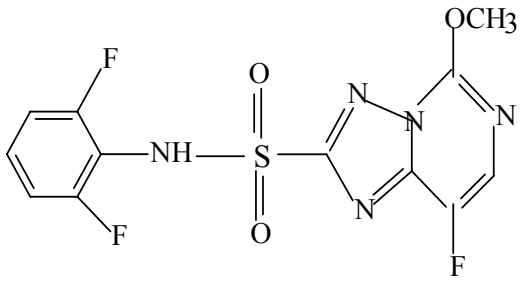
# ANNEX B

# Florasulam

# B.5 Toxicology and metabolism

**B.5.1 Absorption, distribution, excretion and metabolism (toxicokinetics) (Annex IIA 5.1)**




**Florasulam** is a member of triazolopyrimidine sulfonanilides, a class of herbicides known to inhibit the plant enzyme acetolactate synthase, also called acetohydroxyacid synthase, which is the key enzyme in the biosynthesis of the branched chained aminoacids isoleucine, leucine and valine.

Position of radiolabeling : radiolabeling with $^{14}C$ was on the aniline ring or on the triazolo-pyrimidine.

**Findings :**

**Systemic absorption :**
Kinetic parameters were not affected by labeling position and nearly identical for male and female rats.
According to the results reported in table B.5.1.-1, absorption of florasulam after oral administration is extensive and rapid : peak plasma concentrations were attained by 0.5 h and by 1 h post dosing with 10 or 500 mg/kg bw respectively. By 24 h post-dosing, plasma levels had declined in a biexponential manner (2-compartment model). When the dose increased, Cmax was not increased proportionaly to the administered dose and clearance was reduced suggesting a saturation of absorption and / or saturable renal excretion. Binding to plasmatic proteins was observed and was saturable as suggested by an increased volume of distribution (Table B.5.1-2) .
From the excretion data, absorption can be estimated to be high reaching 85 % after oral single or repeated low dose, within the first 24 h decreasing to 77% after high dose (Dryzga et al., 1996).
Absorption can also be extrapolated from the bile cannulated rats which received 10 mg/kg florasulam and were sacrificed after 24 h. From this study, absorption can be estimated to be near 91% of the dose. (Table B.5.1-8).

Dermal absorption :
Florasulam (log Kow = -1.2 (pH7), MW : 360), applied as either the undiluted formulation or as diluted spray solution has shown a low potential for dermal absorption in the rat : an average of 12 % (absorbed dose + treated skin) of the applied radiolabelled dose was absorbed. A value of 12 % for potentially dermal absorption of pure and diluted formulation will be used for calculation of operator exposure (Table B.5.1-3).
According to the notifier, an average of less than 0.5 % of the applied dose was absorbed. The proportion of the dose remaining in the skin beyond 24 h after skin swabbing is significantly more than what was absorbed and excreted, and did not decrease at the 48 and 72 h time points. Therefore, it is considered unlikely that the dose remaining in the skin would be absorbed; it would probably be removed with the natural epidermal turnover.

Comments from the RMS : 72 h after application of formulation, excretion has not come to an end, and it connot be excluded that the amount retained in the application site skin may eventually become systemically available as suggested by the increased urinary excretion observed at 48 and 72 h time points. Moreover, after oral administraiton of florasulam, it appeared from the ADME studies that some affinity for skin was observed. Therefore, the potentially absorbed dose (the amount systemically available plus the amount in the application site skin) should be used for estimation of dermal absorption.
An overage of 12 % (absorbed dose + treated skin) of the applied radio labelled dose was absorbed at 24 h.

**Table B.5.1-1 : Plasma pharmacokinetic parameters of florasulam in rats (Dryzga et al., 1996).**

| **Dose and labeled on** | **1 x 10 mg/kg bw aniline** | | **1 x 10 mg/kg bw pyridine** | **1 x 500 mg/kg bw aniline** | |
|---|---|---|---|---|---|
| Endpoints/sex | _ | _ | _ | _ | _ |
| Cmax $_{0\text{-}24\ h}$ (µg equi./g) | 23.7 | 21.94 | 20.04 | 580.67 | 405.72 |
| Tmax (h) | 0.5 | 0.5 | 0.5 | 1 | 0.5 |
| T1/2 (h)distr. | 0.6 | 0.6 | 0.4 | 1.1 | 1.2 |
| T1/2 (h)elim. | 9.8 | 7.6 | 8.1 | 5.1 | 4.5 |
| AUC (µg eq.h/g) | 26.8 | 26.3 | 23.6 | 3328 | 2523 |
| Volume of distribution (ml/kg) | 449 | 439 | 292 | 852 | 1435 |
| Clearance (ml/min.g) | 7.2 | 7.1 | 6.8 | 2.6 | 3.3 |
| Urinary excretion rate (h) | 3.4 | 3.6 | 3.4 | 4.8 | 4.9 |

**Table B.5.1-2 : Concentration of radioactivity in plasma/RBC as a function of time after oral administration of $^{14}$C labeled florasulam (Dryzga et al., 1996).**

| | **Radioactivity in RBC/plasma (µg equivalents to florasulam $^{14}$C labeled /g) (mean value)** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Dose (mg/kg bw) | 1 x 10 mg/kg bw | | | | 1 x 500 mg/kg bw | | | |
| Time (h) | _ | | _ | | _ | | _ | |
| Tissue | RBC $^{14}$C aniline /pyridine | plasma $^{14}$C aniline/ pyridine | RBC $^{14}$C aniline | plasma $^{14}$C aniline | RBC $^{14}$C aniline | plasma $^{14}$C aniline | RBC $^{14}$C aniline | plasma $^{14}$C aniline |
| 0.5 | 3.97/2.24 | 23.7/20.0 | 5.72 | 21.94 | 41.23 | 463.57 | 40.59 | 405.72 |
| 1 | 0.70/1.16 | 6.93/6.69 | 0.83 | 8.04 | 283.46 | 580.67 | 204.97 | 403.65 |
| 3 | 0.11/0.58 | 0.75/0.60 | 0.12 | 0.69 | 227.62 | 419.95 | 160.99 | 310.58 |
| 5 | 0.08/0.45 | 0.37/0.31 | 0.06 | 0.36 | 96.48 | 156.95 | 174.09 | 130.92 |
| 8 | 0.03/0.49 | 0.22/0.20 | 0.02 | 0.17 | 3.79 | 31.30 | 5.41 | 32.39 |
| 12 | 0.02/0.27 | 0.15/0.13 | NQ | 0.11 | 4.01 | 24.18 | 2.99 | 18.31 |
| 18 | NQ/0.18 | 0.10/0.08 | NQ/ | 0.06 | 1.10 | 8.21 | 1.46 | 6.88 |
| 24 | NQ/0.12 | 0.07/0.06 | NQ | 0.04 | NQ | 4.06 | NQ | 2.87 |
| 48 | NQ/0.04 | 0.03/0.03 | NQ | 0.02 | NQ | 1.30 | NQ | 1.00 |
| 72 | NQ | 0.02/0.02 | NQ | 0.01 | NQ | 0.80 | NQ | 0.77 |
| 168 | NQ | NQ/NQ | NQ | NQ | NQ | NQ | NQ | NQ |

NQ: not quantifiable.

**Table B.5.1-3 : Dermal absorption in rat skin of $^{14}$C labeled formulation of florasulam, after exposure under semi-occluded dressing (Bounds, 1997).**

| µg florasulam/cm² | time | Absorbed dose (urine, feces, cage wash, carcass, tissues, untreated skin) | | Treated skin | | Skin swab/gauze wash/tape strip | | Recovery |
|---|---|---|---|---|---|---|---|---|
| | h | % | µg | % | µg | % | µg | % |
| 1 | 24 | 0.18 | 0.02 | 12.2 | 1..3 | 87.9 | 9.3 | 100.3 |
| 1 | 48 | 0.26 | 0.03 | 21.8 | 23 | 79.8 | 8.4 | 101.9 |
| 1 | 72 | 0.29 | 0.03 | 20.9 | 2.2 | 79.1 | 8.3 | 100.3 |
| 530 | 24 | 0.39 | 20 | 11.1 | 700 | 90.7 | 5720 | 102.2 |
| 530 | 48 | 0.45 | 30 | 9.88 | 620 | 91.6 | 5780 | 102.0 |
| 530 | 72 | 0.13 | 10 | 10.0 | 630 | 92.6 | 5850 | 102.7 |

**Distribution:**

30 min after oral low dose administration, 44 - 61% of the radioactivity was distributed within organs involved in metabolism and excretion and in skin. Radioactivity decreased rapidly at 60 min, representing no more than 32-18% of the administered dose in male and female respectively.
Similar tissue distribution was observed after high dose administration and a higher total dose was reported reaching 82-76% of the dose in male and females, decreasing to 59-38% after 4 hour in male and female respectively (Table B.5.1- 4)(Hansen, 1997).

The average amount of radioactivity remaining in rats 168 h post-dosing was low for all dose groups and was < 0.01% of the dose or non-quantifiable in the majority of tissues. The greatest amount remained in the skin or in the carcass. No sex differences were reported (Table B.5.1.-5).
These data suggest a low potential for bioaccumulation of florasulam.

**Table B.5.1- 4 : Tissue distribution of labeled florasulam after single oral dosing. Sacrifice at Cmax and C1/2 max (Hansen, 1997)**

| | Recovered radioactivity (% of administered dose) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 10 mg/kg | | | | 500 mg/kg | | | |
| time of sacrifice(min) | 30 | 30 | 60 | 60 | 60 | 30 | 240 | 240 |
| | _ | _ | _ | _ | _ | _ | _ | _ |
| Blood | 3.57 | 2.85 | 1.34 | 0.8 | 4.26 | 3.22 | 1.86 | 0.97 |
| Carcass | 10.73 | 9.99 | 6.39 | 2.8 | 22 | 16.76 | 14.5 | 4.68 |
| GI tract/ingesta | 32.34 | 19.88 | 16.85 | 10.68 | 35.14 | 42.19 | 30.62 | 26.25 |
| Kidney | 3.42 | 2.75 | 1.33 | 0.68 | 1.05 | 1.42 | 0.74 | 0.51 |
| Liver | 2.71 | 1.46 | 1.20 | 0.46 | 5.01 | 2.67 | 2.45 | 0.71 |
| Skin | 7.16 | 5.92 | 4.13 | 2.35 | 12.3 | 8.07 | 7.78 | 4.48 |
| Total | 61 | 44.18 | 32 | 18.19 | 82 | 76.73 | 59 | 38 |

**Table B.5.1.-5 : Residual radioactivity in tissues 168 h after oral administration of florasulam in rats (Dryzga et al., 1996)**

| | Residual tissue radioactivity (% of administered dose) | | | |
|---|---|---|---|---|
| **Dose** | **1 x 500 mg/kg bw** | | **15 x 10 mg/kg bw** | |
| | _ | _ | _ | _ |
| Adrenals | NQ | NQ | <0.01 | <0.01 |
| Blood | <0.01 | NQ | <0.01 | <0.01 |
| Bone | NQ | NQ | <0.01 | <0.01 |
| Carcass | NQ | NQ | 0.1 | NQ |
| Duodenum | <0.01 | NQ | NQ | <0.01 |
| Fat | NQ | NQ | NQ | <0.01 |
| Gonads | <0.01 | - | NQ | <0.01 |
| Uterus | - | <0.01 | NQ | NQ |
| GI tract | NQ | NQ | <0.01 | <0.01 |
| Kidneys | <0.01 | <0.01 | <0.01 | <0.01 |
| Liver | <0.01 | NQ | <0.01 | <0.01 |
| Skin | 0.52 | 0.18 | NQ | NQ |
| Lymph nodes | <0.01 | <0.01 | <0.01 | <0.01 |
| Thymus | <0.01 | <0.01 | <0.01 | <0.01 |

NQ : not quantifiable

**Metabolism:**

Metabolites in the urine and feces revealed no evidence of hydrolysis of the sulfonamide bond.
Increasing the dose or repetitive administration resulted in no additional metabolites.
Urine from rats contained 3 radiolabeled peaks at amounts > 3% of the dose. The majority of urinary radioactivity was unchanged parent compound and 2 minor metabolites were OH-phenyl -florasulam and a sulfate conjugate of the OH-phenyl florasulam.
Feces contained 4 radiolabeled peaks. The two major peaks were unchanged parent compound and OH-phenyl-florasulam. The remaining two peaks were not identified but contained <0.32% of the administered dose (Dryzga et al., 1996) (table B..5.1-6)and (fig.B.5.1-1).
Tentatively identified parent florasulam was the major radioactive peak in HPLC profiles of the blood, kidney and liver.
HPLC profiles from liver and kidney extracts showed a total of 8 peaks in both tissues from which, the largest peak was suggested to be unchanged parent compound. Other peaks were not identified. In blood, 2 radioactive peaks were detected from which one corresponded to unchanged parent commpound. In bile, 9 peaks were detected from which one was the parent compound. No other peaks detected in bile represented greater than 0.03% of the administered dose.

**Table B.5.1.-6 :**Metabolism of 14-C labeled florasulam (Dryzga et al., 1996)

| | **Urinary and fecal reconstructed HPLC profile data as percent of administered radioactivity ($^{14}$ Caniline)** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | **Urine** (0-12 h) | | | **faeces** (0-24 h) | | | |
| Peak N° | | 1 | 2 | 3 | 1 | 2 | 3 | 4 |
| Identified as : | Sex | sulfate OH-phenyl-XR-570 | OH-phenyl XR-570 | parent XR-570 | not identified | not identified | OH-phenyl XR-570 | parent XR-570 |
| 1 x 10 mg/kg | _ | 2.77 | 4.96 | 74.94 | 0.18 | 0.14 | 2.23 | 2.72 |
| | _ | ND | 3.75 | 82.33 | ND | 0.14 | 0.42 | 2.66 |
| 15 x 10 mg/kg | _ | 3.43 | 5.59 | 75.37 | ND | ND | 2.11 | 2.71 |
| | _ | ND | 4.29 | 80.72 | ND | 0.25 | 0.59 | 3.61 |
| | | urine (0-24 h) | | | | | | |
| 1 x 500 mg/kg | _ | 3.62 | 2.17 | 71.13 | ND | 0.32 | 2.97 | 11.56 |
| | _ | ND | 2.30 | 74.49 | ND | 0.16 | 0.83 | 10.53 |

ND : not detectable

Excretion:

Within 7 days, a total of 96-99% of the administered radioactivity was recovered after a single oral dose.

The principal route of excretion was urine, which contained 89-92% of the radioactivity. Faecal excretion was 5-7%. The majority of urinary and fecal radioactivity was identified as unchanged florasulam with a urinary elimination half-life values of 3-4 h. (Table B.5.1.-7).

Increasing the dose by a factor of 50 reduced slightly urinary excretion to 81-85% with a concomitant increased faecal excretion to 14-17% of the dose. Elimination half-life value of 5 h was reported at this dose level (Table B.5.1.-1) (Dryzga et al., 1996).

Cmax was reached in bile, 2 h (0.37% of administered dose) after dosing. The bile contained 1% of the administered dose 24 h post-dosing (Hansen, 1997) suggesting that florasulam wass not submitted to an entero-hepatic circulation (Table B.5.1-8).

**Table B.5.1.-7 :Recovery of radioactivity in urines and faeces as a function of time after single low and high dose and after repeated low dose of $^{14}$C labeled florasulam (Dryzga et al., 1996).**

| | **Recovery (% of dose)** | | | | | | |
|---|---|---|---|---|---|---|---|
| **Labeling** | **$^{14}$C- aniline** | | | | | | **$^{14}$C-pyridine** |
| Dose | 1 x 10 mg/kg bw | | 1 x 500 mg/kg bw | | 15 x 10 mg/kg bw | | 1 x 10 mg/kg |
| | _ | _ | _ | _ | _ | _ | _ |
| Urine | | | | | | | |
| 0-12 h | 82.67 | 86.08 | 62.93 | 63.09 | 84.4 | 85.01 | 85.88 |
| 12-24 h | 2.62 | 2.72 | 13.99 | 13.70 | 4.46 | 2.89 | 2.07 |
| 24-36 h | 0.54 | 0.80 | 1.97 | 2.06 | 0.49 | 0.95 | 0.56 |
| 36-48 h | 0.39 | 0.35 | 0.55 | 1.30 | 0.26 | 0.36 | 0.33 |
| 48-72 h | 0.23 | 0.45 | 0.51 | 1.09 | 0.15 | 0.25 | 0.17 |
| 72-168 h | 3.74 | 1.25 | 1.52 | 3.38 | 0.69 | 0.66 | 0.47 |
| Total | 90.19 | 91.66 | 81.47 | 84.61 | 90.45 | 90.11 | 89.47 |
| Faeces | | | | | | | |
| 0-24 h | 5.26 | 3.22 | 14.85 | 11.51 | 4.82 | 4.46 | 5.88 |
| 24-48 h | 0.88 | 2.03 | 1.14 | 1.53 | 0.55 | 0.45 | 0.57 |
| 48-72 h | 0.12 | 0.22 | 0.21 | 0.32 | 0.13 | 0.05 | 0.17 |
| 72-168 h | 0.57 | 0.99 | 0.45 | 0.79 | 0.19 | 0.09 | 0.13 |
| Total | 6.83 | 6.47 | 16.65 | 14.15 | 6.47 | 5.27 | 6.74 |
| | | | | | | | |
| Tissues & carcass | 0.25 | 0.15 | 0.55 | 0.18 | 0.12 | 0.02 | 0.02 |
| Cage wash | 0.83 | 0.96 | 0.53 | 1.24 | 0.21 | 0.45 | 0.18 |
| Total | 99.11 | 99.23 | 99.20 | 100.18 | 97.26 | 95.85 | 96.40 |

**Table B.5.1-8 : Recovery of radioactivity in bile cannulated male rats dosed at 10 mg/kg florasulam, sacrificed 24 h later (Hansen, 1997)**

| **Tissues** | **% administered dose** |
|---|---|
| Faeces | 3.87 |
| Blood | 0.01 |
| Carcass | 0.46 |
| GI tract/ingesta | 5.14 |
| Skin | 2.67 |
| | |
| Tissues & carcass | 8.28 |
| Urine & rinse | 80.97 |
| Bile | 1.00 |

| | |
|---|---|
| Cage wash | 4.95 |
| Total recovery | 98.71 |

**Metabolism in plants and livestock:**

Florasulam is rapidly metabolized by winter wheat plant via hydroxylation in the 4-position of the phenyl ring followed by glucose conjugation (Pillar, 1997)

Lactating goats were given a daily oral dose of 11 mg/kg bw florasulam for 5 consecutive days. 99.8% of the recovered radioactivity was found in urine and faeces from which 82-85 % was in the urine. In the urine, the major component was unchanged florasulam and it can be assumed that sulphonamide bridge cleavage did not occur to any significant extent . Two unknown metabolites were observed in goat liver, kidneys and urine at very low levels and not further characterized (Barnekow, and Huskin, 1994).

When administered orally in the diet to laying hens at doses of 11 mg/kg bw/d, for 5 condecutive days, 99.9% of the dose was rapidly excreted from which 80-95% was parent compound and it can be assumed that sulphonamide bridge cleavage did not occur to any significant extent. One unknown metabolite was observed in hen skin and excreta at very low levels and not further characterized (Barnekow, and Huskin, 1994).

**Overall conclusions :**

After oral administration in rats, absorption of florasulam was rapid and extensive reaching 85-91% of the dose. Increasing the dose by a factor of 50 reduced somewhat the absorption to 77%.

After dermal application, a potentially absorbed dose of 12% was calculated.

30 minutes post-dosing, distribution was large and the amount of radioactivity was highest in gastrointestinal tract, carcass, skin and organs involved in metabolism and excretion e.g.liver and kidney. The average amount of radioactivity remaining in rats 168 h post-dosing was low and <0.01% of the dose. At that time, the greatest amount remained in the skin (0.18-0.52%).

Metabolism of florasulam was minor and limited to hydroxylation of the phenyl ring without affecting the sulfonamide bond.

Excretion reached 96-99% of the dose within 7 day mainly as unchanged florasulam. Urinary excretion was the major route and represented 77-89% of the dose within 24 h after administration while fecal excretion represented 6.5% of the dose. Increasing the dose reduced urinary excretion to 77% with a concomitant increase in fecal excretion to 16%.

In plants , laying hens and lactating goats, florasulam is rapidly and extensively excreted . Metabolism does not occur at a high rate and does not differ from rat.

**Material and methods of the studies:**

-XR-570 : Tissue distribution and metabolism of $^{14}C$-labeled XR-570 in Fischer 344 rats (Dryzga et al., 1996)

**Guidelines :**

Experimental protocol not fully in compliance with test method B, directive 87/302/EEC.

***Deviation from the official protocol :***

Distribution was limited to blood tissue.

**GLP status :** yes (no attest of competent autority).

**Material and methods:**

5 rats (Fischer 344)/group, received by gavage, a single dose of florasulam labeled on the aniline- $^{14}C$ (specific activity : 54.6mCi/mol = Gbq; 99% radiochemical purity) or on the 9th position of the triazolo-pyrimidine (specific activity = 24.2 mCi/mmole, 99% radiochemical purity). Non radiolabeled compound was 99.4% pure (lot n°. 930910) and doses of 10 or 500 mg/kg bw as aqueous suspension in Methocel cellulose ethers were administered.

