| *Group* | **Animals** | **Dose (nominal)** | **Administration and sampling** |
|---|---|---|---|
| | | [Oxadiazin-4-$^{14}$C]<br>368 kBq (9.9 µCi) | Tissues as listed for Group B1 were taken at 4 time points, i.e. $t_{cmax}$, $t_{cmax/2}$, and two additional time points based on the blood kinetics as determined in the corresponding Group B2.<br>Time points: 2, 8, 12, 24 h after dosing. |
| *F5* | 12 females | 0.5 mg/kg [Thiazol-2-$^{14}$C]<br>230 kBq (6.2 µCi) | Single oral dose.<br>Tissues as listed for Group B1 were taken at 4 time points, i.e. $t_{cmax}$, $t_{cmax/2}$, and two additional time points based on the blood kinetics as determined in the corresponding Group B1.<br>Time points: 2, 8, 12, 24 h after dosing. |
| *F6* | 12 females | 100 mg/kg [Thiazol-2-$^{14}$C]<br>2464 kBq (67 µCi) | Single oral dose.<br>Tissues as listed for Group B1 were taken at 4 time points, i.e. $t_{cmax}$, $t_{cmax/2}$, and two additional time points based on the blood kinetics as determined in the corresponding Group D1.<br>Time points: 1, 7, 12, 24 h after dosing. |
| *F7* | 12 females | 0.5 mg/kg<br>[Oxadiazin-4-$^{14}$C]<br>368 kBq (9.9 µCi) | Single oral dose.<br>Tissues as listed for Group B1 were taken at 4 time points, i.e. $t_{cmax}$, $t_{cmax/2}$, and two additional time points based on the blood kinetics as determined in the corresponding Group B2.<br>Time points: 1, 8, 12, 24 h after dosing. |
| *G1* | 4 males | 0.5 mg/kg [Thiazol-2-$^{14}$C]<br>250 kBq (6.8 µCi) | Single oral dose.<br>Urine: 0 - 24, 24 - 48 h. Faeces: 0 - 24, 24 - 48 h.<br>Bile: 0 - 1, 1 - 2, 2 - 4, 4 - 8, 8 - 18, 18 - 24, 24 - 42, 42 - 48.<br>Tissues: Two days after dosing the animals were killed. The gastrointestinal tract and the remaining carcass were saved for radiometry. |
| *G3* | 5 males | 0.5 mg/kg<br>[Oxadiazin-4-$^{14}$C]<br>376 kBq (10.2 µCi) | Single oral dose.<br>Sample collection as in Group G1.<br>Faeces: 0 - 24, 24 - 48 h.<br>Bile: 0 - 0.5, 0.5 - 1, 1 - 2, 2 - 4, 4 - 8, 8 - 18, 18 - 24, 24 - 42, 42 - 48.<br>Tissues: Two days after dosing the animals were killed. The gastrointestinal tract and the remaining carcass were saved for radiometry. |

*Analytical methods:* Radioactivity in urine, plasma, and other liquid samples was measured directly by liquid scintillation counting (LSC). After addition of water the faeces were homogenized manually with a pestle. Radioactivity in blood, bone, lungs, gastrointestinal tract, faeces, and carcass was determined by combustion and LSC. Radioactivity in brain, fat, heart, kidneys, liver, muscle, spleen, gonads, and uterus was determined after digestion with Irgasolve tissue solubiliser by LSC. Thin layer chromatography (TLC) was used to examine the stability of the test substance in the administration solution. Two millilitres of this solution were mixed with methanol (6ml) and with Hionic-Fluor (10 ml) for counting. The pattern of radioactivity was detected with a spark chamber camera (Berta) and quantitation by scraping off the radioactive fractions followed by LSC. Expired $CO_2$ was absorbed into a mixture of ethanolamine/ethylene glycol monomethyl ether 1/2 (v/v). Non-radioactive fractions were located under UV-light at 254 nm. High performance liquid chromatography (HPLC) was used to examine the stability of the test substance in the administration solution. Detection was done with a UV-detector and a radioactivity flow monitor, and quantitation by collecting manually the fractions followed by LSC.

**Findings:** The fate of thiamethoxam , i.e. 3-(2-Chloro-thiazol-5-ylmethyl)-5-methyl-[1,3,5]oxadiazinan-4-ylidene-N-nitroamine, was investigated in rats following different dose regimens using [Thiazol-2-$^{14}$C] and [Oxadiazin-4-$^{14}$C] labelled test substance.

*Animal observations:* The appearance and the behaviour of the animals were observed at defined time points. Group A1: Two hours after the intravenous injection, three animals (no. 95089, 95090 and 95093) incurred minor tail injuries. Hence, blood sampling was stopped after 0.5 and 24 hours for animals 95089 and 95090, respectively. Animal no. 95093 recovered 48 hours after dosing. After seven days, two animals (no. 95091 and 95095) showed encrustations on the neck.
One animal of Group D2 (no. 95079) showed nasal discharge 15 minutes after administration for about 1 hour. Almost all bile-duct cannulated animals showed chromodacryorrhoea during the experiment 8 hours after dosing (Group G3, animals no. 95440 and 95441), 18 hours (Group G3, all animals), 24 hours (Groups G1 and G3, all animals), 42 hours (Group G3, animals no. 95438, 95439 and 95440). Thereafter, all animals recovered. At necropsy two animals (no. 95438 and 95441) of Group G3 showed irritated

white plaque on the right kidney. The weight loss of all the animals (Groups G1, G3) could be attributed to surgery and stress.

*Absorption:* The orally administered test substance was rapidly absorbed, and, since almost 100% of the oral doses were eliminated with the urine in all treatment groups, it is assumed that thiamethoxam was virtually completely absorbed from the gastrointestinal tract irrespective of the dose level, pre-treatment with non-radiolabelled thiamethoxam, the site of label, or the sex of the animals. The experiment with bile-duct cannulated male rats (Groups G1, G3) demonstrated that only a very small portion of the absorbed dose (ca. 4%) was excreted with the bile fluid into the duodenum.

**Table: Absorption after single oral administration [% of dose]**

| Label | [Thiazol-2-$^{14}$C] CGA 293'343 | | | | | | | [Oxadiazin-4-$^{14}$C] CGA 293'343 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Group | B1 [1] | | C1 [1] | | D1 [1] | | G1 [2] | B2 [1] | | D2 [1] | | G3 [2] |
| Sex | male | female | Male | female | male | female | male | male | female | male | female | male |
| Dose [mg/kg] | 0.54 | 0.56 | 0.42 | 0.44 | 91.2 | 98.9 | 0.49 | 0.44 | 0.46 | 100.9 | 104.2 | 0.48 |
| Urine | 91.3 | 93.0 | 96.2 | 94.7 | 95.5 | 96.5 | 81.4 | 92.9 | 95.7 | 96.9 | 99.2 | 86.8 |
| Bile | n.a.[3] | n.a. | n.a. | n.a. | n.a. | n.a. | 3.9 | n.a. | n.a. | n.a. | n.a. | 4.5 |
| Tissues | 0.4 | 0.3 | 0.4 | 0.5 | 0.3 | 0.3 | 1.5 | 0.3 | 0.2 | 0.3 | 0.7 | 1.6 |
| Sum | 91.7 | 93.3 | 96.6 | 95.2 | 95.8 | 96.8 | 86.8 | 93.2 | 95.9 | 97.2 | 99.9 | 92.9 |

[1] 168 hours time interval; [2] bile cannulated male rats, 48 hours time interval; [3] n.a.: not applicable (not collected)

*Excretion:* The routes of elimination were independent of the route of administration (oral and intravenous), the dose level, pre-treatment with non-radiolabelled thiamethoxam, the site of label, and the sex of the animals. The absorbed amount was very rapidly excreted almost exclusively via the kidneys: Within 24 hours 84 to 95% and 3 to 6% of the dose were excreted in the urine and faeces, respectively .

The amount of the dose eliminated with the expired air was insignificant and independent of the site of label. The amount detected in the $CO_2$-traps did not exceed 0.2% of the dose.

The bile-duct cannulated male rats excreted within 48 hours about 4, 84, and 4% in bile, urine and faeces, respectively, independent of the site of the radiolabel (Groups G1 and G3). Hence these results obtained with bile-duct cannulated rats and with intravenously administrated animals clearly show that the small amount eliminated with the faeces is derived from biliary excretion, thus proving complete absorption of thiamethoxam from the gastrointestinal tract (see Table below).

**Table: Excretion of radioactivity following a single intravenous or oral administration [% of dose]**

| Label | | [Thiazol-2-14C] CGA 293'343 | | | | | | | | | [Oxadiazin-4-14C] CGA 293'343 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Group | | A1 | | B1 | | C1 | | D1 | | G1 | B2 | | D2 | | G3 |
| Sex | | male | female | Male | female | male | female | male | female | male | male | female | male | female | male |
| Dose [mg/kg] | | 0.51 | 0.55 | 0.54 | 0.56 | 0.42 | 0.44 | 91.24 | 98.87 | 0.49 | 0.44 | 0.46 | 100.89 | 104.16 | 0.48 |
| Urine | | | | | | | | | | | | | | | |
| | 0 - 24 h | 83.8 | 89.1 | 87.8 | 88.2 | 94.0 | 92.3 | 92.9 | 93.0 | 78.6 | 89.6 | 92.5 | 92.0 | 95.2 | 77.4 |
| | 24 - 48 h | 1.7 | 2.1 | 1.8 | 2.2 | 1.3 | 1.3 | 1.5 | 2.1 | 2.9 | 1.8 | 1.4 | 2.8 | 2.2 | 9.4 |
| | 48 - 168 h | 1.3 | 1.6 | 1.7 | 2.6 | 0.8 | 1.0 | 1.1 | 1.4 | n.a. | 1.6 | 1.8 | 2.1 | 1.7 | n.a. |
| | *Subtotal* | 86.8 | 92.7 | 91.3 | 93.0 | 96.2 | 94.7 | 95.5 | 96.5 | 81.4 | 92.9 | 95.7 | 96.9 | 99.2 | 86.8 |
| Bile | | | | | | | | | | | | | | | |
| | 0 - 24 h | n.a.[1)] | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | 3.8 | n.a. | n.a. | n.a. | n.a. | 4.1 |
| | 24 - 48 h | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | 0.1 | n.a. | n.a. | n.a. | n.a. | 0.3 |
| | *Subtotal* | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | 3.9 | n.a. | n.a. | n.a. | n.a. | 4.5 |
| Faeces | | | | | | | | | | | | | | | |
| | 0 - 24 h | 4.4 | 2.5 | 4.4 | 2.6 | 6.2 | 3.9 | 3.4 | 3.4 | 3.8 | 4.1 | 2.7 | 4.3 | 2.6 | 2.4 |
| | 24 - 48 h | 0.8 | 0.5 | 0.6 | 0.5 | 0.5 | 0.4 | 1.4 | 0.7 | 1.0 | 0.5 | 0.7 | 1.0 | 0.8 | 1.1 |
| | 48 - 168 h | 0.3 | 0.3 | 0.2 | 0.3 | 0.2 | 0.1 | 0.3 | 0.3 | n.a. | 0.4 | 0.7 | 0.4 | 0.6 | n.a. |
| | *Subtotal* | 5.5 | 3.2 | 5.2 | 3.4 | 6.8 | 4.4 | 5.1 | 4.4 | 4.8 | 5.1 | 4.0 | 5.7 | 4.0 | 3.5 |
| Expired air | 0 - 48 h | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | 0.2 | 0.1 | n.a. | n.a. | n.a. | 0.1 | 0.1 | n.a. |
| Cage Wash | | 0.8 | 1.1 | 4.8 | 3.9 | 0.3 | 0.5 | 0.2 | 0.5 | 4.4 | 0.8 | 1.7 | 0.3 | 0.3 | 2.3 |
| **Total Excretion** | | 93.1 | 97.0 | 101.3 | 100.2 | 103.3 | 99.6 | 101.0 | 101.5 | 94.5 | 98.8 | 101.4 | 102.9 | 103.5 | 97.2 |

[1)] n.a. not applicable

*Blood kinetics:* Blood kinetic studies were performed after a single intravenous administration of [Thiazol-2-$^{14}$C] thiamethoxam to male and female rats at the low dose (0.5 mg/kg, Group A1) as well as after oral administration of [Thiazol-2-$^{14}$C] and [Oxadiazin-4-$^{14}$C] to both sexes at the low dose (0.5 mg/kg, Groups B1, B2) and the high dose (100 mg/kg, Groups D1, D2).

After iv dosing maximal blood concentrations averaged around 0.6ppm CGA 293'343 equivalents at the low dose. After oral dosing maximal radioactivity in the blood was reached between 1 to 4 hours after the administration in male and female rats independent of the dose level and the label. Maximal concentrations averaged to 0.18 and about 37 ppm CGA 293'343 equivalents at the low and high dose, respectively.

The radioactivity in the blood decreased rapidly reaching half the maximal values 2 to 3 hours after intravenous and about 7 hours after oral administration in both sexes.

After oral administration the areas under the blood curve ($AUC_{0-24h}$) were in the same range for both labels and both sexes, accounting for an average of 1.4 and 318 $\mu g.h.g^{-1}$ at the low and high dose levels, respectively. Comparison of AUC's at the low and high doses indicate a linear relationship of the bioavailability and the dose in a range of 0.5 to 100 mg/kg.

Independent of the dose and the labels, the bioavailability determined by the $AUC_{0-24h}$ ratio after oral and intravenous administration was about 0.7 and 0.8 for male and female rats, respectively.

The major results are summarized in the Table below.

**Table : Blood kinetics following a single intravenous or oral administration**

| Label | [Thiazol-2-14C] CGA 293'343 | | | | | | [Oxadiazin-4-14C] CGA 293'343 | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Group | A1 | | B1 | | D1 | | B2 | | D2 | |
| Sex | male | female | male | female | male | female | male | female | male | female |
| Dose [mg/kg] | 0.51 | 0.54 | 0.55 | 0.55 | 91.8 | 100.7 | 0.44 | 0.46 | 101.8 | 104.0 |
| Cmax [ppm] | - | - | 0.174 | 0.168 | 43.22 | 34.45 | 0.201 | 0.186 | 35.74 | 32.95 |
| tcmax [h] | 0.25 | 0.25 | 4 | 2 | 2 | 1 | 2 | 1 | 4 | 1 |
| tcmax/2 [h] | 3 | 2 | 8 | 8 | 7 | 7 | 6 | 5 | 9 | 8 |
| $AUC_{0-24h}$ [µg×h/g] | 2.5 | 1.7 | 1.6 | 1.6 | 344.9 | 263.8 | 1.3 | 1.1 | 367.1 | 296.6 |
| Bioavailability % | 100 | 100 | 60 | 90 | 80 | 80 | 60 | 70 | 80 | 90 |

*Depletion from tissues:* The depletion from the tissues is assumed to follow first order kinetics. The half life times in all tissues were in the range of 2 - 6 hours, independent of the dose level, the site of label, and the sex of the animals as shown in the Table below.

