C, and the dissolved oxygen concentration ranged from 8.6 to 8.8 mg/L.

Analytical Results:
Insoluble material was not observed at any time during the test. Mean measured concentrations were 89 to 100% of nominal values and they were stable during the test. Mean measured concentrations were used for all calculations.

Table B.9.2.4.3. Summary of the measured concentrations in the test system

Nominal conc. [µg/L]	Analysed concentration of CGA 279202 [µg a.s./L]		
	0 h	48 h	mean
0 (control)	ND	ND	ND
0 (solvent control)	ND	ND	ND
6.5	6.06	5.92	5.99
12	11.2	10.2	10.7
18	19.9	16.0	18.0
31	29.0	28.2	28.6
50	50.4	47.4	48.9

ND: none detected at or above the limit of quantitation of 1.77 µg/L.

Biological Results:
One hundred percent survival occurred in the control and 95% survival occurred in the solvent control. No sublethal effects were noted in the controls during the exposure period. Sublethal effects, observed as immobilized daphnids, were noted in test vessels containing 28.6 and 48.9 µg/L CGA-279202 during the test. The results are summarised below.

Table B.9.2.4.4. Summary of the toxicity over 48 hours

Mean Measured Concentration of CGA-279,202 (µg/L)		Rep.	Number Alive 0 hr	Number Alive 24 hr	Number Alive 48 hr	Number Affected[1] 0 hr	Number Affected[1] 24 hr	Number Affected[1] 48 hr
ND[2]	(control)	1	10	10	10	0	0	0
		2	10	10	10	0	0	0
ND	(solvent control)	1	10	10	10	0	0	0
		2	10	9	9	0	0	0
5.99		1	10	10	10	0	0	0
		2	10	10	10	0	0	0
10.7		1	10	10	10	0	0	0
		2	10	9	9	0	0	0
18.0		1	10	10	10	0	0	0
		2	10	10	8	0	0	0
28.6		1	10	9	5	0	0	5
		2	10	9	3	0	0	3
48.9		1	10	5	0	0	4	--
		2	10	3	0	0	0	--

[1] Affected daphnids were immobilized.
[2] ND = not detected at or above the limit of quantitation of 1.77 µg/L.

The LC_{50} value after 48 h was determined to be 25.3 µg a.s./L

Conclusions:
Exposure of daphnids to the test substance resulted in a 48 hour-LC_{50} of 25.3 µg a.s./L, with a 95% confidence interval of 21.8 to 29.4 µg a.s./L, based on mean measured concentrations. The 48 hour NOEC is 18.0 µg a.s./L (mean measured).

[Study 3] Acute toxicity of CGA 279202 to the crayfish, *Procambarus acutus acutus* (Ward, T. et al.; 1998, M-052687-01-1)

Objective:
The aim of the study was to assess the 96 h-acute toxicity of CGA 279202 to the crayfish, *Procambarus acutus acutus,* expressed as LC_{50}, in a flow-through test system.

Materials and methods:
Test item: CGA 279202, purity: 96.4%, BASL ID FL-951320; ARS-39718.
The test was conducted over a period of 96 hours with *Procambarus acutus acutus* in deionized water (carbon filtered, adjusted hardness of 40-48 mg/L, aerated and recirculated through particle filters, activated carbon and an UV sterilizer). Oxygen content, pH, conductivity and temperature were measured daily, total hardness was measured at the beginning of the test. A daily photoperiod of 16 hours light and a transition period of 15 min were maintained.

Juvenile crayfish were commercially supplied (Pool Fisheries Inc., Lonoke, Arkansas) acclimated and held for 48 h under test conditions prior testing due to their cannibalistic nature. They were fed a commercial flake food daily (TetraMin®). At the conclusion of the test the mean body length was 52.5 mm and the mean body weight was 2.76 g, corresponding to a loading rate of 1.84 g/L at any time and 0.33 g/L/24 h. Fish were not fed during the 96 hours exposure period.

Two replicates with 10 fish per concentration and control (blank control and solvent control with 0.10 mL dimethylformamide/L) were exposed under flow-through conditions in one 20 L-glass aquarium (with 15 L

water) per replicate to nominal test concentrations of 55, 92, 150, 250, and 420 µg/L. 100 mL-glass beakers were added as shelter for the fish to each aquarium.

The test substance was supplied to the test vessels under flow-through conditions by an intermittent flow proportional diluter which was activated 661 times, resulting in an average of 5.5 volume additions per 24 hours in each test vessel.

Analytical samples were collected from each exposure vessel at the beginning and end of the test. The first replicate was analysed at the beginning of the test and the second replicate was analysed at the end using HPLC-UV.

The numbers of surviving organisms, the occurrence of sublethal effects, and observations of insolubility were determined visually and recorded initially and after 24, 48, 72, and 96 h.

Validity criteria:

Validity criteria (according to OECD 202, 2004)	**Obtained in this study**
Immobilization in the control and solvent control does not exceed 10%	0% in control and solvent control
Dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels	6.6-9.5 mg/L

Results:

Test water had an oxygen concentration of 6.6-9.5 mg/L, pH 7.3-7.6, a temperature of 19.1-19.9°C, a conductivity of 150-170 µmhos/cm and a total hardness of 44 mg/L.

Analytical Results:

Insoluble material was not observed in any test vessel during the test. Mean measured concentrations were 87 to 100% of nominal. The biological results are expressed in terms of mean measured.

Table B.9.2.4.16. Summary of the measured concentrations in the test system

Nominal conc. * [µg/L]	**Analysed concentration of CGA 279202 [µg a.s./L]**		
	0 h	**96 h**	**mean**
0 (control)	ND	ND	ND
0 (solvent control)	ND	ND	ND
45	44	41	43
75	66	63	65
120	110	120	120
200	170	190	180
340	300	310	310

ND = none detected at or above the limit of quantitation of 14 ug/L

* corrected for the 81% recovery in the secondary stock solution

Biological Results:

One hundred percent survival occurred in the control and solvent control, and no sublethal effects were noted in the controls during the exposure period.

Results of the toxicity test could not be interpreted by standard statistical techniques because survival exceeded 50% at all tested concentrations. The 24-, 48-, 72- and 96-h LC_{50} values were reported as greater than the highest tested concentration of test substance. The NOEC is the concentration of test substance that allowed at least 95% survival and did not cause any sublethal effects.

Table B.9.2.4.17 Summary of the toxicity over 96 hours

Mean Measured Concentration of CGA-279,202 (µg/L)		Rep.	Number Alive 0 hr	24 hr	48 hr	72 hr	96 hr	Number Affected 0 hr	24 hr	48 hr	72 hr	96 hr
ND[1]	(control)	1	10	10	10	10	10	0	0	0	0	0
		2	10	10	10	10	10	0	0	0	0	0
ND	(solvent control)	1	10	10	10	10	10	0	0	0	0	0
		2	10	10	10	10	10	0	0	0	0	0
43		1	10	10	10	10	10	0	0	0	0	0
		2	10	10	10	10	10	0	0	0	0	0
65		1	10	10	9	9	9	0	0	0	0	0
		2	10	10	10	10	10	0	0	0	0	0
120		1	10	10	10	10	9	0	0	0	0	0
		2	10	10	9	9	9	0	0	0	0	0
180		1	10	9	9	9	9	0	0	0	0	0
		2	10	9	8	8	8	0	0	0	0	0
310		1	10	10	10	9	8	0	0	0	0	0
		2	10	9	9	9	9	0	0	0	0	0

[1] ND = none detected at or above the limit of quantitation of 14 µg/L.