A third group was given 14 daily oral doses of 10 mg/kg bw of non-radiolabeled compound followed by a single 10 mg/kg bw oral dose on labeled compound on day 15.

Blood was collected at 0.5, 1, 3, 5, 8, 12, 24, 48 and 168 h.

For excretion studies, rats were transferred to metabolic cages and urine and feces were collected for 168 hr.

*Metabolite analysis in urine, plasma or faeces:* identification of metabolites was performed using HPLC separation.

The study is accepted.

-Distribution and metabolism of $^{14}$ C- labeled florasulam in selected tissues at plasma Cmax and $C_{1/2max}$ and in bile following oral administration in Fischer rats 344 (Hansen, 1997)

**Guidelines :**

Experimental protocol in compliance with test method B, directive 87/302/EEC.

This study completes the informations provided in the previous study.

**GLP status : yes (no attest of competent autority).**

**Material and methods:**
Four test groups of 3 Fisher 344 rats/sex/dose received a single oral dose of florasulam uniformly labeled in the aniline ring (specific activity = 54.9 mCi/mmole ; radiochemical purity = 98.5%) of 10 or 500 mg/k as an aqueous suspension in Methocel cellulose ethers (0.5%). Non radiolabeled florasulam was reported to be 99.2% pure. Animals were sacrificed at 30 min (10 mg/kg, group 1&2), 30 and 60 min (500 mg/kg) and groups 3 and 4 sacrificed at 60 min and 4 h.
A fifth group consisted of 3 male rats fitted with indwelling bile-duct cannulas were dosed at 10 mg/kg ; bile and urine were collected 24 h post-dosing. Group 5 was sacrificed at 24 h.
The study is accepted.

- Dermal absorption of $^{14}$C-florasulam in male Fischer 344 rats following exposure to undiluted EF-1343 and a spray solution (Bounds, 1997)

**Guidelines :**
Experimental protocol in compliance with test method B, directive 87/302/EEC.
**GLP status :** yes (no attest of competent autority).
**Material and methods:**
Six groups of 4 Fisher 344 male rats received a single topical application of undiluted florasulam and a spray solution uniformly labeled in the aniline ring (specific activity = 54.6 mCi/mmole ; radiochemical purity = 99%) ; 120 µl was applied to a clipped skin which was semi-occluded for 24 h.Non radiolabeled florasulam was reported to be 99.9% pure.
Rats were killed after 24, 48 or 72 h.
Dose preparations : formulation EF-1343, used as supplied and a dilution of EF-1343 (89.7 mg florasulam/l), that was representative of concentrations that may be applied to field crops. The target spray solution used in this study was therefore 2.39 times more concentrated than the anticipated highest spray concentration that will be used on field crops.
Use rate for EF-1343 : 0.15 l/ha/200l of water giving a spray concentration = 37.5 mg/l

Achieved dose : low dose : 0.0009 mg/cm$^2$
high dose : 0.53 mg/cm$^2$
The study is accepted.

**Figure 1 : Metabolic pathway of Florasulam in rats**

**Figure 2 : Metabolic pathway of Florasulam in winter cereals**

**Figure 3 : Metabolic pathway in lactating goat and laying hen**

**B.5.2 Acute toxicity including irritancy and skin sensitization (Annex IIA 5.2)**

**B.5.2.1 Acute oral toxicity (Annex IIA 5.2.1)**

- Rat, 1000, 3000 or 6000 mg/kg bw (Gilbert and Yano, 1995)

**Findings :**
*Mortality :* at 6000 mg/kg bw mortality occurred for 1 male (day 7) and 2 females (day 2 and day 7).
*Body weight :* transient decrease observed day 1 and day 2 after dosing.
*Clinical observations* : salivation, urine and fecal soiling in the perineal area were observed at 1000 mg/kg bw onwards. Symptoms occurred within 5 hours after dosing and continued through test day 3.
*Ne cropsy :* in died animals, congestion of viscera was observed. In rats surviving the observation period, there were no compound-related lesions.

**Conclusions :**
$LD_{50}$ male >5000 mg/kg bw
$LD_{50}$ female>5000 mg/kg bw
$LD_{50}$ combined >5000 mg/kg bw.

**Guidelines :**
Experimental protocol not fully in compliance with method B.1 Annex V of Directive 84/449/EEC or OECD 401.
*Deviation from official protocol* : exact value of $LD_{50}$ not determined. The compound is administered as a 30 or 50% suspension : it is not clearly explained which suspension is used .
**GLP :** Yes (no attest of competent autority)
**Material and Methods :**
5 F344 rats/sex/group received a single oral dose by gavage of florasulam (B.n°.TSN100298 ; 99.2%) as a 30 or 50% suspension in 0.5% Methocel at1000, 3000 or 6000 mg/kg bw.
The study is accepted.

- Mice, 600, 2000 or 5000 mg/kg bw (Brooks, 1997)

**Findings :**
*Mortality :* at 5000 mg/kg bw , all male mice survived for 14 days after dosing and 2 females were found dead on test day 2.
*Body weight :* not affected by treatment.
*Clinical observations* : at 5000 mg/kg bw, 1 male mouse had urine soiling of perianal region on dosing day.
*Necropsy :* there were no treatment-related gross pathologic observations.

**Conclusions :**
Florasulam is slightly more toxic for females than for males.
$LD_{50}$ male >5000 mg/kg bw
$LD_{50}$ female>5000mg/kg bw
$LD_{50}$ combined >5000 mg/kg bw.

**Guidelines :**
Experimental protocol not fully in compliance with method B.1 Annex V of Directive 84/449/EEC or OECD 401.
*Deviation from official protocol* : exact value of $LD_{50}$ not determined.Variable volumes were used at the different dose levels.Animals were not fasted prior to dosing.
GLP : Yes (no attest of competent autority)
**Material and Methods :**
5 CD mice/sex/group received a single oral dose of florasulam, by gavage (b.n° XDE-570 ; 99.3%) as a 50% suspensiopn in Methocel A4M at 600, 2000 or 5000 mg/kg bw.
The study is accepted.

**B.5.2.2 Acute percutaneous toxicity (Annex IIA 5.2.2)**

- Rabbit, 2000 mg/kg bw , semi-occluded dressing for 24 h. (Gilbert, 1995a)

**Findings :**
*Mortality:* all animals survived.

*Clinical signs* : no signs of systemic toxicity were observed.
*Dermal observations:* on day 2, edema and erythema were observed in all animals. Edema was resolved in all animals by test day 3 and erythema was resolved in all animals by test day 10.
Male recovered completely on day 4, while females recovered completely day 9.
*Body weight*: not altered.
*Necropsy findings*: all tissues were examined and were within normal limits.

**Conclusions :**
$LD_{50}$ male > 2000mg/kg bw
$LD_{50}$ female > 2000 mg/kg bw
$LD_{50}$ combined > 2000mg/kg bw

**Guidelines:**
Experimental protocol in compliance with method B.3 Annex V of Directive 92/69/EEC.Limit test.
**GLP :** Yes (no attest of competent autority)
**Material and Methods :**
5 New Zealand white rabbits/sex were dermally exposed to florasulam (B.n°XDE-570; 99.2%) moistened with distilled water at 2000 mg/kg bw, applied on approximately 10% of the surface area, under semi-occluded dressing for 24 h.
The study is accepted.

### B.5.2.3 Acute inhalation toxicity (Annex IIA 5.2.3)

-Rat, inhalation, aerosol, by nose-only exposure, 5 mg/l, 4 hour (Clements and Cieszlak, 1995)

Findings :
*Mortality* : all animals survived the 4-hour exposure and the 14 day post-exposure observation period.
*Clinical signs:* no particular clinical signs were observed.
*Body weight:* on the day following the exposure, a mean body weight loss of 2.4% for males and 1.5% for females was noted.
*Necropsy:* all tissues were examined and were within normal limits.

Conclusions :
4-hour $LC_{50}$ > 5 mg/l
$$\left(\frac{5 \times 7\,l \times 1000\ mg/kg\ bw \times 4\ hours}{333} > 420\ mg/kg\ bw\right)$$

**Guidelines :**
Protocol in compliance with method B.2, Annex V of directive 92/69/EEC. Limit test at 0.5 mg/l.
**GLP :**yes (no attest of competent authority)
**Material and Methods :**
5 F344 strain rats/sex were exposed nose only in an inhalation chamber to a 5mg/l florasulam (B.n°. TSN100511, lot 940714; 99.3%)aerosol.
The MMAD was 4.07µm and was within 2% of the 1-4 µm targeted range for respirable atmosphere. Approximatively 5% of the total mass was less than 1 micron in size and 72% was less than 6 microns in size.
The study is accepted.

### B.5.2.4 Skin irritation (Annex IIA 5.2.4)

- 6 Rabbit, semi- occlusive dressing, 500 mg/kg bw, 4 hours (Gilbert, 1995b)

**Findings**
There was a slight irritation at the skin of 1 animal during the observation periods which recovered on day 8.
There was no changes in animals body weights.
Evaluation of the data according to the EU methodology, gave the following results:

< Score erythema > 24+48+72 h = 0.166
< Score oedema > 24+48+72 h = 0

**Conclusions :**

Florasulam is not a skin irritant.

**Guidelines :**
Protocol in compliance with method B.6 of directive 92/69/EEC or OECD guideline 404(1981-92).
**GLP :** Yes (no attest of competent autority)
**Material and Methods :**
6 male New Zealand white rabbits were exposed to 500 mg of florasulam wetted with 0.2 ml distilled water (B.n°.XDE570, TSN100298 ; 99.2%) under semi-occluded dressing for 4 hours.
The study is accepted.

### B.5.2.5 Eye Irritation (Annex IIA 5.2.5)

- Rabbit eyes, 100 mg (Gilbert, 1995c)

**Findings :**
Evaluation of the data, according to the EU methodology, gave the following results for the unrinsed eyes of 6 rabbits :

< Score cornea opacity > 24+48+72h = 0
< Score iris > 24+48+72 h =0
< Score redness > 24+48+/72h =0
< Score chemosis > 24+48+72 h=0

**Conclusions :**
Florasulam is not irritating to eyes.

**Guidelines :**
Protocol in compliance with method B.5 of Directive 92/69/EEC.
**GLP :** yes (no attest of competent autority)
**Material and Methods :**
100 mg (B.n°.XDE570, TSN100298 ; 99.2%) was placed into the right eye of each of 3male and 3 female New Zealand white rabbits .
The study is accepted.

### B.5.2.6 Skin sensitization (Annex IIA 5.2.6)

- Guinea pig, Maximization test (Johnson, 1996)

**Findings :**
*Clinical signs*: no signs of ill health or toxicity were observed.
*Body weight* were not altered.
*Skin sensitization*:
Induction: after intradermal injections, necrosis was recorded at sites receiving Freund's Complete Adjuvant in test and control animals. Slight irritation was seen in animals at site receiving florasulam and in control animals at sites receiving Alembicol D.
After topical application, slight erythema was observed in control and treated animals at the site of application. However, after challenge, there were no dermal reactions seen in any of the 20 test and 10 control animals.

**Conclusions :**
Florasulam is not a sensitizer.

**Guidelines :**
Protocol in compliance with method B.6, Annex V, Directive 96/54/EEC and method B.6, Annex V, Directive 84/449/EEC (Maximisation test)
GLP : yes (no attest of competent autority)
**Material and Methods :**
10 Albino guinea pigs (Dunkin-Hartley)/sex were exposed to Florasulam (B.n°.XDE570, TSN100298 ; 99.2%)
In the control group 5 animals/sex were used.
Preliminary study indicated that 1% w/v Florasulam in Alembicol D was the maximum injectable concentration. For topical

induction administration, the maximum practicable concentration was a 100% solution ; for intradermal induction, 1% solution was used, and 100 and 50% in Alembicol D for topical challenge. The study is accepted.

- Guinea pig, Buehler test (Gilbert, 1995d)

**Findings :**
*Clinical signs*: no signs of ill health or toxicity were observed.
*Body weight* were not altered.
*Skin sensitization*: florasulam did not cause delayed contact hypersensitivity in guinea pigs.

**Conclusions :**
Florasulam is not a sensitizer.

**Guidelines :**
Protocol in compliance with method B.6, Annex V, Directive 96/54/EEC and method B.6, Annex V, Directive 84/449/EEC (Buehler test)
**GLP :** yes (no attest of competent autority)
**Material and Methods :**
10 male Albino guinea pigs (Hartley) were exposed to Florasulam (B.n°.XDE570, TSN100298 ; 99.2%)
In the control group 5 animals were used.
Preliminary study indicated that 0.4 g of neat Florasulam was the maximum non-irritating dose level. In the induction phase, 0.4g florasulam moistened with 0.2 ml distilled water was applied to the left side. Challenge,was performed 2 weeks later with 0.4 g florasulam moistened with 0.2 ml distilled water .
The study is accepted.

**B.5.2.7 Summary of acute toxicity including irritancy and skin sensitization (Annex IIA 5.2)**

In rat and mice, acute oral toxicity of florasulam was low. However, some death occurred in rats, at 6000 mg/kg bw and at 5000 mg/kg bw in mice, females being more sensitive than males. Transient clinical signs such as salivation, urine and fecal soiling in the perineal area suggest toxic effects towards the autonomic (parasympathomimetic) system. Local signs such as erythema and edema which completely resolved on day 10 were observed after dermal application. Female rabbits were also more sensitive than males.
Florasulam is not a skin and eye irritant, it is not a sensitizer and is not classified.

**Table B.5.2.7-1 : Summary of acute toxicity of florasulam.**

| Type of test | Batch n°, purity | Results | Classification | References |
|---|---|---|---|---|
| Acute oral, rat | B.n°.TSN100298 ; 99.2% | $LD_{50}$ combined > 5000 mg/kg bw | - | Gilbert and Yano, 1995 |
| Acute oral, mice | b.n° XDE-570 ; 99.3% | $LD_{50}$ combined > 5000 mg/kg bw | - | Brooks, 1997 |
| Rabbit, dermal, semi-occluded | B.n°XDE-570; 99.2% | $LD_{50}$ > 2000 mg/kg bw | - | Gilbert, 1995a |
| Rat, aerosol, nose only inhalation, 4 hours | B.n°. TSN100511, lot 940714; 99.3% | $LC_{50}$ > 5 mg/l (= 420 mg/kg bw) | - | Clements and Cieszlak, 1995 |
| Rabbit, skin irritation | B.n°.XDE570, TSN100298 ; 99.2% | Not irritant | - | Gilbert, 1995b |
| Rabbit, eye irritation | B.n°.XDE570, TSN100298 ; 99.2% | Not irritant | - | Gilbert, 1995c |
| Maximisation test | B.n°.XDE570, TSN100298 ; 99.2% | Not sensitizer | - | Johnson, 1996 |
| Buehler test | B.n°.XDE570, TSN100298 ; 99.2% | Not sensitizer | - | Gilbert, 1995d |

**B.5.3 Short-term toxicity (Annex IIA 5.3)**

**B.5.3.1 Oral 14-day toxicity (Annex IIA 5.3.1)**

-Rats, diet, at 100, 500 or 1000 mg/kg bw/d ,14 days (Szabo and Davis, 1993)

**Findings :**

Main findings are described in Table B.5.3.1-1. The MTD was reached.
There were no deaths and no particular clinical signs .
*Bodyweight* was reduced and probably related to a lower food consumption, as a result of minor degree of unpalatibility .
*Hematology:* hematocrit (PCV) and hemoglobin were significantly reduced but were within the range for normalcy for the Fisher 344 rats.
*Clinical chemistry*: the reported decrease in AP may result from fasting of animals because the intestinal isoenzyme is an important component of serum enzyme activity.
*Organ weight and histopathological examination* :
Altered kidney weights , observed at top dose for male and female rats , were accompanied by an increased incidence of degeneration/regeneration of renal tubules in males at 1000 mg/kg bw and in females at 500 and 1000 mg/kg bw/d. Nuclear pleomorphism (karyomegaly and anisokaryocytosis) was present in male and females at 500 and 1000 mg/kg bw/d. Additionally, animals had multifocal necrosis of proximal tubule epithelial cells.
Increased liver weight was not accompanied by any histopathological change .

**Table B.5.3.1-1 : 28 days subacute study in rats.**

| **Endpoints/dose (mg/kg bw/d)** | **0** | | **100** | | **500** | | **1000** | |
|---|---|---|---|---|---|---|---|---|
| | _ | _ | _ | _ | _ | _ | _ | _ |
| Body weight | | | | | | | ↘7% | ↘10% |
| Food cons. | | | | | | | ↘8% | ↘6% |
| Hematology: | | | | | | | | |
| PCV | | | | | | | ↘4% | ↘2% |
| Hb | | | | | | | ↘3% | ↘2% |
| RBC | | | | | | | ↘4% | ↘1% |
| Clinical chemistry : | | | | | | | | |
| AP | | | | | | | ↘19% | |
| Urinalysis: | | | | | | | | |
| pH | | | | | | | ↘ | |
| Relative organ weights : | | | | | | | | |
| kidneys | | | | | | | ↗12.5 % | ↗11% |
| liver | | | ↗4% | | ↗4% | | ↗1.4% | ↗5.1% |
| Microscopic examination of kidneys : nbre affected rats | | | | | | | | |
| pleomorphism, nuclear proximal tubule, diffuse | | | | | 2/5 | 5/5 | 5/5 | 5/5 |
| necrosis | | | | | | | 1/5 | 4/5 |
| statistically significant for equality of variance using Bartlett's test and ANOVA test | | | | | | | | |

**Conclusions :**
NOAEL = 100 mg/kg bw/d

**Guidelines :**
Experimental protocol not fully in compliance with test method B.7, Annex V, of Directive 92/69/EEC.
***Deviation from official protocol :*** animals were observed once daily for morbidity and mortality. Weight of adrenals, thymus, epididymes and spleen were not taken. Histopathology was limited to liver and kidneys.
GLP : yes (no attest of competent authority)
**Material and Methods :**
5 F344 rats/sex/dose, received in the diet florasulam (B.n°.AGR 291939, 92.2 %)) at 0, 100, 500 or 1000mg/kg bw/d, seven times per week, 2 weeks.
The study is accepted.

-Mice, diet , 100, 500, 1000 mg/kg bw, 14 days (Svabo and Davis, 1992)

**Findings :**
*Clinical signs and mortality* : no systemic treatment-related effects and no mortality were noted during the study. While male food consumption was increased (25%, 19%, 26%), body weights were not affected. At the contrary, females which strongly reduced their food consumption (40-50%) for each dose, had their bodyweight marginally decreased (6% at the top dose). This small body weight reduction observed in females correlated with a non significant reduction of liver and kidney weight without histopathological lesions.

**Conclusion:**
NOAEL= 1000 mg/kg bw/d

**Guidelines :**
Experimental protocol not fully in compliance with test method B.7, Annex V, of Directive 92/69/EEC, 84/449/EEC or OECD guideline 407(1981-1995).
*Deviation from official protocol :* animals were observed once daily for morbidity and mortality. Water consumption was not measured. Weight of thymus, adrenals, epididymes and spleen were not taken. Histopathology was limited to liver and kidneys.Hematology was not measured in all animals, for technical reasons.
Statistical analysis was performed but the significance of the results is not reported in the tables.
**GLP :** yes (no attest of competent authority)
**Material and Methods :**
5 B6C3F1 mice /sex/dose, received in the diet florasulam (B.n°.AGR 291939, 92.2 %) at 0, 100, 500 or 1000mg/kg bw/d, for 2 weeks.
The study is accepted.

- Dog, diet, 0, 50, 150 or 450 mg/kg bw/d, 4 weeks (Sullivan and Cronin Singleton, 1995a)

**Findings:**
*A preliminary study* was performed in order to assess palatibility (Dalgard, 1995).
Food consumption at a target level of 1000 mg/kg bw/d was not sufficient to sustain life. At target levels of 550, 500 and 450 mg/kg bw/d, body weight declined steadily. Food consumption showed a modest increase at 550 mg/kg bw/d but declined steadily at 500 and 450 mg/kg bw/d. Target levels of 250 and 350mg/kg bw/d resulted in a compound consumption value of 290 mg/kg bw/d.
*Main study:*
At the high dose level, actual food consumption was reduced to 56% of the target and male and females were therefore exposed at 250mg/kg bw/d.
*Clinical signs* at the top dose were yellowing of mucous membranes, transient absence of fecal output or diarrhea and vomiting (once time).
*Ophtalmoscopic examination* revealed no abnormalities.
*Body weight* was decreased but not at the same extent as food consumption reduction .
*Hematological parameters* were not affected.
*Clinical chemistry* : the effects are compatible with dose dependent hepatocellular injury and cholestasis, and are supported by histopathologic lesions in the liver at doses $\geq$ 150 mg/kg bw/d. Livers had bile duct hyperplasia and one male had also bile stasis and hepatocellular necrosis (Table B.5.3.1-2).