**Table: Depletion of residual activity from selected tissues**

| Half life time [h][1)] | | | | | | |
|---|---|---|---|---|---|---|
| Label | [Thiazol-2-$^{14}$C] | | | | [Oxadiazine-4-$^{14}$C] | |
| Group | F1 | F2 | F5 | F6 | F3 | F7 |
| Sex | male | male | female | female | male | female |
| Dose [mg/kg] | 0.54 | 98.97 | 0.53 | 100.56 | 0.58 | 0.58 |
| Time interval [h] | 2 - 24 h | 7 - 24 h | 2 - 24 h | 1 - 24 h | 2 - 24 h | 1 - 24 h |
| Blood | 3.5 | 3.0 | 3.5 | 3.8 | 3.9 | 3.3 |
| Bone | 4.1 | 3.7 | 3.8 | 4.1 | 4.9 [1)] | 3.6 |
| Brain | 3.2 | 2.6 | 3.4 | 3.3 | 5.0 | 3.2 |
| Fat (abdominal) | 3.8 | 2.9 | 3.9 | 3.6 | 5.7 | 3.5 |
| Heart | 3.1 | 2.5 | 3.1 | 2.5 | 4.0 | 3.0 |
| Kidneys | 3.1 | 2.8 | 3.4 | 3.1 | 3.7 | 3.1 |
| Liver | 4.0 | 3.7 | 3.9 | 4.4 | 4.8 | 3.9 |
| Lungs | 3.1 | 3.0 | 3.1 | 3.5 | 3.4 | 3.0 |
| Muscle (skeletal) | 3.0 | 2.4 | 3.1 | 3.3 | 4.1 | 2.9 |
| Ovaries | n.a[2)] | n.a. | 4.0 | 3.5 | n.a. | 3.5 |
| Plasma | 3.2 | 2.8 | 3.5 | 3.6 | 3.7 | 3.2 |
| Spleen | 3.3 | 2.5 | 3.5 | 3.6 | 3.5 | 3.2 |
| Testes | 3.0 | 2.6 | n.a. | n.a. | 4.0 | n.a. |
| Uterus | n.a. | n.a. | 3.6 | 3.5 | n.a. | 3.1 |
| Carcass | 4.1 | 3.7 | 4.5 | 4.7 | 5.0 | 4.0 |

[1)] Half life time [t½] for the time interval 8 - 24 h; [2)] n.a.: not applicable

*Tissue residues:* Tissue residues were determined in male and female rats at 4 different time points after oral administration of [Thiazol-2-$^{14}$C] thiamethoxam at both dose levels and [Oxadiazin-4-$^{14}$C] thiamethoxam at the low dose level. As a consequence of the rapid depletion, the tissue residues were very low seven days after oral administration of the low dose (0.5 mg/kg; Groups B1, C1, and B2). As shown in the summary table (see Table below), the tissue residues did not exceed 0.0033 ppm thiamethoxam equivalents (liver). The other tissue residues were close to or even below the limit of determination (LQ). In some tissues, e.g. bone, the residues were below the limit of detection.

Pre-treatment with 14 consecutive daily oral administrations of non-radiolabelled thiamethoxam (0.5 mg/kg) followed by a single oral dose of [Thiazol-2-$^{14}$C] labelled test substance (Group C1) did not influence the pattern of tissue residues.

At the high dose level (100 mg/kg; Groups D1, D2) the tissue residues were proportionally higher, i.e. about 200 times higher. The highest values were found again in the liver. The liver residues in male rats were slightly higher after administration of the [Oxadiazin-4-$^{14}$C] label, i.e. 0.56 ppm, than after dosing of the [Thiazol-2-$^{14}$C] label (0.37 ppm), while female rats showed similar concentrations at both labels. However, since the residue levels in all the other tissues at the high dose and in all tissues at the low dose were essentially the same for both labels, a significant label difference cannot be stated.

**Table : Tissue residues seven days after a single oral administration**

| Tissue residues [ppm CGA 293'343 equivalents] | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Label | [Thiazol-2-$^{14}$C] CGA 293'343 | | | | | | [Oxadiazine-4-$^{14}$C] CGA 293'343 | | | |
| Group | B1 | | C1 | | D1 | | B2 | | D2 | |
| Sex | male | female | male | female | male | female | male | female | male | female |
| Dose [mg/kg] | 0.54 | 0.56 | 0.42 | 0.44 | 91.2 | 98.9 | 0.44 | 0.46 | 100.9 | 104.2 |
| Blood | 0.0011 | 0.0010 | 0.0014 | < LQ[1)] | 0.199 | 0.181 | = LQ | = LQ | 0.149 | 0.154 |
| Bone | < LD[2)] | < LD | = LD | < LD | 0.028 | 0.024 | < LQ | = LQ | 0.019 | 0.019 |
| Brain | = LQ | = LQ | < LQ | < LQ | 0.021 | 0.020 | < LQ | < LQ | 0.016 | 0.016 |
| Fat (abdominal) | < LD | < LQ | < LQ | < LQ | 0.020 | 0.019 | < LQ | < LQ | 0.011 | 0.012 |
| Heart | 0.0007 | 0.0007 | = LQ | = LQ | 0.045 | 0.049 | 0.0006 | = LQ | 0.037 | 0.037 |
| Kidneys | 0.0015 | 0.0012 | 0.0010 | 0.0009 | 0.134 | 0.129 | 0.0007 | 0.0006 | 0.078 | 0.066 |
| Liver | 0.0033 | 0.0018 | 0.0020 | 0.0016 | 0.373 | 0.240 | 0.0016 | 0.0009 | 0.557 | 0.251 |
| Lungs | < LQ | < LQ | = LD | < LD | 0.075 | 0.078 | = LD | = LD | 0.054 | 0.108 |
| Muscle (skeletal) | = LQ | < LQ | < LQ | < LQ | 0.035 | 0.031 | = LQ | < LQ | 0.030 | 0.031 |
| Ovaries | n.a.[3)] | < LQ | n.a. | < LQ | n.a. | 0.056 | n.a. | < LQ | n.a. | 0.048 |
| Plasma | < LQ | < LQ | = LD | = LD | 0.053 | 0.052 | < LQ | < LQ | 0.037 | 0.043 |
| Spleen | 0.0010 | 0.0012 | 0.0009 | 0.0008 | 0.057 | 0.059 | < LQ | < LQ | 0.048 | 0.053 |
| Testes | 0.0006 | n.a. | 0.0007 | n.a. | 0.026 | n.a. | < LQ | n.a. | 0.022 | n.a. |
| Uterus | n.a. | < LQ | n.a. | < LQ | n.a. | 0.041 | n.a. | < LQ | n.a. | 0.042 |
| Carcass | 0.0017 | 0.0016 | 0.0014 | 0.0021 | 0.211 | 0.246 | 0.0012 | 0.0007 | 0.237 | 0.745 |
| Total residues [% of dose] | 0.40 | 0.31 | 0.39 | 0.48 | 0.29 | 0.27 | 0.33 | 0.17 | 0.31 | 0.72 |

[1)] LQ: Limit of determination; [2)] LD: Limit of detection; [3)] n.a.: not applicable

At the low dose level the limits of determination (LQ) were 0.0004-0.008 ppm (blood, brain, fat, heart, kidneys, liver, muscle, plasma, spleen and testes), 0.006-0.0011 ppm (bone, lungs and uterus), 0.0024-0.0031 ppm (ovaries).

*Conclusion:* Thiamethoxam orally administered at a low and high dose, i.e. at 0.5 and 100 mg/kg body weight, respectively, was rapidly and completely absorbed from the gastro-intestinal tract into the systemic circulation, as estimated from the amount renally excreted, the amount retained in tissues, and the amount eliminated in the bile fluid. The complete absorption is confirmed by the fact that the excretion pattern is identical after intravenous and oral administration of the test substance.

Maximal blood concentrations were reached 1 to 4 hours after administration independent of the dose level, the site of label, and the sex of test animals. The maximal values ranged between 0.17 - 0.20 ppm and 33 - 43 ppm at the low and high dose, respectively. Thereafter, the residues in blood depleted very quickly, reaching half maximal values approximately 8 hours after dosing. The areas under the blood concentration-time curve (AUC) were the same for both sexes and both labels and showed a linear relationship with the dose.

Tissue residues were determined in male and female rats at 4 different time points after oral administration of [Thiazol-2-$^{14}$C] thiamethoxam at both dose levels and of [Oxadiazin-4-$^{14}$C] thiamethoxam at the low dose level. The depletion from the tissues is assumed to follow first order kinetics. The half life times in all tissues were in the range of 2 - 6 hours, independent of the dose level, the site of label, and the sex of the animals. Seven days after oral administration of the low dose, the tissue residues were very low irrespective of pre-treatment with non-radiolabelled test substance, the site of label, and the sex of the animals. The highest values were found in the liver, ranging from 0.001 to 0.003 ppm thiamethoxam equivalents. Seven days after oral administration of the high dose, the tissue residues were about 200-fold higher than in low dose-treated animals, i.e., in linear relationship with the dose.

The routes of elimination were independent of the route of administration (oral and intravenous), the dose level, pre-treatment with non-radiolabelled thiamethoxam, the site of label, and the sex of the animals. The absorbed material was readily excreted predominantly in the urine. Within 24 hours, about 90% of the dose was excreted via kidneys and about 4% with the faeces. Bile-duct cannulated male rats which received a low dose of either label excreted - independent of the label - within 48 hours 4%, 84%, and 4% of the dose in bile fluid, urine, and faeces, respectively. These results demonstrate that, in rats, the amount eliminated in the faeces originates from biliary excretion.

In conclusion, thiamethoxam is rapidly and completely absorbed, rapidly distributed in the body, and rapidly eliminated. The toxicokinetics are not influenced by the route of administration, the dose level in a range of 0.5 to 100 mg/kg body weight, pre-treatment, the site of label, and the sex of the animals.

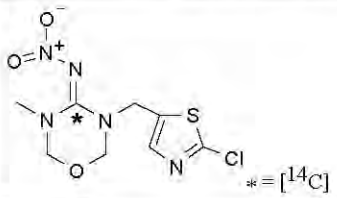
| **Evaluation by Competent Authorities** | |
|---|---|
| | **EVALUATION BY RAPPORTEUR MEMBER STATE** |
| **Date** | February 2005 |
| **Materials and Methods** | [redacted] |
| **Results and discussion** | [redacted] |

| | |
|---|---|
| | [redacted] |
| **Conclusion** | [redacted] |
| **Reliability** | [redacted] |
| **Acceptability** | [redacted] |
| **Remarks** | |

| | | |
|---|---|---|
| 98/8 Doc IIIA section No. | 6.2 / 02 | Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study |
| 91/414 Annex Point addressed | II 5.1.2 / 01 | Studies on absorption, distribution, excretion and metabolism in mammals - Dermal absorption - Dermal absorption in vivo |

| | |
|---|---|
| 1. Annex point(s) | IIA, 5.1.2 Studies on absorption, distribution, excretion and metabolism in mammals - Dermal absorption - Dermal absorption in vivo |
| 2. Reference point (location) in dossier | Volume 7, Section 3, Annex IIA, points 5.1.2 |
| 3. Authors (year)<br>Title<br><br>Owner, Date | [redacted]<br>Dermal Absorption of [Oxadiazin-4-$^{14}$C] CGA 293'343 Formulated as Cruiser 350FS (A-9700 B) in the Rat (invivo).<br>Syngenta Crop Protection AG, unpublished report No. 027AM12, April 11, 2002<br>Syngenta File No. 293343/1464 |
| 4. Testing facility | [redacted] |
| 5. Dates of work | October 16, 2001 – March 15, 2002 |
| 6. Test substance | ISO common name: Thiamethoxam<br>*Non-labeled compound:* (CGA 293'343) [redacted]<br>[*Oxadiazin-4-$^{14}$C*] *-labeled compound:* [redacted] |
| 7. Test method | OECD Guideline for Testing of Chemicals; Percutaneous Absorption: In Vivo method, Draft Document, June 1996.<br>Deviations - none. |
| 8. GLP | Yes (laboratory certified by the Eidgenössisches Departement des Inneren (Federal Department of Home Affairs), Bern, Switzerland) |

**Material and methods:** Test substance: [Oxadiazin-4-$^{14}$C] CGA 293343

| | |
|---|---|
| Structure<br>(* position of label) |  <br>*= [$^{14}$C] |
| Batch:<br>Purity:<br>Specific Activity: | [redacted]<br>500 kBq/mg (Groups P1 and L1)<br>30 kBq/mg (Groups P2 and L2) |
| Non-radiolabeled Substance: CGA 293343, batch [redacted] | |

The test substance was formulated according to protocol A-9700 B as CRUISER® 350 FS. The formulation was prepared separately for both dose levels. Details are given in the table below.

Table 5.1.2.1-1 **Formulation of [Oxadiazin-4-$^{14}$C] CGA 293343**

| Group | Dose Level | [$^{14}$C]-CGA 293343 | Blank Formulation | Total Amount |
|---|---|---|---|---|
| P1, L1 | low | 56 mg | 136 mg | 192 mg |
| P2, L2 | high | 980.7 mg | 2325 mg | 3305.7 mg |

The low dose of 20 g a.i./l reflects concentrations recommended for the use in treatment of wheat seeds (35 g a.i./2.1 l applied to 100 kg seeds) and potato tuber (5 g a.i./214.3 ml applied to 100 kg tubers). The high dose represents the undiluted formulation (350 g a.i./l). The formulated test substance of the low dose level was mixed with 2650 µl water and the high dose level was applied undiluted.

An aliquot of 100 µl was applied to a shaved dorsal skin of approx. 8 week old Hanlbm: WIST (SPF) rats (about 250 g). The application area was confined by a double O-ring (10 cm²) glued to the shaved skin using cyanoacrylate adhesive. In order to prevent loss of the test substance, the O-ring was covered with a permeable tape (non-occlusive conditions). Ingestion of the test substance was prevented by a collar around the rat's neck.

After an exposure period of 6 hours, which is recommended in the OECD guidelines and is based on the anticipated exposure period of a plant or field worker, the cover tape was removed and retained for analysis. The remaining test substance was removed from the application site by washing 5 times with a mild soap solution using cotton swabs. The moist skin area was dried with cotton swabs after each washing step. Finally, a fresh cover was applied to the O-ring except for animals of Subgroups P1t1 and P2t1. In order to prevent the detachment of the glued O-ring during the experimental period of 3 and 7 days, a bandage was applied around the body of the animals of subgroups t3 and t4.

**Table: Experimental scheme**

| Dose Level (Group) | Dose [mg / cm²] | Subgroup | Animals | Collection Period [h] |
|---|---|---|---|---|
| Low Dose (P1, skin stripping) | 0.197 | P1t1 | 4 males | 0 - 6 |
| | | P1t2 | 4 males | 0 - 24 |
| | | P1t3 | 4 males | 0 - 72 |
| | | P1t4 | 4 males | 0 - 168 |
| Low Dose (L1, radioluminography) | 0.197 | L1t3 | 1 male | 0 - 72 |
| | | L1t4 | 1 male | 0 - 168 |
| High Dose (P2, skin stripping) | 3.72 | P2t1 | 4 males | 0 - 6 |
| | | P2t2 | 4 males | 0 - 24 |
| | | P2t3 | 4 males | 0 - 72 |
| | | P2t4 | 4 males | 0 - 168 |
| High Dose (L2, radioluminography) | 3.72 | L2t3 | 1 male | 0 - 72 |
| | | L2t4 | 1 male | 0 - 168 |

The dermal absorption of the test substance during a 6-hours exposure period was determined. In order to estimate the depletion of the test substance associated with the application site after washing, the amount of radioactivity remaining in/on the skin at the application site was measured at three additional time points, i.e. 24, 72 and 168 hours after application of the test substance.