The 24-, 48-, 72-, and 96-hour LC_{50s} for crayfish are all greater than 310 µg a.s./L (mean measured), the highest tested concentration and the apparent water solubility limit under conditions of the test.
The 96-hour NOEC is 65 µg a.s./L (mean measured).

Conclusions:
The 24-, 48-, 72- and 96-h LC_{50} values were reported as greater than the highest tested concentration of test substance, namely >310 µg a.s./L, based on mean measured concentrations. The 96-hour NOEC is 65 µg a.s./L.

[Study 4] Acute flow-through mollusc shell deposition test with CGA 279202 (Boeri, R. et al.; 1996, M-032088-01-1)

Objective:
The aim of the study was to assess the 96 h-acute toxicity of CGA 279202 to the eastern oyster, *Crassostrea virginica*, expressed as EC_{50} concerning shell growth after 96 h, under flow-through conditions.

Materials and methods:
Test item: CGA 279202, Technical ID: FL-941274, ARS 31748, purity: 96.0% (reanalysed 95.5%), Batch No.: P.405009.

The test was conducted over a period of 96 hours with *Crassostrea virginica* in unfiltered, natural seawater (salinity of 32-33 ppt, aerated). Oxygen content, pH, salinity and temperature were measured daily. The test was conducted at a 16 h light and 8 h dark photoperiod with a transition period of 15 minutes.

Juvenile eastern oysters were commercially obtained (P. Cummins Oyster Company, Inc., Stevensville, Maryland) and kept for 11 d under test conditions (21.6-22.6°C, dissolved oxygen concentration ≥ 6.4 mg/L). Oysters were 25 - 35 mm in height (measured along the long axis) prior to grinding. Immediately prior to the test initiation, each oyster was ground with a rotary grinder to remove approximately 3 - 5 mm of shell and form a smooth edge. The test media was unfiltered sea water collected at Marblehead, Massachusetts. Routine sampling of the water did not identify any impurities. During the test, oysters were supplied with live marine phytoplankton to supplement the existing food in the unfiltered, natural seawater that was used as dilution water.

Two replicates with 10 oysters each were applied per concentration and control (seawater control and solvent control: 0.1 mL dimethylformamide/L) and were exposed under flow-through conditions in 20 L-glass aquaria (with 15 L water, and intermittent flow resulting in 8.4 volume additions per 24 h and 0.53 L per oyster per h) to nominal test concentrations of 10, 18, 29, 49, and 80 µg a.s./L.

Analytical determination of test substance concentration was performed with samples collected from each replicate test vessel after 0 and 96 hours. Samples collected from replicate 1 test vessels at initiation and replicate 2 test vessels at termination were analysed using HPLC with UV detection.

The numbers of surviving organisms and the occurrence of sublethal effects were determined visually and recorded after 24, 48, 72 and 96 hours.

The EC_{50} calculation was performed using the mean measured concentrations of test substance and new shell growth at 96 hours. The EC_{50} and associated 95% confidence interval were calculated using a weighted least squares non-linear regression technique. The slope of the 96 hour dose response curve was calculated by the probit method using percent of solvent control shell growth. The no observed effect concentration was calculated using TOXSTAT 3.3.

Validity criteria:

Validity criteria (according to OECD 202, 2004)	**Obtained in this study**
Immobilization in the control and solvent control does not exceed 10%	0% in control and solvent control
Dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels	5.6 to 7.5 mg/L

Results:

The salinity ranged from 32-33 ppt, the pH ranged from 8.1 to 8.2, temperature ranged from 21.1 to 21.7°C, and the dissolved oxygen concentration ranged from 5.6 to 7.5 mg/L.

Analytical Results:

Insoluble material was not observed at any time during the test. Mean measured concentrations were 92 to 99% of nominal values and were stable during the test.

Table B.9.2.4.8. Summary of the measured concentrations in the test system

Nominal conc. [µg/L]	Analysed concentration of CGA 279202 [µg a.s./L]		
	0 h	96 h	mean
0 (control)	ND	ND	ND
0 (solvent control)	ND	ND	ND
10	9.51	10.1	9.81
18	17.8	15.8	16.8
29	27.9	29.3	28.6
49	46.9	43.4	45.2
80	77.6	71.9	74.8

ND: none detected at or above the limit of quantitation of 7.15 µg/L.

Biological Results:
No mortality occurred in the control and in the solvent control. No sublethal effects were noted in any test vessel during the exposure period. One oyster died in the highest test concentration.
The shell deposition by oysters exposed in the solvent control were significantly different from the control oyster shell deposition when compared using a parametric "t" test ($\alpha = 0.05$), and treatment data were therefore compared to the solvent control data.
The EC_{50} value after 96 h was determined to be 34.9 µg a.s./L. Mean measured concentrations were used for all calculations.

Table B.9.2.4.9. Summary of the toxicity over 96 hours

Shell growth at longest finger (mm) per control and treatment group [in µg/L]							
	Control	Solvent c.	9.81	16.8	28.6	45.2	74.8
Mean	2.9	3.3	2.8	2.7 [A]	1.4 [A]	1.0 [A]	0.2 [A]
Std.dev.	0.5	0.6	0.8	0.6	0.7	0.8	0.4
% of control	-	113	97	94	48	35	7
% of solvent control	-	-	85	82	43	31	6

[A] Mean is significantly different than the solvent control at the 95% confidence level.

Conclusions
Exposure of eastern oysters to the test substance resulted in a 96 hour-EC_{50} for shell growth of 34.9 µg a.s./L, with a 95% confidence interval of 19.7 to 62.0 µg a.s./L, based on mean measured concentrations. The 96 hour NOEC is 9.81 µg a.s./L.
However, mortality is a more relevant endpoint for classification purposes. Only one oyster died at the highest concentration so the LC_{50} is > 74.8 µg a.s./L.

4.3.3 Algal growth inhibition tests

[Study 1] Growth inhibition test of CGA 279202 tech. to green algae (*Scenedesmus subspicatus*) in a static system (Grade, R., 1995, M-032098-01-1)

Detailed study summary and results:
Objective:
The aim of the study was to assess the 72 h toxicity of CGA 279202 to green algae, *Scenedesmus subspicatus*, expressed as inhibition of algal growth, under static test conditions.

Materials and methods:
Test item: CGA 279202, purity 96.4±0.5%, Batch No.: P.405009, solubility in water: 0.6 mg/L at 20°C.
The test was conducted over a period of 72 hours with green algae (*Scenedesmus subspicatus* strain 86.81 SAG) in growth medium (according to guideline).

Scenedesmus from Collection of Algal Cultures (Göttingen, Germany) was kept under test conditions prior testing.

Three replicates for each test concentration (nominal 0.0020, 0.0044, 0.0096, 0.021, 0.046, 0.10 and 0.22 mg/L) and six for the control and solvent control (0.0088 mg TWEEN 80/L) were applied. The test was performed under static conditions in 100 mL Erlenmeyer flasks (50 mL test solution) with cotton stoppers on a lab shaker. Temperature was set to 24±1°C, continuous illumination (8000 lux) was provided. Initial cell density was 9900 cells/mL.