**Table B.5.3.1-2 : 28 days subacute study in dogs.**

| **Endpoints/dose** | **0** | | **50 mg/kg bw/d** | | **150 mg/kg bw/d** | | **250 mg/kg bw/d** | |
|---|---|---|---|---|---|---|---|---|
| | _ | _ | _ | _ | _ | _ | _ | _ |
| Body weight (mean value) | | | ↘11% | ↗3% | ↘10% | ↘6% | ↘24% | ↘18% |
| Food cons. (mean value) | | | | ↘11% | ↘28% | ↘5% | ↘80% | ↘50% |
| Clinical chemistry:2 dogs /sex | | | | | | | | |
| AP | | | ↗2 | ↗1 | ↗2 | ↗2 | ↗2 | ↗2 |
| AST | | | | | | | ↗1 | ↗1 |
| ALT | | | | | | | ↗1 | ↗1 |
| Bilirubin, cholesterol, glucose | | | | | | | ↗1 | |
| Albumin | | | | | ↘2 | ↘1 | ↘2 | ↘2 |
| Organ weight (relative)mean 2 dogs | | | | | | | | |
| kidneys | | | | ↘7% | ↗11% | ↘4% | ↘23% | ↗6% |
| liver | | | ↗14% | | ↗31% | ↗12% | ↗16% | |
| Histopathology: 2 dogs /sex | | | | | | | | |
| Liver: bile duct hyperplasia | | | | | 1 | | 1 | 2 |
| necrosis | | | | | | | 1 | |
| inflammation | | | | | | | 1 | |
| Kidneys: | | | | | | | | |
| tubules vacuolation | | | | | | | | 1 |

**Conclusion:**
NOAEL < 50 mg/kg bw/d

**Guidelines :**
Experimental protocol not fully in compliance with test method B.7, Annex V, of Directive 92/69/EEC, 84/449/EEC or OECD guideline 407(1981-1995).
*Deviation from official protocol :* Water consumption was not measured.. Histopathology was not performed for brain, bone marrow, trachea, prostate, urinary bladder, lymphe nodes,and peripheral nerves.Only liver and kidneys were weighed.
**GLP :** yes (no attest of competent authority)
**Material and Methods :**
*Preliminary study*: 1 Beagle dog /sex/dose, received in the diet florasulam (XDE-570, lot n° 930910; 99.2 %) at a target level of 1000 mg/kg bw/d. Since the dogs refused to eat the diet in quantities that were adequate to maintain their body weights, they were placed on normal diet and used for a subsequent trial 4 weeks later.The following doses were given : 250 mg/first week, increasing to 350 mg for 2 weeks; another group received 550mg/kg bw/d for 35 days, , a group received 450 mg/kg bw for 2 weeks and one group received 500 mg/kg bw for 4 weeks.
*Main study* : 2 Beagle dog /sex/dose, received in the diet florasulam (TSN 100511, lot n° 940714; 99.3 %) at a target level of 0, 50, 150 or 450 mg/kg bw/d for 4 weeks.
Corrected doses for males : 0, 60, 148, 267 mg/kg bw/d
for females : 0, 50, 161, and 238 mg/kg bw/d.
The study is accepted.

**B.5.3.2.1 Oral 90-day toxicity (rat) (Annex IIA 5.3.2)**

- Rats (F344) , diet, 1000, 3000, or 6000 ppm, for 13 weeks followed by a reversibility period of 4 week (Redmond and Johnson, 1996)

**Findings:**

The main findings, affecting more specifically male rats of the top dose are described in Table B.5.3.2.1-1. The MTD was reached.

*Hematology* : Anemia was evidenced by the change in red blood cell parameters in male rats given 1000mg/kg bw/d, correlated to histopathologic observations of slight decreased extramedullary hematopoieisis in the spleen and was attributed to florasulam. Reduced WBC count may be consecutive to restricted food consumption.

*Clinical chemistry* : Reduced AP, total protein and triglycerides may have been secondary to decreased body weight and therefore considered of no toxicological significance. Although a slight hypercholesterolemia may suggest hepatotoxicity , liver microscopic lesions were not in evidence. Glycemia was increased .

Increased $K^+$ and lower phosphorus were within historical control range.

*Ophtalmology :* no treatment-related effects.

*Urinalysis*: urine pH reflect excretory processes rather than a pathologic effect. Effects on gravity was compound related.

*Histopathology*:

Renal lesions differed between sexes :

Collecting ducts : the lesion was characterized by hypertrophy of cells (inner stripe of outer zone of medulla). A dose-responsive manner was observed for males. Females appeared to be affected to a lesser degree than males. Urine acidification suggest that the affected cell type was the intercalated cells. Cellular enlargement was characterized by electron microscopy as an increased number and size of mitochondria.

Descending proximal tubules (outer stripe of outer zone of medulla) : necrosis and/or degeneration of tubular epithelium with a variable regenerative response, varying widely among rats. This effect appeared typical of acute to subacute necrosis with regeneration rather than a 13 week old lesion.

Tubules of the papilla : Small foci of mineralized debris, probably due to a dystrophic mineralization of tubular debris from the tubular lesions were also observed in female rats .

Atrophy of adipose tissue at the top dose correlated with the grossly noted decrease of adipose tissue as well as the lower body weights.

*Recovery period*: all effects observed during the subchronic study lessened or were absent at the end of the 4-week recovery period (Table B.5.3.2.1-2).

**Table B.5.3.2.1-1 : 90 days toxicity study in F344 rats with florasulam .**

| **Endpoints/dose** | **0** | | **20** | | **100** | | **500** | | **800(_) , /1000(_) mg/kg bw/d** | |
|---|---|---|---|---|---|---|---|---|---|---|
| | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ |
| Mortality | | | no mortality | | | | | | | |
| Clinical signs | | | | | | | | | perineal soiling | |
| Body weight | | | ↘5% | | | | | ↘ 6% | ↘17% | ↘12% |
| Food cons.(not significant) | | | (↘ 4%) | | | | | (↘ 12%) | (↘ 9%) | (↘ 8%) |
| B W gain | | | | | | | ↘ | ↘ | ↘ | ↘ |
| Ophthalmology | no changes related to treatment | | | | | | | | | |
| Hematology: | | | | | | | | | | |
| Hematocrit | | | | | | | ↘3% | | ↘7% | |
| Hb | | | | | | | ↘2.5% | | ↘6% | |
| RBC | | | | | ↘2% | | ↘4% | | ↘8% | |
| Total WBC | | | | | | | | | | ↘28% |
| Clinical chemistry: | | | | | | | | | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| AP | | | | | | | | | ↘22% | |
| total proteins | | | | | | | | | ↘4% | |
| triglycerides | | | | | | | | | ↘20% | |
| glucose | | | | | | | | | | ↘16% |
| cholesterol | | | | | | | | | ↗15% | |
| electrolytes | | | | | | | | | ↗11% K+ | ↗12% P |
| Urinalysis: pH | | | | | | | ↘ | ↘ | ↘ | ↘ |
| specific gravity | | | | | | | | | ↘ | |
| Organ weight: relative | | | | | | | | | | |
| heart | | | | | | | | | ↗11% | |
| kidney | | | | | | | ↗15% | ↗17% | ↗37% | ↗30% |
| Histopathology : nb affected animals | | | | | | | | | | |
| kidneys : | | | | | | | | | | |
| Collecting ducts hypertrophy | | | | | | | | | | |
| very slight | | | | | | | 10 | 8 | 3 | 6 |
| slight | | | | | | | | | 7 | 3 |
| Papilla : mineralization: v slight | | | | | | | | | | 9 |
| Proximal tubules:necrosis with degeneration /regeneration | | | | | | | | | | |
| | | | | | | | | 3 | | 5 |
| Cortical tubules: dege/regen. V slight | 7 | 1 | 7 | 2 | 4 | 0 | 5 | 4 | 3 | 3 |
| Spleen: reduced extramedullary hematopoieisis | | | | | | | | | | |
| | | | | | | | | | 6 | |
| Mesenteric tissue : atrophy, adipose tissue | | | | | | | | | | |
| | | | | | | | | | 7 | 8 |
| Statistically significant intergroup difference↗ or ↘ : p <0.05 : according to Bartlett's test , parametric Anova, non parametric Anova, Dunnett's test, Wilcoxon rank-sum test, Outlier test | | | | | | | | | | |

Table B.5.3.2.1-2: Main effects after 4 week of the reversibility period

| Endpoints/dose | 0 | | 800/1000 mg/kg bw/d | |
|---|---|---|---|---|
| Sex | _ | _ | _ | _ |
| Body weight | | | ↘11% | (↘4%) |
| B W gain | | | ↘ | (↘) |
| Hematology | | | recovery | recovery |
| electrolytes | | | ↗4% K+ | |
| Organ weight : relative | | | | |
| heart | | | ↗4% | ↗5% |
| kidneys | | | ↗11% | ↗15% |
| liver | | | | ↗10% |
| Histopathology: | | | | |

| | | | | |
|---|---|---|---|---|
| kidneys collecting ducts hypertrophy: | | | recovery | |
| Cortical tubules | | | | increased incidence very slight degeneration/regeneration |
| proximal tubules necrosis with regen. | | | recovery | |
| renal papilla | | | | mineralized debris |
| mesenteric tissue: atrophy adipose tissue | | | 1 | 4 |
| spleen reduced hematopoieisis | | | | present but resolving |
| Statistically significant intergroup difference↗ or ↘ ; or not significant () was assessed by Dunnett's test | | | | |

**Conclusions :**
NOAEL = 100 mg/kg bw/d.

**Guidelines :**
Experimental protocol not fully in compliance with test method B, annex V, directive 87/302/EEC .
*Deviation to official protocol :* adrenals were not weighed.
**GLP status:** yes (no attest of competent authority).
**Material and methods:**
10 rats (F344 strain)/sex/dose were exposed to (TSN 100298, lot n° 930910; 99.2 %) in diet at 0, 20, 100, 500 or 800 (female) 1000 (male) mg/kg bw/d for 90 days. A 4 -week recovery period for the high-dose groups (10 rats/sex/dose + control group) was included.

Daily dose intake for males: 22, 112, 550, 1111 mg/kg bw/d.
For females : 21, 106, 528, 843 mg/kg bw/d.
The study is accepted.

- Rats (CD) , diet, 100, 500 or 1000 mg/kg bw/d, for 13 weeks (Liberacki, et al., 1996)

**Findings:**
The MTD was reached.
Decreased food consumption and body weights, increased relative kidney weights , renal histopathologic changes, and incidence of clinical signs, observed at 1000 mg/kg bw/d were consistent with the data generated in F344 rats previously reported.
*Kidney histopathology* : hypertrophied collecting duct cells (inner stripe of outer zone of medulla) in response to florasulam was higher than in F344 rats. Proximal convoluted tubules presented a tubular degeneration/regeneration (not observed in F344 rats) along with tubular necrosis (females) and papillary necrosis, centered at the corticomedullary junction especially males. Papillary necrosis with secondary hyperplasia of transitional epithelium of papilla was noted in males at top dose, varying in severity in rats dying spontaneously and was likely the cause of death (Table B.5.3.2.1-3).

No effects were observed at 100 mg/kg bw/d.

**Table B.5.3.2.1-3 : 90 days toxicity study in CD rats with florasulam .**

| **Endpoints/dose** | **0** | | **100** | | **500** | | **1000 mg/kg bw/d** | |
|---|---|---|---|---|---|---|---|---|
| | _ | _ | _ | _ | _ | _ | _ | _ |
| Mortality | | | | | | | 3 | |
| Clinical signs: perineal and/or facial soiling | | | | | | | all animals | |
| Thin, rough haircoat Reddish urine | | | | | 1 | 1 | only males | |
| Body weight | | | | | | | ↘41% | ↘25% |
| Food cons. | | | | | ↘5 | ↘8% | ↘33% | ↘39% |
| Organ weight: relative | | | | | | | | |
| Kidney: (not significant) | | | | | (↗7%) | (↗5%) | ↗72% | ↗36% |
| Histopathology of kidney : nbre affected animals | | | | | | | | |
| Pelvic epithelium : hyperplasia | | | | | 1/10 | 0/10 | 7/7 | 1/10 |
| Collecting ducts : hypertrophy | | | | | | | | |
| very slight | 0/10 | 0/10 | 0/10 | 0/10 | 6/10 | 9/10 | 0/7 | 3/10 |
| slight | 0/10 | 0/10 | 0/10 | 0/10 | 4/10 | 1/10 | 3/7 | 4/10 |
| moderate | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 | 7/10 | 3/10 |
| Papilla :necrosis | | | | | | | 5/7 | 1/10 |
| papilla, mineralization | 0/10 | 2/10 | 0/10 | 3/10 | 0/10 | 2/110 | 0/7 | 2/10 |
| Proximal convoluted tubules : degeneration/regeneration | | | | | | | | |
| Very slight | 8/10 | 2/10 | 8/10 | 2/10 | 7/10 | 2/10 | 0/7 | 1/10 |
| slight | 0/10 | 0/10 | 1/10 | 0/10 | 1/10 | 0/10 | 7/7 | 4/10 |
| moderate | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 | 3/10 | 0/10 |
| Proximal convoluted tubules necrosis | | | | | | | 0/7 | 3/10 |
| statistically significant intergroup difference(↗ or ↘ : $p < 0.05$) according to Dunnett's test, Wilcoxon rank-sum test | | | | | | | | |

**Conclusions :**
NOAEL = 100 mg/kg bw/d.

**Guidelines :**
Experimental protocol not fully in compliance with test method B, annex V, directive 87/302/EEC .
*Deviation to official protocol :* hematology, clinical chemistry, urinalysis and ophtalmology not performed ; organ weight and histopathology limited to kidneys. This study was designed to establish dose levels for the multigeneration reproduction study.
**GLP status:** yes (no attest of competent authority).
**Material and methods:** 10 (CD strain) rats/sex/dose were exposed to florasulam (lot n°.940714, 99.3%) in the diet at 0, 100, 500, 1000/570 mg/kg bw/day for 90 days.
The study is accepted.

- Mice, diet, 0, 20, 100 , 500 or 1000 mg/kg bw/d, 13 week (Redmond and Johnson, 1996)

**Findings:**
*Mortality and clinical signs* : one female mouse given 20 mg/kg bw/d died on day 7 and no clinical signs suggest a compound-related effect. There were no treatment-related observations during the study suggestive of systemic

toxicity at any dose level.
*Body weight and food consumption* of male and female mice were not significantly altered. No treatment-related changes in cumulative body weight gain were observed at any dose level.
Normal hematological parameters were observed.
*Clinical chemistry* : blood urea nitrogen (↘15%) was reduced for male mice at 500 and 1000 mg/kg bw/d and cholesterol for mice at 500 mg/kg bw/d was also reduced. These decreases were not toxicologically significant.
*Urinalysis* was not performed.
*Organ weight*: there were no statistically identified differences of absolute or relative organ weights from control mean.
*Histopathologic examination* of kidneys showed a very slight, bilateral multifocal hypertrophy of epithelial cells of collecting ducts in all male mice at 500 and 1000 mg/kg bw/d and in 8/10 females at 1000 mg/kg bw/d. Morphologically, this is essentially the same lesion reported in the Fischer 344 rat study. These cells had granular pale eosinophilic cytoplasm.This renal change is probably an adaptative response. The hypertrophied cells are compatible with intercalated cells.

**Conclusion:**
NOAEL = 100 mg/kg bw/d

**Guidelines**: experimental protocol not fully in compliance with test method B, annex V, directive 87/302/EEC or OECD guideline 408 (1981).
*Deviation from official protocol* : adrenals were not weighed**.**
**GLP status**: yes (no attest of competent authority)
**Material and methods:**
10 B6C3F1 mice/sex/dose received florasulam (TSN 100298, lot 930910 ; 99.2%) in the diet at 0, 20 , 100, 500 or 1000 gm/kg bw/d for 13 weeks.
Achieved intake :
Male: 0, 22, 110, 549, 1129 mg/kg bw/d
female :0, 20, 101, 503, 1007 mg/kg bw/d
The study is accepted.

### B.5.3.2.2 Oral 90-day toxicity (dog) (Annex IIA 5.3.2)

- Dogs, 3 months, 0, 200, 2000, 3300 ppm in diet (Sullivan and Singleton, 1995b)

**Findings:**
Main findings are described in table B.5.3.2.2-1.
Treatment-related effects were increased AP activity, likely of hepatic origin, in males and females given > 50mg/kg bw/d. Increased liver weight was observed in both sexes at the top dose. Slight hepatic vacuolation was observed at 50 and 100 mg/kg bw/d.
*Kidney histopathologic analysis* showed a slight hypertrophy of epithelial cells lining renal collecting ducts , in animals given 50 or 100 mg/kg bw/d. The effect was consistent with an intercalated cells hypertrophy. They were not associated with any cellular degeneration or necrosis, and there was no progression in the severity . There were no significant changes in urinary parameters .

**Table B.5.3.2.2.-1 : 90 day dog study with florasulam.**

| **Endpoint/dose** | **0** | | **5** | | **50** | | **100 mg/kg bw/d** | |
|---|---|---|---|---|---|---|---|---|
| | _ | _ | _ | _ | _ | _ | _ | _ |
| Mortality | | | No mortality | | | | | |
| Clinical signs: | | | Occasional diarrhea and vomiting | | | | | |
| Body weight (not statistically significant) | | | | | | | | (↘6%) |
| Ophtalmoscopy | | | No effect | | | | | |
| Hematology | No treatment related effects | | | | | | | |
| Clinical chemistry | | | | | | | | |
| AP | | | | | ↗209% | ↗226% | ↗548% | ↗340% |
| A/G ratio | | | | | | | ↘16% | ↘13% |
| Urinalysis | | | No treatment related effects | | | | | |
| Organ weight: relative | | | | | | | | |
| liver | | | | | | | ↗26% | ↗26% |
| Histopathology : nbre affected animals | | | | | | | | |
| Kidney : hypertrophy epithelial cells , collecting ducts : | | | | | | | | |
| very slight | 4 | 0 | 3 | 0 | 2 | 1 | 0 | 3 |
| slight | 0 | 0 | 0 | 1* | 2 | 3 | 4 | 3 |
| Liver : slight vacuolation | 1 | 0 | 0 | 0 | 1 | 2 | 3 | 1 |
| statistical analysis : significant ↗ or ↘ (a< 0.05) Bonferroni corrected P-value ;<br>* this dog had congenital absence of 1 kidney | | | | | | | | |

**Conclusion:**
NOAEL = 5 mg/kg bw/d, not taking into account the kidney effects observed in one dog which had a congenital defect, and the very slight degree of hypertrophy seen as a spontaneous change in this species.

**Guidelines:** Experimental protocol in compliance with test method B, Annex V, of directive 87/302/EEC or OECD guideline 409 (1981)
**GLP status:** yes (no attest of competent authority)
**Material and methods:**
4 Beagle dog/dose/sex received florasulam in diet (TSN 100511, lot n°. 940714 ; 99.3%) at 0, 5, 50 or 100 mg/kg bw/day for 90 days.
Converted dose for males : 6, 56, 104 mg/kg bw/dThe study is accepted.
For females : 5, 55, 94 mg/kg bw/d

**B.5.3.2.3 Oral 1 year toxicity (dog) (Annex IIA 5.3.2)**

- Dogs, diet, 0, 0.5, 5 or 100/50 mg/kg bw/d for 1 year (Stebbins and Haut, 1997)

**Findings:**
The high dose concentration was reduced from 100 to 50 mg/kg bw/d on test day 105, due to treatment related decrease in body weight and food consumption in the high dose animals.
Main findings are described in table B.5.3.2.3-1.
Hematology and clinical chemistry parameters were measured after 90 day and 1 year.
*Hematology :*
After 90 day, at 100 mg/kg bw/d, red blood cell parameters were affected essentially for males (↘RBC, hemoglobin, hematocrit) suggesting anemia ; platelet count was increased. In females, a slight macrocytosis was also observed, affecting 2 females, and 1 female had slight anisocytosis and hypochromasia.

These parameters were again evaluated on day 123 and were more strongly affected. An improvement was noted by 6 months and parameters were comparable to controls at the end of the 12 month period.
*Clinical chemistry* : Serum ALT and AP were strongly increased, and after reduction of the dose, AP remained significantly elevated while ALT returned to normal values. Albumin, total proteins (not significantly) were reduced. All these effects , observed in male and/or female dogs , are probably related to hepatocellular injury not associated to a strong organ hypertrophy or abnormal histopathology. Hypocholesterolemia at top dose, not reaching statistical significance, may result from fasting.
*Organ weights* were not significantly altered.
*Histopathology* : kidneys of 3 dogs from the high dose group had very slight to slight hypertrophy of individual epithelial cells of the collecting ducts in the inner and outer stripe of the outer zone of the medulla (above the very slight degree of hypertrophy seen as spontaneous change in this species). The hypertrophic intercalated cells seen in the high dose animals represent a slight exacerbation of a spontaneous lesion and are consistent with intercalated cells. They were not associated with any cellular degeneration or necrosis, and there was no progression in the severity of the alteration at comparable dose levels from 13 weeks to one year.

A treatment-related effect of adrenals, characterized as a slight vacuolation of the *zona reticularis* and *zona fasciculata,* was present in animals of the high dose group. It was not associated with inflammation, necrosis or clinical chemistry changes. The vacuolation was consistent with fatty changes. This effect was considered of uncertain toxicological importance.