The association of the remaining test substance with the skin at the application site was investigated after sacrifice of the rats of Groups P1 and P2 by skin stripping in order to separate the stratum corneum from the viable epidermis. At necropsy, the cover and the O-ring was carefully removed and extracted with solvents. The upper skin layer, i.e. the stratum corneum and the fur grown within the duration of the experiment, was removed from the application site by gluing a tape with cyanoacrylate adhesive on the top of the skin treated area. After a drying period of about 3 minutes the tape was snatched off from the application site. This operation was repeated one to two times until the stratum corneum was removed from the application site. The tape strips were dissolved with tissue solubilizer.

The distribution of residues in the treated skin area was investigated by whole body radioluminography of the rats of Groups L1 and L2 at two time points, i.e. 72 and 168 hours after application. After sacrifice, the animals were immediately frozen as a whole and embedded as a block in gelatine. The frozen block was cut in a cryostat microtome. Sections of 40 µm thickness were sliced off down to the level of interest, i.e. whole body sections at three different levels per animal. At each level of interest two slices for

radioluminography were obtained. Prior to sectioning a visual picture was made from each level of interest in order to compare the visual image with the radioluminogram. The removed sections of 40 µm thickness, fixed on transparent adhesive tape, were lyophilized. In order to visualize the radioactivity, the animal sections, together with blood calibration standards, were exposed to phosphor imaging plates processed on a Bio-Imaging Analyser.

Urine and faeces were collected from all animals individually during periods of 0-6, 6-24 hours and thereafter in daily intervals until 168 hour after application. Serial blood was collected at 0.5, 1, 2, 4, 6, 8, 24 and 48 hours after application from animals of Subgroups P1t4 and P2t4 from the tail vein by cutting the tip of the tail.

Radioactivity was determined in terminal blood, serial blood, urine, faeces, cage wash, skin wash, tape strips, treated skin, non-treated skin, extracts of cover and O-ring, gastrointestinal tract and residual carcass. Additionally, TLC analyses of the dosing formulation and of skin wash were performed.

**Findings**: During 6 hours of exposure to formulated [Oxadiazin-4-$^{14}$C] CGA 293343, only 0.55% and 0.06% of the dose were systemically absorbed, i.e. recovered in excreta, blood, non-treated skin, gastrointestinal tract and remaining carcass, at the low and high dose level, respectively (Subgroups P1t1 and P2t1). The penetration rates were calculated to be 0.18 µg $cm^{-2}$ $h^{-1}$ at the low dose level and 0.38 µg $cm^{-2}$ $h^{-1}$ at the high dose level. The ratio of the penetration rates between the 19-fold higher concentration of the undiluted formulation as compared to the concentration used for seed or tuber treatment accounted only for a factor of 2.1.

In all subgroups about 84 - 89% and 94 - 98% of the applied low and high dose, respectively, could be removed by the skin wash at the end of exposure and another 0.1 – 1.1% were recovered from O-ring and cover.

After the washing procedure, 3.6% and 0.49% of the low and high dose, respectively, remained in/on the treated skin (Subgroups P1t1 and P2t1). The result of skin stripping of the washed treated skin revealed that the remaining dose in/on the application site was almost completely associated with the hairs and the upper skin layers (stratum corneum) and not with the remaining treated skin (viable epidermis and dermis). The amount of radioactivity associated with the hairs and the stratum corneum did not significantly decrease within 168 hours after application and did not lead to a significant increase of the systemic absorption determined.

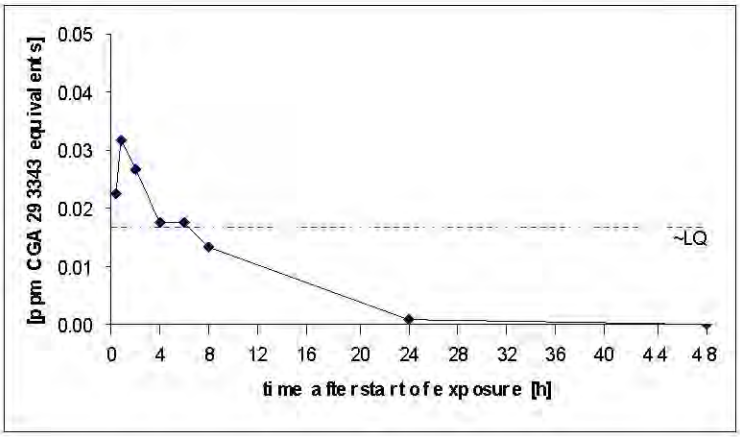
Slight changes, i.e. changes in the amount of radioactivity associated with the treated skin (apparent increase up to 4.3% in Subgroups P1t2, P1t3 and P1t4 and decrease to 0.31% in Subgroup P2t4) and in the amount of CGA 293343 systemically absorbed (increase to 1.06% in Subgroup P1t4 and to 0.11% in Subgroup P2t4) were within the experimental variation. Therefore, it is concluded that at both dose levels the majority of test substance associated with the application site after washing was not available for further systemic absorption.

The removed radioactivity at the end of exposure at both dose levels was analyzed as unchanged CGA 293343.

At the low dose level, the mean blood radioactivity level increased rapidly and reached a maximum of only 0.032 ppm CGA 293343 equivalents at 1 hour after application. Thereafter, the radioactivity in blood decreased to a level at the limit of determination (ca. 0.017 ppm) at 4-8 hours after application and then to a blood level below the limit of detection (ca. 0.006 ppm) at 24 hours after application.

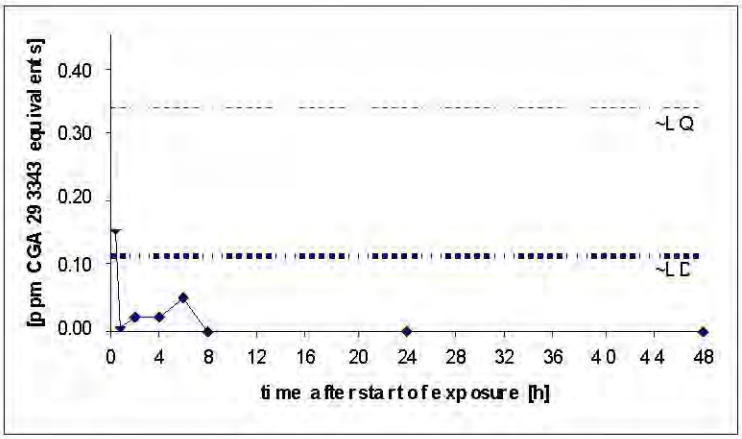
At the high dose level, the mean radioactivity concentration in blood was at all sampling time points below the limit of detection.

**Figure: Mean Blood Residues after Dermal Application**




Low Dose Level

(Subgroup P1t4, LQ ca. 0.017 ppm)




High Dose Level

(Subgroup P2t4, LQ ca. 0.34 ppm)

The small amount of systemically absorbed test substance was rapidly excreted, almost entirely in the urine.

The experimental recoveries were between 89.4% and 98.6% of the applied dose (Groups P1 and P2).

Semiquantitative whole-body radioluminography 72 and 168 hours after dermal application (Groups L1 and L2) confirmed the results of the examinations in Groups P1 and P2. Whereas at both time points no radioactivity was detected in the tissues or body fluids of the animals, the majority of the low remaining radioactivity after washing and depletion was associated with the hairs and the upper layer of the epidermis. Despite a four-day longer depletion period, at both dose levels no difference in the roughly estimated amount of radioactivity in/on the treated skin area was observed in subgroups t4 as compared to subgroups t3. This reveals again that the majority of radioactivity associated with the application site after skin wash was not available for systemic absorption.

**Table 5.1.2.1-5 Summary Table [percent of dose]**

| Group | P1 | | | | P2 | | | |
|---|---|---|---|---|---|---|---|---|
| Dose Level | Low Dose 197.1 µg/cm² | | | | High Dose 3724 µg/cm² | | | |
| Subgroup | P1t1 | P1t2 | P1t3 | P1t4 | P2t1 | P2t2 | P2t3 | P2t4 |
| Sacrifice Time | (6 h) | (24 h) | (72 h) | (168 h) | (6 h) | (24 h) | (72 h) | (168 h) |
| Urine 0 - 6 h | 0.31 | 0.32 | 0.23 | 0.39 | 0.03 | 0.01 | 0.04 | 0.03 |
| 6 - 24 h | - | 0.26 | 0.28 | 0.33 | - | 0.02 | 0.04 | 0.04 |
| 24 - 48 h | - | - | 0.06 | 0.09 | - | - | 0.01 | 0.01 |
| 48 - 72 h | - | - | 0.04 | 0.04 | - | - | < 0.01 | < 0.01 |
| 72 - 96 h | - | - | - | 0.04 | - | - | - | < 0.01 |
| 96 - 120 h | - | - | - | 0.03 | - | - | - | < 0.01 |
| 120 - 144 h | - | - | - | 0.03 | - | - | - | < 0.01 |
| 144 - 168 h | - | - | - | 0.02 | - | - | - | < 0.01 |
| *Subtotal* | 0.31 | 0.58 | 0.60 | 0.96 | 0.03 | 0.04 | 0.09 | 0.10 |
| Faeces 0 - 6 h | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 |
| 6 - 24 h | - | 0.03 | 0.03 | 0.03 | - | < 0.01 | < 0.01 | < 0.01 |
| 24 - 48 h | - | - | 0.01 | 0.02 | - | - | < 0.01 | < 0.01 |
| 48 - 72 h | - | - | < 0.01 | < 0.01 | - | - | < 0.01 | < 0.01 |
| 72 - 96 h | - | - | - | < 0.01 | - | - | - | < 0.01 |
| 96 - 120 h | - | - | - | < 0.01 | - | - | - | < 0.01 |
| 120 - 144 h | - | - | - | < 0.01 | - | - | - | < 0.01 |
| 144 - 168 h | - | - | - | < 0.01 | - | - | - | < 0.01 |
| *Subtotal* | < 0.01 | 0.03 | 0.05 | 0.08 | < 0.01 | < 0.01 | < 0.01 | 0.01 |
| Cage Wash | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 |
| *Total Excretion* | 0.32 | 0.62 | 0.65 | 1.05 | 0.04 | 0.05 | 0.10 | 0.11 |
| Residues | | | | | | | | |
| Whole Blood | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 |
| Skin (Non-treated) | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 |
| Gastrointestinal Tract | 0.05 | 0.02 | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 |
| Remaining Carcass | 0.17 | 0.03 | < 0.01 | < 0.01 | 0.02 | 0.01 | < 0.01 | < 0.01 |
| *Total Residues* | 0.23 | 0.05 | < 0.01 | 0.01 | 0.03 | 0.02 | < 0.01 | < 0.01 |
| **Systemic Absorption** | **0.55** | **0.66** | **0.65** | **1.06** | **0.06** | **0.07** | **0.10** | **0.11** |
| Tape Strips | 3.22 | 4.29 | 4.17 | 4.19 | 0.45 | 0.42 | 0.40 | 0.31 |
| Remaining Skin (Treated) | 0.39 | 0.07 | 0.13 | 0.02 | 0.04 | < 0.01 | < 0.01 | < 0.01 |
| **Skin (Treated)** | **3.60** | **4.35** | **4.30** | **4.21** | **0.49** | **0.43** | **0.40** | **0.31** |
| Skin Wash | 84.12 | 88.14 | 88.37 | 89.40 | 95.70 | 96.71 | 97.94 | 93.45 |
| O-Ring and Cover | 1.12 | 0.33 | 0.09 | 0.76 | 0.51 | 0.45 | 0.19 | 0.15 |
| **Dislodged Dose** | **85.24** | **88.47** | **88.46** | **90.17** | **96.21** | **97.16** | **98.13** | **93.60** |
| **Total Recovery** | **89.39** | **93.49** | **93.41** | **95.44** | **96.76** | **97.65** | **98.64** | **94.02** |

**Conclusion**: In summary, during an exposure period of 6 hours after dermal application of CGA 293343 formulated as CRUISER® 350 FS (A-9700 B) and depletion periods up to 7 days, only 0.55% - 1.06% of the low dose, i.e. the concentration used for seed or tuber treatment, and 0.06 - 0.11% of the high dose, i.e. the undiluted formulation, penetrated through rat skin. About 4% and 0.4% of the low and high dose, respectively, remained at the application site after washing and was almost totally associated with the hairs and the stratum corneum and was not available for systemic absorption.

| Evaluation by Competent Authorities | |
|---|---|
| | **EVALUATION BY RAPPORTEUR MEMBER STATE** |
| **Date** | February 2005 |
| **Materials and Methods** | [redacted] |
| **Results and discussion** | [redacted] |
| **Conclusion** | [redacted] |
| **Reliability** | [redacted] |
| **Acceptability** | [redacted] |
| **Remarks** | [redacted] |

| | | |
|---|---|---|
| 98/8 Doc IIIA section No. | 6.2 / 03 | Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study |
| 91/414 Annex II Point addressed | 5.1.2 / 02 | Studies on absorption, distribution, excretion and metabolism in mammals - Dermal absorption - Dermal absorption in vitro |

| | |
|---|---|
| 1. **Annex point(s)** | **IIA, 5.1.2 Studies on absorption, distribution, excretion and metabolism in mammals - Dermal absorption - Dermal absorption in vivo** |
| 2. **Reference point (location) in dossier** | Volume 7, Section 3, Annex IIA, points 5.1.2 / 01 |
| 3. **Authors (year)**<br>**Title**<br><br>**Owner, Date** | [redacted]<br>The Percutaneous Penetration of [Oxadiazin-4-$^{14}$C] CGA 293343 Formulated as CRUISER® 350 FS (A-9700 B) Through Rat and Human Split-Thickness Skin Membranes (in vitro);<br>Syngenta Crop Protection AG, unpublished report to study No. 027AM11, 26.04.2002<br>Syngenta File No. CGA293343/1469 |
| 4. **Testing facility** | [redacted] |
| 5. **Dates of work** | 05.09.2001 to 01.10.2001 |
| 6. **Test substance** | ISO common name: Thiamethoxam<br>*Non-labeled compound:* (CGA 293'343) [redacted]<br>[*Oxadiazin-4-$^{14}$C*] *-labeled compound:* [redacted] |
| 7. **Test method** | OECD Guideline for Testing of Chemicals, Skin Absorption: in vitro Method, Draft New Guideline 428, December 2000;<br>Deviations: None |
| 8. **GLP** | Yes (laboratory certified by the Eidgenössisches Departement des Inneren (Federal Department of Home Affairs), Bern, Switzerland) |

**Materials and Methods**: The test substance was formulated according to protocol A-9700 B as CRUISER® 350 FS. The formulation was prepared separately for both dose levels. Details are given in Table 1.

**Table 1 Formulation of [Oxadiazin-4-$^{14}$C] CGA 293343**

| Dose Level | | [$^{14}$C]-CGA 293343 | Blank Formulation | Total Amount |
|---|---|---|---|---|
| A1 | low | 56 mg | 136 mg | 192 mg |
| A2 | high | 980.7 mg | 2325 mg | 3305.7 mg |

The low dose of 20 g a.i./l reflects concentrations recommended for the use in treatment of wheat seeds (35 g a.i./2.1 l applied to 100 kg seeds) and potato tuber (5 g a.i./214.3 ml applied to 100 kg tubers). The high dose represents the undiluted formulation (350 g a.i./l). The formulated test substance of the low dose level was mixed with 2650 µl water and the high dose level was applied undiluted.