Composite samples (about 150 ml per concentration and control) of each test concentration were drawn by mixing identical volumes of the test solutions taken from the test vessels. Samples of test solutions were taken immediately before exposure and after 72 hours exposure. All samples were analyzed using HPLC with UV-detection.

Temperature was continuously measured, pH was measured at test start and after 72 h. Cell densities were measured at 24, 48 and 72 hours exposure on a "TOA" cell counter.

Validity criteria:

Validity criteria (according to OECD 201, 2011)	Obtained in this study
The biomass in the control should increase exponentially by a factor of at least 16 during the test (specific growth rate of 0.92/day)	Blank control: Factor 143 during 0-72 h Solvent control: Factor 129 during 0-72 h
The mean coefficient of variation for section-by-section specific growth rates (days0-1, 1-2, 2-3) in controls must not exceed 35%	Blank control: 12.0% Solvent control: 12.2%
The coefficient of variation of average specific growth rates during the whole test period in control replicates must not exceed 7% (*P.subcapitata*, *D.subspicatus*) or 10% (other species)	Blank control: 2.65% Solvent control: 4.52%

Results:
The pH was between 7.6-7.8, temperature was maintained at 23.0±1.0°C.

Analytical Results:
The analytical measurements of trifloxystrobin are summarised in table B.9.2.6.3. It is evident that concentrations were not within ± 20% of nominal. Therefore, the toxicity endpoint is based on mean measured concentrations.

Table B.9.2.6.3. Summary of the measured concentrations in the test system

Nominal conc. [mg/L]	Measured concentrations of CGA 279202					
	0 h [mg/L]	0 h [%]	72 h [mg/L]	72 h [%]	Arithmetic mean [mg/L]	Geometric mean [mg/L]
Blank	<0.0002	-	<0.0002	-	<0.0002	<0.0002
Vehicle	<0.0002	-	<0.0004	-	<0.0004	<0.0004
0.0020	0.00111	56	0.00096	48	0.00104	0.00103
0.0044	0.00182	41	0.00202	46	0.00192	0.00192
0.0096	0.00246	26	0.00229	24	0.00238	0.00237
0.021	0.0141	67	0.0177	84	0.0159	0.0158
0.046	0.0167	36	0.0241	52	0.0204	0.0201
0.10	0.0399	40	0.0319	32	0.0359	0.0357
0.22	0.0629	29	0.0587	27	0.0608	0.0608

Biological Results:
The following values were calculated based on mean measured concentrations.

Table B.9.2.6.4. Toxicity (72 h) of trifloxystrobin to algae

Conc. act. mean [mg/L]	Mean cell densities [cells/mL*10000]			Inhibition (areas under growth curves)		Inhibition (growth rates)	
	24 h	48 h	72 h	Mean A	IA 0-72 h [%]	Mean µ	Iµ 0-72 h [%]
Blank	4.1	23.9	141.2	2306	0.0	69	0.0
Vehicle	4.5	28.0	127.3	2248	2.5	67	2.3
0.00104	4.4	26.0	141.8	2372	0.0	69	0.1
0.00192	4.1	24.2	135.0	2241	2.8	68	1.1
0.00238	4.9	21.5	88.5	1635	29.1	62	10.1
0.0159	4.1	5.0	14.3	330	85.7	37	46.7
0.0204	3.4	3.4	6.3	180	92.2	26	62.7
0.0359	2.4	2.8	4.7	123	94.7	22	68.7
0.0608	1.6	2.5	3.8	85	96.3	19	73.0

Endpoint	EC_{50} (0-72 h) [mg/L]	95% conf. limit [mg/L]	Slope	NOEC (0-72 h) (5%level) [mg/L]
Inhibition (growth rate)	E_rC50 = 0.016	0.014-0.019	0.64	NOE_rC 0.00192
Inhibition (areas under growth curves)	E_bC50 = 0.0053	0.0037-0.0078	1.04	NOE_bC 0.00192

The EC_{50} values were calculated according to the maximun likelihood method, probit model.

Conclusions:
The E_rC_{50} (0-72h) for *Scenedesmus subspicatus* exposed to CGA 279202 is 0.016 mg a.s./L based on mean measured concentrations. The NOEC was determined to be 0.00192 mg a.s./L.

Recalculation: Herno, V.; 2017, M-582093-01-1
The endpoints were re-calculated on the basis of geomean measured concentrations, in addition EC_{10} and EC_{20} values are provided.

Endpoint (with 95% confidence interval) [mg/L]	Growth rate	Yield	Biomass
EC_{50}	0.0174 (0.0148-0.0203)	0.0044 (0.0033-0.0063)	0.0053 (0.0041-0.0071)
EC_{20}	0.0049 (0.0035-0.0063)	0.0020 (0.0014-0.0026)	0.0023 (0.0016-0.0030)
EC_{10}	0.0025 (0.0016-0.0036)	0.0013 (0.0008-0.0018)	0.0015 (0.0009-0.0020)

Based on recalculated endpoints the new proposed endpoint is an E_rC_{50} of 0.0174 mg a.s./L (geometric mean measured) and an E_bC_{50} of 0.0053 mg a.s./L (geomentric mean measured). The E_rC_{10} is 0.0025 mg a.s./L (geometric mean measured) and the NOEC is 0.00192 mg a.s./L (geometric mean measured).

4.3.4 *Lemna* sp. growth inhibition test

[Study 1]

Valid study not available.

4.4 Chronic toxicity

4.4.1 Fish early-life stage (FELS) toxicity test

[Study 1] Early life-stage toxicity of CGA 279202 to rainbow trout (*Oncorhynchus mykiss*) using newly fertilized "green" eggs in a flow-through system (Anonymous, 1997h, M-032080-02-1)

Detailed study summary and results:

The study was conducted in accordance with US-EPA guideline 72-4(a), OECD guideline 210, and in accordance with GLP.

Objective:
The aim of this early life stage toxicity test was to establish chronic toxicity levels of CGA 279202 using the most critical and sensitive life stage of the whole life cycle of rainbow trout (*Oncorhynchus mykiss*), in a flow-through system.

Materials and methods:
Test item: CGA 279202, purity: 96.4%, Batch No.: P.405009, solubility in water: 0.6 mg/L at 20°C, log P_{OW} = 4.3, hydrolysis at 25°C: >1000 d at pH 5, 34 d at pH 7, and 15 h at pH 9.

Test organisms: The test lot of unfertilized rainbow trout eggs and milt was obtained from [redacted]. The eggs and milt were each collected from three individual adults, acclimated to the test temperature without allowing contact with water and combined after a vitality check. Mean fish biomass to solution ratio (loading) in the control at the end of the test was 1.01 g/L or 0.17 g/L per 24 hours.

Approximately four hours after fertilization, 42 embryos were transferred into each replicate egg incubator cup (suspended in aquaria, 13 cm diameter x 8 cm height, stainless steel screen). In addition, 42 fertilized eggs were placed in additional incubation cups in the controls for determining viability (fertilization).
20 L-glass aquaria with 15 L water were used as test vessels, each with 42 eggs, three replicates per treatment and control/solvent control. They were not aerated.

In addition to the test item treatments, a blank control (dechlorinated tap water, carbon fine filtered) and a solvent control (83.6 mg dimethylformamide/L) were established.