**Table B.5.3.2.3-1: 1 year toxicity study of florasulam in dogs.**

| **Endpoint/dose** | **0** | | **0.5** | | **5** | | **100/50*** | |
|---|---|---|---|---|---|---|---|---|
| | _ | _ | _ | _ | _ | _ | _ | _ |
| Mortality | | | No mortality | | | | | |
| Clinical signs: | | | | | | | thin appearance | |
| Body weight : day 105 | | | | | | | ↘10% | ↘15% |
| : end of 1 year | | | | | | | normal | ↘16% |
| Food consumption | | | | | | | ↘ 17% day 1-120 | ↘25% day 1-120 |
| Hematology: | | | | | | | data 90 day/ 1 year | |
| WBC | | | | | | | ↗40%/↗17% | -/↘10% |
| RBC | | | | | | | ↘14%/↘9% | ↘5%/↗6% |
| Hb | | | | | | | ↘15%/↘10% | ↘5%/↗10% |
| Hematocrit | | | | | | | ↘14%/↘9% | -/↗12% |
| Platelet count | | | | | | | ↗35%/↗18% | ↗33%/↗6% |
| MCV | | | | | | | -/ - | -/↗5% |
| Ophtalmoscopy | | | No effect | | | | | |
| Urinalysis | No treatment related effects | | | | | | | |
| Clinical chemistry | | | | | | | | |
| AP | | | | | | | ↗277%/↗241% | ↗263%/↗453% |
| ALT | | | | | | | ↗390% - | ↗269%/ - |
| Albumin | | | | | | | ↘15%/↘15% | ↘25%/↘8% |
| | | | | | | | ↘13%/↘10% | ↘10%/↘4% |
| Urinalysis | | | No treatment related effects | | | | | |
| Organ weight: | | | no statistically significant ↗ of relative liver weight (_ : 7% _: 16%) | | | | | |
| Histopathology : nbre affected animals | | | | | | | | |
| kidney hypertrophy epithelial cells collecting ducts | | | | | | | | |

| **Endpoint/dose** | **0** | | **0.5** | | **5** | | **100/50*** | |
|---|---|---|---|---|---|---|---|---|
| | _ | _ | _ | _ | _ | _ | _ | _ |
| very slight | 2 | 2 | 2 | 0 | 2 | 0 | 1 | 1 |
| slight | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Adrenal glands : slight vacuolation | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 |
| Statistical analysis : Barlett's test , Dunnett's test , analysis of variance<br>- : no effect<br>* : day 1-105, 100 mg/kg bw/d; day 106-365, 50 mg/kg bw/d | | | | | | | | |

**Conclusion :** NOAEL = NOEL = 5 mg/kg bw/d.

**Guidelines:** Experimental protocol not fully in compliance with test method B, Annex V, directive 87/302/EEC or OECD guideline 409 (1981).
***Deviation from official protocol*** : gamma Gt and ornithine decarboxylase was not measured; urinary analysis was not performed at 3 months. Urinary volume was not measured.
**GLP status:** yes (no attest of competent authority)
**Material and methods:**
4 Beagle dog/dose/sex received florasulam in the diet (TSN 100511; lot n°. 940714, 99.3%) at 0,0.5,5 or 100 mg/kg bw/d for 52 weeks.On test day 105, for reasons of animal welfare, due to significant decreased body weight, it was necessary to reduce the high dose to 50 mg/kg bw/d.
Time-weighed average dose-leves over the coarse of the study in these animals was : 0, 0.5, 5.0, and 70 mg/kg bw/d.
The study is accepted.

**B.5.3.3.1 28-day inhalation toxicity (rat) (Annex IIA 5.3.3)**

In the acute inhalation toxicity studies of florasulam there was no mortality even when they were treated at the highest dose required by the guidelines. The vapour pressure of florasulam is very low as 1 x $10^{-5}$ Pa at 25°C and therefore a short-term inhalation study is not required.

**B.5.3.3.2 90-day inhalation toxicity (rat) (Annex IIA 5.3.3)**

No data, not necessary.

**B.5.3.3.3 Percutaneous 28-day toxicity (rat) (Annex IIA 5.3.3)**

- Rat, dermal doses of 100, 500 or 1000 mg/kg bw/d, 28 day (Scorrichini and Kociba, 1997)

**Findings:**
Dermal application of florasulam resulted in no treatment-related systemic effects in male and female rats. Very slight, transient dermal irritation(day 23) at the application site was observed only in male rats receiving applications of 1000 mg/kg bw /d.
Some hematological parameters reached statistical differences (WBC↗32% for males ; for female :Hb↘ 4%, HTC↘ 8% and ↘ 8% RBC at 100 mg/kg bw/d and ↘ 5% Hb at 1000mg/kg bw/d)) and they were considered representative of normal variation, and in the absence of any clear cut dose response pattern or histopathological correlate, were considered unrelated to treatment with florasulam .Clinical chemistry and electrolytes were normal. Urinalysis was normal. Altered adrenal weight (↗ 6% at top dose) was not associated with histopathological changes and therefore not considered related to treatment.
Kidney histopathology did not reveal any treatment related effect.

**Conclusion:**
NOAEL syst = 1000 mg/kg bw/d

NOAEL irritation = 500 mg/kg bw.

**Guidelines:** Experimental protocol in compliance with test method B9 Annex V, directive 92/69/EEC or OECD guideline 410 (1981).
**GLP status:** yes (no attest of competent authority)
**Material and methods:**
5 F344 rats/dose/sex received dermal dose of florasulam as a suspension in aqueous 0.5% Methocel (TSN 100511; lot n°. 940714, 99.3%) at 0, 100, 500 or 1000 mg/kg bw/d for 6 hours. Animals were sacrificed on day 29.
The study is accepted.

### B.5.3.3.4 Percutaneous 90-day toxicity (rat) (Annex IIA 5.3.3)

No data, not necessary.

### B.5.3.4 Summary of short-term toxicity (Annex IIA 5.3)

In rat and mice, oral administration of florasulam induced a decreased food intake, probably related to food unpalatibility, and a reduced body weight.
Target organs were kidneys in which a minor functional impact was observed. Kidney proximal tubule epithelial cells presented nuclear pleomorphism and multifocal cell necrosis. These effects were noted, after 2-3 week exposure, at 500 mg/kg bw/d onwards.
In dogs, hepatocellular injury and cholestasis were reported at 150 mg/kg bw/d onwards. Some kidney tubule vacuolation was seen.

After 13 week oral exposure of rats, at 500 mg/kg bw/d onwards, different parts of the nefron were target of florasulam:
- renal collecting ducts (probably Type A intercalated cells) were hypertrophied , in male and females F344 and CD rats, severity was higher in CD rats.
- descending proximal tubules necrosis with degeneration/regeneration of a higher portion of the nephron, in females CD and F344 rats. This effect is typical of acute necrosis with regeneration rather than a 13 week old rat lesion.
- In the tubules of papilla, small foci of mineralized debris were reported in females F344 rats, while in CD rats, papillary necrosis with secondary hyperplasia was observed..

While intercalated cell hypertrophy and urine acidification were reversible, mineralization in papillae and degeneration/regeneration in cortical tubules did not recover in female F344 high dose group.

Bilateral multifocal hypertrophy of epithelial cells of collecting ducts was also observed in mice kidneys at a very slight degree suggesting probably an adaptative response. This lesion was morphologically the same as that reported in Fisher 344 rats. Anemia and decreased extramedullary hematopoiesis in the spleen were also evidenced.
For these studies, a NOAEL = 100 mg/kg bw/d is acceptable.

Dogs were the most sensitive species. Anemia was observed and increased serum enzymes suggested hepatotoxicity at 50 mg/kg bw/d. Renal hypertrophy and adrenal vacuolation were also evident. The hypertrophic intercalated cells, seen in dogs, seems to represent a slight exacerbation of a spontaneous lesion, as this effect was not associated with any cellular degeneration or necrosis. In dogs, the lowest NOAEL was 5 mg/kg bw/d.

The NOAEL, LOAEL and relevant toxic effects for florasulam are given in Table B.5.3.4-1. From these repeated dose, subchronic experiments, a reference NOAEL of 5 mg/kg bw/d can be derived for short-term repetitive exposures.

**Table B.5.3.4-1 : Summary of short term-toxicity of florasulam**

| Type of test | Compound and test substance purity | Results | | | References |
|---|---|---|---|---|---|
| | | NOAEL (mg/kg bw /day) | LOAEL (mg/kg bw/day) | Critical endpoints | |
| Rat, oral, 14 day | B.n°.AGR 291939, 92.2 % | 100 | 500 | renal tubules alterations : nuclear pleomorphism | Szabo and Davis, 1993 |
| Mice, oral, 14 day | B.n°.AGR 291939, 92.2 | 1000 | - | small body weight reduction | Svabo and Davis, 1992 |
| Dog, oral, 28 day | TSN 100511, lot n° 940714; 99.3 % | <50 | 50 | ↗AP,↘ body weight | Sullivan and Cronin Singleton, 1995a |
| Rat, F344 oral, 90 day | lot n° 930910; 99.2 % | 100 | 500 | renal collecting duct hypertrophy ↘ body weight, altered urinary parametes, anemia | Redmond and Johnson, 1996 |
| Rat, CD oral, 90 day | lot n° 940714; 99.3 % | 100 | 500 | renal collecting duct hypertrophy | Liberacki, et al., 1996 |
| Mice, oral, 90 day | lot 930910 ; 99.2% | 100 | 500 | renal collecting duct hypertrophy | Redmond and Johnson, 1996 |
| Dog, oral, 90 day | lot n°. 940714 ; 99.3% | 5 | 50 | kidney : slight hypertrophy of collecting duct cells, slight liver vacuolation ; ↗AP | Sullivan and Singleton, 1995b |
| Dog, oral, 1 year | TSN 100511; lot n°. 940714, 99.3% | 5 | 50 | ↗AP ; kidney : slight hypertrophy of collecting duct cells ; adrenal vacuolation ; anemia | Stebbins and Haut, 1997 |
| Rabbit, dermal , 28 day | TSN 100511; lot n°. 940714, 99.3% | 1000 | - | - | Scorrichini and Kociba, 1997 |

**B.5.4 Genotoxicity (Annex IIA 5.4)**

**B.5.4.1 In vitro genotoxicity testing (Annex IIA 5.4.1)**

**B.5.4.1.1 In vitro genotoxicity testing in bacterial cells.**

**- *S. typhimurium* strains (0.333; 1.0; 3.33; 10.0; 33.3; 100 µg/plate) and *E. coli* -WP2uvrA (10.0; 33.3; 100; 333;1000; 3330 µg/plate) with and without S9 (Lawlor, 1995)**

**Findings:**
In both original and confirmatory experiment, florasulam did not cause increases in the number of revertants with any of the tester strain either with or without S9.
Toxicity appeared at about 33.3 µg/plate (*S. typhimurium*) and about 3330 µg/plate (*E.coli*).

**Conclusion:**
Florasulam is not mutagenic in these experimental conditions.

**Guidelines:Protocol** not fully in compliance with test method B.14 of directive 92/69/EEC
*Deviations from protocol:* no untreated control and no uvrB check
**GLP status:**The study is GLP
**Materials and methods:**
Three tests were performed (range-finding, original and confirmatory). 0.1ml overnight grown bacteria were exposed to Florasulam (99.2%; B.n.930910) at final concentrations of 0; 10.0; 33.3; 100; 333;1000; 3330 _g/plate ±S9 (E.coli WP2uvrA), and 0; 0.333; 1.00; 3.33; 10.0; 33.3; 100 _g/plate ±S9 (S. typhimurium TA100, TA1535, TA98, TA1537) in preincubation (20 min.) followed by incubation during 48h. Post-mitochondrial supernatant was obtained from Aroclor1254-induced _ Sprague Dawley rat, and used for preparation of metabolic activation mixture (10% S9 v:v).
Positive control was obtained by treating with appropriate reference mutagens as a function of strain and exogeneous activation [2-aminoanthracene for each strain (+S9); sodium azide for TA100 and TA1535, 4-nitroquinoline-N-oxide for WP2uvrA, 2-nitrofluorene for TA98, and ICR-191 for TA1537 (-S9)].
Negative control was obtained by treating with the vehicle (DMSO). Florasulam dosage was checked analytically. Criterium for determining positive response: if compound caused a 3-fold increase of spontaneous revertants, in a dose-responsive and reproducible way.
The study is accepted.

**B.5.4.1.2 Gene mutation test in mammalian cells (Annex IIA 5.4.1)**

- HPRT assay in CHO cells at 187.5; 375.0; 750.0; 1500; 3000 mg/ml (±S9) (Linscombe, 1995)

**Findings:**
In a preliminary toxicity assay, florasulam was assayed at concentrations ranging from 23.4 to 3000 µg/ml. The highest concentration was based upon solubility limitations. No cytotoxicity was observed in the absence of S9. In the presence of S9, a slight toxicity was noted at 750, 1500 and 3000 µg/ml (table 5.4.1.2-1).

No effect of the compound on the cloning efficiency was observed. An increased mutant frequency was observed in experiment 1 at the highest dose, but this was not confirmed in the second experiment.

**Table 5.4.1.2-1: HPRT assay with florasulam (data mean values of replicates)**

| Dose (µg/ml) | Relative cell survival in % [(a)] | | cloning efficiency in % [(b)] | | mutant frequency/$10^6$ survivors | |
|---|---|---|---|---|---|---|
| | exp1 | exp2 | exp1 | exp2 | exp1 | exp2 |
| -S9 mix | | | | | | |
| DMSO | 100.0 | 100.0 | 55.2 | 74.0 | 4.3 | 2.0 |
| 187.5 | 125.0 | 116.9 | 49.1 | 78.4 | 6.7 | 3.8 |
| 375.0 | 110.3 | 110.2 | 50.7 | 59.4 | 5.5 | 3.0 |
| 750.0 | 106.5 | 96.1 | n.a. | 64.8 | n.a. | 3.5 |
| 1500.0 | 129.6 | 102.2 | 67.8 * | 62.3 | 8.1 * | 1.6 |
| 3000.0 | 128.8 | 96.8 | 53.3 | 63.0 | 10.0 | 1.2 |
| pos. control | 39.0 | 50.4 | 24.5 * | 27.1 | 449 * | 106.2 |
| +S9 mix | | | | | | |
| DMSO | 100.0 | 100.0 | 55.5 | 72.4 | 3.8 | 0.3 |
| 187.5 | 102.5 | 106.2 | 60.2 | 72 | 6.3 | 3.4 |
| 375.0 | 85.8 | 106.2 | 64.7 | 67.2 | 3.9 | 1.1 |
| 750.0 | 96.2 | 101.2 | 58.8 | 64.3 | 0.9 | 5.8 |
| 1500.0 | 94.3 | 86.4 | 56.9 | 65.8 | 3.6 | 2.0 |
| 3000.0 | 16.0** | 98.4 | 51.7** | 61.4 | 11.7** | 1.3 |
| pos. control | 70.9 | 94.2 | 50.0 | 50.0 | 82.7 | 60.0 |

n.a. inadequate number of cells at the first subculture;
*: only one replica present (insufficient cells); **: precipitation of the test compound in medium, but cleared upon sonication.

**Conclusion:**
Florasulam is not mutagenic in these experimental conditions.

**Guidelines:** Protocol partly in compliance with test method B.17 of directive 92/69/EEC
*Deviations from protocol:* concentration-related toxic effect is not observed
**GLP status:**The study is GLP.
**Materials and methods:**
CHO cells (CHO-K1-BH4) were incubated in the presence of Florasulam (99.2%, B.n°: TSN100298, dissolved in DMSO, 1% end-concentration) at dose-levels of 0;187.5; 375; 750; 1500; 3000 µg/ml (±S9). S9 was obtained from Aroclor1254-induced _ Sprague Dawley rat.
Positive controls: ethyl methane sulfonate at the dose-level of 621 µg/ml (-S9) or 3-methylcholanthrene at the dose-level of 4 µg/ml (+S9). Negative control : vehicle. HGPRT+ cells were treated for 4h, and grown for 6-8 days in medium with 6-thioguanine in order to express induced mutants. Cloning efficiency was assessed in medium without hypoxanthine (7-9d growth). Mutant clones were expressed relative to total viable clones. The spontaneous mutant frequencies in the solvent-treated controls were within the historical control data range, and also the positive controls gave the expected response. Criterium for determining positive response: if compound caused a statistically significant, dose-responsive, reproducible increase of mutation frequency.
The study is accepted.

### B.5.4.1.3. In vitro chromosome aberration assay (Annex IIA 5.4.1)

-Rat lymphocytes ,30; 100; 300 µg/ml (-S9); 300; 1000; 3000 µg/ml (+S9) (Linscombe, 1995)

**Findings:**
In the first experiment, mitotic index dropped by about 30% at 300 µg/ml in absence of S9. In presence of S9, no cytotoxicity was observed (precipitation of substance limitating factor at 3000 µg/ml).The relative number of metaphases with chromosomal aberrations was not increased by florasulam, both in presence or in absence of the metabolic activation. The corresponding vehicle controls had aberration incidences well within those of the historical

control values, while positive controls responded as expected.

**Conclusion:**

Florasulam is not mutagenic in these experimental conditions.

**Guidelines:**

Protocol in compliance with test method B.10 of directive 92/69/EEC .

**GLP status:** The study is GLP.

**Materials and methods:**

Whole blood of _ Sprague Dawley rats was set up RPMI 1640 medium (+10% heat-inactivated FBS) 48h before treatment. Florasulam (99.2%, B.n°: TSN100298) dissolved in DMSO (final conc. 1% v:v), was added at dose-levels of 0.0; 30; 100; 300 µg/ml (-S9) and 0.0; 300; 1000; 3000 µg/ml (+S9). Compound concentration was checked analytically. S9 was obtained from Aroclor1254-induced _ Sprague Dawley rat. Positive control : Mitomycin C (MMC) at the dose-level of 0.05 or 0.075 µg/ml (-S9) or cyclophosphamide (CP) at the dose-level of 6.0 µg/ml (+S9). Negative control :vehicle (DMSO). Exposure time was 4h (+S9), followed by 20h (assay1) or 44h (assay2) incubation without flurazulam, or 24h (-S9) followed by immediate harvest (assay1) or 24h incubation without flurazulam (assay2, only vehicle control and top dose).About 3h before harvest, cells were treated with colcemid (0.2µg/ml). Cultures were terminated by hypotonic shock followed by fixation (methanol:acetic acid, 3:1). Mitotic Index (M.I.) was determined (on at least 1000 cells/concentration/culture), as wel as structural and numerical aberrations (on at least 100 cells/concentration/culture).Two independent experiments were set up, with duplicate cultures. Criterium for determining positive response: if compound caused a statistically significant ($\chi^2$,$p<0.01$), dose-responsive reproducible increase of mutation frequency. The study is accepted.

### B.5.4.2 *In vivo* genotoxicity testing (somatic cells) (Annex IIA 5.4.2)

### B.5.4.2.1. *In vivo* mammalian bone-marrow micronucleus test (Annex IIA 5.4.2)

-Mice , gavage, 1250; 2500; 5000 mg/kg bw/d (Lick, 1995)

**Findings:**

The MTD was reached. At the highest dose, 2 _ died at d 2. One of the animals had slight visceral congestion, and one showed decrease activity prior to death. Additional observations include a roughened haircoat (1_, 2500 mg/kg bw) and perineal soiling (1_, 5000 mg/kg bw, 1_, 2500 mg/kg bw).

The treatments did not have an observable effect on the ratio of PCE/NCE.

A slight dose-dependent increase in MN was noted at 24h harvest time, both in _ and in _ without reaching statistical significance. This effect was not observed at 48 h.

**Conclusion:**

Florasulam is not clastogenic in these experimental conditions.

**Guidelines:** Protocol in compliance with test method B.12 of directive 92/69/EEC

**GLP status:** The study is GLP.

**Materials and methods:**

5 mice/sex/dose/sampling time (CD-1(ICR) BR) received by gavage Florasulam (99.2%, B.n°: TSN100298) dissolved in corn oil at the dose levels 0; 1250; 2500 and 5000 mg/kg b.w.These doses were determined in a previous tolerability test. An analytical verification of administered samples revealed good agreement with calculated doses.Animals were sacrificed 24h, 48h and 72 h after dosing. Positive control : cyclophosphamide, at the dose-level of 120 mg/kg (48h sacrifice). Negative control : vehicle (corn oil). Femural bone marrow cells were harvested. Criteria for determining positive response: if the mean number of micronucleated polychromatic erythrocytes is statistically different from controls, as determined byDunnetts's t-test ($p<0.01$). The study is accepted.

### B.5.4.3 Summary of genotoxicity (Annex IIA 5.4)

Florasulam was tested for its mutagenic potential *in vitro* and negative results were observed in the bacterial point mutation assay as well as in the mammalian cell assay. Negative results were also reported in its ability to induce *in vitro* chromosomal damage in rat lymphocytes. Florasulam did not induce micronuclei in mouse bone-marrow after *in vivo* administration.
In conclusion, florasulam is devoid of genotoxic effects *in vitro* and *in vivo*.