**Table 2 Experimental Plan**

| Dose Level | Group (Species) | Concentr. [mg·cm$^{-3}$] | Applied Dose [mg·cm$^{-2}$] | Number of Replicates | Collection period [h] |
|---|---|---|---|---|---|
| low (A1) | Q1 (rat) | 18.8 | 0.18 | 6 | 0 - 24 |
| low (A1) | Q2 (human) | 18.8 | 0.18 | 7 | 0 - 24 |
| high (A2) | Q1 (rat) | 368 | 3.45 | 7 | 0 - 24 |
| high (A2) | Q2 (human) | 368 | 3.45 | 7 | 0 - 24 |

Male rats (Hanlbm: WIST (SPF)), ca. 9 weeks old, were used for the preparation. After sacrifice, the fur on the dorsal site was clipped and the full thickness skin excised. Abdominal cadaver skin from Caucasian donors (male and female, 66 and 89 years old, resp.) was obtained from the "Institut für Pathologie, Kantonsspital Basel", Switzerland.

The subcutaneous fat was carefully removed from the full thickness skin preparations and then skin sections of about 200 µm thickness were cut off from the top using a dermatome.

One automated flow-through cell system was used per species with seven diffusion cells each placed in one aluminium manifold connected to a water bath (31 - 33°C). Each diffusion cell consisted of a donor and receptor chamber. The area of skin membrane exposed to the donor chamber was 0.64 cm$^2$. The receptor chambers were connected to a multi-channel peristaltic pump (ca. 3 ml/h). During the given time intervals, the effluent from the cell was collected directly into vials on a fraction collector.

Pieces of the skin membranes (ca. 1.8 x 1.8 cm$^2$) were cut and mounted in the diffusion cells between the donor and receptor chamber in such a way that the stratum corneum was exposed to the air and the basal part of the skin membrane was in direct contact with the receptor fluid. For an equilibration period of 0.5 - 1 hour, a saline solution (0.9% NaCl, w/v) was pumped through the receptor chamber.

The integrity of the skin membranes was checked by applying 50 µl tritiated water (ca. 190'000 dpm) to the skin membrane surface. The donor chamber was covered with Parafilm (occluded conditions). The cumulative penetration was determined over 6 hours by collecting hourly fractions. The permeability coefficient (Kp) of each skin membrane was calculated for the 3 - 6 hours interval. Rat skin membranes with Kp > $3.5 \cdot 10^{-3}$ cm·h$^{-1}$ and human skin membranes with Kp > $2.5 \cdot 10^{-3}$ cm·h$^{-1}$ were excluded.

After 6 hours the Parafilm cover was removed from the donor cell chamber and the cells left overnight with the saline flowing through the receptor chamber. Thereafter, the receptor fluid was changed to ethanol/water (1/1, v/v) and delivered for about 1 hour.

Then a 6 µl aliquot of the formulated test substance was applied to the surface of each skin membrane. The donor chamber was covered with a permeable tape (non-occluded conditions). The perfusates were collected at ambient temperature (0 - 6 hours: 1 h intervals; 6 - 24 hours: 2 h intervals).

Twenty four hours after application the skin membrane surface was rinsed with ethanol/water (1/1, v/v; 10 ml), the skin membrane was removed from the in-line cells and dissolved in tissue solubilizer and the cells were washed with ethanol/water (1/1, v/v; 200 ml).

Radioactivity was determined in perfusates, skin rinse, cell wash and skin membranes. Additionally, TLC analyses of the dosing formulation and of skin rinse were performed.

**Findings:** The formulated test substance was found to be stable at the time of application as checked by TLC.

The integrity of the skin membranes was proven by measuring permeability to tritiated water (see Table 3).

**Table 3 Mean Permeability Coefficient (Kp) of Tritium Water [cm·h$^{-1}$·10$^{-3}$]**

| Test System | Rat Skin Membranes (Group Q1) | Human Skin Membranes (Group Q2) |
|---|---|---|
| Low Dose Level (A1) | 0.96 ± 0.52 | 0.91 ± 0.48 |
| High Dose Level (A2) | 1.10 ± 0.65 | 1.13 ± 0.34 |

During 24 hours after application of formulated [Oxadiazin-4-$^{14}$C] labeled CGA 293343, only 0.8% and 1.2% of the dose penetrated through rat split-thickness skin membranes at the low and high dose level, respectively. The human split-thickness skin membranes showed a lower permeability of CGA 293343. Within 24 hours after application, only 0.2% and 0.4% of the low and high dose, respectively, penetrated through human skin membranes.

A species difference in respect to the penetration of formulated CGA 293343 is also reflected by the flux. The flux at steady state conditions was determined to be about 0.06 $\mu g \cdot cm^{-2} \cdot h^{-1}$ and 1.18 $\mu g \cdot cm^{-2} \cdot h^{-1}$ through rat skin membranes and about 0.01 $\mu g \cdot cm^{-2} \cdot h^{-1}$ and 0.20 $\mu g \cdot cm^{-2} \cdot h^{-1}$ through human skin membranes at the low and high dose level, respectively. This results in a human : rat ratio of the flux of about 1 : 4.8 at the low dose level and 1 : 5.8 at the high dose level.

The percutaneous penetration (*in vitro*) of CGA 293343 through rat and human skin membranes is summarised in Table 4:

**Table 4 Summary of results**

| **Test System (Group)** | **Rat Skin Membrane (Q1)** | | | | **Human Skin Membrane (Q2)** | | | |
|---|---|---|---|---|---|---|---|---|
| Dose Level | A1 (low) | | A2 (high) | | A1 (low) | | A2 (high) | |
| Applied Dose [$\mu g \cdot cm^{-2}$] | 176.6 | | 3451 | | 176.6 | | 3451 | |
| Applied Volume [µl] | 6 | | 6 | | 6 | | 6 | |
| Application Area [$cm^2$] | 0.64 | | 0.64 | | 0.64 | | 0.64 | |
| Concentration [$mg \cdot cm^{-3}$] | 18.8 | | 368.1 | | 18.8 | | 368.1 | |
| Penetration within | $\mu g \cdot cm^{-2}$ | %of dose | $\mu g \cdot cm^{-2}$ | %of dose | $\mu g \cdot cm^{-2}$ | %of dose | $\mu g \cdot cm^{-2}$ | %of dose |
| 6 h | 0.39 | 0.22 | 19.4 | 0.56 | 0.10 | 0.06 | 8.2 | 0.24 |
| 12 h | 0.78 | 0.44 | 28.8 | 0.83 | 0.17 | 0.10 | 12.8 | 0.37 |
| 24 h | 1.45 | 0.82 | 40.8 | 1.18 | 0.34 | 0.19 | 15.0 | 0.44 |
| Flux [$\mu g \cdot cm\text{-}2 \cdot h^{-1}$] | 0.062 | | 1.179 | | 0.013 | | 0.204 | |

The 19-fold higher concentration of the undiluted formulation as compared to the concentration used for seed or tuber treatment led to a corresponding 19-fold and 16-fold higher penetration rate (flux) in rat and human skin membrane, respectively.

The radioactivity in the skin rinse at the end of exposure at both dose levels and for both species was analysed as unchanged CGA 293343.

**Table 5Recovery [% of Dose]**

| Test System | | Rat Skin Membrane (Q1) | | Human Skin Membrane (Q2) | |
|---|---|---|---|---|---|
| Dose Level | | A1 (low) | A2 (high) | A1 (low) | A2 (high) |
| Applied Dose [$\mu g \cdot cm^{-2}$] | | 176.6 | 3450.6 | 176.6 | 3450.6 |
| Perfusates (0-24 h) | | 0.82 | 1.18 | 0.19 | 0.44 |
| Remaining Dose | | | | | |
| | Cell Wash | 0.19 | 0.43 | 0.08 | 2.42 |
| | Skin Rinse | 95.71 | 75.19 | 99.92 | 69.00 |
| | Skin Membrane | 0.50 | 12.40 | 0.23 | 17.73 |
| | Subtotal | 96.40 | 88.02 | 100.24 | 89.15 |
| Recovery | | 97.22 | 89.20 | 100.42 | 89.59 |

The experimental recoveries were between 89.2% and 100.4% of the applied dose.

**Conclusion:** In summary, the penetration of [14C]-CGA 293343 formulated as CRUISER® 350 FS through rat and human skin membranes was very low. It penetrates through rat skin membrane faster and to a higher extent than through human skin membrane, at concentrations recommended for seed or tuber treatment as well as at the concentration of the undiluted formulation. The human: rat ratio of the flux is about 1:4.8 at the low dose level and 1:5.8 at the high dose level.

| **Evaluation by Competent Authorities** | |
|---|---|
| | **EVALUATION BY RAPPORTEUR MEMBER STATE** |
| **Date** | March 2005 |
| **Materials and Methods** | [redacted] |

| | |
|---|---|
| | [redacted] |
| Results and discussion | [redacted] |
| Conclusion | [redacted] |
| Reliability | [redacted] |
| Acceptability | [redacted] |
| Remarks | [redacted] |

| | | |
|---|---|---|
| **98/8 Doc IIIA section No.** | **6.2 / 04** | **Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study** |
| **91/414 Annex Point addressed** | **II 5.1.3 / 01** | **Studies on absorption, distribution, excretion and metabolism in mammals - Metabolism in Rats** |

| | |
|---|---|
| **1. Annex point(s)** | **IIA, 5.1.3 Studies on absorption, distribution, excretion and metabolism in mammals - Metabolism in Rats** |
| **2. Reference point (location) in dossier** | Volume 7, Section 3, Anex IIA, point 5.1.3/01 |
| **3. Authors (year) Title Owner, Date** | [redacted]<br>The Metabolism of [Thiazol-2-$^{14}$C] and [Oxadiazin-4-$^{14}$C] CGA 293'343 in the Rat.<br>Syngenta Crop Protection AG, unpublished report No. 027AM02, September 18, 1998 and November 05, 1998 (Amendment no. 1 to the Report) |
| **4. Testing facility** | [redacted] |
| **5. Dates of work** | November 13, 1995 - September 11, 1998, and October 01, 1998 - November 02, 1998 (Amendment no. 1 to the report). |
| **6. Test substance** | ISO common name: Thiamethoxam<br>*Non-labeled compound:* [redacted]<br>[*Thiazol-2-$^{14}$C*] - *labeled compound:* Batch numbers.: [redacted]<br>[*Oxadiazin-4-$^{14}$C*] - *labeled compound:* Batch numbers.: [redacted] |
| **7. Test method** | OECD 417 ≡ FIFRA § 85-1 ≡ Japan MAFF ≡ 94/79/EEC<br>Deviations - none |
| **8. GLP** | Yes (laboratory certified by the Eidgenössisches Departement des Inneren (Federal Department of Home Affairs), Bern, Switzerland ) |

**Material and methods:**
*Origin of specimens:* To elucidate the metabolic pathways of thiamethoxam in male and female rats, the samples analysed in this study were collected in the previous toxicokinetic study [redacted] P., 1996; see 6.2 / 01) as listed in theTable below.

**Table: Identification of pooled samples used for metabolite identification and quantification**

| Group | Animals | Treatment [mg/kg] | Specimen Type | Sampling Time [h] | [% of Dose] |
|---|---|---|---|---|---|
| A1 | 4 males | [thiazol-2-$^{14}$C]: 0.51 i.v., 229 kBq (6.2 µCi) | urine<br>faeces | 0 - 24<br>0 - 24 | 83.8<br>4.4 |
| | 5 females | [thiazol-2-$^{14}$C]: 0.55 i.v., 229 kBq (6.2 µCi) | urine<br>faeces | 0 - 24<br>0 - 24 | 89.1<br>2.5 |
| B1 | 5 males | [thiazol-2-$^{14}$C]: 0.54 p.o., 278 kBq (7.5 µCi) | urine<br>faeces | 0 - 24<br>0 - 24 | 87.8<br>4.4 |
| | 5 females | [thiazol-2-$^{14}$C]: 0.56 p.o., 278 kBq (7.5 µCi) | urine<br>faeces | 0 - 24<br>0 - 24 | 88.2<br>2.6 |
| C1 | 5 males | [thiazol-2-$^{14}$C]: 0.42 p.o.*, 205 kBq (5.6 µCi) | urine<br>faeces | 0 - 24<br>0 - 24 | 94.1<br>6.2 |
| | 5 females | [thiazol-2-$^{14}$C]: 0.44 p.o.*, 205 kBq (5.6 µCi) | urine<br>faeces | 0 - 24<br>0 - 24 | 92.3<br>3.9 |
| D1 | 5 males | [thiazol-2-$^{14}$C]: 91.2 p.o., 6590 kBq (178 µCi) | urine<br>faeces | 0 - 24<br>0 - 48 | 92.9<br>4.8 |
| | 5 females | [thiazol-2-$^{14}$C]: 98.9 p.o., 6590 kBq (178 µCi) | urine<br>faeces | 0 - 24<br>0 - 48 | 93.0<br>4.2 |
| B2 | 5 males | [oxadiazin-4-$^{14}$C]: 0.44 p.o., 288 kBq (7.8 µCi) | urine<br>faeces | 0 - 24<br>0 - 24 | 89.6<br>4.1 |
| | 5 females | [oxadiazin-4-$^{14}$C]: 0.46 p.o., 288 kBq (7.8 µCi) | urine<br>faeces | 0 - 24<br>0 - 24 | 92.5<br>2.7 |
| D2 | 5 males | [oxadiazin-4-$^{14}$C]: 100.9 p.o., 6422 kBq (174 µCi) | urine<br>faeces | 0 - 24<br>0 - 48 | 92.0<br>5.3 |
| | 5 females | [oxadiazin-4-$^{14}$C]: 104.2 p.o., 6422 kBq (174 µCi) | urine<br>faeces | 0 - 24<br>0 - 48 | 95.2<br>3.4 |
| G1 | 4 males*** | [thiazol-2-$^{14}$C]: 0.49 p.o., 250 kBq (6.8 µCi) | urine<br>faeces<br>bile | 0 - 24<br>0 - 48<br>0 - 24 | 78.6<br>4.8<br>3.8 |
| G3 | 5 males*** | [oxadiazin-4-$^{14}$C]: 0.48 p.o., 376 kBq (10.2 µCi) | urine<br>faeces<br>bile | 0 - 48<br>0 - 48<br>0 - 42 | 86.8<br>3.6<br>4.5 |

* pre-treatment with daily oral doses (0.5 mg/kg) of non-radiolabelled CGA 293'343 for 14 days
*** bile-duct cannulated animals

All samples were stored at or below -18°C. The stability of metabolites during storage was checked by analysis after collection of the specimen and at the beginning and at the end of the analytical study. It was considered, that storage has not affected the results of the present study.

*Analytical methods:* 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.
Analysis and separation of metabolites was performed by thin layer chromatography (TLC), high performance liquid chromatography (HPLC), and high voltage electrophoresis. The pattern of radioactivity on TLC plates was detected with a spark chamber camera (*Berta*) or a Bio-Imaging Analyser (*BAS 2000*). Quantitative data for fractions on TLC were obtained by scraping off radioactive zones followed by LSC or alternatively by further processing of the images and electronic integration of the radioactive fractions on *TINA* software. Non-radioactive fractions on TLC plates were located under UV-light at 254 nm. Pre-purification of urine was carried out by flash chromatography and solid phase extraction. MS- and NMR-spectroscopy were used for structure elucidation of metabolites. In addition selected metabolites were identified by comparison with authentic reference substances.