The water/test solution was delivered to the test vessels at a flow rate of 2160 L/24 h, which was equivalent to the addition of six replicate chamber volumes in a 24 hour period. Trout embryos were maintained in darkness until day 7 post-hatch. After this time a 16:8-hour light:dark photoperiod was maintained with a 30 min transition period.

Egg mortality, as discerned by a distinct change in coloration, was recorded on working days and dead eggs were removed to prevent fungal growth. Eggs without visible neural keel were removed on day 19. When hatching commenced on day 32, the number of embryos hatched in each incubation cup was recorded daily until day 35. Hatch was actually complete by day 35, since >95% hatch in the blank control had occurred. Day 35 was designated day 0 of the 60 day post-hatch growth period. The number of larval fry was impartially reduced to 20 per replicate on day 35.

The fry were released from the incubation cups into the growth chambers without exposure to the air by submerging the cups on study day 15 post-hatch - after >95% were swimming up on that day - and the egg cups were removed.

The food used during the study included commercially prepared Salmon Starter and brine shrimp nauplii.
Abnormal (sublethal) behavioural or physical changes (loss of equilibrium, erratic swimming, loss of reflex, excitability, discoloration, or change in appearance or behaviour) and mortality were monitored by visually inspecting each growth chamber on working days and recording the data. Survival data were collected for statistical analysis at the end of the test. Dead test organisms were removed when first observed.

At test termination on study day 95 (60 days post-hatch), all surviving fish were sacrificed in tricaine methanesulfonate (MS-222) and measured for total length, and weighed by analytical balance. After drying for 24 hours at 60°C, the fish were reweighed for the determination of the dry weights.

Chemical analysis
Samples of each test concentration and the control were taken for the determination of the actual concentrations of the test at day 0, 7, and weekly thereafter as well as at test termination. In addition, samples were taken before the start of the exposure to check and adjust the diluter system. Samples were analysed by HPLC-UV.

Water quality parameters were measured before the start of the test, at the start and throughout the test. Measurement of conductivity, alkalinity and hardness were made weekly in one replicate of the control and in the highest concentration with surviving organisms at the start and at the end of exposure. Furthermore, total hardness was determined in the various concentrations at the start of the test. TOC and suspended solids were measured before the start of the test, at the start and at the end of the test. Temperature ranged from 9.5 to 10.6°C during day 0-35 and 11.7 to 12.6°C during days 0 - 60 post-hatch. Oxygen saturation ranged from 81 to 103% over the period of the test. The specific conductance ranged from 381 to 443 µS/cm, total alkalinity from 2.91 to 3.24 mVal/L, hardness from 170 to 199 mg $CaCO_3$/L, the TOC ranged from 0.6 to 39 mg C/L and suspended solids were below 2 mg/L.

Statistical analysis
Results of the toxicity test were interpreted by standard statistical techniques using SAS Version 6.11. and the procedures "ECOS" by *Fisch and Strehlau, 1996*[1], and "AQUA" by *Kremers, 1991*[2], using SAS PROC GLM and PROC NLIN.

Validity criteria:

Validity criteria (according to OECD 210, 2013)	**Obtained in this study**
Dissolved oxygen concentration in the control and test vessels is > 60% throughout the test	81 to 103%
Water temperature does not differ more than ± 1.5°C between chambers/successive days and is within the recommended range for the test species (10 ± 1.5°C)	9.5 to 10.6°C during day 0-35 and 11.7 to 12.6°C during days 0 - 60 post-hatch
Chemical analysis is performed	Yes
Survival of fertilised eggs and post-hatch success in controls is at least 75% for *O. mykiss*	Mean 94, 97 and 94% larvae survival at hatch, at swim up (18 d post-hatch) and at test end (60 d post-hatch) in control groups

All validity criteria as specified in OECD 210 (2013) were met. A minor deviation was noted for temperature during the post-hatching phase. As the temperature was in range of the other OECD protocol recommendations (e.g. OECD 203) for this species, was in a range of 1°C, and as no effects were reported in controls, this is not likely to have influenced study findings.

Results:

Chemical analysis:
The chemical analysis of the water is summarised in tables B.9.2.2.1 to B.9.2.2.4.

[1] Fisch, R.D. and G.A. Strehlau, 1996: ECOS: Statistische Analyse von Oekotoxikologie-Daten. Draft report of Mathematical Applications in Biology and surveys. ScS, IS 7.0, Ciba-Geigy AG, July 1996.

[2] Kremers, W., 1991:SAS Program for Analysis of Aquatic Data, Ciba-Geigy

The nominal concentrations were: 0.00069, 0.0012, 0.0022, 0.0040, 0.0072, 0.013 mg test substance/L. Insoluble material was not observed and the substance appeared in true solution in all test vessels and at all times and concentrations.
Table B.9.2.2.1 to B.9.2.2.4 indicate that concentrations were not maintained with in ± 20% of nominal, and therefore the concentrations are expressed as arithmetic mean measured concentrations, as per OECD 210 (2013). The overall arithmetic mean measured concentrations for each test level, averaged from the measured concentrations of CGA 279202 at the beginning, at weekly intervals and at the end of the exposure period were: not detected at or above the analytical detection limit of 0.00005 mg/L in the blank and vehicle control, 0.00093 ± 0.00037, 0.0014 ± 0.00027, 0.0025 ± 0.00039, 0.0043 ± 0.00060, 0.0077 ± 0.0014 and 0.015 ± 0.0017 mg test substance/L in the treated groups.

Table B.9.2.2.1 Summary of concentrations of trifloxystrobin

Nominal conc. [mg a.s./L]	**Measured concentrations**							
	0 d		**7 d**		**14 d**		**21 d**	
	Mean A,B,C		**A**		**B**		**C**	
	[mg a.s./L]	**% of nominal**	**[mg a.s./L]**	**% of nominal**	**[mg a.s./L]**	**% of nominal**	**[mg a.s./L]**	**% of nominal**
Control	<0.00005	100	<0 00005	100	<0 00005	100	<0.00005	100
Vehicle control	<0.00005	100	<0.00005	100	<0 00005	100	<0.00005	100
0.00069	0.00054	78.3	0.00070	101.4	0.00063	91.3	0.00063	91.3
0.0012	0.00107	89.2	0.00150	125	0.00140	116.7	0.00130	108.3
0.0022	0.00187	85	0.00260	118.2	0.00220	100	0.00240	109.1
0.0040	0.00320	80	0.00420	105	0.00410	102.5	0.00390	97.5
0.0072	0.00520	72.2	0.00690	95.8	0.00660	91.7	0.00670	93.1
0.013	0.01193	91.8	0.01490	114.6	0.01490	114.6	0.01460	112.3

Table B.9.2.2.2 Summary of concentrations of trifloxystrobin

Nominal conc. [mg a.s./L]	**Measured concentrations**							
	28 d		**35 d**		**43 d**		**50 d**	
	A		**B**		**C**		**A**	
	[mg a.s./L]	**% of nominal**	**[mg a.s./L]**	**% of nominal**	**[mg a.s./L]**	**% of nominal**	**[mg a.s./L]**	**% of nominal**
Control	<0 00005	100	<0 00005	100	<0.00005	100	<0.00005	100
Vehicle control	<0 00005	100	<0.00005	100	<0 00005	100	<0.00005	100
0.00069	0.00062	89.9	0.00036	52.2	0.00077	111.6	0.00073	105.8
0.0012	0.00155	129.2	0.00100	83.3	0.00140	116.7	0.00115	95.8
0.0022	0.00230	104.5	0.00250	113.6	0.00260	118.2	0.00230	104.5
0.0040	0.00430	107.5	0.00420	105	0.00420	105	0.00410	102.5
0.0072	0.00710	98.6	0.00760	105.6	0.00830	115.3	0.00730	101.4
0.013	0.01675	128.8	0.01740	133.8	0.01790	137.7	0.01495	115