Table 5.4.3-1 Summary of genotoxicity studies

| Type of test | Result | Purity, batch n° | References |
|---|---|---|---|
| *In vitro* genotoxicity tests: | | | |
| *S. typhimurium* (TA100, TA1535, TA 98, TA1537) and *E.coli* (WP2uvrA) ± S9, DMSO | negative | 99.2% TSN100298 | Lawlor, 1995 |
| HPRT, CHO-K1-BH4, DMSO | negative | 99.2% TSN100298 | Linscombe, 1995a |
| Chromosome aberration assay, rat lymphocytes, DMSO | negative | 99.2% TSN100298 | Linscombe, 1995b |
| *In vivo* genotoxicity tests: | | | |
| mouse, micronucleus bone marrow assay 1250, 2500, 5000 mg/kg bw, in corn oil | negative | 99.2% TSN100298 | Lick, 1995 |

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

**B.5.5.1 Long-term (2 years) oral toxicity in the rat (Annex IIA 5.5)**

**-Rats (F344) , diet, 0, 10, 125, 250, 500 mg/kg bw/d , 104 weeks (Johnson, Haut and Stebbins, 1997)**

**Findings:**
Main findings are described in table B.5.5.1-1 and table B.5.5.1-2. The MTD was reached.
*Mortality:* There was no statistically identified difference in mortality for either males or females.
*Ophhalmology :* No compound related effect.
***Haematology* :**
Red blood cell parameters (RBC count, hemoglobin and hematocrit ↘ 5%) were decreased for males given 500 mg/kg bw/d at both 6 and 12 months sampling time. These parameters were not affected at 18 mths and were actually increased in the terminal sample after 24 months. Similar effects were also reported in previous short-term studies and are considered as treatment-related.
The white blood cell count was also decreased at the 12 month sampling for males top dose group. Platelet count was reduced at 24 month. These findings , as they were not repeatably present in this or other studies, were considered to be spurious findings. Additionally, histopathologic examination of the bone marrow after 12 and 24 months did not have any histopahtologic effects which correlate with these altered cell counts.
*Clinical chemistry* : Although some parameters were affected for rats receiving the highest dose levels , these effects were not repeatably present and may have been secondary to the body weight and growth differences at the top dose levels. There were no effects upon serum urea nitrogen, creatinine or electrolytes, with the possible exception of slighlty increased bicarbonate levels in high dose level males, in light of the renal effects found histopathologically.
*Urinalysis*: although the specific gravity was consistently less than controls for the males given 500 mg/kg bw/d, it was always well above the specific gravity of renal ultrafiltrate indicating that the kidneys of these rats were still able to concentrate urine. Urine pH, protein and ketone levels were consistently affected for males at the 2 top doses. These parameters were not affected at 10 mg/kg bw/d. Only urinary pH was affected for females.
*Organ weight:* the absolute and relative increase of kidney weight for males given 500 mg/kg bw/d was considered to be related to treatment. The effect on organ weight for other organs is considered to be related to decreased body weight rather than a specific organ toxicity. Similar effects were observed after 12 month exposure.
*Histopathology* : the only effects attributed to florasulam were noted in the kidmey.
The primary renal histopathological effect was hypertrophy of individual cells within the collecting ducts of rats given 125 mg/kg bw/d or higher florasulam. Although this effect appeared to be more prominent after 24 months than after 12 months, it did not appear to compromise renal function.
A possible second renal effect due to the compound was the degree of renal tubular degeneration with regeneration. This diagnosis was used at the 12 month necropsy interval to denote the earliest manifestations of the spontaneous chronic renal disease of the Fischer 344 rat. At the top dose, male rats had a slightly lesser degree of tubular degeneration than controls although the extent of this difference was equivocal. However, although females had less tubular degeneration with regeneration, those given 250 mg/kg bw/d appeared to have slightly higher incidence of very slight grade than control.
In fact, treated rats tended to have less geriatric renal disease (chronic progressive glomerulonephropathy) based upon histopathologic grades and urinary protein levels in the treated rats. Male rats given 500 mg/kg bw/d also had low incidence of reactive hyperplasia of the transitional epithelium and 3 had unilateral renal papillary necrosis that were condidered related to treatment. Male rats given 250 mg or 500 mg/kg bw/d had increased mineralization within the renal papilla but this was of minimal degree and was considered to possibly be dystrophic mineralization of sloughed cells within loop of Henle.
While the underlying cause of the hypertrophy of the intercalated cells is unknown, it is important to note that the continued ingestion of florasulam did not result in apparent deterioration of renal function nor in renal tumors. Although renal disease is quite common in aged Fischer 344 rats, particularly males, it appeared to be of somewhat lesser severity in rats receiving the highest doe levels of florasulam for 24 months. Morphologically, there was a greater degree of intercalated cell hypertrophy after 24 months than was present at 12 months, but this change, and the urine acidification, have both been demonstrated to be reversible.

A few other statistically-identified non-neoplastic histopathologic observations were considered to be geriatric conditions unrelated to florasulam ingestion. These observations included : heart degeneration, kidney mineralization, liver dilatation, basophilic foci in liver and mesenteric tissues for which the statistical identification was considered to represent normal variability in the occurrence of common geriatric changes.

*Neoplasms*: there were no effects attributed to florasulam ingestion. Overall, neoplasms were found in the majority of rats as is common in this strain of rats near the end of their lifespan. Some neoplasms were found in the majority of rats (e.g.interstitial cell adenomas of the testes, pituitary adenomas) from all dose groups. In general, treated rats tended to have somewhat fewer neoplasms.

**Table B.5.5.1-1 : Main findings reported in rats after 104 week treatment with florasulam**

| **Dose/ endpoint** | **0** | | **10** | | **125** | **250** | | **500 mg/kg bw/d** |
|---|---|---|---|---|---|---|---|---|
| | _ | _ | _ | _ | _ | _ | _ | _ |
| Mortality: | | | | | | | | |
| week 0-52 | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 2/50 | 0/50 | 1/50 |
| week53-104 | 13/50 | 10/50 | 17/50 | 12/50 | 11/50 | 16/50 | 15/50 | 16/50 |
| Clinical signs: | | | | | | | | |
| soiling | 6/50 | 14/50 | 9/50 | 18/50 | 45/50 | 46/49 | 50/50 | 49/49 |
| thin | | 4/50 | | 6/50 | 8/50 | | 10/50 | |
| Body weight : at day 365 at day 730 | | | | | | | ↘9% | ↘16% ↘13% |
| bw gain | | | | | | (↘3%) | (↘14.4%) | (↘22.5%) |
| Food intake | | | ↘8% | | | ↘6% | | ↘8% |
| Haematology | | | | | | | | |
| RBC | | | | | | | | ↗15% |
| Hb | | | | | | | | ↗10% |
| Platelets | | | | | | | | ↘29% |
| Clinical chemistry: at 12/24 months | | | | | | | | |
| AP | | | | | | | | ↘11%/- |
| globulin | | | | | | | | /↘19% |
| cholesterol | | | | | | | | /↘40% |
| P | | | | | | | ↗11%/ | ↘8%/- |
| $HCO_{3-}$ | | | | | | | | /↗6% |
| triglycerides | | | | | | | | -/↘48% |
| albumin | | | | | | | -/↘10% | -/↗7% |
| Urinalysis : 24 mth | | | | | | | | |
| specific gravity | | | | | | | | ↘ |
| pH | | | | | ↘ | ↘ | ↘ | ↘ |
| protein / ketones | | | | | | ↘ | | ↘ |
| epithelial cells in urine sediment | | | | | | | | ↗ |
| Organ weights:12 mth/24 mth | | | | | | | | |
| heart (r) | | | | | | | -/↗12% | ↗10%/- |
| | | | | | | | | ↗24%/↗22 |

| | | | | |
|---|---|---|---|---|
| kidneys (r) | | | -/↗11% | % |
| testes (r) | | | | ↗16%/- |
| liver(r) | | | | -/↘14% |

Statistically significant ↗ or ↘ by Barlett's test and analysis of variance and Dunnett's test or Wilcoxon Rank Sum test. () : there were no statistical comparison of means.

**Table B.5.5.1.-2 : Macropathology and histopathology in animals treated with florasulam**

| **Dose (mg/kg bw/d)/ endpoint** | **0** | | **10** | | **125** | **250** | | **500** |
|---|---|---|---|---|---|---|---|---|
| | _ | _ | _ | _ | _ | _ | _ | _ |
| Kidney collecting duct : individual cells, hypertrophy : | | | | | | | | |
| very slight 12 mth | 36432 | 36432 | 36432 | 36432 | 36432 | 36437 | 36437 | 36434 |
| 24 mth | 0/50 | 0/50 | 0/50 | 0/50 | 28*/50 | 29*/50 | 39*/50 | 11*/50 |
| slight 12 mth | | | | | | | | 36440 |
| 24 mth | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 12*/50 | 0/50 | 20*/50 |
| moderate 24 mth | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 18*/50 |
| **Papilla** : hyperplasia : transition al epithelium multifocal, slight | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10* |
| **Papilla**: necrosis, unilateral | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| **Papilla** : mineralization tubule multifocal very slight | 2 | 0 | 5 | 0 | 0 | 12* | 0 | 34* |
| slight | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 5* |
| **Tubules:** degeneration/regeneration multifocal | | | | | | | | |
| very slight at 12 month | 36440 | 36433 | 36441 | 36435 | 36432 | 36442 | 36438 | 36440 |
| slight at 12 month | 36434 | 36433 | | | | 36432 | | 36432 |
| **Geriatric effects :** | | | | | | | | |
| **Chronic progreessive glomerulonephropathy:** | | | | | | | | |
| very slight | 9 | 32 | 6 | 38 | 37 | 13 | 28 | 22* |
| slight | 14 | 7 | 15 | 2 | 7 | 19 | 6 | 21* |
| moderate | 15 | 6 | 16 | 4 | 2 | 16 | 4 | 3* |
| severe | 7 | 0 | 11 | 0 | 0 | 0 | 0 | 1* |
| **liver:** hepatocytes;basophilic focus of cell.alteration | 14/50 | 21/50 | 8/50 | 13/50 | 16/50 | 15/50 | 15/50 | 23*/50 |
| liver, dilatation or cystic spaces-peliosis, focal | 3/50 | 2/50 | 6/50 | 2/50 | 1/50 | 13*/50 | 0/50 | 2/50 |
| **peripheral nerve** degeneration very slight | 28/50 | 43/50 | 2/50 | 7/50 | 9/50 | 5/50 | 35*/50 | 25/50 |
| **mesenteric tissue** blood vessels mineralization | 39/50 | 4/50 | 15/50 | 0/50 | 1/50 | 12/50 | 3/50 | 48*/50 |
| **myocardium** degeneration with/wo | 7/50 | 33/50 | 3/50 | 5/50 | 7/50 | 3/50 | 35/50 | 18/50* |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| inflammation v.slight | | | | | | | | |
| **kidneys:** multifocal tubule mineralization very slight | 5/50 | 41/50 | 8/50 | 49*/50 | 50*/50 | 9/50 | 41/50 | 1/50 |

At 24 mth :*statist. significant by Yate's pairwise Chi square

Conclusion :
NOAEL = 10 mg/kg bw/d and LOAEL = 125 mg/kg bw/d
Florasulam was not carcinogenic under the conditions of the study.

Guidelines :
Experimental protocol not fully in compliance with test method B, annex V, directive 87/302/EEC or OECD guideline 453 (1981). *Deviation from official protocol* : haematological examination and urinalysis were not performed at 3 months. Urinary volume was not measured and Gamma GT and Ornithine decarboxylase were not measured.
GLPstatus : yes (no attest of competent authority)
Material and methods : 65 rats (Fischer 344) sex/dose were fed diets containing florasulam (99.3%, lot n°.940714) at concentrations of 0,10, 125 and 250 mg/kg bw/d for fermales and 0, 10, 250 and 500 mg/kg bw/d for males. Florasulam was stable in rodent chow for greater than 30 days.10 animals/dose/sex were necropsied after 1 year. 5 other animals/sex/dose were sacrified after 12 months for chronic neurotoxicity assessment.
Achieved dosages:
For males : 10.15; 254.28 and 505.88 mg/kg bw/d
For fermales : 10.18; 127.23 and 253.76 mg/kg bw/d.

**B.5.5.2 Carcinogenicity study in the rat (Annex IIA 5.5)**

See point B.5.5.1.

**B.5.5.3 Carcinogenicity study in the mouse (Annex IIA 5.5)**

- Mice, diet, 0, 50, 500 or 1000 mg/kg bw/d, for 2 years (Quast, Haut, and Kociba, 1997)

Findings:
Main findings are described in table B.5.5.3-1 . The MTD was reached in this study.
*Mortality* : there were no statistically identified differences in the mortality rate.
*Clinical signs*: observations noted including the in-life palpable mass findings were those which normally occur in this strain of aged mice and were unrelated to treatment.
*Ophtalmology* : most findings for the eyes during the in-life examination were secondary to pressure associated with an inflammatory process or development of a benign adenoma of the posterior orbital lacrimal gland. There were no compound-related changes in the eyes of any mice on test.
*Clinical chemistry:* the significantly decreased cholesterol (↘18% at 12 month) and triglycerides(↘37% at 12 month) values in male top dose were consistent with significantly decreased body weight noted at necropsy in this dose group and significantly decreased liver weight (↘10%). Clinical chemistry and electrolyte values were unaffected and were without any indication of altered renal function in either dose group of males.
*Organ weight, histopathology:*
At 12 month,in the high dose male mice, statistically significant decreased heart (↘11%) and liver weight and increased adrenal weight (↗27%) were unassociated with any histopathologic findings .
At 24 month necropsy, there were fewer organ weight differences and except for kidney weight, they were considered to be a reflection of slightly lower body weight .
Decrease in kidney weight , reported after 12 months at 500 mg/kg (↘10% ,a and ↘9 % , r) and at 1000 mg/kg bw/d (↘12%(a)) and after 24 month, in male mice at 500 (↘7% r) and 1000 mg/kg bw/d (↘5%, r) were correlated with microscopic changes :
- After 12 month, microscopic changes were characterized as bilateral decreases in vacuolation in the tubules, and a a decrease in the degree of renal tubular degeneration with regeneration. In female mice, the middle and high dose group appeared to have a slight decrease in the incidence of this normally occurring disease process which is more frequently observed in male mice than in females.
In liver, altered tinctorial properties of centrilobular hepatocytes were observed and reflective of the amount of

glycogen present in centrilobular hepatocytes. The decreased amount of hepatocellular glycogen in all high dose male was consistent with their lower body weight, and not considered an indication of hepatotoxicity. The lower incidence for this same observation in the control, low dose, and middle dose male mice reflected the normal variation in the amount of hepatic glycogen in a few of these nonfasted mice, and is a normal physiologic change.

- After two years, the primary microscopic kidney effects due to florasulam included statistically significantly increased hypertrophy of individual collecting duct cells (both sexes), decreased vacuolization of cortical epithelial cells (male only) and decreased tubular degeneration/regeneration(both sexes) of the 500 and 1000 mg/kg bw/d. The hypertrophic cells were consistent with intercalated cells with increased cytoplasmic volume containing numerous mitochondria.

*Incidence of neoplasms:* overall, neoplasms were found in a number of mice, as is common in this strain, as they are near the end of their lifespan. In general, the liver and lung are common sites for spontaneously occurring neoplasms, especially in males. The incidence of neoplams in these two tissues were generally decreased in a dose-related manner.
The incidence of benign adenoma in the lacrimal/Harderian gland of high dose males appears to be slightly elevated although not statistically significant. The apparent effect was due to an unusually low control value based upon the laboratory historical control data.
There were no neoplasms in any dose group which were identified as statistically increased in either male or female mice.

**Table B.5.5.3-1: Carcinogenicity study of florasulam in mice.**

| **Endpoints/dose** | **0** | | **50** | | **500** | | **1000 mg/kg bw/d** | |
|---|---|---|---|---|---|---|---|---|
| | _ | _ | _ | _ | _ | _ | _ | _ |
| Mortality day 365 | 1/50 | 2/50 | 0/50 | 2/50 | 0/50 | 1/50 | 1/49 | 3/50 |
| Day 746 | 10/50 | 13/50 | 3/50 | 14/50 | 8/50 | 21/50 | 6/49 | 21/50 |
| Clinical signs | | | no compound related effects | | | | | |
| Body weight | | | | | | | ↘3.4% | ↘5.3% |
| Bw gain (no stat.performed) | | | | | | | ↘11.3% | ↘13% |
| Food consumption | | | transient decreased food consumption, no significant effect at end | | | | | |
| Haematology: | | | no significant effects | | | | | |
| Histopathology at 12 months: | | | | | | | | |
| kidneys: hypertrophy collecting ducts, multifocal ,any severity: | | | | | | | | |
| | 0 | 0 | 0 | 0 | 10 | 10 | 10 | 10 |
| kidneys, vacuolation, decreased, tubules, bilateral, any severity: | | | | | | | | |
| | 1 | 0 | 1 | 0 | 5 | 0 | 9 | 0 |
| kidneys, degeneration/regeneration, tubules, focal or multifocal, very slight: | | | | | | | | |
| | 7 | 7 | 8 | 5 | 7 | 3 | 6 | 3 |
| liver: altered tinctorial properties, centrilobular | | | | | | | | |
| | 3 | 0 | 2 | 0 | 2 | 0 | 10 | 0 |
| Histopathology at 24 months | | | | | | | | |
| kidneys: hypertrophy, collecting duct, individual cells | | | | | | | | |
| very slight | 0 | 0 | 0 | 0 | 41* | 18* | 27* | 34* |
| slight | 0 | 0 | 0 | 0 | 0 | 0 | 20* | 0 |
| renal tubule,cortex decreased vacuolization | | | | | | | | |
| slight | 11 | 0 | 17 | 0 | 27* | 0 | 14 | 0 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| moderate | 13 | 3 | 9 | 0 | 20 | 0 | 33* | 0 |
| Degeneration, tubule, with regeneration | | | | | | | | |
| very slight | 20 | 8 | 17 | 4 | 33* | 4 | 32* | 1* |
| slight | 30 | 0 | 31 | 0 | 16* | 0 | 17* | 0 |
| lacrimal/Harderian gland: | | | | | | | | |
| benign adenoma | 2/50 | 4/50 | 1/50 | 2/50 | 4/50 | 3/50 | 7/49 | 3/50 |
| historical control :1/50 → 9/50 ; mean = 6/50 | | | | | | | | |

Statistically significant ↘ or ↗ by Barlett's test, analysis of variance (ANOVA) , Dunnett's test or the Wilcoxon Rank-Sum tet. * Statistically significant by Yate's pairwise Chi-square .

**Conclusion:**
Florasulam is not carcinogenic for mice.
NOAEL = 50 mg/kg bw/d.

**Guidelines:**
Experimental protocol not fully in compliance with test method B, annex V, directive 87/302/EEC .
*Deviation from the official protocol* : hematology was performed on 10 mice /sex/dose instead of blood smears on all animals.
**GLP status:** yes (no attest of competent authority)
Material and methods:
50 (Charles River, B6C3F1) mice/sex/dose received florasulam (lot n°.940714, 99.3%) in the diet at 0, 50, 500 or 1000mg/kg bw/d, over a period of 24 months. Groups of 10 males and 10 females for interim sacrifice after treatment for 12 months were allocated at each dose.
Stability in diet was controlled.
Achieved intake :
Male: 0, 50.49, 505.21, and 1008.66 mg/kg bw/d
female : 0, 50.88, 496.5, and 1019.42 mg/kg bw/d

### B.5.5.4 Mechanism of action and supporting data (Annex IIA 5.5)

Comments reported in the 2 year rat carcinogenicity study (Johnson, Haut and Stebbins, 1997)

The underlying mechanism for hypertrophy of a selected cell population within the collecting duct is unknown.
The collecting duct is composed of two major groups of cells, the principal cells and the intercalated cells. Intercalated cells, which constitute approximately 25-40% of the epithelium of the collecting duct, are distinguished by their size (tall, dome-shaped cells) and staining characteristics (often referred to as dark cells due to their denser, mitochondria rich cytoplasm as compared to principal cells).
Intercalated cells are functionally involved in acid-base regulation. These appears to be two distinct populations of intercalated cells within the collecting ducts of rats, rabbits and mice.

- The α-intercalated cell, which is abundant in the outer medullary collecting duct but is also present in the cortical collecting duct, is involved in acid secretion into urine and $HCO_3$- resorption.
- β-intercalated cells found only in cortical collecting ducts, are responsible for $HCO_3$- secretion into the urine.

α-intercalated cells contain high levels of acid transporter molecules (H+ ATPase ; H+K+ ATPase) in the apical cell membranes (i.e. the site adjacent the urine) and the mitochondria are intimately associated with abundant H+ ATPase-containing vesicles. When rats were treated with NH4Cl for 15 days to induce a steady state of acidosis, ultrastructural examination revealed a decrease in the number of H+ ATPase-containing vesicles due to their apparent merging with the apical cell membrane. Conversely, β-intercalated cells have an apical $HCO_3$-/Cl- transporter with a basolateral H+ ion transporter.

From the histologic and ultrastructural appearance of the hypertrophied cells, the site within the collecting duct which they were present, and from the urine pH changes, it is extremely likely that the cells noted to be affected due to florasulam ingestion are Type A intercalated cells. Hypertrophy of Type A intercalated cells has been reported as a physiologic response to several factors affecting acid-base homeostasis, including acute respiratory acidosis and

chronic metabolic acidosis. Other potential mechanisms of Type A- intercalated cell hypertrophy include hypokalemia, altered levels of adrenal mineralocorticoids, carbonic anhydrase inhibition and $HCO_3$-/Cl- exchange in the basolateral membrane.
Lastly, florasulam may have acted directly upon the type A intercalated cells by some unknown mechanism to cause the hypertrophy along with secondary functional effects.