**Findings:** The metabolite patterns determined quantitatively by two-dimensional TLC are shown in the Tables below. The urinary pattern was complex and essentially independent of sex, dose, pre-treatment with non-radiolabelled thiamethoxam and route of administration. Very minor label-specific fractions were observed. The faecal pattern was similar but less complex than that found for urine, with some quantitative variations but generally independent of sex, dose, pre-treatment and route of administration and only slightly dependent on the position of label. The bile pattern was even less complex and

independent of the position of label with some quantitative variations. All the excreta patterns were dominated by two major fractions accounting in total for 80 - 90% of dose.
Ultimately 12 metabolites were isolated from the urine of the high dosed male and female animals and identified by spectroscopic means. In addition 13 metabolites were identified by co-chromatography with authentic reference substances.
The major fraction in urine, accounting for about 70 - 80% of the dose, was identified as unchanged thiamethoxam.
Based on the structures of these metabolites the metabolic pathways of thiamethoxam were derived:

- cleavage of the oxadiazine ring to the corresponding nitroguanidine compound (major pathway)
- reduction of the nitroguanidine group to a hydrazine followed by either acetylation or pyruvate conjugation (minor pathway)
- cleavage of the nitroguanidine group yielding a guanidine derivative (minor pathway)
- hydrolysis of the guanidine group to the corresponding urea (minor pathway)
- demethylation of the guanidine group (minor pathway)
- substitution of the chlorine of the thiazole ring by glutathione (minor pathway)
- cleavage between the thiazole and oxadiazine ring (minor pathway)

Cleavage between the thiazole- and oxadiazine ring occurs to a very small extent. It is initiated either by attack of glutathione on the bridging methylene group as long as the nitroguanidine moiety is intact, rendering a good leaving group, or alternatively by oxidative dealkylation. The former gives rise to a thiazole-5-ylmethyl-glutathione derivative and a nitroguanidine compound whereas the latter produces ultimately the respective carboxylic acid derivative of thiazole and the corresponding cleavage counterpart.

Three hydrazine derivatives as metabolites have been found in the rat. Two of these derivatives were detectable in rat excreta in small amounts. All hydrazine derivatives detected are acylated (pyruvate or acetate) and they are incorporated in an enamine structure suggesting a reduced reactivity of the hydrazine moiety.

The glutathione derivatives are prone to further degradation of the glutathione moiety resulting in various sulphur-containing metabolites (e.g. mercapturates, sulphides, and sulphoxides).

Both the thiazole and oxadiazine moieties are susceptible to oxidative attack. The previous toxicokinetic study (Müller, T. and Stampf, P., 1996, see section 5.1.1) renders evidence that small but measurable amounts of radioactivity were exhaled after administration of either label, most probably as $CO_2$. These minor pathways proceed rapidly to small molecules, which may, at least partially, enter the general metabolism.

The various sulphur-containing metabolites and small metabolites from thiazole- and oxadiazine-ring degradation are probably the reason for the complex metabolite pattern detected in urine.

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

The degradation resulted in metabolites which together with unchanged thiamethoxam were eliminated very rapidly. The administered dose was almost completely absorbed, and was degraded partially and eliminated almost completely via urine. Excretion via bile and ultimately via faeces was of very minor importance. Enterohepatic circulation was negligible.

In conclusion, the degradation of thiamethoxam accounted for about 20 - 30% of the applied dose. The metabolic pathways are independent of the route of administration, the dose level in the range of 0.5 and 100 mg/kg body weight, pre-treatment, and the sex of the animals, within the limits of this study. Due to the rapid absorption and excretion, it is assumed that exposure time of thiamethoxam to biotransformation enzymes is reduced resulting in excretion of high amounts of the unchanged compound.

**Table: Quantitative metabolite pattern of rat urine after administration of thiamethoxam [% of dose]**

| Label | [Thiazol-2-$^{14}$C] | | | | | | | | | [Oxadiazin-4-$^{14}$C] | | | | | Assignment of Metabolites* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Group** | **A1** (single, i.v.) | | **B1** (single, p.o.) | | **C1** (multiple, p.o.) | | **D1** (single, p.o.) | | **G1** | **B2** (single, p.o.) | | **D2** (single, p.o.) | | **G3** | |
| **Sex** | male | female | male | female | male | female | male | female | male | male | female | male | female | male | |
| **Dose** [mg/kg] | 0.51 | 0.55 | 0.54 | 0.56 | 0.42 | 0.44 | 91.2 | 98.9 | 0.49 | 0.44 | 0.46 | 100.9 | 104.2 | 0.48 | |
| Metabolite Fraction | | | | | | | | | | | | | | | |
| U1 | 0.3 | 0.4 | 0.4 | 0.3 | 0.6 | 0.6 | 0.4 | 0.2 | 0.7 | 0.2 | 0.1 | 0.2 | 0.2 | 0.3 | |
| U2 | ⟩1.1 | ⟩1.2 | ⟩1.5 | ⟩1.3 | ⟩1.8 | ⟩1.4 | 0.7 | 0.6 | ⟩1.4 | ⟩1.2 | ⟩1.1 | 0.6 | 0.6 | ⟩0.9 | NOA 407475 |
| U3 | 1.6 | 1.7 | 2.2 | 1.8 | 2.4 | 2.0 | 1.4 | 0.9 | 1.9 | 1.5 | 1.1 | 1.3 | 1.0 | 1.2 | 5U/16U/17U/NOA 421276 |
| U4 - U11 | 71.7 | 76.2 | 69.5 | 76.1 | 76.3 | 81.2 | 1.0 | 0.6 | 68.7 | 74.2 | 82.4 | 1.2 | 0.9 | 76.2 | |
| U12 | --- | --- | 0.1 | --- | 0.2 | --- | 73.6 | 81.8 | --- | --- | --- | 72.3 | 82.6 | --- | 1U (CGA 293343) |
| U13 | ⟩0.5 | ⟩0.5 | 0.4 | 0.4 | 0.2 | 0.2 | 0.1 | 0.1 | --- | ⟩1.0 | ⟩0.8 | --- | --- | --- | 6U |
| U14 | 7.1 | 7.5 | 0.3 | 0.4 | 0.3 | 0.3 | 0.4 | 0.4 | --- | 10.5 | 6.4 | 0.3 | 0.2 | 0.6 | 4U |
| U15 | 0.8 | 0.9 | 10.8 | 6.6 | 10.2 | 5.5 | 0.2 | 0.2 | 5.1 | 1.2 | 0.5 | 1.0 | 0.7 | 7.2 | 3U/NOA 405217 |
| U16 | --- | --- | 1.3 | 0.5 | 1.1 | 0.6 | 12.2 | 6.9 | 0.5 | --- | --- | 13.1 | 8.0 | 0.6 | 2U** |
| U17 | 0.6 | 0.7 | 0.3 | 0.1 | 0.2 | --- | 2.0 | 0.6 | --- | --- | --- | 1.9 | 0.9 | --- | 18U |
| U18 - U19 | 0.1 | 0.2 | 0.9 | 0.6 | 0.6 | 0.5 | <0.1 | --- | 0.5 | --- | --- | <0.1 | --- | --- | |
| U20 | --- | --- | 0.1 | 0.1 | 0.2 | 0.2 | 0.9 | 0.6 | --- | --- | --- | --- | --- | --- | 14U |
| U21 | | | 0.1 | 0.1 | --- | --- | 0.1 | 0.1 | --- | --- | --- | <0.1 | <0.1 | --- | 15U |
| U22 | | | | | | | <0.1 | 0.1 | | | | --- | --- | | |
| **Total** | **83.8** | **89.1** | **87.8** | **88.2** | **94.1** | **92.3** | **92.9** | **93.0** | **78.6** | **89.6** | **92.5** | **92.0** | **95.2** | **86.8** | |

* NOA 421275, CGA 204261, 19U, NOA 408445, CGA 330050 chromatograph between U2 and U3, U7 and U12, U15 and U16, U16 and U17, U17 and U21, respectively, each accounting for <0.1% of dose in group D1 and/or D2.

** CGA 282149, CGA 353042, CGA 340575 correspond to metabolite fraction U16, accounting for 0.2, 0.1, and <0.1% of dose in group D2, respectively.

**Table : Quantitative metabolite pattern of rat faeces after administration of thiamethoxam [% of dose]**

| **Label** | **[Thiazol-2-$^{14}$C]** | | | | | | | | | **[Oxadiazin-4-$^{14}$C]** | | | | | **Assignment of Metabolites** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Group** | **A1** (single, i.v.) | | **B1** (single, p.o.) | | **C1** (multiple, p.o.) | | **D1** (single, p.o.) | | **G1** | **B2** (single, p.o.) | | **D2** (single, p.o.) | | **G3** | |
| **Sex** | male | female | male | female | male | female | male | female | male | male | female | male | female | male | |
| **Dose** [mg/kg] | 0.51 | 0.55 | 0.54 | 0.56 | 0.42 | 0.44 | 91.2 | 98.9 | 0.49 | 0.44 | 0.46 | 100.9 | 104.2 | 0.48 | |
| Metabolite Fraction of Extract | | | | | | | | | | | | | | | |
| F1 | } 0.5 | } 0.3 | } 0.5 | } 0.4 | } 0.8 | } 0.4 | 0.4 | 0.2 | } 0.3 | } 0.4 | } 0.3 | } 0.6 | } 0.3 | } 0.3 | NOA 407475 |
| F2 | | | | | | | 0.3 | 0.2 | | | | | | | |
| F3 | | | 0.4 | 0.3 | 0.3 | 0.2 | } 0.5 | } 0.3 | 0.2 | 0.3 | 0.2 | } 0.2 | } 0.1 | } 0.1 | NOA 421275 |
| F4 | --- | --- | --- | --- | --- | --- | | | --- | --- | --- | | | | |
| F5 - F8 | 0.4 | 0.2 | 0.4 | 0.3 | 0.6 | 0.3 | 0.5 | 0.4 | 0.3 | 0.4 | 0.3 | 0.6 | 0.3 | 0.3 | |
| F9 | 1.3 | 0.7 | 0.8 | 0.4 | 1.5 | 1.8 | 0.8 | 1.5 | 3.3 | 0.7 | 0.6 | 0.9 | 0.8 | 2.1 | 1U (CGA 293343) |
| F10 | --- | --- | --- | --- | --- | --- | 0.1 | 0.1 | --- | --- | --- | 0.1 | <0.1 | --- | 3U |
| F11 | 0.2 | 0.1 | 0.2 | 0.1 | 0.4 | 0.2 | 0.2 | 0.2 | 0.3 | 0.2 | 0.1 | 0.3 | 0.1 | 0.3 | 2U |
| F12 | --- | --- | --- | --- | 0.1 | --- | 0.1 | <0.1 | --- | 0.1 | <0.1 | 0.1 | <0.1 | --- | 18U |
| F13 | --- | --- | --- | --- | --- | --- | <0.1 | <0.1 | --- | --- | --- | --- | --- | --- | 14U |
| *Sum* | *2.4* | *1.3* | *2.3* | *1.5* | *3.6* | *2.9* | *2.8* | *2.8* | *4.4* | *2.2* | *1.5* | *2.6* | *1.7* | *3.1* | |
| Non-extractable | 2.1 | 1.2 | 2.1 | 1.1 | 2.5 | 0.9 | 2.0 | 1.4 | 0.4 | 2.0 | 1.2 | 2.6 | 1.7 | 0.5 | |
| **Total** | **4.4** | **2.5** | **4.4** | **2.6** | **6.2** | **3.9** | **4.8** | **4.2** | **4.8** | **4.1** | **2.7** | **5.3** | **3.4** | **3.6** | |

**Table: Quantitative metabolite pattern of rat bile after administration of thiamethoxam [% of dose]**

| Label | [Thiazol-2-$^{14}$C] | [Oxadiazin-4-$^{14}$C] | Assignment to Metabolite |
|---|---|---|---|
| **Group** | **G1** | **G3** | |
| **Sex** | male | male | |
| **Dose** [mg/kg] | 0.49 | 0.48 | |
| Metabolite Fraction | | | |
| B1 | 2.2 | 2.9 | |
| B2 | 0.2 | 0.2 | |
| B3 | 1.2 | 1.1 | 1U (CGA 293'343) |
| B4 | --- | 0.1 | 3U |
| B5 | 0.2 | 0.2 | 2U |
| **Total** | **3.8** | **4.5** | |

| **Evaluation by Competent Authorities** | |
|---|---|
| | **EVALUATION BY RAPPORTEUR MEMBER STATE** |
| **Date** | March 2005 |
| **Materials and Methods** | [redacted] |
| **Results and discussion** | [redacted] |
| **Conclusion** | [redacted] |
| **Reliability** | [redacted] |
| **Acceptability** | [redacted] |
| **Remarks** | |

| | |
|---|---|
| 98/8 Doc IIIA section No. 6.2 / 05 | Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study |
| 91/414 Annex II Point addressed 5.1.3 | Studies on absorption, distribution, excretion and metabolism in mammals |

| | |
|---|---|
| 1. Annex point(s) | IIA, 5.1.3 Studies on absorption, distribution, excretion and metabolism in mammals |
| 2. Reference point (location) in dossier | Volume 7, Section 3, Anex IIA, point 5.1.3 |
| 3. Authors (year) Title Owner, Date | [redacted]<br>The Metabolism of [Thiazol-2-$^{14}$C] CGA 293'343 after Multiple Oral Administration to Mice<br>Syngenta Crop Protection AG, unpublished report No. 027AM09, November 03, 1998 incl. Amendment 1, August 23, 2000<br>Syngenta File No. CGA293343/0800 and CGA293343/1278 (Amendment 1) |
| 4. Testing facility | [redacted] |
| 5. Dates of work | July 29, 1998 to October 30, 1998 |
| 6. Test substance | Thiamethoxam<br>Non-labeled compound: Batch number: [redacted]<br>*[Thiazol-2-$^{14}$C] - labeled compound:* Batch number: [redacted] |
| 7. Test method | Not applicable – investigative study |
| 8. GLP | Yes - laboratory certified by the Eidgenössisches Departement des Inneren (Federal Department of Home Affairs), Bern, Switzerland; |

**Material and methods:** Male mice, housed in 3 groups of 4 animals each, received daily doses of [$^{14}$C]-thiamethoxam for 14 consecutive days. The test substance was orally administered at a dose level of 118 mg/kg bw and urine and faeces were collected daily until 72 hours after the last dose. Expired $^{14}CO_2$ was trapped during the first three days of the treatment.

For metabolite quantitation and identification urine and faeces specimens from selected time intervals were pooled prior to analysis (see Table below).

For the profiling of the urine, each pooled urine sample was analysed by two-dimensional - TLC in one or two different solvent systems and metabolites were compared with known reference substances and metabolites identified in the rat metabolism study.

The faeces pools were extracted exhaustively with acetonitrile followed by extractions with methanol/water (4:1 v/v). The extracts of each faeces pool were analysed by 2-dimensional TLC in two different TLC solvent systems.

Faeces extracts of pool F1 and F2 (see Table 1) were used to isolate an unknown metabolite by RP-18 HPLC and preparative TLC on silica gel. The structure of this metabolite was proposed based on results from mass spectroscopy.