Table B.9.2.2.3 Summary of concentrations of trifloxystrobin

Nominal conc. [mg a.s./L]	**Measured concentrations**							
	57 d		**63 d**		**71 d**		**78 d**	
	B		**C**		**A**		**B**	
	[mg a.s./L]	**% of nominal**	**[mg a.s./L]**	**% of nominal**	**[mg a.s./L]**	**% of nominal**	**[mg a.s./L]**	**% of nominal**
Control	<0 00005	100	<0 00005	100	<0 00005	100	<0.00005	100
Vehicle control	<0.00005	100	<0.00005	100	<0.00005	100	<0.00005	100
0.00069	0.00098	142	0.00140	202.9	0.00130	188.4	0.00120	173.9
0.0012	0.00135	112.5	0.00160	133.3	0.00170	141.7	0.00140	116.7

0.0022	0.00230	104.5	0.00250	113.6	0.00270	122.7	0.00220	100
0.0040	0.00410	102.5	0.00420	105	0.00450	112.5	0.00410	102.5
0.0072	0.00700	97.2	0.00770	106.9	0.00845	117.4	0.00720	100
0.013	0.01540	118.5	0.01570	120.8	ND	-	ND	-

ND: not determined

Table B.9.2.2.4 Summary of concentrations of trifloxystrobin

Nominal conc. [mg a.s./L]	**Measured concentrations**					
	85 d		**92 d**		**95 d**	
	C		**A**		**Mean A,B,C**	
	[mg a.s./L]	**% of nominal**	**[mg a.s./L]**	**% of nominal**	**[mg a.s./L]**	**% of nominal**
Control	<0 00005	100	<0 00005	100	<0.00005	100
Vehicle control	<0.00005	100	<0.00005	100	<0.00005	100
0.00069	0.00130	188.4	0.00140	202.9	0.00143	207.2
0.0012	0.00140	116.7	0.00180	150	0.00200	166.7
0.0022	0.00280	127.3	0.00315	143.2	0.00340	154.5
0.0040	0.00470	117.5	0.00530	132.5	0.00580	145
0.0072	0.00880	122.2	0.01035	143.8	0.01045	145.1
0.013	ND	-	ND	-	ND	-

ND: not determined

Biological results:
The biological effects of trifloxystrobin are summarised in table B.9.2.2.5.

Survival
Ninety four percent of the control and solvent control embryos hatched, and 94% of the fish in the control and solvent control survived the 60-day post hatch exposure. Control and solvent control fish had an average weight of 818 mg and an average length of 45 mm.
There was 100% survival in pooled control at 48 hours. Percent hatch in the pooled control and test concentrations 0.00093, 0.0014, 0.0025, 0.0043, 0.0077 and 0.015 mg/L trifloxystrobin was 90, 94, 99, 95, 99, 92 and 24 respectively, being significantly lower than control at the highest concentration tested.
At swim up (18 days post-hatch), there was 97, 98, 98, 95, 98, 88 and 0% survival in the pooled control, 0.00093, 0.0014, 0.0025, 0.0043, 0.0077 and 0.015 mg a.s./L treatment groups, respectively with survival at the two highest concentrations being significantly lower than the blank control according to the Tukey's Test ($P \leq 0.05$).
The survival rate in the same groups after 60 days was 94, 98, 93, 95, 97, 82 and 0% respectively, that was significantly lower than the blank control at the highest concentration. A statistically non-significant decrease was evident at 0.0077 mg a.s./L treatment group, although this appears to be driven by a decrease in replicate A.

Table B.9.2.2.5 Summary of the toxicity over 96 hours

Mean Measured Concentrations mg a.s./L	Replicate	Hatching success on day 18 (number of fry)	Swim up day 18 (number of fry)	Survival at swim-up 18 days post-hatch (%)	Survival at 60 Days post-hatch (%)	Bodyweight day 60 (mg)		Length day 60 (mm)	
Control	A	25	20	100	100	812	± 134	44.0	± 2.7
	B	37	19	95	90	882	± 233	46.7	± 4.4
	C	35	19	95	90	804	± 195	43.6	± 4.1
Vehicle	A	29	19	95	90	841	± 179	46.5	± 3.8
	B	34	20	100	100	765	± 204	42.8	± 4.1
	C	36	19	95	95	809	± 138	46.6	± 2.8
Mean		**32.7**	**19.3**	**97**	**94**	**819**	**± 113**	**45.0**	**± 4.0**
0.00093	A	33	20	100	100	847	± 189	45.1	± 3.2
	B	35	20	100	100	829	± 178	46.3	± 3.3
	C	34	19	95	95	795	± 221	43.3	± 4.2
Mean		**34**	**19.7**	**98**	**98**	**824**	**± 193**	**44.9**	**± 3.7**
0.0014	A	37	20	100	95	918	± 168	46.8	± 2.3
	B	34	20	95	90	848	± 187	44.7	± 3.2
	C	36	20	100	95	1009	± 146	46.9	± 2.5
Mean		**35.7**	**20**	**98**	**93**	**925**	**± 178**	**46.2**	**± 2.8**
0.0025	A	31	19	95	95	765	± 252	44.3	± 4.6
	B	36	19	95	95	984	± 157	46.4	± 2.7
	C	36	19	95	95	825	± 169	44.1	± 3.0

Time to Hatch

Hatch began on day 32 of the study and continued until day 35. From days 32 to 35 the rate of hatch was reduced in the 0.0077 and the 0.015 mg a.s./L. This was confirmed statistically for day 34 with significant lower values in concentration 0.015 mg a.s./L. The decreased rate of hatching the 0.0077 mg a.s./L treatment group was statistically non-significant, the delay lasting approximately 1 day.

Hatching Success

Percent hatch in the pooled control and in test concentrations 0.00093, 0.0014, 0.0025, 0.0043, 0.0077 and 0.015 mg a.s./L was 90, 94, 99, 95, 99, 92 and 24%, respectively. Statistically significant reductions were observed at 0.015 mg a.s./L.

Swim-Up

Newly hatched fry began swimming up from the bottom of the incubation cup at 13 days post-hatch. The number of fry swimming up in each chamber was recorded for days 13 to 18 post-hatch and for days 13 to 15 post-hatch. Swim-up was complete by day 18 post-hatch (no observations made on days 16 and 17). A statistically significant decrease in swim-up was noted at 0.0077 mg a.s./L on days 13-15; a statistically non-significant decrease was observed on day 18.

Morphological and Behavioural Abnormalities

Symptoms noted after hatching in two individual organisms were crippled ("bent") fish (blank control and concentration 0.0077 mg a.s./L). No other abnormalities were observed, nor fungus, parasites or bacteria on fins or the skin.

Effects on Length and Weight

Length and weight data have been summarized by replicate in Table B.9.2.2.6.