**B.5.5.5 Summary of long-term toxicity and carcinogenicity (Annex IIA 5.5)**

Florasulam identified in rats body weight, body weight gain, and the kidney as target organs. Urine analysis revealed a treatment-related decrease of urinary pH for males receiving 250 or 500 mg/kg bw/d and for females receiving 125 or 250 mg/kg bw/d; urine specific gravity was decreased in high dose males, with lower levels of urinary protein and ketones. Kidney weight were increased in high dose males at 1 and 2 year and in high dose females at 2 year. At and above dose levels of 125 mg/kg bw</d, hypertrophy of cells within the collecting duct of the kidney were observed upon histopathological examination after 1 year, and more prominent after 2 year. In males given 250 or 500 mg/kg bw/d, mineralization of sloughed epithelial cells within loops of Henle was also reported. Unilateral papillary necrosis was reported in males. These effects were not associated with a deterioration of the renal function.
Florasulam was not carcinogenic in rats. The NOAEL in this study is 10 mg/kg bw/d.

In mice, at 500 and 1000 mg/kg bw/d, florasulam produced consistent microscopic effects, with the kidney being the only target organ identified. Hypertrophy of collecting duct cells was observed in the inner and outer stripe of the renal medulla, affecting sometimes cells in the proximal portion of the papilla. There was also an increased incidence of decreased cytoplasmic vacuolation of the cortical epithelial cells which may have contributed to the lower kidney weights. Clinical chemistry was not indicative of altered renal function. Treated mice were found to have less spontaneous occurring geriatric renal disease compared to their respective control.
In mice, florasulam was not carcinogenic and the NOAEL is 50 mg/kg bw/d.

The underlying mechanism for collecting duct cell hypertrophy is not fully understood. It is however suggested that α-intercalated cells are involved.

**Table B.5.5.5-1 : Summary of long-term toxicity and carcinogenicity**

| Type of test<br>Test species | Test substance purity | Results | | | References |
|---|---|---|---|---|---|
| | | NOAEL (mg/kg bw/ day) | LOAEL (mg/kg bw/day) | Critical endpoints | |
| Rat, 104 week | 99.3% ; lot n°. 940714 | 10 | 125 | ↗ clinical signs, urinary pH↘, cell hypertrophy collecting duct | Johnson, Haut and Stebbins, 1997 |
| Mice, diet, 78 week | lot n°.940714, 99.3% | 50 | 500 | ↘ kidney weight; ↗ hypertrophy collecting duct individual cells; renal tubule, cortex ↘ vacuolation | Quast, Haut, and Kociba, 1997 |

**B.5.6 Reproductive toxicity (Annex IIA 5.6)**

**B.5.6.1.1 Two generation reproductive toxicity in the rat (Annex IIA 5.6.1)**

- Rat, diet, 0, 10, 100, 500 mg/kg bw/d , 2 generation study (Liberacki, Carney and Kociba, 1997)

**Findings:**

- *Adult data :* Main adult data are reported in Table B.5.6.1.1-1.
All P1 adults survived to the scheduled necropsy. All P2 adults survived , with the exception of a single 100 mg/kg bw/d female resulting from a diffuse inflammation of the uterus. Due to the isolated nature of the occurrence , this death was not considered treatment-related.
*Clinical signs* were noted and included perineal soiling and reddish urine. Reddish urine was also noted for 1 male at 100 mg/kg bw/d , and, as no gross pathologic correlate was made and sporadic occurrences of reddish urine have been noted in control animals from other reproduction studies, the toxicologic significance of this single observation was uncertain.
At 500 mg/kg bw/d : parental effects consisted of decreased *food consumption* and significantly lower body weights of P2 males and P1/P2 females during most the pre-mating, gestation and lactation periods. Body weight gains of this group P1 and P2 females were also significantly lower than the controls during gestation (days 0-21).
Significant increases in relative kidney weight occurred in group P1 and P2 adults.
*Histopathologically,* hypertrophy of the renal tubular collecting ducts, observed in the kidneys of most P1 and P2 rats was considered to represent an adaptative response rather than a pathologic effect.
Necrosis and/or inflammation of the renal papilla with resultant hemorrhagic casts in the lumen of the urinary bladder occurred in a few P1 and P2 rats.
No treatment-related effects were observed in P1 or P2 males or females administered 10 or 100 mg/kg bw/d.
Reproductive parameters were not affected.

*Offsprings* (Table B. 5.6.1.1-2):
No treatment-related *clinical observations* or physical alterations were observed for the F1 pups in any of the dose groups during the P1/F1 lactation period.
At 500 mg/kg bw/d, transient decreases in *body weight* were attributed, in part, to the decreased food consumption of the P1 and P2 maternal animals early in their respective lactation periods. Neonatal survival was not adversely affected at any dose level in either generation.
No adverse effects were observed on pup body weights at dose levels of 10 or 100 mg/kg bw/d.
There were no treatment-related gross pathologic observations at any dose level in F1/F2 weanlings.

**Table B.5.6.1.1-1: 2-generation study in rats : adult data.**

| **Dose (mg/kg bw/d)/ endpoint** | **0** | **10** | **100** | **500** |
|---|---|---|---|---|
| P1/P2 adults: | _ _ | _ _ | _ _ | _ _ |
| Mortality | | no death | | |
| Clinical signs : perineal soiling P1<br>P2 | | | | 5/30 14/30<br>9/30 9/30 |
| reddish urine P1<br>P2 | | | 1/30 | 2/30<br>1/30 |
| Food consump. premating period P1<br>P2 | | | | ↘(±10%)<br>↘(±10%) |
| Food consump. Gestation period P1<br>P2 | | | | ↘7-11%<br>↘6-13% |
| Food consump. Lactation period P1<br>P2 | | | | ↘17%d1-4<br>↘23%d1-4<br>↘18%d4-7 |
| Body weight : mean for P1<br>P2 | | | | ↘6-8%d34-69<br>↘7-10%d27-69<br>↘7-13%d27-139 |
| Body weight during gestation P1<br>P2 | | | | ↘8-11%<br>↘9-14% |
| Body weight during lactation P1<br>P2 | | | | ↘7-15%<br>↘8-17% |

| **Dose (mg/kg bw/d)/ endpoint** | **0** | **10** | **100** | **500** |
|---|---|---|---|---|
| P1/P2 adults: | _ _ | _ _ | _ _ | _ _ |
| Reproductive indices, gestation survival, pup survival indices, pup sex ratio, gestation lenght, time to mating:<br>P1 adult / F1 litters and P2 adult/F2 litters | | not affected | | |
| Organ weight - P1 adults and P2 adults : | | | | |
| kidney weight (r)P1<br>P2 | | | | ↗<br>↗ ↗ |
| Histopathologic observations P1/P2: | | | | |
| Kidney hypertrophy, renal tubules, collecting ducts, inner stripe of outer zone,renal medulla, multifocal, very slight | | | | |
| P1 | | | | 25/30 21/30 |
| P2 | | | | 24/30 22/30 |
| Renal papilla : necrosis bilateral, multifocal, slight | | | | |
| P1 | | | | 1/30 |
| P2 | | | | 4/30 |
| inflammation, bilateral, multifocal, slight | | | | |
| P1 | | | | 1/30 |
| P2 | | | | 3/30 |
| Urinary bladder : hemorrhagic casts in lumen | | | | |
| P1 | | | | 1/30 |
| P2 | | | | 2/30 |

Statistically significant ↗ or ↘ according Fisher's exact test; Barlett's test, Kruskal-Wallis test, Dunnet-type or Scheffé-type test.

**Table B. .5.6.1.1-2 : Mean litter data in F1 and F2 rats pups**

| **Dose (mg/kg bw/d)/ endpoint** | **0** | | **10** | | **100** | | **500** | |
|---|---|---|---|---|---|---|---|---|
| | _ | _ | _ | _ | _ | _ | _ | _ |
| F1 pup body weight lactation day 4 | | | | | | | ↘7% | |
| F1 pup body weight lactation day 7<br>F2 | | | | | | | ↘10% ↘9%<br>↘15% ↘12% | |

Statistically significant ↗ or ↘ according Fisher's exact test; Barlett's test, Kruskal-Wallis test, Dunnet-type or Scheffé-type test.

**Conclusion:**
NOAEL reproduction toxicity > 500 mg/kg bw/d.
NOAEL syst.toxicity = 100mg/kg bw/d

**Guidelines:** Experimental protocol in compliance with method B, Annex V, directive 87/302/EEC or OECD guideline 416 (1983).
**GLP status :** yes (no attest of competent authority)
**Material and methods:**
30 rats(CD Sprague-Dawley) /sex/dose were treated via diet with florasulam (lot n°. 940714, 99.3%) for 2 successive generations at 0, 10, 100 or 500 mg/kg bw/d.

**B.5.6.1.2 Supplementary studies (Annex IIA 5.6.1)**

No data, not necessary.

**B.5.6.2.1 Teratogenicity test by the oral route in the rat (Annex IIA 5.6.2)**

*Preliminary study* : Rat, 0, 100, 500, 750 mg/kg bw/d, by gavage, from day 6 to day 15 (Liberacki, Breslin and Stebbins, 1996)

**Material, methods and findings:**

10 female rats CD/dose received by gavage florasulam (lot.n°100511; 99.3%) at 0, 100, 500, or 1000 mg/kg bw/d as a aqueous suspension in an aqueous solution of 0.5% Methocel A4M from day 6 through day 15 of gestation inclusive. Due to excessive maternal toxicity at 1000 mg/kg bw/d, two additional groups of 10 rats were added to the study at doses of 0 and 750 mg/kg bw/d. Animals were sacrificed on day 16 of gestation.

Main results are reported in Table B.5.6.2.1-1.

Five dams administered 1000 mg/kg bw/d were found dead prior to scheduled necrospy. Clinical observations made on 2/5 dams prior death included excessive chromorhinorrhea and/or decreased activity. The remaining dams were sacrificed without further data collection on day 13 of gestation.The top dose was reduced to 750 mg/kg bw/d.

One dam was found dead at 750 mg/kg bw/d and upon gross examination, the death was attributed to a probable lymphoreticular tumor and uterine hemorrhage and was therefore, not considered treatment-related.

**Table B.5.6.2.1-1. Maternal and litter data.**

| Endpoints | Dose (mg/kg bw/day) | | | | |
|---|---|---|---|---|---|
| Maternal data: | 0* | 100 | 500 | 750 | 1000 |
| mortality | 0/0 | 0 | 0 | 1, day 15 | 3 day 10, 2 day13 |
| % pregnant | 9/10/10/10 | 9/9 | 10/10 | 10/10 | |
| Food consumption day 6-12 | | | | ↘8-10% | ↘28% |
| Body weight during gestation | | | | (not sign ↘3-4%) | ↘8% |
| Body weight gain during gestation | | | | ↘32% d 6-9 | ↘36% d 9-12 |
| kidney weight (a,r) | | | | ↗ 12%, ↗ 16% | not measured |
| Litter data: | | | | | |
| n°viable litters | 9 | 9 | 10 | 9 | |
| n° corpora lutea/dam | 14.6/13.1 | 15.9 | 14.3 | 13.6 | |
| n°implantation/dam | 13.1/12.7 | 13.4 | 13.5 | 13.3 | |
| % preimplantation loss | 9.9/4.5 | 15.2 | 6.9 | 3.0 | |
| % implantation resorbed | 8.5/3.1 | 9.1 | 3.0 | 4.2 | |
| % litters with resorption | 77.8/40.0 | 55.6** | 30.0** | 33.3 | |
| resorptions/litters with resorptions | 1.4/1.0 | 2.2 | 1.3 | 1.7 | |

Statistical key :↗ or ↘ according to Bartlett's test for equality of variances ; analysis by Dunnett's test or Wilcoxon rank-sum test with Bonferroni's correction ; Fisher exact probability test;
* this study has 2 control groups.
**decreased compared to control value

***Main study :***

Rat, gavage,0, 50, 250 or 750 mg/kg bw/d, day 6-15 (Liberacki and Carney, 1997)

Findings : main findings are described in table B. 5.6.2.1-2. The MTD was reached in this study.
*Clinical signs*: an increased frequency relative to controls for the surviving 750 mg/kg bw/d dams were salivation immediatly following dosing which resolved within 10 minutes, and perieal soiling. Upon gross examination of dams found dead, all 4 were noted to be pregnant with normal developing embryos respective to gestational age. Gavage error was the probable cause of 3 death as suggested by the dark or firm aspect of lungs. The fourth dam had a decreased amount of fat and a dilated renal pelvis ; the cause of death was not determined and may have been treatment-related.
Maternal toxicity was observed in dams administered the top dose as evidenced by decreased *food consumption, body weight and body weight gain.*
No significant maternal effects were observed at 50 and 250 mg/kg bw/d.
*Average fetal body weight* at 250 and 750 mg/kg bw/d were slightly reduced. However, these differences were minor in nature and showed no evidence of a dose-response that one would expect based on the 3-fold increase in dose level between the 2 doses. Comparison of these values with current historical control data revealed that the weights in these groups were well within the control range and that the control group from this study is at the upper end of the expected control range.
There were no statistically significant differences in the incidence of any fetal alteration in any of the dose groups.

**Table B.5.6.2.1-2. Maternal and litter data.**

| **Endpoints** | **Dose (mg/kg bw/day)** | | | |
|---|---|---|---|---|
| Maternal data: | 0 | 50 | 250 | 750 |
| mortality | | | | 1 sacrified d 10, 1 d 9, 2 d13 |
| % pregnant | 100 | 96.0 | 96.0 | 100 |
| Food consumption | | | | ↘ d 6-9, 9-12, 12-16 |
| Body weight gain during gestation | | | | ↘10% d 0-21 |
| Body weight | | | | ↘ ±5% d 9,12,16,19 |
| kidney weight (a,r) | | | | ↗ 7%, ↗ 12% |
| Litter data: | | | | |
| fetal body weight | 5.5 | 5.49 | 5.30 ↘4% | 5.27 ↘4% (historical control : 5.03-5.6) |
| n°viable litters | 25 | 24 | 24 | 23 |
| n° corpora lutea/dam | 16.7 | 16.5 | 16.3 | 16.0 |
| n°implantation/dam | 14.0 | 13.7 | 14.0 | 13.5 |
| % preimplantation loss | 15.4 | 15.8 | 13.8 | 14.2 |
| % implantation resorbed | 4.6 | 6.1 | 5.1 | 8.0 |
| % litters with resorption | 44.0 | 58.3 | 50.0 | 56.5 |
| resorptions/litters with resorptions | 1.5 | 1.4 | 1.4 | 1.9 |
| Variations and malformations: n° fetus/n°litter | | | | |
| external examination n°fetus(litters)examined | 333 (205) | 308 (24) | 319(24) | 286(23) |
| visceral examination n°fetus(litters)examined | 173 (25) | 161 (24) | 165 (24) | 148(23) |
| skeletal examination: n°fetus(litters)examined | 160 (25) | 147 (24) | 154 (24) | 138(23) |

| | | | | |
|---|---|---|---|---|
| total malformed skeletal | 1/1 | 0/0 | 5/4 | 1/1 |
| total malformed visceral | 0/0 | 1/1 | 0/0 | 0/0 |
| skull, delayed ossification | 6/5 | 4/4 | 15/7 | 11/7 |
| misplaced suture line | 0/0 | 0/0 | 0/0 | 1/1 |
| cervical delayed ossification | 50/19 | 66/19 | 61/17 | 62/23 |
| ribs: delayed ossification | 1/1 | 1/1 | 7/5 | 8/4 |
| class II wavy ribs | 0/0 | 0/0 | 5/4 | 0/0 |
| calloused ribs | 1/1 | 0/0 | 0/0 | 1/1 |
| sternebrae: delayed ossification | 17/10 | 14/12 | 12/9 | 27/12 |
| hydronephrosis | 0/0 | 1/1 | 0/0 | 0/0 |
| microphthalmia | 1/1 | 1/1 | 1/1 | 0/0 |
| Statistical key :↗ or ↘according to Bartlett's test for equality of variances ; analysis by Dunnett's test or Wilcoxon rank-sum test with Bonferroni's correction ; Fisher exact probability test; () not significant<br>* this study has 2 control groups.<br>**decreased compared to control value | | | | |

**Conclusion:**
NOAEL maternal toxicity = 250 mg/kg bw/d
NOAEL embryonal/fetal toxicity > 750 mg/kg bw/d

**Guidelines :** experimental protocol in compliance with test method B, annex V, directive 87/302/EEC or OECD guideline 414 (1981)
**GLP status :** yes (no attest of competent authority)
**Material and methods:.**
25-27 bred female rats CD/dose received by gavage florasulam (lot.n°940714; 99.3%) at 0, 50, 250, or 750 mg/kg bw/d as a suspension in an aqueous solution of 0.5% Methocel A4M from day 6 through day 15 of gestation inclusive. Animals were sacrificed on day 21 of gestation.
Stability of florasulam in food : at least 13 days.
The study is accepted.

### B.5.6.2.2 Teratogenicity test by the oral route in the rabbit (Annex IIA 5.6.2)

*Preliminary study* :

- Rabbit, gavage, from day 7-19, at 0,100, 300, 600 or 1000 mg/kg bw/d (Zablotny and Quast, 1996)

**Material, methods and findings:**
7 Adult females time-mated New-Zealand white rabbits/dose received by gavage, florasulam (99.3% ; lot n° 940714) as a suspension in an aqueous vehicle of 0.5% Methocel A4M on days 7-19 of gestation at doses of 0, 100, 300, 600 or 1000 mg/kg bw/d. As excessive maternal toxicity was observed at 1000 mg/kg bw/d, this group was terminated on gestation day 17. Animals were sacrificed on day 20.
At 1000 mg/kg bw/d, at necropsy of dead animals, 1 rabbit had edematous lungs and hemorrhage in vaginal wall, the second was normal and uterus contained 9 normal appearing fetuses. The third dam had five normal appearing fetuses and 1 early resorption. The only necropsy finding was edema in the lungs. Food consumption, body weight gain and fecal output were decreased. The deaths were treatment-related.
At 600 mg/kg bw/d, similar symptoms were observed as at 1000 mg/kg bw/d, the uterus contained 8 normal appearing fetuses and 2 early resorptions. The death was considered treatment-related.
Kidney weight was increased in all groups, but without dose response relationship and the organ weight values among the treated groups were comparable to historical control means. Therefoer, these differences were not considered treatment-related.
There were no treatment-related effects on pregnancy rates, numbers of corpora lutea, implantations, resorptions or litter size .

Table **B.5.6.2.2 -1 :Teratogenicity test by the oral route in the rabbit**

| **Endpoints/ dose (mg/kg bw/d)** | **0** | **100** | **300** | **600** | **1000** |
|---|---|---|---|---|---|
| Mortality | | | | 1 | 3 |
| Clinical signs | | | | | ↘ amount of feces,↘ activity |
| Food consumption | | | | ↘ 12%d 10-11 | ↘ 31% d 8-9 |
| body weight gain mean | | | | | ↘ d7-10 & 13-16 |
| body weight | | | | | ↘ d 16 |
| kidney weight : r | | ↗ 8% | ↗ 8% | ↗ 9% | |

Statistical key :↗ or ↘according to Bartlett's test for equality of variances ; analysis by Dunnett's test or Wilcoxon rank-sum test with Bonferroni's correction ; Fisher exact probability test; () not significant

***Main study* :**

- Rabbit, gavage, from day 7-19, at 0, 50, 250 or 500 mg/kg bw/d (Zablotny andCarney, 1997)

**findings:**

Main findings are described in table B.5.6.2.2.-2

A single abortion of 5 normal appearing fetuses was reported in 1 dam at 250 mg/kg bw/d . This effect was considered a spontaneous occurrence and was considered to be within the historical control range.

At 500 mg/kg bw/d, 1 dam aborted secondary to pneumonia and uterus contained 5 normal fetuses and 2 empty implantation sites from aborted fetuses ; another rabbit was found dead due to a ruptured esophagus and the uterus contained 7 normal fetuses. These effects were not considered treatment-related.

No treatment-related effects on food consumption were observed. The increased food consumption reported at 500 mg/kg bw/d was not associated with increased body weight or body weight gain and therefore, not considered treatment related. The lower body weight gain reported at 250 mg/kg bw/d was not considered treatment-related as there were no effects on body weight or body weight gain at 500 mg/kg bw/d.

There were no significant treatment-related effects on final body weights or absolute or relative liver or kidney weights at any dose level tested.

Reproductive parameters were not affected.

There were no statistically or biologically significant treatment-related differences in the incidence of any fetal alteration in any of the dose groups. All variations and malformations observed in fetuses expsoed to florasulam occurred at low frequencies, were not dose-related and fell within the range of New Zealand rabbit historical control data from the laboratory.