**Table 1 Identification of pooled samples used for metabolite identification and quantitation**

| Dosing Group | Administration | Sex | Number of Mice | Type of Specimen | Pool # | Sampling Time [h] | Amount [% of dose[1)] ] |
|---|---|---|---|---|---|---|---|
| N1 | p.o. 14 consecutive daily doses | male | 12 | urine | U1 | 0-24 | 66.0 |
| | | | | | U2 | 72-96 | 80.3 |
| | | | | | U3 | 144-168 | 66.9 |
| | | | | | U4 | 216-240 | 84.1 |
| | | | | | U5 | 288-312 | 77.8 |
| | | | | | U6 | 360-384 (24-48h after final dose) | 3.8 |
| | | | | faeces extract | F1 | 0-24 | 6.0 |
| | | | | | F2 | 72-96 | 8.4 |
| | | | | | F3 | 144-168 | 14.7 |
| | | | | | F4 | 216-240 | 25.2 |
| | | | | | F5 | 288-312 | 15.9 |
| | | | | | F6 | 360-384 (24-48h after final dose) | 2.8 |

[1)] of a daily dose

**Findings:** *Absorption and excretion in male mice:* The administered dose was readily and rapidly excreted, as approximately 73% of the first dose was excreted in urine & faeces within 24 h and only minor amounts of radioactivity were found in the excreta on day 2 and 3 after the last administration (360-384h timepoint). The applied radioactivity was predominantly excreted via the kidneys. Throughout the duration of the experiment, a total of 71.8% of the administered dose was recovered in the urine and 18.8% in the faeces. Radioactivity in expired air was determined during the first three days of administration and was low (<0.3% of the daily administered dose). A steady state of excretion was reached within 3 days after the first administration. The rate of excretion in per cent of the daily dose is summarized in Table 5.1.5-2.

The excretion expressed in per cent of the total dose is given in Table 2 and 3.

**Table 2 Excretion of radioactivity after multiple oral dosing of $^{14}$C-thiamethoxam at a high dose level to male mice (% of daily dose)**

| Group N1 (% of daily dose) | | | | | |
|---|---|---|---|---|---|
| *Subgroup* | 1 | 2 | 3 | **Mean** | SD |
| *Dose [mg/kg bw and day]* | | 118.2 | | | |
| *Urine* | | | | | |
| 0 -24 h | 71.73 | 49.35 | 77.02 | 66.03 | 14.69 |
| 24 - 48 h | 49.27 | 58.45 | 68.76 | 58.83 | 9.75 |
| 48 - 72 h | 66.87 | 72.60 | 67.57 | 69.01 | 3.13 |
| 72 - 96 h | 78.81 | 84.33 | 77.82 | 80.32 | 3.51 |
| 96 - 120 h | 57.17 | 60.13 | 53.24 | 56.85 | 3.46 |
| 120 - 144 h | 50.00 | 67.13 | 52.40 | 56.51 | 9.28 |
| 144 - 168 h | 62.37 | 76.79 | 61.56 | 66.91 | 8.57 |
| 168 - 192 h | 68.67 | 78.31 | 68.26 | 71.75 | 5.69 |
| 192 - 216 h | 54.41 | 77.83 | 60.19 | 64.14 | 12.20 |
| 216 - 240 h | 80.23 | 88.14 | 84.03 | 84.13 | 3.96 |
| 240 - 264 h | 90.22 | 87.17 | 66.12 | 81.17 | 13.12 |
| 264 - 288 h | 96.61 | 87.58 | 79.86 | 88.02 | 8.38 |
| 288 - 312 h | 78.46 | 86.00 | 68.87 | 77.77 | 8.59 |
| 312 - 336 h | 95.46 | 82.89 | 57.78 | 78.71 | 19.18 |
| 336 - 360 h * | 10.24 | 11.28 | 7.27 | 9.60 | 2.08 |
| 360 - 384 h * | 3.45 | 3.62 | 4.21 | 3.76 | 0.40 |
| *Faeces* | | | | | |
| 0 -24 h | 7.33 | 5.50 | 8.14 | 6.99 | 1.35 |
| 24 - 48 h | 43.62 | 39.21 | 22.11 | 34.98 | 11.36 |
| 48 - 72 h | 10.22 | 16.89 | 16.11 | 14.41 | 3.65 |
| 72 - 96 h | 8.59 | 9.76 | 11.43 | 9.93 | 1.42 |
| 96 - 120 h | 16.07 | 17.01 | 23.12 | 18.73 | 3.83 |
| 120 - 144 h | 10.28 | 21.03 | 32.47 | 21.26 | 11.10 |
| 144 - 168 h | 10.92 | 13.18 | 25.52 | 16.54 | 7.86 |
| 168 - 192 h | 17.25 | 12.06 | 15.36 | 14.89 | 2.63 |
| 192 - 216 h | 17.01 | 16.70 | 24.25 | 19.32 | 4.27 |
| 216 - 240 h | 35.88 | 18.59 | 27.85 | 27.44 | 8.65 |
| 240 - 264 h | 16.86 | 15.58 | 23.20 | 18.55 | 4.08 |
| 264 - 288 h | 12.76 | 11.50 | 18.02 | 14.09 | 3.46 |
| 288 - 312 h | 22.79 | 8.91 | 21.10 | 17.60 | 7.57 |
| 312 - 336 h | 9.93 | 13.00 | 28.94 | 17.29 | 10.21 |
| 336 - 360 h * | 4.19 | 2.51 | 13.69 | 6.80 | 6.03 |
| 360 - 384 h * | 1.40 | 1.00 | 6.82 | 3.07 | 3.25 |
| Expired Air | | | | | |
| 0 -24 h | 0.16 | 0.19 | 0.17 | 0.17 | 0.01 |
| 24 - 48 h | 0.21 | 0.32 | 0.22 | 0.25 | 0.06 |
| 48 - 72 h | 0.25 | 0.28 | 0.23 | 0.26 | 0.03 |

* % of daily dose calculations are based on administered radioactivity at 312 h

**Table 3 Excretion of radioactivity after multiple oral dosing of $^{14}$C-thiamethoxam at a high dose level to male mice (% of cumulative dose)**

| | | Group N1 (% of total dose) | | | | |
|---|---|---|---|---|---|---|
| *Subgroup* | | 1 | 2 | 3 | Mean | SD |
| *Dose [mg/kg bw and day]* | | | 118.2 | | | |
| *Urine* | | | | | | |
| | 0 -24 h | 6.36 | 4.02 | 5.49 | 5.29 | 1.18 |
| | 24 - 48 h | 4.35 | 4.74 | 4.87 | 4.65 | 0.27 |
| | 48 - 72 h | 5.87 | 5.85 | 4.76 | 5.49 | 0.63 |
| | 72 - 96 h | 5.27 | 6.90 | 5.57 | 5.92 | 0.87 |
| | 96 - 120 h | 3.81 | 4.91 | 3.80 | 4.18 | 0.64 |
| | 120 - 144 h | 3.33 | 5.48 | 3.74 | 4.18 | 1.14 |
| | 144 - 168 h | 4.17 | 6.28 | 4.41 | 4.95 | 1.16 |
| | 168 - 192 h | 4.54 | 4.76 | 4.84 | 4.71 | 0.15 |
| | 192 - 216 h | 3.65 | 4.79 | 4.32 | 4.25 | 0.57 |
| | 216 - 240 h | 5.35 | 5.40 | 6.00 | 5.59 | 0.36 |
| | 240 - 264 h | 6.03 | 5.35 | 4.73 | 5.37 | 0.65 |
| | 264 - 288 h | 6.52 | 5.43 | 5.77 | 5.91 | 0.56 |
| | 288 - 312 h | 5.28 | 5.32 | 4.96 | 5.19 | 0.19 |
| | 312 - 336 h | 6.36 | 5.07 | 4.12 | 5.19 | 1.12 |
| | 336 - 360 h | 0.68 | 0.69 | 0.52 | 0.63 | 0.10 |
| | 360 - 384 h | 0.23 | 0.22 | 0.30 | 0.25 | 0.04 |
| | ***Subtotal*** | **71.82** | **75.21** | **68.22** | **71.75** | **3.50** |
| *Faeces* | | | | | | |
| | 0 -24 h | 0.65 | 0.45 | 0.58 | 0.56 | 0.10 |
| | 24 - 48 h | 3.85 | 3.18 | 1.57 | 2.86 | 1.17 |
| | 48 - 72 h | 0.90 | 1.36 | 1.14 | 1.13 | 0.23 |
| | 72 - 96 h | 0.57 | 0.80 | 0.82 | 0.73 | 0.14 |
| | 96 - 120 h | 1.07 | 1.39 | 1.65 | 1.37 | 0.29 |
| | 120 - 144 h | 0.69 | 1.72 | 2.32 | 1.57 | 0.83 |
| | 144 - 168 h | 0.73 | 1.08 | 1.83 | 1.21 | 0.56 |
| | 168 - 192 h | 1.14 | 0.73 | 1.09 | 0.99 | 0.22 |
| | 192 - 216 h | 1.14 | 1.03 | 1.74 | 1.30 | 0.38 |
| | 216 - 240 h | 2.39 | 1.14 | 1.99 | 1.84 | 0.64 |
| | 240 - 264 h | 1.13 | 0.96 | 1.66 | 1.25 | 0.37 |
| | 264 - 288 h | 0.86 | 0.71 | 1.30 | 0.96 | 0.31 |
| | 288 - 312 h | 1.53 | 0.55 | 1.52 | 1.20 | 0.56 |
| | 312 - 336 h | 0.66 | 0.80 | 2.07 | 1.17 | 0.77 |
| | 336 - 360 h | 0.28 | 0.15 | 0.98 | 0.47 | 0.44 |
| | 360 - 384 h | 0.09 | 0.06 | 0.49 | 0.21 | 0.24 |
| | *Subtotal* | **17.69** | **16.10** | **22.73** | **18.84** | **3.46** |
| *Expired Air* | | | | | | |
| | 0 -24 h | 0.01 | 0.02 | 0.01 | 0.01 | < 0.01 |
| | 24 - 48 h | 0.02 | 0.03 | 0.02 | 0.02 | < 0.01 |
| | 48 - 72 h | 0.02 | 0.02 | 0.02 | 0.02 | < 0.01 |
| | *Subtotal* | **0.05** | **0.07** | **0.05** | **0.06** | **< 0.01** |
| *Cage Wash* | | **3.45** | **3.98** | **3.99** | **3.81** | **0.31** |
| **Total Recovery** | | **93.01** | **95.36** | **95.00** | **94.46** | **1.27** |

*Metabolism:* The quantitative analysis of the excreted radioactivity in mouse urine and faeces indicates that 30 - 60% of the daily administered thiamethoxam was excreted as metabolites. The major reaction involved in the biotransformation of thiamethoxam in mice is the cleavage of the oxadiazine ring to result in the corresponding nitroguanidine compound CGA 322'704 (for IUPAC names see Table 5.1.5-7). The second major metabolite, CGA 265'307, is formed by demethylation of CGA 322'704. Substitution of the chlorine of the thiazole ring in thiamethoxam by glutathione and subsequent reactions on the glutathione moiety resulted in metabolite R6 found in mouse faeces. Minor pathways (<1% of the daily dose for each metabolite) include the cleavage of the nitroguanidine group of thiamethoxam to result in the corresponding guanidine, yielding metabolite NOA 407'475. Cleavage of the nitroguanidine group and the oxadiazine ring yielded metabolite NOA 421'275. Demethylation of NOA 421'275 yielded metabolite NOA 421'276. Hydrolysis of the guanidine group of CGA 265'307 yielded the respective urea derivative NOA 404'617. Substitution of the chlorine of the thiazole ring by glutathione and subsequent degradation resulted in the formation of metabolite NOA 402'988. Metabolite 4U is formed by hydrolysis of NOA 421'276. Further reactions of NOA 421'276 with glutathione and subsequent metabolic transformations led to the formation of metabolite 6U, a N-acetylcysteinyl derivative of the thiazole moiety of thiamethoxam.

Unchanged thiamethoxam, and the metabolites CGA 265'307 and CGA 322'704 accounted for the majority of the urinary radioactivity, independent of the sampling interval and the type of excreta analysed. Minor metabolites that were detected in mouse urine are NOA 402'988, NOA 407'475, NOA 404'617, 4U, 6U, NOA 421'275 and NOA 421'276. In addition to these metabolites two unknown non-polar metabolites were detected in mouse urine (R14 and R15). Quantitative results are presented in Table 4.

2D-TLC analysis of the faeces extracts revealed the presence of unchanged thiamethoxam and of the metabolites CGA 322'704, CGA 265'307, NOA 407'457, NOA 402'988, NOA 421'275, NOA 407'475 and NOA 421'276. Metabolite R6, accounting for 1.9-2.6% of the daily dose in mouse faeces, was isolated and a structure proposed based on results from mass-spectrometry. Quantitative results of the metabolite quantitation in mouse and rat faeces are presented in Table 5.

**Table 4 The quantitative distribution of metabolites in urine after single (rat) and multiple (mouse) oral administration of $^{14}$C-thiamethoxam at a high dose level (% of dose)**

| Species | Rat**** | Mouse | | | | | |
|---|---|---|---|---|---|---|---|
| Dose regimen | Single oral | multiple oral dose | | | | | |
| Group/Sex | D1 male | N1 male | | | | | |
| Dose | 91.2 | 118.2 | | | | | |
| Sampling interval | 0-24 | 0-24 | 72-96 | 144-168 | 216-240 | 288-312 | 360-384 *** |
| Urinary excretion [% of daily dose] | 92.9**** | 66.0 | 80.3 | 66.9 | 84.1 | 77.8 | 3.8 |
| Metabolite/ Fraction | % of daily dose | | | | | | |
| CGA 293'343 | 73.34 | 40.38 | 37.85 | 33.38 | 40.59 | 39.15 | 1.17 |
| CGA 322'704 | 12.00 | 7.80 | 12.32 | 10.56 | 12.33 | 11.12 | 0.54 |
| CGA 265'307 | 1.91 | 9.41 | 17.86 | 13.13 | 17.66 | 15.92 | 1.12 |
| NOA 402'988 | 0.89 | 0.17 | 0.26 | 0.15 | 0.20 | 0.23 | 0.05 |
| NOA 404'617 | 0.07 | 0.11 | 0.12 | 0.19 | 0.72 | 1.61 | 0.08 |
| NOA 407'475 | 0.69 | 0.37 | 0.54 | 0.54 | 0.56 | 0.42 | 0.05 |
| NOA 421'276* | 0.04 | 0.13 | n.a. | n.a | n.a | 0.22 | n.a |
| NOA 421'275* | 0.33 | 0.02 | n.a. | n.a. | n.a. | 0.07 | n.a. |
| 6U | 0.09 | 0.07 | 0.07 | 0.14 | 0.17 | 0.16 | 0.01 |
| 4U | 0.46 | 0.20 | 0.22 | 0.19 | 0.28 | 0.22 | 0.01 |
| R14 | n.d. | 0.18 | 0.27 | 0.15 | 0.14 | 0.14 | 0.01 |
| R15 | n.d. | 0.47 | 0.57 | 0.49 | 0.66 | 0.54 | 0.06 |
| U5 | 0.37 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| U6 | 0.08 | 0.13 | 0.24 | 0.24 | 0.29 | 0.22 | 0.02 |
| U7 | 0.35 | 0.30 | 0.43 | 0.27 | 0.24 | 0.19 | 0.01 |
| U8 | 0.08 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| U9 | 0.33 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| U10 | 0.06 | 0.14 | 0.15 | 0.13 | 0.13 | 0.09 | 0.00 |
| Polar Unresolved | 0.30 | 4.66 | 8.12 | 6.00 | 8.14 | 6.73 | 0.46 |
| Remainder | 1.49 | 1.47 | 1.29 | 1.35 | 2.04 | 0.75 | 0.17 |
| | | | | | | | |
| Total | 92.9 | 66.0 | 80.3 | 66.9 | 84.1 | 77.8 | 3.8 |

data less than 0.005% are reported as 0.00%
* estimated
*** per cent of daily dose calculations are based on dose administered at 312 h
**** figures taken from the toxicokinetic and metabolism study in rats
n.a. = not analysed
n.d. = not detected