Mean Total Length

For the pooled control and the 0.00093, 0.0014, 0.0025, 0.0043, 0.0077 mg a.s/L levels with surviving fish were 45.0, 44.9, 46.2, 45.0, 45.1, 44.9 mm, respectively. No significant length reductions were detected for any test level when compared to the control.

Mean Wet Weight
For the pooled control and the 0.00093, 0.0014, 0.0025, 0.0043 and 0.0077 mg a.s./L levels with surviving fish were 819, 824, 925, 858, 916 and 869 mg, respectively. No reductions in wet weight were detected for any test level when compared to the control.

Table B.9.2.2.6. Summary on survival of rainbow trout:

Biological endpoint	**NOEC [mg/L]**	**LOEC [mg/L]**	**MATC [mg/L]**
Survival			
At 48 h	0.015	>0.015	>0.015
At hatch	0.015	>0.015	>0.015
At swim-up (18 d post-hatch)	0.0043	0.0077*	0.0058
At 30 d post-hatch	0.0077	0.015	0.011
At the end of exposure (60 d post-hatch)	0.0077	0.015	0.011
Growth at the end of the test			
Total length	0.0077	0.015	0.011
Wet weight	0.0077	0.015	0.011
Dry weight based on mean of fish in replicates	0.0077	0.015	0.011
Other observed effects			
Healthy embryos at 48 hours	0.015	>0.015	>0.015
Healthy embryos at hatch	0.015	>0.015	>0.015
Healthy fish at swim-up (18 d post-hatch)	0.0077	0.015	0.011
Healthy fish at 30 d post-hatch	0.0077	0.015	0.011
Healthy fish at end of exposure 60 d post-hatch)	0.0077	0.015	0.011
Hatching success	0.0077	0.015	0.011
Time to hatch	0.0077	0.015	0.011
Time to swim-up and first feeding	**0.0043**	**0.0077****	**0.058**

*The reduction of survival was observed after 18 days post-hatch (swim-up), but not after 30 and 60 days post-hatch. ** The delay in time to swim up was statistically significantly decreased on days 13-15; the decrease was not statistically significant by day 18.

Table B.9.2.2.7 Summary of EC_{10} and EC_{20}

Estimated EC-values	**EC_{10} (95 % Confidence interval) [mg/L]**	**EC_{20} (95% Confidence interval) [mg/L]**
Survival at swim-up	0.0076 (0.0072-0.010)	0.0080 (0.0077-0.011)
Survival at 30 d post-hatch	0.0077 (0.0070-0.0087)	0.0081 (0.0076-0.0092)
Survival at the end of the test	0.0075 (0.0068-0.0083)	0.0079 (0.0075-0.0089)

Conclusion:

Based on the transient effects on survival and time to hatch, the no observed effect concentration for fish exposed for 95 days to trifloxystrobin is 0.0077 mg a.s./L (based on mean measured concentrations).
However, the statistically significant delay in time to swim up at 0.0077 mg a.s./L, although reported to be transient, is considered to be treatment related. On this basis it was considered that the NOEC is 0.0043 mg a.s./L. The EC_{10} and EC_{20} for survival at the end of the test are 0.0075 and 0.0079 mg a.s./L, respectively (mean measured).

4.4.2 Fish short-term toxicity test on embryo and sac-fry stages

4.4.3 Aquatic Toxicity – Fish, juvenile growth test

4.4.4 Chronic toxicity to aquatic invertebrates

[Study 1] Chronic toxicity of CGA 279202 to the daphnid, *Daphnia magna* (Boeri, R. et al.; 1996, M-032097-01-1)

Detailed study summary and results:

The study was conducted in accordance with US EPA 72-4(b), and in accordance with GLP.

Objective:
The aim of the study was to establish chronic toxicity levels of CGA 279202 to the freshwater invertebrate *Daphnia magna* in a 21-d exposure test, under flow-through conditions.

Materials and methods:
Test item: CGA 279202 tech., purity 96.4%, Batch No.: P.405009, BASL ID No.: FL-951320, ARS-39718.
The test was performed in deionised water (hardness of 160-180 mg $CaCO_3$/L; pH adjusted to 7.5-7.8; aerated and passed through a particle filter, UV sterilizer and activated carbon). Oxygen content, pH, conductivity and temperature were measured daily. The test was conducted at a 16 h light and 8 h dark photoperiod with a 15 min transition period.

Daphnids were commercially obtained (Aquatic BioSystems, Inc., Fort Collins, Colorado) and were kept under test conditions 12 d prior testing. The culture was supplied with a yeast/trout chow suspension and the freshwater alga, *Selenastrum capricornutum*, daily before the start of the test and at least twice per day during the test. Juvenile daphnids less than 24 h old were used for the test.

Daphnids were exposed in 1 L-glass vessels (with 800 mL test solution, mean flow rate of 26 volume additions/24 h). The solvent control contained 0.10 mL dimethylformamide/L.

Analytical determination of CGA 279202 concentrations was performed with samples collected from each test vessel on days 0, 7, 14, and 21. All samples were analysed using HPLC with UV/VIS-detection.

Investigated endpoints were survival of first generation daphnids (on day 21), sublethal effects as immobilization, changes in behaviour or appearance (daily), the time to first brood, the number of young per female (daily from onset of reproduction), and the length and the dry weight of surviving daphnids (on day 21). Progeny and dead organisms were removed daily.

Statistical analysis
Results of the toxicity test were interpreted by standard statistical techniques using TOXSTAT 3.3. Control and solvent control data were pooled and in all cases no differences were observed with a parametric "t" test ($\alpha = 0.05$). If the assumptions of data normality were met (survival, young production, dry weight, and length of first generation survivors; by a Chi-aquare and a Bartlett´s test), a parametric one-way ANOVA and Bonferroni's test were used to compare treatments to the pooled control means. Otherwise (day of first brood), a nonparametric Kruskal-Wallis test was used.

Validity criteria

Validity criteria (according to OECD 211, 2012)	**Obtained in this study**
Parent mortality does not exceed 20% at the end of the test	5% in control and solvent control, respectively
Mean number of living offspring produced per parent animal surviving at the end of the test is ≥60 in controls	62 per control female

Results:
The conductivity ranged from 540 to 610 µmhos/cm (mean = 570 µmhos/cm) during the test.

Chemical analysis:
The chemical analysis of the test item is summarised in table B.9.2.5.1. Insoluble material was not observed in any test vessels during the test. Concentrations were maintained within ± 20% of the nominal and therefore concentrations are reported as mean measured concentrations.

Table B.9.2.5.1. Measured concentrations of trifloxystrobin

Nominal conc. [µg/L]	**Measured concentration [µg/L]**				
	Day 0	**Day 7**	**Day 14**	**Day 21**	**Mean measured concentration [µg/L]**
Control	n.d.	n.d.	n.d.	n.d.	n.d.
Solvent control	n.d.	n.d.	n.d.	n.d.	n.d.
3.2	2.97 3.17	2.67 2.57	2.53 2.60	2.58 3.01	2.76
6.5	6.23 6.37	5.85 5.50	6.08 5.84	6.26 5.72	5.98
13	11.4 11.7	12.3 13.1	11.8 11.3	12.2 11.9	12.0
25	25.1 25.5	23.6 25.8	-[A]	-	25.0
50	50.9 50.4	52.8 48.1	-	-	50.6

n.d.: no test substance detected at or above the quantitation limit of 1.79 µg/L
[A] All daphnids exposed to 25 and 50 µg/L were dead prior to day 7.