Table **B.5.6.2.2 -2 :Teratogenicity test by the oral route in the rabbit**

| **Endpoints/ dose (mg/kg bw/d)** | **0** | **50** | **250** | **500** |
|---|---|---|---|---|
| **Mortality** | 0 | 0 | 0 | 2 |
| **Clinical signs** | | no compound related signs | | |
| **Food consumption** | | | | ↗26% d24-25 |
| **body weight gain** mean | | | ↘ d10-13 | |
| **body weight** | | | | |
| N° pregnant | 20/20 | 19/20 | 20/20 | 20/20 |
| n° aborted | 0 | 0 | 1 d22 | 1 d 17 |
| n° viable litters | 20 | 20 | 19 | 18 |
| n° corpora lutea/dam | 9.7 | 9.9 | 9.8 | 9.9 |
| n° implantation/dam | 8.6 | 8.8 | 8.6 | 8.2 |

| | | | | |
|---|---|---|---|---|
| %preimplantation loss | 10.8±11 | 10.6±11 | 11.1±13 | 17.1±20 |
| n° resorption/litter | 0.3 | 0.3 | 0.2 | 0.4 |
| % implantation resorbed | 2.9 | 3.6 | 2.4 | 4.8 |
| % litters with resorptions | 20.0 | 31.6 | 21.1 | 33.3 |
| mean n° fetuses/litter | 8.3 | 8.5 | 8.4 | 7.8 |
| fetal body weight (g) | 38.99 | 37.33 | 38.94 | 38.87 |
| sex ratio (m/f) | 52:48 | 56:44 | 55:45 | 47:53 |
| gravid uterine weight (g) | 501.0±92 | 487.5±69 | 492.7±80 | 456.95±102 |
| **Fetal alterations**: | | | | |
| external, visceral, skeletal examination n°fetuses (n°litters) examined | 166(20) | 162 (19) | 160(19) | 140(18) |
| ventricular septal defect | | 1fetus/1litter | | |
| fused thoracic centra | | | 1 fetus/1 litter | |
| rudimentary tail | | | | 1 fetus/1litter |
| retroesophagial right subclavian artery | | | | 1 fetus/1litter |
| **Statistical key :↗ or ↘according to Bartlett's test for equality of variances ; analysis by Dunnett's test or Wilcoxon rank-sum test with Bonferroni's correction ; Fisher exact probability test** | | | | |

**Conclusion:**
There was no evidence of teratogenicity at dose levels up to and including 500 mg/kg bw/d.
NOAEL maternal toxicity > 500 mg/kg bw/d
NOAEL fetal toxicity > 500 mg/kg bw/d.

**Guidelines:**
Experimental protocol in compliance with test method B, annex V, directive 87/302/EEC or OECD guideline 414 (1981).
GLP status : yes (no attest of competent authority)
**Material and methods:**
20 Adult females time-mated New-Zealand white rabbits/dose received by gavage, florasulam (99.3% ; lot n° 940714) as a suspension in an aqueous vehicle of 0.5% Methocel A4 M on days 7-19 of gestation at doses of 0, 50, 250 or 500 mg/kg bw/d. Animals were sacrificed on day 28. Stability of florasulam in diet : at least 15 days.
The study is accepted.

### B.5.6.3 Summary of reproductive toxicity and teratogenicity (Annex IIA 5.6)

In the two generation study, parental animals receiving florasulam at the highest dose(500 mg/kg bw/d) had decreased food consumption and body weight during most of the pre-mating, gestation and lactation periods. An increased relative kidney weight was observed in P1 and P2 adults. Necrosis and/or inflammation of the renal papilla with the resultant hemorrhagic cast in the lumen of the urinary bladder occurred in some top dose P1 and P2 rats. There were no abnormalities in the reproductive performance of parental animals and the reproductive index of offspring.
In rats, maternal toxicity was observed at 750 mg/kg bw/d as suggested by the decreased body weight, food consumption and increased kidney weights. No adverse embryonal/fetal effects were observed at any dose level tested.
In rabbits, no maternal toxicity was observed and there was no evidence of teratogenicity at dose levels up to and including 500 mg/kg bw/d.

**Table B.5.6.3-1 : Summary of reproductive toxicity and teratogenicity of florasulam**

| **Type of test**<br>**Test species** | **Test substance purity** | **Results** | | | **References** |
|---|---|---|---|---|---|
| | | **NOAEL (mg/kg b w/ day)** | **LOAEL (mg/kg b w /day)** | **Critical endpoints** | |
| Rat, 2 generation study | lot n°. 940714, 99.3% | reprotox > 500<br>syst.toxicity =100 | -<br>500 | ↗Kidney weight, necrosis and/or inflammation renal papilla, hemorrhagic cast in urinary bladder | Liberacki, Carney and Kociba, 1997 |
| rat, developmental | lot.n°940714; 99.3% | maternal tox = 250<br><br>developmental tox > 750 | 750<br><br>- | ↘food consumption, body weight, ↗kidney weight | Liberacki and Carney, 1997 |
| Rabbit, developmental | lot.n°940714; 99.3% | maternal tox > 500<br><br>developmental tox > 500 | 600<br><br>- | range finding study: mortality, ↘ transient food consumption | Zablotny andCarney, 1997 |

**B.5.7 Neurotoxicity (Annex IIA 5.7)**

**B.5.7.1 Acute neurotoxicity (Annex IIA 5.7)**

- Rat, single oral administration of florasulam at 0, 200, 1000 or 2000 mg/kg bw by gavage (Mattsson, Guirk and Yano, 1997)

**Findings:**
Main results are explained in table B.5.7.1-1.
*FOB* : Although the number of findings were few and within that expected by chance, the results suggested male high dose rats had slightly depressed activity and decreased responsiveness to sharp noise on the day of treatment (day1). The other findings were sparse, did not form patterns, and were considered to be due by chance.
Body weight, grip performance, landing foot splay and rectal temperature did not show a treatment-related effect.
*Motor activity:* the decreased motor activity of high dose males on the day of treatment was considered a minimal treatment-related effect.
*Neuropathology:* the resulst of the histopathologic evaluation indicate that florasulam had no effect on the histology of the central and peripheral nervous system.

**Table B.5.7.1-1 Neurotoxicity study in rats after acute doses of florasulam**

| **Endpoints/dose (mg/kg bw)** | **0** | | **200** | | **1000** | | **2000** | |
|---|---|---|---|---|---|---|---|---|
| Content of FOB | _ | _ | _ | _ | _ | _ | _ | _ |
| Hand-held observations: | | | | | | | | |
| perineal staining | | | | | 7/10 d2 | | 4/10 d2<br>2/10 d3 | |
| Open field observations: | | | | | | | | |
| Day 1:<br>level of activity (ave.rank) | 2.9 | 3.2 | 2.6 | 3.5 | 2.9 | 3.0 | 2.3 | 3.6 |
| responsiveness to sharp noise | 3.1 | 2.8 | 2.8 | 2.9 | 2.9 | 2.8 | 2.6 | 3.0 |
| Day 8 :<br>level of activity (ave.rank) | 2.3 | 2.7 | 2.5 | 2.7 | 2.1 | 2.9 | 2.3 | 3.4 |
| urination (ave.rank) | 1.5 | 1.0 | 1.8 | 1.2 | 1.5 | 1.3 | 2.1 | 1.0 |

Bold : average rank difference from control was 0.5 rank or greater or a ranked observation was statistically different from control, Test of proportions, a=0.02.Significant pairs marked by bold print.

**Conclusion:**
NOAEL neurotoxicity = 200 mg/kg bw

**Guidelines:**
Experimental protocol in compliance with OECD guideline 424 (1997)
**GLP status:** yes (no attest of competent authority)
**Material and methods:**
10 Fischer 344 rats /sex/dose received by gavage a single dose florasulam (TSN 100511; 99.3%) in methylcellulose at 0, 200, 1000 or 2000 mg/kg bw. The solution was stable for 2 weeks. Animals were observed for FOB and motor activity test once prior to florasulam (day -7), on day 1 dosing day, on day 8 and 15. Rats were sacrified after 2 weeks and pathology and neuropathology was performed.

The study is accepted.

**B.5.7.2 Chronic neurotoxicity (Annex IIA 5.7)**

- Rat, diet , 1 year , at 0, 10, 250 or 500 mg/kg bw (Shankar and Johnson, 1996)

**Findings :**
*Cagesides*: no treatment-related effects were observed.
The only FOB observations related to treatment were urinary perineal soiling and possibly a slight increase in open-field urination in high dose male and female rats and in middle-dose male rats.
*Body weights* : a significant effect of treatment over time occurred in males but not in females.
*Body weight gains*: the decreases reported in males attained toxicological significance at 500 mg/kg bw/d.
Treatment did not affect grip performance, landing foot splay or rectal temperature.
*Motor activity* was not altered and no treatment related differences were found in auditory brainstem response.
No gross or *histopathologic* lesions were observed in the central and peripheral nervous system tissues.

**Table B.5.7.2-1 Summary of treatment related effects in rats- 1 year oral feeding florasulam in diet.**

| Endpoints/dose: mg/kg bw/d | month of effect | 0 | 10 | 250/125(m,f) | 500/250(m,f) |
|---|---|---|---|---|---|
| Clinical observations: | | | | | |
| perineal soiling | 6, 9, 12 | - | - | + (m,f) | + (m,f) |
| FOB conducted on 0,3,6,9,12 mth | | | | | |
| Body weights | 6, 9, 12 | - | - | - | + (m) |
| body weight gain | 6, 9, 12 | - | - | - | + (m) |
| hand-held observations: perineal soiling | 3,6,9,12 | - | - | + (m) | + (m, f) |
| open field observations: urination increased | | - | - | possible effect | possible effect |
| grip performance | none | | | | |
| landing foot splay | none | | | | |
| rectal temperature | none | | | | |
| Motor activity test : conducted on 0, 3,6,9,12 mth | | | | | |
| activity differences | none | | | | |
| Auditory brain stem response: waveform differences | none | | | | |
| Neuropathology : after 12mth | | | | | |
| lesions | none | | | | |

**Conclusion:**
NOAEL neurotoxicity = 250 mg/kg bw/d. There were no effects of florasulam on any parameter that would suggest a neurotoxic effect.

**Guidelines:**

Experimental protocol in compliance with OECD guideline 424 (1997)
**GLP status:** yes (no attest of competent authority)
**Material and methods:**
5 Fischer 344 rats /sex/dose received by gavage a single dose florasulam (TSN 100511; 99.3%) indiet at 0, 10, 250 or 500 mg/kg bw/d for males and at 0, 10, 125 or 250 mg/kg bw/d for females . Animals were observed for neurotoxicity once prior to florasulam , on months 3, 6, 9 and 12 by FOB, froelimb and hindlimb grip strenght, landing foot splay and automated mototr activity test. Diet was prepared weekly.
The study is accepted.

### B.5.7.3 Summary of neurotoxicity (IIA 5.7)

Florasulam caused a minor, transient depression of activity and depression of reactivity to noise stimulus on the day of dosing in male rats at 2000 mg/kg bw. Perineal soiling occurred in some high and mid dose female rats. These effects disappeared after day 3.
After 1 year feeding, there were no effects of florasulam on any parameters measured in rats, that would suggest a neurotoxic effect.

Table B.5.7.3-1 : Summary of neurotoxicity studies of florasulam

| Type of test Test species | Test substance purity | Results | | | References |
|---|---|---|---|---|---|
| | | NOAEL (mg/kg bw/day) | LOEAEL (mg/kg bw/day | Critical endpoints | |
| Rat, acute | lot n°. TSN 100511; 99.3% | 200 | 1000 | Perineal soiling | Mattsson, Guirk and Yano, 1997 |
| Rat, chronic | lot n°. TSN 100511; 99.3% | systemic toxicity = 10<br>neurotoxicity = 250/500 | 125/250 | Perineal soiling | Shankar and Johnson, 1996 |

### B.5.8 Further toxicological studies (Annex IIA 5.8)

No data, not necessary.

### B.5.9 Medical data (Annex IIA 5.9)

#### B.5.9.1 Report on medical surveillance on manufacturing plant personnel (Annex IIA 5.9.1)

There have been five laboratory scale productions of florasulam and two manufacturing pilot plant productions. The laboratory productions made a total of 9 kg of florasulam from 1992, using five workers. Pilot plant operations produced 130 kg of florasulam in 1993 and 560 kg in 1994, using three workers on each occasion. Among all the individuals involved, both in the laboratory and the pilot plant, there were no reported health effects. Routine health surveillance comprises a two-year programme of questionnaire, physical testing and examination, blood testing (comprising haematology, serum chemistry, hepatic function and renal function) and a medical consultation. Health

surveillance revealed no adverse health effects associated with production of florasulam.
In the laboratory and greenhouses, nine personnel were involved in trials with florasulam intermittently from 1991 until 1996. These scientists wore protective clothing, but respiratory protection and eye protection were not always used. In European field trials, 36 company field scientist and three contractors worked with florasulam from 1990. In each year from 1990 to 1997, and in each of six countries, up to two hectares of cereals were treated with florasulam. Personal protective equipment was not always used. All of these laboratory, greenhouse and field personnel participated in a programme of annual health surveillance, including physical examination, blood testing and medical consultation. No health effects were either reported or discovered in any of the personnel.

**B.5.9.2 Report on clinical cases and poisoning incidents (Annex IIA 5.9.2)**

There have been no reports of cases of exposure of humans to florasulam. There is no information on florasulam in open literature because the use of florasulam has been confined to company internal trials and there has been no exposure of the general population.

**B.5.9.3 Observations on exposure of the general population and epidemiological studies (Annex IIA 5.9.3)**

The use of florasulam has been confined to company internal trials and there has been no exposure of the general population.

**B.5.9.4 Clinical signs and symptoms of poisoning and details of clinical tests (Annex IIA 5.9.5)**

In animal studies, the acute toxicity of florasulam, by oral, dermal and inhalation routes, is low and no macroscopic evidence of specific organ effects could be found in any experimental animals. No specific symptoms of poisoning have been defined and no specific diagnostic test of poisoning is known. However, in repeated exposures in mammals, the liver and kidney are the main target organs. The most sensisitive indicator of toxicity was increased serum alkaline phosphatase in the dog. This elevation reverted to normal levels upon reduction or cessation of exposure. Therefore, although there is no example of human exposure as yet, a reversible elevation of liver enzymes in the serum could be a relevant diagnostic pointer in cases of suspected poisoning with florasulam.
Poisoning should be suspected after evidence of a gross exposure to the technical compound, or neat formulated florasulam or an undiluted formulation containing florasulam. The primary route of excretion of florasulam is, unchanged, in the urine.

**B.5.9.5 First aid measures - Therapeutic regimes (Annex IIA 5.9.5)**

First aid measures are non-specific and supportive, as for any substance of low toxicity.
In the event of ingestion, observation is appropriate. In cases where it is certain that there has been an ingestion of more than about 140 gr of florasulam less than an hour earlier, physicians will wish to consider carrying out gastric lavage, provided that the airway can be protected. This advice is based upon the assumption that it would be desirable to remove florasulam from the stomach when ingestion has exceeded 2 g/kg bw in a 70 kg person.
In the event of contamination of the skin, either with the active substance or with formulated product, the affected area should be washed with soap and water and rinsed with clean water.
In the event of contamination, or suspected contamination, of the eyes, either with the active substance or with the formulated product, the eyes should be irrigated with water or physiological saline immediatly, with irrigation continuing for at least five minutes.
There is no specific antidote to florasulam.

**B.5.9.6.1 Expected effects and duration of poisoning as a function of the type, level and duration of exposure or ingestion (Annex IIA 5.9.6)**

Animal testing has failed to show any specific organ or tissue effects following acute exposure to florasulam. However, in repeated exposures in mammals, the liver and kidney are the main target organs. The most sensitive

indicator of toxicity was increased serum alkaline phosphatase activity in the dog. This elevation would revert to normal levels upon cessation of exposure. Therefore, in cases of massive or prolonged exposure to, or absorption of, florasulam, it would be prudent to investigate hepatic and renal function.

B.5.9.6.2 Expected effects and duration of poisoning as a function of varying time periods between exposure or ingestion and commencement of treatment (Annex IIA 5.9.6)

From the animal studies, it appears that florasulam is rapidly absorbed and excreted, mainly as unchanged compound in urine, within 24 h following absorption. The compound does not accumulate. After acute intoxication, no specific effects are expected. After repeated exposure, target organs will be probably kidney and liver.

**B.5.10 Summary of mammalian toxicology and proposed ADI, AOEL and drinking water limit (Annex IIA 5.10)**

*Absorption, distribution, metabolism, excretion* :

After oral administration in rats, absorption of florasulam was rapid and extensive reaching 85-91% of the dose. Increasing the dose by a factor of 50 reduced somewhat the absorption to 77%.
After dermal application, a potentially absorbed dose of 12% was calculated.
30 minutes post-dosing, distribution was large and the amount of radioactivity was highest in gastrointestinal tract, carcass, skin and organs involved in metabolism and excretion e.g.liver and kidney. The average amount of radioactivity remaining in rats 168 h post-dosing was low and <0.01% of the dose. At that time, the greatest amount remained in the skin (0.18-0.52%).
Metabolism of florasulam was minor and limited to hydroxylation of the phenyl ring without affecting the sulfonamide bond.
Excretion reached 96-99% of the dose within 7 day mainly as unchanged florasulam. Urinary excretion was the major route and represented 77-89% of the dose within 24 h after administration while fecal excretion represented 6.5% of the dose. Increasing the dose reduced urinary excretion to 77% with a concomitant increase in fecal excretion to 16%.
In plants , laying hens and lactating goats, florasulam is rapidly and extensively excreted . Metabolism does not occur at a high rate and does not differ from rat.

*Acute toxicity :*

In rat and mice, acute oral toxicity of florasulam was low, however, some death occurred in rats, at 6000 mg/kg bw and at 5000 mg/kg bw in mice, females being more sensitive than males. Transient clinical signs such as salivation, urine and fecal soiling in the perineal area suggest toxic effects towards the autonomic (parasympathomimetic) system .
Florasulam caused a minor, transient depression of activity and depression of reactivity to noise stimulus on the day of dosing in male rats at 2000 mg/kg bw. These effects, as well as perineal soiling disappeared after day 3.
Local signs such as erythema and edema which completely resolved on day 10 were observed after dermal application. Female rabbits were also more sensitive than males.
Florasulam is not a skin and eye irritant, it is not a sensitizer and is not classified.

*Short-term toxicity:*

In rat and mice, oral administration of florasulam induced a decreased food intake, probably related to food unpalatibility, and a reduced body weight. Target organs were kidneys in which a minor functional impact was observed. Kidney proximal tubules presented nuclear pleomorphism and multifocal necrosis. These effects were noted, after 2-3 week exposure, at 500 mg/kg bw/d onwards.
In dogs, hepatocellular injury and cholestasis were reported at 150 mg/kg bw/d onwards. Some kidney tubule vacuolation was seen.

After 13 week oral exposure of rats, at 500 mg/kg bw/d onwards, different parts of the nefron were target of florasulam:

- renal collecting ducts (probably Type A intercalated cells) were hypertrophied , in male and females F344 and CD rats, severity was higher in CD rats.
- descending proximal tubules necrosis with degeneration/regeneration of a higher portion of the nephron, in females CD and F344 rats. This effect is typical of acute necrosis with regeneration rather than a 13 week old rat lesion.
- In the tubules of papilla, small foci of mineralized debris were reported in females F344 rats, while in CD rats, papillary necrosis with secondary hyperplasia was observed..

While intercalated cell hypertrophy and urine acidification were reversible, mineralization in papillae and degeneration/regeneration in cortical tubules did not recover in female F344 high dose group.

Bilateral multifocal hypertrophy of epithelial cells of collecting ducts was also observed in mice kidneys at a very slight degree suggesting probably an adaptative response. This lesion was morphologically the same as that reported in Fisher 344 rats. Anemia and decreased extramedullary hematopoiesis in the spleen was also evidenced.
For these studies, a NOAEL = 100 mg/kg bw/d is acceptable.

Dogs were the most sensitive species. Anemia was reported and increased serum enzymes suggested hepatotoxicity at 50 mg/kg bw/d. Renal hypertrophy and adrenal vacuolation were also evident. The hypertrophic intercalated cells, seen in dogs, seems to represent a slight exacerbation of a spontaneous lesion, as this effect was not associated with any cellular degeneration or necrosis. In dogs, the lowest NOAEL was 5 mg/kg bw/d.
From these repeated dose, subchronic experiments, a reference NOAEL of 5 mg/kg bw/d can be derived for short-term repetitive exposures.

*Genotoxicity:*

Florasulam was tested for its mutagenic potential *in vitro* and negative results were observed in the bacterial point mutation assay as well as in the mammalian cell assay. Negative results were also reported in its ability to induce *in vitro* chromosomal damage in rat lymphocytes. Florasulam did not induce micronuclei in mouse bone-marrow after *in vivo* administration.
In conclusion, florasulam is devoid of genotoxic effects *in vitro* and *in vivo*.

*Long-term toxicity :*

Florasulam identified in rats body weight, body weight gain, and the kidney as target organs. Urine analysis revealed a treatment-related decrease of urinary pH for males receiving 250 or 500 mg/kg bw/d and for females receiving 125 or 250 mg/kg bw/d; urine specific gravity was decreased in high dose males, with lower levels of urinary protein and ketones. Kidney weight were increased in high dose males at 1 and 2 year and in high dose females at 2 year. At and above dose levels of 125 mg/kg bw/d, hypertrophy of cells within the collecting duct of the kidney were observed upon histopathological examination after 1 year, and more prominent after 2 year. In males given 250 or 500 mg/kg bw/d, mineralization of sloughed epithelial cells within loops of Henle was also reported. Unilateral papillary necrosis was reported in males. These effects were not associated with a deterioration of the renal function.
Florasulam was not carcinogenic in rats. The NOAEL in this study is 10 mg/kg bw/d.

In mice, at 500 and 1000 mg/kg bw/d, florasulam produced consistent microscopic effects, with the kidney being the only target organ identified. Hypertrophy of collecting duct cells was observed in the inner and outer stripe of the renal medulla, affecting sometimes cells in the proximal portion of the papilla. There was also an increased incidence of decreased cytoplasmic vacuolation of the cortical epithelial cells which may have contributed to the lower kidney weights. Clinical chemistry was not indicative of altered renal function. Treated mice were found to have less spontaneous occurring geriatric renal disease compared to their respective control.
In mice, florasulam was not carcinogenic and the NOAEL is 50 mg/kg bw/d.