**Table 5 The quantitative distribution of metabolites in faeces after single (rat) and multiple (mouse) oral administration of $^{14}$C- thiamethoxam at a high dose level (% of dose)**

| Species | Rat* | Mouse | | | | | |
|---|---|---|---|---|---|---|---|
| Dose regimen | single oral | multiple oral dose | | | | | |
| Group/Sex | D1male/female | N1 male | | | | | |
| Dose | 95.05 | 118.2 | | | | | |
| Sampling interval | 0-24 | 0-24 | 72-96 | 144-168 | 216-240 | 288-312 | 360-384 *** |
| % of daily dose | 4.5* | 6.99 | 9.93 | 16.54 | 27.44 | 17.6 | 3.07 |
| Faeces extract [% of daily dose] | 2.8 | 6.0 | 8.4 | 14.7 | 25.2 | 15.9 | 2.80 |
| Metabolite/ Fraction | % of daily dose | | | | | | |
| CGA 293'343 | 1.17 | 0.91 | 0.97 | 3.53 | 7.84 | 4.56 | 0.98 |
| CGA 322'704 | 0.19 | 0.27 | 0.49 | 1.68 | 3.19 | 1.81 | 0.42 |
| CGA 265'307 | 0.04 | 0.59 | 1.93 | 3.86 | 6.32 | 3.31 | 0.72 |
| NOA 402'988 | 0.02 | 0.02 | 0.11 | 0.09 | 0.15 | 0.11 | 0.04 |
| NOA 404'617 | 0.00 | 0.01 | 0.08 | 0.17 | 0.29 | 0.23 | 0.05 |
| NOA 421'276 | 0.30 | 0.19 | 0.36 | 0.33 | 0.45 | 0.32 | 0.06 |
| NOA 421'275 | 0.00 | 0.05 | 0.07 | 0.07 | 0.15 | 0.08 | 0.01 |
| NOA 407'475 | 0.38 | 0.06 | 0.05 | 0.05 | 0.09 | 0.08 | 0.01 |
| R4 | n.d. | 0.03 | 0.03 | 0.02 | 0.04 | 0.03 | 0.00 |
| R5 | n.d. | 0.42 | 0.58 | 0.57 | 0.82 | 0.57 | 0.07 |
| R6 | n.d. | 2.15 | 1.98 | 2.05 | 2.57 | 1.93 | 0.07 |
| R8 | n.d. | 0.43 | 0.38 | 0.42 | 0.63 | 0.38 | 0.03 |
| R14 | n.d. | 0.01 | 0.03 | 0.03 | 0.05 | 0.04 | 0.01 |
| R15 | n.d. | 0.01 | 0.08 | 0.19 | 0.25 | 0.16 | 0.04 |
| R21 | n.d. | 0.01 | 0.03 | 0.02 | 0.02 | 0.01 | 0.00 |
| R22 | n.d. | 0.05 | 0.04 | 0.04 | 0.01 | 0.02 | 0.00 |
| | | | | | | | |
| Remainder | 0.7 | 0.8 | 1.2 | 1.6 | 2.3 | 2.3 | 0.3 |
| | | | | | | | |
| Total | 2.8 | 6.0 | 8.4 | 14.7 | 25.2 | 15.9 | 2.8 |

data less than 0.005% are reported as 0.00%
* mean D1 male and D1 female; figures are taken from the toxicokinetic and metabolism studies in rats
*** per cent of daily dose calculations are based on dose administered at 312 h ; n.d. = not detected

*Comparison of excretion and metabolism in mice and rats:* An about 4-fold increased faecal excretion of radioactivity in mice as compared to rats revealed a species difference in the routes of excretion. About 19% of the total administered radioactivity was found in the mouse faeces and only 5.1% of the dose was recovered in the rat faeces. The data are summarized in Table 6 and compared with the respective data from the rat toxicokinetics study.

**Table 6 Species difference in metabolism between rats and mice**

| | Excretion in % of total applied dose | |
|---|---|---|
| | Rat[1)] | Mouse |
| Group | D1 male | N1 |
| Dose | single oral, 91.2 mg/kg bw | multiple oral, 14 x 118.2 mg/kg bw |
| Urine | 95.49 | 71.75 |
| Faeces | 5.14 | 18.84 |
| Expired air | 0.18 | 0.06[2)] |
| Cage wash | 0.18 | 3.81 |
| Tissue residues | 0.29 | not determined |
| Total recovery | 101.28 | 94.46 |

1) data are taken from the rat toxicokinetic study; see section 5.1.3

2) collection period 0-72 h after the first administration

Since the relative amounts excreted in mouse urine and faeces did not change considerably over the treatment period it is assumed that the different excretion pattern is attributed to a species difference rather than to the different dose regimen. Of the daily administered thiamethoxam dose, 30-60% was excreted metabolised in mice excreta, yet only 20-30% of the dosed thiamethoxam was biotransformed in rats.

**Conclusion:** Comparison of metabolite pattern in urine and faeces extracts of mice and rats revealed that the major metabolic pathways are essentially the same with some quantitative variations. The formation of polar metabolites, excreted via urine, was more pronounced in the mouse (rat 0.3% of the dose, mouse

4.7-8.0% of the daily dose). All major and almost all minor metabolites found in rat excreta were also detected in mouse excreta. A more complex pattern of polar metabolites was detected in urine of mice.

| Evaluation by Competent Authorities | |
|---|---|
| | **EVALUATION BY RAPPORTEUR MEMBER STATE** |
| **Date** | March 2005 |
| **Materials and Methods** | [redacted] |
| **Results and discussion** | [redacted] |
| **Conclusion** | [redacted] |
| **Reliability** | [redacted] |
| **Acceptability** | [redacted] |
| **Remarks** | |

| | |
|---|---|
| 98/8 Doc IIIA section No. 6.2 / 06 | Metabolism studies in mammals. Basic toxicokinetics, including a dermal absorption study |
| 91/414 Annex II Point addressed 5.1.3 | Studies on absorption, distribution, excretion and metabolism in mammals |

| | |
|---|---|
| 1. Annex point(s) | IIA, 5.1.3 Studies on absorption, distribution, excretion and metabolism in mammals |
| 2. Reference point (location) in dossier | Volume 7, Section 3, Anex IIA, point 5.1.3 |
| 3. Authors (year) Title Owner, Date | [redacted]<br>**Status report**: The Metabolism of [Thiazol-2-$^{14}$C] CGA 293'343 after Multiple Oral Administration to Mice – Further identification of metabolites<br>Syngenta Crop Protection AG, unpublished report No. 027AM09, 10. 11. 1999<br>(Interim status of work on the reopened previously reported study of [redacted] (1998b) The Metabolism of [Thiazol-2-$^{14}$C] CGA 293'343 after Multiple Oral Administration to Mice, Syngenta Crop Protection, Basel, Switzerland, unpublished report to study No. 027AM09, November 03, 1998; Dates of experimental work: July 29, 1998 to October 30, 1998).<br>Syngenta File No. CGA293343/1204<br>Amendment 1, 23.08.2000, Syngenta File No. CGA293343/1278 |
| 4. Testing facility | [redacted] |
| 5. Dates of work | . |
| 6. Test substance | ISO common name: Thiamethoxam<br>Non-labeled compound: Batch number: [redacted]<br>*[Thiazol-2-$^{14}$C] - labeled compound:* Batch number.: [redacted] |
| 7. Test method | Not applicable – investigative study |
| 8. GLP | Yes - laboratory certified by the Eidgenössisches Departement des Inneren (Federal Department of Home Affairs), Bern, Switzerland; |

**Material and methods**: Male mice, housed in 3 groups of 4 animals each, received daily doses of [$^{14}$C]-thiamethoxam for 14 consecutive days. The test substance was orally administered at a dose level of 118 mg/kg bw and urine and faeces were collected daily until 72 hours after the last dose. Expired $^{14}CO_2$ was trapped during the first three days of the treatment.

For metabolite quantitation and identification, urine and faeces specimens from selected time intervals were pooled prior to analysis.

**Findings**: Several additional minor metabolites were isolated and identified (italics in Table 1).

The mouse metabolite R15 was isolated and identified as CGA 359683. CGA 359683 is the precursor of the glycine conjugate NOA 408445 which was detected in the rat.

The mouse metabolite R14 was isolated and identified as 3-(2-chloro-thiazol-5-ylmethyl)-[1,3,5]oxadiazinan-4-ylideneamine. This metabolite was already detected in the goat (metabolite N1).

Metabolite MO1 was isolated and identified as a glucuronic acid conjugate of CGA 349208.

Metabolite MO2 was isolated and identified as an N-acetyl cysteine conjugate of CGA 349208.

Metabolite MO3 was isolated and identified as an N-acetyl cysteine conjugate of CGA 265307.

CGA 359683 and R14 were isolated at levels lower than 1% of the urinary radioactivity and metabolites MO1, MO2 and MO3 at levels lower than 0.1% of the urinary radioactivity.

Furthermore the presence of several metabolites already found in the original study was confirmed, i.e. NOA 421276, NOA 407475, 4U, and Mouse R6.

No new metabolic pathway was detected.

The major reaction involved in the biotransformation of CGA 293343 in the mouse is the cleavage of the oxadiazine ring to result in the corresponding nitroguanidine compound CGA 322704. Another major metabolite, CGA 265307, is formed by demethylation of CGA 322704. Substitution of the chlorine of the thiazole ring in CGA 293343 and CGA 265307 by glutathione and subsequent reactions resulted in metabolite Mouse R6 and metabolite MO3, respectively.

Minor pathways include the cleavage of the nitroguanidine group to result in the corresponding guanidine yielding metabolite NOA 407475. Demethylation of NOA 407475 yielded metabolite N1. Cleavage of the nitroguanidine group and the oxadiazine ring of metabolite NOA 407475 yielded metabolite NOA 421275. Demethylation of NOA 421275 yielded metabolite NOA 421276. Hydrolysis of the guanidine group of CGA 265307 yielded the respective urea derivative NOA 404617. Metabolite 4U is formed by hydrolysis of NOA 421276. Cleavage between the thiazole and oxadiazine moieties occurs to a very small extent as shown by the presence of minor metabolites 6U, MO1, MO2, CGA 359683 and NOA 402988.

Based on the metabolites identified, the metabolism of thiamethoxam in the mouse proceeds by the same major pathways as in the rat.

**Table 1 Nomenclature of identified metabolites of thiamethoxam in the mouse**

| Metabolite | IUPAC Name | Concentration (% of daily dose) |
|---|---|---|
| CGA 265307 | N-(2-chloro-thiazol-5-ylmethyl)-N'-nitro-guanidine | 10.0-24.0 |
| CGA 322704 | N-(2-chloro-thiazol-5-yl-methyl)-N'-methyl-N''-nitro-guanidine | 8.1-15.5 |
| NOA 402988 | 2-methylsulfanyl-thiazole-5-carboxylic acid | 0.2-0.4 |
| NOA 404617 | 1-(2-chloro-thiazol-5-ylmethyl)-3-nitro-urea | 0.1-1.8 |
| NOA 407475 | 3-(2-chlorothiazol-5-ylmethyl)-5-methyl-[1,3,5] oxadiazinan-4-ylideneamin | 0.4-0.6 |
| NOA 421275 | N-(2-chloro-thiazol-5-ylmethyl)-N'-methyl-guanidine | 0.1-0.2 |
| NOA 421276 | N-(2-chloro-thiazol-5-ylmethyl)-guanidine | 0.3-0.6 |
| 4U | (2-chloro-thiazol-5-ylmethyl)-urea | 0.2-0.3 |
| 6U | 2-acetylamino-3-(2-chloro-thiazol-5-ylmethylsulfanyl)-propionic acid | 0.1-0.2 |
| R6 | 2-acetylamino-3-[5-(5-methyl-4-nitroimino-[1,3,5]oxadiazinan-3-ylmethyl)-thiazol-2-ylsulfanyl]-propionic acid | 1.9-2.6 |
| *R14* | *3-(2-chloro-thiazol-5-ylmethyl)-[1,3,5]oxadiazinan-4-ylideneamine* | *0.01* |
| *R15: CGA 359683* | *2-chloro-thiazole-5-carboxylic acid* | *0.04* |
| CGA 349208 | (2-chloro-thiazol-5-yl)-methanol | |
| *MO1* | *glucuronic acid conjugate of* CGA 349208 | |
| *MO2* | *N-acetyl cysteine conjugate of* CGA 349208 | |
| *MO3* | *N-acetyl cysteine conjugate of* CGA 265307 | |

**Conclusion:** Comparison of metabolite pattern in urine and faeces extracts of mice and rats revealed that the major metabolic pathways are essentially the same. While several additional minor metabolites were identified, no new metabolic pathway was detected. The conclusions of the original study remain unchanged.

| **Evaluation by Competent Authorities** | |
|---|---|
| | **EVALUATION BY RAPPORTEUR MEMBER STATE** |
| **Date** | March 2005 |
| **Materials and Methods** | [redacted] |

| | |
|---|---|
| | [redacted] |
| **Results and discussion** | [redacted] |
| **Conclusion** | [redacted] |
| **Reliability** | [redacted] |
| **Acceptability** | [redacted] |
| **Remarks** | |

| | | | |
|---|---|---|---|
| **98/8 Doc IIIA section No.** | | **6.3.1 / 01** | **Repeated dose toxicity (oral)** |
| **91/414 Point addressed** | **Annex** | **II 5.3.1 / 01** | Short-term toxicity - oral 28-day studies |

| | |
|---|---|
| **1. Annex point(s)** | **IIA, 5.3.1 Short-term toxicity - Oral 28-day studies - dogs** |
| **2. Reference point (location) in dossier** | Volume 7, Section 3, Annex IIA, point 5.3.1 / 01 |
| **3. Authors (year) Title Owner, Date** | [redacted] CGA 293'343 tech. - 28-day range finding toxicity study in Beagle dogs. Syngenta Crop Protection AG, unpublished report No. 942106, June 19, 1996. |
| **4. Testing facility** | [redacted] |
| **5. Dates of work** | March 06, 1995 - April 02/03, 1995 |
| **6. Test substance** | ISO common name: Thiamethoxam; [redacted] |
| **7. Test method** | OECD 409 (adopted for the purpose of the test)<br>Deviations - The most important deviations were the duration of treatment (28 day instead of 90), the number of animals per sex and group (2 animals sex/group instead of 4), and the limited histopathological examination (no histopathology, except for nerve tissue, was performed). |
| **8. GLP** | No |

**Material and methods:** Thiamethoxam [redacted] was administered orally for 28 days to groups of 2 male and 2 female beagle dogs, by admixture in the diet, at concentrations of 0, 300, 1000 and 3000ppm. Mortality and clinical signs were checked daily, individual body weights and food consumption were recorded weekly. Haematology, clinical chemistry and urinalysis investigations, and ophthalmologic examinations were performed on all animals pre-dose and at the end of the treatment period. All animals were subjected to necropsy and *post mortem* examination, major organs were weighed and selected tissues examined histopathologically.