Biological Results:
The biological effects of trifloxystrobin are summarised in table B.9.2.5.2.
Survival of the F0 generation was statistically significantly decreased at concentrations of 12.0 ug a.s./L and above. The same pattern was noted on day to first brood. For the average number of young per surviving adult, average dry weight of adults and average length of adults at day 21, statistically significant decreases were noted at concentrations of 5.98 µg a.s./L and above.

B.9.2.5.2. Summary of biological endpoints measured in 21-day flow-through toxicity test using *Daphnia magna*

Endpoint	Mean measured concentration mg CGA 279202/L						
	Control	Solvent Control	0.0027	0.0060	0.012	0.025	0.0506
Percent survival day 21	95	95	95	93	48*	0**	0**
Mean day of first brood	10	12	9.0	9.5	**	**	**
Mean young per surviving daphnid	62	57	57	47*	0**	0**	0**
Mean dry wt of surviving adults (mg)	0.61	0.53	0.53	0.39*	0.19*	0**	0**
Mean length of surviving adults (mm)	3.9	3.8	3.9	3.5*	2.7*	0**	0**

* statistically different to pooled controls (p=0.05)
** assumed to be statistically different to pooled controls,

Sublethal effects, other than visually observed size differences, or immobilization of offspring, were not observed at any time during the test and did not require statistical analysis. EC_{10} and EC_{20} endpoints were calculated for survival of the F0 generation, mean dry weight and mean bodylength of adult on day 21.

Conclusion:
The 21-day EC_{50} for adult immobilisation, based on mean measured concentrations was 0.0098 mg trifloxystrobin/L. The 21-day NOEC, based on mean measured concentrations, was 0.0027 mg trifloxystrobin/L, based on mean number of young per surviving *Daphnia*, mean dry weight and mean length.

Recalculation: Herno, V.; 2017, M-582255-01-1
Owing to the lack of a dose-response relationship, the EC_{20} and EC_{10} could not be calculated for mean time to first brood or number of young produced. The endpoints that were calculable are summarized in table B.9.2.5.3.

Table B.9.2.5.3. Summary of the toxicity endpoints

Biological endpoint	NOEC	LOEC	MATC	EC_{50}	EC_{20}	EC_{10}
	[µg/L]					
First generation survival	5.98	12.0	8.47	9.98	6.71	5.30
Mean time to first brood	5.98	12.0	8.47	-	-	-
Number of young produced	2.76	5.98	4.06	-	n.c.	n.c.
Mean dry weight of first generation	2.76	5.98	4.06	-	4.59	3.28
Mean length of first generation	2.76	5.98	4.06	-	9.50	6.81

n.c. cannot be calculated due to the lack of dose-response

The NOEC is 0.00276 mg a.s./L (mean measured). The EC_{10} and the EC_{20} for mean dry weight in the F1 generation are 0.00328 and 0.00459 mg a.s./L (mean measured), respectively.

4.4.5 Chronic toxicity to algae or aquatic plants

[See short-term toxicity]

4.5 Acute and/or chronic toxicity to other aquatic organisms

OECD TG 218: Sediment-Water Chironomid Toxicity Using Spiked Sediment and

OECD TG 219: Sediment-Water Chironomid Toxicity Using Spiked Water

[Study 1] Toxicity test of CGA 279202 tech. on sediment-dwelling *Chironomus riparius* (syn. *Chironomus thummi*) under static conditions (Grade, R., 1998, M-033988-01-1)

Detailed study summary and results:

The study was conducted according to a proposed BBA Guideline 1995 and in compliance with GLP.

Objective:
The aim of the study was to determine the effects of CGA 279202 tech. on the day of first emergence, the time distribution (peak) of emergence of male and female midges, and the total number of fully emerged male and female midges in a 28-d exposure test, under static test conditions.

Materials and methods:
Test item: CGA 279202, purity 95.6%, Batch No.: P.405009, solubility in water: 0.61 mg/L at 25°C, vapour pressure: 3.4×10^{-6} Pa at 25°C.

The toxicity of technical trifloxystrobin (purity 95.6%) to the sediment-dwelling larvae of the midge *Chironomus riparius* was assessed under static conditions in 28-day study. The sediment-water system comprised units of 1 litre glass beakers containing about 1.5 cm of artificial sediment and a water column of 8 cm at the start and 6 cm at the end (samples taken for chemical analysis).

Following a range finding study, first instar midge larvae (2-3 days old) were exposed to 6 nominal aqueous concentrations of trifloxystrobin ranging from 0.0125 to 0.40 mg a.s./L. Application of test material was made to the water column of the sediment-water systems 24 hours after the larvae had been added.

Each test vessel contained 20 larvae, and a total of 3 replicates per test concentration. In addition there were three blank control and three vehicle (DMF) controls. The *Chironomus* larvae were fed with a fish flake food (dry ground powder of TETRAMIN), before and during the test (1 mg food per day and larvae). Visual assessments (behaviour, mortalities, emergence) were made daily. The number, time and sex of emerged adults were recorded.

Oxygen content and pH were measured weekly, conductivity and temperature were measured from freshly prepared medium and temperature was measured continuously. Environmental conditions were: water temperature was 18.9 to 24 °C, photoperiod of 16 h light (800 lux) and 8 h dark, pH was 8.1 to 8.3 at start and 8.0 to 8.5 at termination. The conductivity was 679-681 µS/cm and the water hardness corresponded to 252-258 mg $CaCO_3$/L. Oxygen content ranged from 7.4 to 9.6 mg/L

Chemical analysis
Analytical determination of CGA 279202 and of its main metabolite in sediment was performed with samples collected from each test vessel on days 0, 7, 14, and 28. All samples were analysed using HPLC with UV-detection.

Statistical analysis
ER and GR are transformed prior to statistical analysis, using an arcsine-squareroot transform. Dunnett tests (a = 5%) for these three parameters are performed to calculate NOEC and LOEC. ECx and 95%-confidence

limits are calculated by logit analysis for emergence rate and development rate (Finney, D.Y. 1978: Statistical Methods in Biological Assay, C.Griffin & Co Ltd, London).

Dunnett tests (a = 5%) were performed to calculate NOEC and LOEC. ECx and 95%-confidence limits were calculated by logit analysis for emergence rate and development rate.

Validity criteria:

Validity criteria (according to OECD 219, 2004)	Obtained in this study
Emergence in controls must be at least 70% at the end of the test	At least 75% in control and solvent control
C. riparius emergence should occur between 12 and 23 days after insertion to the test vessels	Between 12-20 days in control and solvent control
pH should be in the range 6-9, oxygen concentration should be at least 60% of the air saturation value	Oxygen content ranged from 7.4 to 9.6 mg/L. The pH was 8.0 to 8.5.
Water temperature should not differ by more than ±1°C	Water temperature was ranging from 18.9 to 24°C. However, this had no impact on control performance.

Results:

Chemical analysis:

The chemical analysis of the sediment and water are summarized in table B.9.2.5.4.