**Findings:** Preliminary diet analyses demonstrated the stability at room temperature of thiamethoxam in diet. Analysis of diets prepared for the study demonstrated mean achieved concentrations to be in the range 91.0 - 97.3% of nominal concentrations. Mean achieved dose levels were 0, 10.0, 31.6 and 47.7 mg/kg bw/day in males and 0, 10.7, 32.6 and 43.0 mg/kg bw/day in females, in order of rising diet concentration.

No deaths attributable to thiamethoxam occurred during the study although one male dosed at 3000ppm died on day 15 from intussusception of the small intestine. Clinical signs of an adverse effect of treatment at 3000ppm were restricted to occasional vomiting during week 1 in some animals. Marked body weight loss and reduced food consumption occurred in both sexes at 3000ppm. Ophthalmoscopic examinations revealed no evidence of ocular toxicity.

**Table: Mean body weight, body weight gain and food consumption**

| Dose level [ppm] | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 300 | 1000 | 3000 | 0 | 300 | 1000 | 3000 |
| **Body weight [kg]** | | | | | | | | |
| - week 0 | 8.400 | 9.850 | 9.700 | 9.100 | 9.150 | 9.400 | 9.650 | 10.400 |
| - week 4 | 9.350 | 10.400 | 10.350 | 7.600 | 9.800 | 9.650 | 10.000 | 8.700 |
| **Body weight gain [kg]** | 0.950 | 0.550 | 0.650 | -1.500 | 0.650 | 0.250 | 0.350 | -1.700 |
| Food consumption [kg] | | | | | | | | |
| - overall week 1-4 | 1.400 | 1.400 | 1.400 | 0.506 | 1.400 | 1.400 | 1.400 | 0.559 |
| % of control value | 0 | 0 | 0 | -63.86 | 0 | 0 | 0 | -60.07 |

Treatment-related effects on haematology and clinical chemistry occurred in both sexes at 3000ppm only. Haematological evaluation revealed leucopaenia in both sexes and slightly elevated red blood cell count, haemoglobin level and haematocrit value in the male survivor at 3000ppm. Plasma levels of urea and creatinine were slightly elevated compared with controls at 4 weeks and with pre-dose levels in females and the surviving male at 3000ppm. Creatinine levels were higher than controls in males at 300 and 1000ppm but pre-dose levels were also higher than controls and a treatment-related effect is not indicated. ALAT activities at 3000ppm were elevated in males and reduced in females. There were no treatment-related effects on urine physiological parameters or cellular and chemical constituents of urine.

**Table: Treatment-related haematology and clinical chemistry findings at week 4**

| Sex | Parameter/unit | 0ppm | 300ppm | 1000ppm | 3000ppm |
|---|---|---|---|---|---|
| **Haematology** | | | | | |
| Male | WBC [G/l] | 9.190 | 9.595 | 8.125 | 2.600 |
| | Neut [G/l] | 4.705 | 4.330 | 4.340 | 0.520 |
| | Eos [G/l] | 0.300 | 0.300 | 0.205 | 0.095 |
| | Lymph [G/l] | 3.535 | 4.380 | 3.150 | 1.955 |
| | RBC [T/l] | 6.150 | 6.560 | 5.960 | 7.465 |
| | Hb [mmol/l] | 8.300 | 9.100 | 8.300 | 9.950 |
| Female | WBC [G/l] | 12.20 | 10.28 | 10.72 | 6.853- |
| | Neut [G/l] | 6.865 | 5.985 | 5.645 | 3.648- |
| | Eos [G/l] | 0.305 | 0.170 | 0.300 | 0.728 |
| | Lymph [G/l] | 4.275 | 3.695 | 4.260 | 2.255 |
| | RBC [T/l] | 6.635 | 6.600 | 6.82 | 6.620 |
| | Hb [mmol/l] | 9.250 | 9.300 | 9.750 | 9.450 |
| **Blood Chemistry** | | | | | |
| Male | Urea [mmol/l] | 3.705 | 3.250 | 4.180 | 5.170 |
| | Creatinine [µm/l] | 67.70 | 78.85 | 78.00 | 75.30 |
| | ALAT [U/l] | 49.60 | 33.30 | 28.50- | 98.85 |
| Female | Urea [mmol/l] | 3.695 | 3.895 | 3.910 | 5.715 |
| | Creatinine [µm/l] | 77.70 | 70.45 | 75.30 | 93.75 |
| | ALAT [U/l] | 56.25 | 46.25 | 34.80- | 24.80- |

- negative trend (Jonckheere)

No treatment-related gross lesions were evident at necropsy, but effects on organ weights occurred at 3000ppm (Table below). Thymus weights and ratios were markedly reduced in both sexes. Lower absolute liver weights at 3000ppm are considered to be secondary to lower body weights. Non-dose-related differences in organ weights are considered incidental to treatment with thiamethoxam.

Treatment-related histopathological changes occurred at 3000ppm only (Table below). Minimal pigment accumulation in hepatic Kupffer cells, minimal to moderate atrophy of the marginal zone splenic white pulp and minimal to marked thymic atrophy occurred in all animals. The observed leucopaenia at 3000ppm can be correlated with the latter two histopathological findings, which may be a direct effect of thiamethoxam or secondary to marked body weight loss. Therefore, the aetiology of these histopathological findings is equivocal. All other histopathological observations showed no dose-relationship or occurred at similar incidence and severities in treated and control groups. No histological changes were detected in the brain or kidneys to correlate with observed clinical chemistry or organ weight changes.

**Table: Treatment-related organ weight changes**

| Parameter | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 300 | 1000 | 3000 | 0 | 300 | 1000 | 3000 |
| **Carcass** [kg] | 8.95 | 10.38 | 10.05 | 7.10 | 9.17 | 9.26 | 9.56 | 7.72 |
| **Liver** | | | | | | | | |
| - absolute[1] | 322.2 | 333.5 | 318.2 | 267.0 | 254.4 | 346.4 | 315.2 | 205.8 |
| - relative[2] | 3.603 | 3.217 | 3.168 - | 3.761 | 2.775 | 3.773 | 3.299 | 2.666 |
| **Thymus** | | | | | | | | |
| - absolute | 9.107 | 11.94 | 10.20 | 5.647 | 12.54 | 9.780 | 16.14 | 3.824 |
| - relative | 1.018 | 1.150 | 1.015 | 0.795 | 1.364 | 1.049 | 1.687 | 0.495 |

[1] organ weight in grams; [2] % body weight; - / + negative / positive trend (Jonckheere)

**Table: Treatment-related histopathological findings**

| Findings / Dose Level [ppm] | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 300 | 1000 | 3000 | 0 | 300 | 1000 | 3000 |
| Animals in study | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Liver Kupffer cells - pigmentation | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 2 |
| Splenic white pulp marginal zone - atrophy | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 2 |
| Thymus - atrophy | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 2 |

**Conclusion:** Thiamethoxam at 3000ppm diet provides daily dose levels that are clearly toxic, and the results are a suitable basis on which to establish dose levels for a 90-day study.

No-observed-effect-level (NOEL): 1000ppm in both sexes, equivalent to dose levels of 31.6 mg/kg bw/day (males) and 32.6mg/kg bw/day (females), based on the clear general systemic toxicity seen at 3000ppm.

## Evaluation by Competent Authorities

### EVALUATION BY RAPPORTEUR MEMBER STATE

**Date** March 2005

**Materials and Methods** [redacted]

**Results and discussion** [redacted]

**Conclusion** [redacted]

| | |
|---|---|
| | [redacted] |
| **Reliability** | [redacted] |
| **Acceptability** | [redacted] |
| **Remarks** | |

| | | | |
|---|---|---|---|
| **98/8 section No.** | **Doc IIIA** | **6.3.1 / 02** | **Repeated dose toxicity (oral)** |
| **91/414 Point addressed** | **Annex II** | **5.3.1 / 02** | Short-term toxicity - oral 28-day studies |

| | |
|---|---|
| **1. Annex point(s)** | **IIA, 5.3.1 Short-term toxicity - Oral 28-day studies - rats** |
| **2. Reference point (location) in dossier** | Volume 7, Section 3, Annex IIA, point 5.3.1 / 02 |
| **3. Authors (year) Title Owner, Date** | [redacted]<br>CGA 293'343 tech. - 28-days range finding study in rats (administration in food).<br>Syngenta Crop Protection AG, unpublished report No. 942088, May 05, 1995. |
| **4. Testing facility** | [redacted] |
| **5. Dates of work** | October 05, 1994 - November 03, 1994 |
| **6. Test substance** | ISO common name: Thiamethoxam,<br>[redacted] |
| **7. Test method** | OECD 407 (adopted for the purpose of the test)<br>Deviations - none |
| **8. GLP** | No (laboratory certified by the Eidgenössisches Departement des Inneren (Federal Department of Home Affairs), Bern, Switzerland) |

**Material and methods:** Thiamethoxam ([redacted] was administered orally for 28 days to groups of 5 male and 5 female Tif: RAIf (SPF) rats, by admixture in the diet, at concentrations of 0, 100, 1000, 2500 and 10000 ppm. Mortality and clinical signs were checked daily, individual body weights and food and water consumption were recorded weekly. Haematological, clinical chemistry and urinalysis investigations were performed on all animals towards the end of the treatment period. All animals were subjected to necropsy and *post mortem* examination, major organs were weighed and selected tissues examined histopathologically. In a separate investigation, measurement of replicative DNA synthesis was performed on liver samples from the male animals. The results of this separate investigation are presented in section 5.8.2(Supplementary studies on the active ingredient).

**Findings:** Preliminary analysis of diets demonstrated the stability and homogeneity of distribution of thiamethoxam. Analysis of test diets demonstrated achieved concentrations to be in the range 98 - 104% nominal concentrations. Mean achieved daily dose levels were 0, 8.0, 81.7, 198.6 and 710.6mg/kg bw in males and 0, 8.7, 89.3, 210.6 and 762.6mg/kg bw in females in order of rising diet concentration.

No deaths occurred and no clinical signs of a reaction to treatment were evident during the administration period. Body weight gain was reduced by 29% in males at 10000ppm but females at this level were not affected. Minimal retardation of weight gain occurred in males at 2500ppm. Food consumption was markedly reduced at 10000ppm in males throughout the treatment period but in females was only slightly reduced during the first week.

**Table: Body weight, body weight gain and food consumption**

| | Males | | | | | Females | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Dose level [ppm]** | **0** | **100** | **1000** | **2500** | **10000** | **0** | **100** | **1000** | **2500** | **10000** |
| **Body weight [g]:** | | | | | | | | | | |
| - week 1 | 218.6 | 217.5 | 220.0 | 214.6 | 187.5* | 168.4 | 171.3 | 174.8 | 175.8 | 159.5 |
| - week 4 | 325.0 | 327.2 | 329.2 | 315.1 | 262.7* | 211.0 | 228.7 | 235.5 | 226.1 | 201.0 |
| Body weight gain [g] | 106.4 | 109.7 | 109.2 | 100.5 | 75.2 | 42.6 | 57.4 | 60.7 | 50.3 | 41.5 |
| % gain (relative to control) | 0 | +3.1 | +2.6 | -5.5 | -29.3 | 0 | +34.7 | +42.9 | +18.1 | -2.6 |
| **Food consumption [g]:** | | | | | | | | | | |
| - week 1 | 135.8 | 136.9 | 138.7 | 132.0 | 82.8* | 98.9 | 108.6 | 110.1 | 114.2* | 78.5* |
| - week 4 | 152.0 | 158.8 | 158.3 | 152.8 | 138.1 | 116.1 | 131.1* | 133.1 | 120.8 | 111.0 |
| - overall week 1-4 | 592.8 | 594.7 | 609.7 | 582.0 | 457.5 | 416.8 | 476.2 | 496.6 | 468.9 | 394.2 |
| % (relative to control) | 0 | +0.3 | +2.9 | -1.8 | -22.8 | 0 | +14.3 | +19.1 | +12.5 | -5.4 |

* $p \leq 0.05$ (Wilcoxon)

There were no treatment-related effects on haematological profiles at dose levels up to and including 10000ppm (Table below). Isolated statistically significant differences from the controls, having no relationship to dose, are considered incidental to treatment. Slightly higher platelet counts at 10000ppm remained within the historical control range and are also considered to be of no toxicological significance. Cholesterol levels showed a dose-related increase in male groups treated at ≥1000ppm, and in females treated at 10000ppm. Other treatment-related effects were confined to 10000ppm and are considered to be minor sequelae to renal and hepatic morphological changes. Slight changes occurred in plasma urea levels (increased) and $Na^+$ concentrations (reduced) in both sexes, and in males ASAT activity was elevated and total protein and albumin levels were slightly decreased. All other clinical chemistry findings showed no relationship to dose level and are considered incidental to treatment with thiamethoxam. There were no effects on any urinary physiological parameters or on cellular and chemical constituents.

**Table: Treatment-related haematology and clinical chemistry findings**

| **Sex / parameter** | | **0 ppm** | **100 ppm** | **1000 ppm** | **2500 ppm** | **10000 ppm** |
|---|---|---|---|---|---|---|
| Males | Plt [$10^9$/l] | 997.8 | 1058 | 1056 | 1077 | 1228*+ |
| | Chol [mmol/l] | 1.696 | 2.064 | 2.074* | 2.144* | 2.432* |
| | ASAT[U/l] | 55.44 | 56.56 | 52.32 | 58.04 | 75.04*+ |
| | Alb [g/l] | 35.27 | 35.03 | 34.92 | 35.46 | 33.24* |
| | Urea [mmol/l] | 6.154 | 6.026 | 6.062 | 5.738 | 7.302 |
| | Na [mmol/l] | 141.8 | 141.3 | 140.3 | 141.3 | 139.4*+ |
| Females | Plt [$10^9$/l] | 1013 | 1026 | 985.0 | 1025 | 1195 |
| | Chol [mmol/l] | 2.064 | 1.986 | 1.994 | 2.216 | 3.503*+ |
| | ASAT [U/l] | 61.28 | 56.20 | 56.18 | 50.08 | 53.68 |
| | Alb [g/l] | 35.66 | 36.29 | 36.55 | 36.27 | 34.64 |
| | Urea [mmol/l] | 6.676 | 6.042 | 6.020 | 6.078 | 8.017* |
| | $Na^+$ [mmol/l] | 140.4 | 140.4 | 140.1 | 140.5 | 139.6* |

* $p \leq 0.05$ (Wilcoxon); + positive trend (Jonckheere)

Treatment-related effects on organ weights occurred in both males and females at ≥1000ppm (Table below). Liver weights were increased in both sexes at 10000ppm. Relative kidney weights were increased in males at 2500ppm, and in females at 1000 and 10000ppm. Reduced absolute adrenal and thymus weights in males at 10000ppm are