The actual measured concentrations of trifloxystrobin in the water phase were 0.009, 0.021, 0.046, 0.101, 0.212 and 0.416 mg/L at day 0 (1-3 h after application). At the end of the test (day 28) levels of trifloxystrobin in the water phase were below the limit of detection (stated to be 0.0024 mg/L) in all test concentrations. Therefore, the test concentrations are based on mean measured initial concentrations.
Levels of the metabolite CGA 321113 in the water phase were <0.0016, 0.004, 0.006, 0.006, 0.003 and 0.006 at test day 0 (1-3 h after application). At the end of the test (day 28) water concentrations of CGA 321113 had increased to 0.004, 0.012, 0.025, 0.056, 0.12 and 0.2 mg/L.

Sediment from the two highest test concentrations was analysed on days 0, 7 and 28. At the nominal concentration of 0.21 mg trifloxystrobin/L, the measured concentrations of trifloxystrobin plus CGA 321113 in the sediment (including interstitial water) were 0.10, 0.22 and 0.23 mg/kg sediment (wet) on days 0, 7 and 28 respectively. At the nominal concentration of 0.4 mg trifloxystrobin/L the measured concentrations of trifloxystrobin plus CGA 321113 in the sediment (including interstitial water) were 0.17, 0.79 and 0.36 mg/kg sediment (wet) on days 0, 7 and 28 respectively.

Table B.9.2.5.4. Summary of the measured concentrations in the test system

Nominal conc. [mg/L]	Measured concentration in water samples [mg/L]									
	Day 0		Day 1		Day 7		Day 14		Day 28	
	a.s.	met.	a.s.	met.	a.s.	met.	a.s.	met.	a.s.	met.
Control	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Solvent control	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
0.0125	0.009	n.d.	n.d.	0.007	n.d.	0.009	n.d.	0.007	n.d.	0.004
0.025	0.021	0.004	n.d.	0.018	n.d.	0.016	n.d.	0.017	n.d.	0.012
0.05	0.046	0.006	0.005	0.034	n.d.	0.038	n.d.	0.034	n.d.	0.025
0.1	0.101	0.006	0.032	0.058	n.d.	0.078	n.d.	0.070	n.d.	0.056
0.2	0.212	0.003	0.127	0.044	n.d.	0.150	n.d.	0.140	n.d.	0.120
0.4	0.416	0.006	0.279	0.061	0.048	0.130	0.015	0.180	n.d.	0.200

n.d.: not detected

a.s.: active substance CGA 279202; met.: soil metabolite CGA 321113

Table B.9.2.5.5. Summary of the measured concentrations in the test system

Nominal conc. [mg/L]		Amount of raw sediment used for analysis [g]	Absolute amount determined in the sediment [mg]		Volume of interstitial water [mL]	Absolute amount determined in interstitial water [mg]	
			a.s.	met.		a.s.	met.
Day 0	Blank	29.5	LOD	n.d.	2.7	<LOD	n.d.
	0.2	27.9	0.0029	0.0004	1.8	<LOD	n.d.
	0.4	28.8	0.0039	n.d.	2.4	<LOD	n.d.
Day 7	Blank	28.9	LOD	n.d.	2.7	<LOD	n.d.
	0.2	28.5	0.0058	0.0014	2.3	<LOD	0.00023
	0.4	28.7	0.0218	0.0012	2.0	0.00008	0.00018
Day 28	Blank	28.8	LOD	n.d.	2.7	<LOD	n.d.
	Solvent	29.3	LOD	n.d.	2.8	<LOD	n.d.
	0.2	29.5	0.0019	0.0044	2.7	<LOD	0.00084
	0.4	29.9	0.0059	0.0058	2.9	<LOD	0.00133

Biological results:

The biological results are summarised in table B.9.2.5.6.

Statistically significant effects were observed at the highest treatment of 0.4 mg a.s./L for development rate and emergence rate

The final mean percentage emergence figures were 86.6, 81.6, 81.6, 81.6, 80, 71.6 and 60 in the blank control, vehicle control 0.025, 0.05, 0.10, 0.2 and 0.4 mg a.s./L trifloxystrobin groups respectively, values for the 0.0125 mg a.s./L group are not included due to sampling errors.

An assessment of larval weight was made on exposure day 11 however since only low numbers of larvae were measured no statistical analysis was performed on the data.

From the sample assessed, there was no evidence of any dose related reduction in larval weight although these measurements are considered to be of limited value.

Mean development rates (reciprocal of development time) were 0.07634, 0.07637, 0.07546, 0.07375, 0.0744, 0.07537 and 0.06424 in the blank control, vehicle control, 0.025, 0.05, 0.10, 0.2 and 0.4 mg trifloxystrobin groups, respectively; values for the 0.0125 mg a.s./L group are not included.

Table B.9.2.5.6. Summary of the toxicity following 28 days of exposure

Group (µg a.s./L)	Number emerged by day 28	Number of males	Emergence rate	Development rate (± SD)
Control	17	4	0.85	0.071648
Control	16	10	0.8	0.080744
Control	19	8	0.95	0.076655
Vehicle control	15	5	0.75	0.072590
Vehicle control	18	6	0.9	0.074630
Vehicle control	16	10	0.8	0.081919
Mean	**16.83**	**7.16**	**0.84**	**0.076 (0.0043)**
21.0	17	9	0.85	0.073754
21.0	14	7	0.7	0.074308
21.0	18	12	0.9	0.078345
Mean	**16.33**	**9.33**	**0.82**	**0.075 (0.0025)**
46.0	16	10	0.8	0.071153
46.0	16	7	0.8	0.075070
46.0	17	8	0.85	0.075050
Mean	**16.33**	**8.33**	**0.82**	**0.073 (0.0023)**
101.0	14	5	0.7	0.070747
101.0	16	12	0.8	0.077088
101.0	18	8	0.9	0.075407
Mean	**16.00**	**8.33**	**0.8**	**0.074 (0.0034)**
212.0	13	6	0.65	0.075504
212.0	17	8	0.85	0.072593
212.0	15	8	0.75	0.078025
Mean	**15.00**	**7.33**	**0.75**	**0.075 (0.0028)**
410.0	13	5	0.65	0.061117
410.0	10	6	0.5	0.065838
410.0	13	8	0.65	0.065790
Mean	**12.00**	**6.33**	**0.60****	**0.064 (0.0031)*****

***Dunnett test <0.01 ** Dunnett test <0.001

Endpoint	EC_{50} [mg/L]	95% conf. limit	Statistic. method	NOEC [mg/L]	LOEC [mg/L]
Emergence rate	0.56	none	Logit	0.20	0.40
Development rate	0.45	none	Logit	0.20	0.40

Conclusion:
The 28-day EC_{50} based on emergence rate was 0.56 mg a.s./L. The 28-day EC_{50} based on developmental rate is 0.45 mg a.s./L. The corresponding NOEC values are 0.2 mg a.s./L.

Recalculation: Herno, V.; 2017, M-582256-01-1

Endpoint	EC_{10} [mg/L]	95% conf. limit	EC_{20} [mg/L]	95% conf. limit	NOEC [mg/L]	LOEC [mg/L]
Emergence rate	0.14	0.08-0.21	0.32	(0.21-0.67)	0.21	0.41
Development rate	No calculation can be performed: lack of dose-response relationship				0.21	0.41

The 28-day EC_{10} and EC_{20} for emergence rate are 0.14 and 0.32 mg a.s./L respectively. It was not possible to calculate EC_{10} and EC_{20} for development rate as there was no dose-response relationship. The NOEC for emergence rate and development rate is 0.21 mg a.s./L.

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