The underlying mechanism for collecting duct cell hypertrophy is not fully understood. It is however suggested that α-intercalated cells are involved.

*Reproduction toxicity:*

In the two generation study, parental animals receiving florasulam at the highest dose(500 mg/kg bw/d) had decreased food consumption and body weight during most of the pre-mating, gestation and lactation periods. An increased relative kidney weight was observed in P1 and P2 adults. Necrosis and/or inflammation of the renal papilla with the resultant hemorrhagic cast in the lumen of the urinary bladder occurred in some top dose P1 and P2 rats. There were no abnormalities in the reproductive performance of parental animals and the reproductive index of offspring.
In develommental studies in rats, maternal toxicity was observed at 750 mg/kg bw/d as suggested by the decreased body weight, food consumption and increased kidney weights. No adverse embryonal/fetal effects were observed at any dose level tested.
In rabbits, no maternal toxicity was observed and there was no evidence of teratogenicity at dose levels up to and

including 500 mg/kg bw/d.

*Neurotoxicity:*

Florasulam caused a minor, transient depression of activity and depression of reactivity to noise stimulus on the day of dosing in male rats at 2000 mg/kg bw. Perineal soiling occurred in some high and mid dose female rats. These effects disappeared after day 3.
After 1 year feeding, there were no effects of florasulam on any parameters measured in rats, that would suggest a neurotoxic effect.

### B.5.10.1 Acceptable daily intake (ADI)

An ADI can be calculated from the lowest NOAEL of 5 mg/kg bw/d, identified in the 1 year dog study. The effects at the next highest dosage of 50 mg/kg bw/d were an increase in serum AP activity, of hepatic origin, hypertrophy of renal collecting duct cells and adrenal gland vacuolation.
Applying an assessment factor of 100 (10 for interspecies variation x 10 for intraspecies variation), the acceptable daily intake becomes :

ADI = 0.05 mg/kg bw/day

The applicant proposes to use the next higher dose of 50 mg/kg bw/d as being a NOAEL, because the increased PA activity was without any histopathological correlate, the renal hypertrophy was only slightly more pronounced than the spontaneous form of the lesion seen in this species and the adrenal vacuolation also represents a slight exacerbation of a spontaneous lesion.

### B.5.10.2 Acceptable operator exposure (AOEL)

The AOEL short-term for man is calculated on the basis of an internal NOAEL from a sub-chronic animal experiment, taking into account the apparent degree of absorption, and applying an assessment factor, chosen in function of the critical effect observed in the animal experiments.
There is no evidence that florasulam is mutagenic, carcinogenic, neurotoxic, teratogenic or a reproduction toxin.

It appears from the animal experiment that a subchronic exposure to 5 mg/kg bw/d (lowest NOAEL, taken from the 90 day, dog study) of florasulam will not result in any toxic effect. At the next highest dosage, AP was increased, hepatic vacuolation as well as a slight hypertrophy of epithetial cells lining renal collecting ducts were observed. The oral absorption seems to be near 100%. Applying an assessment factor of 100 for extrapolation to man. The acceptable operator exposure level, expressed as an internal, systemic dose becomes:

AOEL systemic = 0.05 mg/kg bw/d

The notifier proposed to use the same study but taking an assessment factor of 25, this gives an AOEL =0.2 mg/kg bw/d.

Although a 28-day dermal study with rats is available, it was not considered appropriate to derive a dermal AOEL, since no tratment-related effects were observed up to the highest dose level.

### B.5.10.3 Acute reference dose.

Florasulam has been assessed in acute toxicity studies in rats and mice, in an acute neurotoxicity study in rats and in developmental studies in rats and rabbits. In all of these studies, florasulam showed a very low acute toxicity. It can be concluded from the submitted data, that there is no concern with regard to acute intake by consumers. Therefore, there is no need for derivating an ARfD.

Is intended for use on winter and spring cereals and will have one or two applications per season.

**B.5.11 Acute toxicity including irritancy and skin sensitization of the preparations (Annex IIIA 7.1)**

**B.5.11.1 Acute oral toxicity (Annex IIIA 7.1.1)**

-Rat, CD strain, limit test at 2000 mg/kg b.w. (Johnson, 1996a)

**Findings:**
*Mortality* : there was no death.
*Clinical signs*: there was no sign of reaction to treatment.
*Body weight* : was not affected.
*Necropsy* : no significant macroscopic lesions.

**Conclusion:**
$LD_{50}$ oral rats > 2000 mg/kg bw

**Guidelines**: Protocol in compliance with test method B.1 of directive 92/69/EEC
**GLP status**: The study is GLP (no attest of competent authority).
**Materials and methods**:
5 rats/sex (CD) reveived a single dose by gavage of EF-1343 (suspension of 50g/l of florasulam) (B.n° B767-42) in purified water, at a dose level of 2000 mg/kg/bw.
The study is accepted.

-Rat, Fischer 344 strain, limit test at 5000 mg/kg b.w. (Haut and Brooks , 1997a)

**Findings:**
*Mortality:* none
*Clinical signs:* 1_: and 1_ rat had fecal soiling of the perineal region on the day of dosing, which resolved by day 2. Urine soiling of the perineal region was also observed on the same female rat but was resolved by test day 3.
*Body weight:* not altered
*Necropsy findings:* none.

**Conclusion:**
$LD_{50}$ oral rats > 5000 mg/kg bw

**Guidelines:**Protocol in compliance with test method B.1 of directive 92/69/EEC
**GLP status:**The study is GLP (no attest of competent authority).
**Materials and methods:**
5 rats/sex (Fisher 344) reveived a single dose by gavage of EF-1343 (suspension of 50g/l of florasulam) (B.n° B767-7-A) neat test material, at a dose level of 5000 mg/kg/bw.
The study is accepted.

-Mice, limit test at , 5000 mg/kg b.w. (Haut and Brooks, 1997b)

**Findings:**
*Mortality* : there were no death.
*Clinical signs*: no effect.
*Body weight:* all animals gained body weight during the study.
*Necropsy:* no treatment related macroscopic findings.

**Conclusion:**
$LD_{50}$ oral mice > 5000 mg/kg bw

**Guidelines:** Protocol not fully in compliance with test method B.1 of directive 92/69/EEC

***Deviation from protocol:*** animals not fasted prior to dosing.
**GLP status:** The study is GLP (no attest of competent authority)**.**
**Materials and methods:**
5 mice/sex (CD-1) reveived a single dose by gavage of EF-1343 (suspension of 50g/l of florasulam) (B.n° B767-7-A) neat test material, at a dose level of 5000 mg/kg/bw.
The study is accepted.

### B.5.11.2 Acute percutaneous toxicity (Annex IIIA 7.1.2)

-Rat, EF-1343, 2000 mg/kg bw , occlusive dressing (Johnson, 1996b)

**Findings:**
*Mortality:* there was no death
*Clinical signs*: there were no systemic signs nor local signs of reaction to treatment.
*Body weight* : were not altered
*Necropsy* : no significant macroscopic lesions were observed.

**Conclusion:**
$LD_{50}$ >2000 mg/kg b.w.

**Guidelines:** Protocol not fully in compliance with test method B3 of directive 92/69/EEC
*Deviation from protocol:* animals are not caged individually
GLP status:The study is GLP (no attest of competent authority).
**Materials and methods:**
5 rats/sex (CD) were exposed to EF-1343 (suspension of 50g/l of florasulam) (B.n° B767-42), at a dose level of 2000 mg/kg/bw (undiluted compound) by dermal occlusive application for 24h.
The study is accepted.

### B.5.11.3 Acute inhalation toxicity to rats (Annex IIIA 7.1.3)

Based on the intended uses and methods of application of Primus SC, an inhalation study on the undiluted formulation is not triggered . Primus SC is a liquid formulation. It will be applied to cereals by field crop or knapsack sprayers that do not generate a significant proportion (>1% on a weight basis) of particles or droplets of diameter < 50 µm.

### B.5.11.4 Skin irritation (Annex IIIA 7.1.4)

The acute percutaneous $LD_{50}$ of Primus SC was greater than 2000 mg/kg bw and this EC limit test dose caused no irritation of the skin at the treated site.
As no reactions occurred at the dose site in any animal in the percutaneous toxicity study, EC test guideline (method B.4., 92/69 EEC) indicate that, under these circumstances, the test material should not be tested further in animals for dermal irritation.

### B.5.11.5 Eye Irritation (Annex IIIA 7.1.5)

- 3 Rabbit , 0.1 ml of undiluted substance (Johnson, 1996c)

Findings:
Evaluation of the data, according to EU methodology:

<Score corneal opacity>$_{24+48+72}$ = 0/0/0
<Score iris>$_{24+48+72}$ = 0/0/0
<Score erythema>$_{24+48+72}$ = 0.3/0/0

<Score chemosis>$_{24+48+72}$ = 0/0/0

Conclusion:
**EF-1343** has no eye irritating properties

**Guidelines:** Protocol in compliance with test method B.5 of directive 92/69/EEC
**GLP status:** The study is GLP (no attest of competent authority).
Materials and methods:
A preliminary irritation screen was carried out using a single rabbit and 0.1 ml of Primus SC was instilled followed by irrigation. In the absence of severe irritation response in the screen animal , the main study was undertaken.
3 _ rabbits (NZW) were exposed to EF-1343 **(suspension of 50g/l of florasulam) (B.n° B767-42)** by instillation of 0.1 ml of the undiluted test substance into the everted lid of the right eye. **The study is accepted.**

-6 Rabbit, 0.1 ml, (Haut and Brooks, 1997c)

Findings:
Evaluation of the data, according to EU methodology:
<Score corneal opacity>$_{24+48+72}$ = 0
<Score iris>$_{24+48+72}$ = 0
<Score redness>$_{24+48+72}$ = 0.055
<Score chemosis>$_{24+48+72}$ = 0

Conclusion:
**EF-1343** is not irritating to eyes.

Guidelines: Protocol in compliance with test method B.5 of directive 92/69/EEC
GLP status: The study is GLP (no attest of competent authority).
Materials and methods:
3 rabbits/sex (NZW) were exposed to EF-1343 (suspension of 50g/l of florasulam) (B.n° B767-7-A) by instillation of 0.1 ml of the undiluted test substance into the everted lid of the right eye. An ocular anesthetic was used for both eyes of each rabbit after discomfort was observed in the first animal.
The study is accepted.

### B.5.11.6 Skin sensitization (Annex IIIA 7.16)

-Guinea Pig, modified Buehler test, pure compound (Johnson, 1996d)

Findings
No significant dermal response was observed in either the test or control animals, challenged with Primus SC.

Conclusion:
EF-1343 is not a sensitizer.

**Guidelines:** Protocol not fully in compliance with test method B.6 of directive 92/69/EEC.
*Deviation from official protocol:* induction must be performed on day 0, 6-8 and 13-15.
**GLP status**: The study is GLP (no attest of competent authority).
Materials and methods:
In the preliminary irritation test (30%, 50%,75% and 100%, dilutions in purified water, during 6h), it was established that no irritating properties appeared with the undiluted compound.
10 guinea pigs (Dunkin-Hartley)/sex were used for test group and 5/sex for the control group.
*Induction phase*: 0.5 ml undiluted test substance (EF-1343 **(suspension of 50g/l of florasulam), B.n° B767-42**) or vehicle (purified water) was applied to the shaved skin, for 6h, on day 1, 3, 5, 8, 10, 12, 15, 17 and 19.
*Challenge:*

On day 29, animals were treated by occluded application of 0.5 ml undiluted test compound or water.
The study is accepted.

**B.5.11.7 Supplementary studies for combinations of plant protection products (tests as at points 7.1.1 to 7.1.6) (Annex IIIA 7.1.7)**

No data. Not necessary.

**B.5.12 Dermal absorption (Annex IIIA 7.3)**

**B.5.12.1 Dermal absorption, in vivo in the rat (Annex IIIA 7.3)**

A study was performed in 4 male Fischer 344 rats/ group following a single topical application (24 h semi-occluded,120 µl applied on area of dorsal skin of 12 $cm^2$) of undiluted formulation and a spray dilution, chosen to represent concentrations that will be applied to field crops. Animals were killed after 24, 48 or 72 h. The proposed highest use rate is 0.5 L product/ha (7.5 g florasulam) in 200 l of water equivalent to a spray solution of 37.5 mg florasulam/ L.

- Diluted formulation (nominally 100 mg florasulam/L) that was representative of concentrations that may be applied to field crops.
The target spray solution concentration was therefore, 2.67 times more concentrated than the anticipated highest spray concentration that will be used on field crop.
- Concentrated formulation , Primus SC which was used as supplied.

Findings:
Primus SC has shown a very low potential for dermal absorption in the rat : an average of less than 0.5% of the applied radiolabelled dose was absorbed.The proportion of the dose remaining in the skin beyond 24 h after skin swabbing is significantly more than was absorbed and excreted, and did not decrease at the 48 and 72 h time points.Therefore, it is considered unlikely that the dose remaining in the skin would be absorbed ; it would probably be removed with the natural epidermal turnover.

**Comments from the RMS:**
72 h after application of formulation , excretion has not come to an end, and it cannot be excluded that the amount retained in the application site skin may eventually become systemically available as suggested by the increased urinary excretion observeed at 48 and 72h time points. Moreover, after oral administration of florasulam, it appeared from the ADME studies that some affinity for skin was observed. Therefore, the potentially absorbed dose (the amount systemically available plus the amount in the application site skin) should be used as a value for dermal absorption. **An average of 12% (absorbed dose + treated skin) of the applied radiolabelled dose was absorbed.**

**Table B.5.12.1-1 : Dermal absorption in rat skin of $^{14}$C labeled formulation of florasulam (Bounds, 1997).**

| Achieved dose µg/$cm^2$ | Target dose µg/$cm^2$ | Absorbed dose | | Treated skin | | Skin swab/gauze wash/tape strip | | Recovery |
|---|---|---|---|---|---|---|---|---|
| 24 h kill | | % | µg | % | µg | % | µg | % |
| 0.90 | 1 | 0.18* | 0.02 | 12.2 | 1.3 | 87.9 | 9.3 | 100.3 |
| 530 | 500 | 0.38** | 0.02 | 11.1 | 0.7 | 90.7 | 5.72 | 102.2 |
| 48 h kill | | | | | | | | |
| 0.90 | 1 | 0.26* | 0.028 | 21.8 | 2.32 | 79.8 | 8.44 | 101.9 |
| 530 | 500 | 0.44** | 0.03 | 9.88 | 0.62 | 91.6 | 5.78 | 102.0 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 72 h kill | | | | | | | | |
| 0.90 | 1 | 0.29* | 0.032 | 20.9 | 2.2 | 79.13 | 8.3 | 100.3 |
| 530 | 500 | 0.13** | 0.01 | 10.0 | 0.63 | 92.55 | 5.85 | 102.7 |

* : express radioactivity in urine ; levels of radioactivity in feces, cage wash, carcass, tissues, untreated skin, liver, kidneys, blood were below limit of detection.
**urine + cage wash + untreated skin + carcass ; liver, kidneys, feces below detection limit.

Conclusion :
A value of 12% for potentially dermal absorption of pure and diluted formulation will be used for calculation of operator exposure.

**B.5.12.2 Comparative dermal absorption, *in vitro* using rat and human skin Annex IIIA 7.3)**

No data. Not necessary.

**B.5.13 Toxicological data on non active substances (Annex IIIA 7.4 and point 4 of the introduction)**

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**B.5.14 Summary of toxicity of formulation PRIMUS SC**

Table B.5.14-1 : Summary of acute toxicity including irritancy and skin senstisation of PRIMUS SC.

| Type of test | $LD_{50}$ (mg/kg b.w.) | batch n° | Classification | Reference |
|---|---|---|---|---|
| Rat, oral | >5000 | B767-7-A | - | Haut and Brooks, 1997a |
| Mouse, oral | >5000 | B767-7-A | | Haut and Brooks, 1997b |
| Rat, dermal | >2000 | B767-42 | - | Johnson, 1996b |
| Rabbit, eye irritation | not irritant | B767-7-A | - | Haut and Brooks, 1997bc |
| Guinea pig, skin sensitisation (Buehler) | not sensitiser | B767-42 | - | Johnson, 1996d |

**B.5.15 Exposure data (Annex IIIA 7.2)**

**B.5.15.1 Estimation of operator exposure(Annex IIIA 7.2.1.1)**

PRIMUS SC is intended for use on winter and spring cereals and will have one or two applications per season. Potentially, Primus SC could be applied during a five-month period each year. Applicators could experience frequent exposure for more than three months in any 12-month period. However, evidence suggest that this scenario is unlikely to occur in practice.
The formulation PRIMUS SC (florasulam (50g/l) is designed for spray application (tractor mounted boom with air assisted sprayers). It is recommended to apply a preparation with 30, or 100 l water/ha.
Model calculations were made on the basis of UK-POEM and German model .

**Applications parameters :**

**Table B.5.15.1-1 : Application parameters .**

| Application technique | Crop | Max. application rate (kg a.s./ha) | Water vol. (L/ha) | Treatment area (ha) |
|---|---|---|---|---|
| field crop sprayer / tractor mounted | winter and spring cereals | 0.0075 | 0.15 | 50(UK) 20(G) |

**The following parameters were taken into account for exposure estimates :**

| | UK POEM | GERMAN MODEL |
|---|---|---|
| Use rate: | 7.5 g a.s./ha | 7.5 g a.s./ha |
| Number ha treated/day: | 50 | 20 |
| Application equipment: | tractor mounted with cab | field crop tractor mounted |
| Body weight: | 60 kg | 70 kg |
| AOELsytemic: | 0.05 mg/kg bw/day | Itol = 3.5 |
| | | Dtol =42 |
| Dermal absorption: | 12% | 12% |

**Protective equipment :**
Calculations were made for scenarios without protective equipment.

**Expected operator exposures :**
**Table B.5.15.1-2 : Estimated operator exposure (mg/person/day) according to the UK POEM model**

| Type of protection: none Dermal absorption : 12% | Dermal absorbed dose (mg/person/day) | | Inhalation exposure (mg/person/day) | Total absorbed dose (m g/ person/day) |
|---|---|---|---|---|
| | Mix/load | Spray | Spray | |
| 30 l/ha | 0.48 | 0.373 | 0.0045 | 0.857 |
| 100 l/ha | 0.48 | 1.246 | 0.015 | 1.741 |

**Table B.5.15.1-3 : Estimated external operator exposure (mg/person/day) according to the German model**

| Type of protection/ dermal absorption | Dermal exposure (mg/person/day) | | | Inhalation exposure (mg/person/day) | | | Total external exposure (mg/person/ day) |
|---|---|---|---|---|---|---|---|
| | Mix/load | Spray | Total | Mix/load | Spray | Total | |
| Type of protection : none | | | | | | | |
| 12% | 0.36 | 0.306 | 0.666 | 0.00009 | 0.00015 | 0.00024 | 0.66624 |

Comparision of estimated and tolerable exposure :

Table B.5.15.1-4 : Exposure as a proportion of AOEL -UK model

| **Total systemic exposure - 60 kg person (mg/kg bw/day)** | | **% of AOEL** |
|---|---|---|
| **no PPE worn** | | **no PPE worn** |
| 30 l/ha | 0.0142 | 28 |
| 100 l/ha | 0.0290 | **58** |

**Table B.5.15.1-5 : Total degree of exposure E - German model**

| **Total exposure - 70 kg person (mg/kg bw/day)** | **E** |
|---|---|
| no PPE worn | no PPE worn |
| 0.00951 | 0.106 |

**Conclusions :**
The operator exposure (% AOEL, degree of exposure E) without protective equipment is acceptable.

**B.5.15.2 Measurement of operator exposure (Annex IIIA 7.2.1.2)**

No data. Not necessary.

**B.5.15.3 Estimation of bystander exposure (Annex IIIA 7.2.2)**

It can be assumed that bystanders may be present during the field use of PRIMUS SC and can therefore be considered to be exposed mainly by the airborne route but also by dermal route.
Usind the data from operator exposure calculated in the UK-POEM model, estimation of bystander exposure can estimated :
Dermal and inhalation exposure of the operator during spraying reaches (0.373 + 0.0045 mg/person/d) 0.3775 mg/perwson/d which represents 0.00629 mg/kg bw/d = 12% of the AOEL , when a solution of 0.075 mg/kg (100 l/ha) Primus SC is applied.
With the higher concentration of 0.25 mg/l (30 l/ha), exposure of bystander will represent 1.261 mg/person/d or 0.0210 mg/kg bw/d (42% of AOEL) .
**Conclusion :**
The bystander exposure is considered as acceptable.

**B.5.15.4 Estimation of worker exposure (Annex IIIA 7.2.3.1)**

The active substance is used as herbicide in cereals at stage 2-3 leaves to stem elongation. Reentry of the worker to the crop is not expected.

**B.5.15.5 Measurement of worker exposure (Annex IIIA 7.2.3.2)**

No data. Not necessary.

**B.5.16 References relied on**

**Toxicology and metabolism of the active substance (Annex IIA 5)**

| Annex point(s) 91/414/EEC | Author, Title, Test institute, Report number/Study ID, Date of report For publications: reference | Dow AgroSciences Report No. | GLP GEP | Published Protected |
|---|---|---|---|---|
| IIA 5.1.2 | Bounds, SVJ, XDE-570: Dermal Absorption of [$^{14}$C] XDE-570 in Male Fischer 344 Rats Following Exposure to Undiluted EF-1343 and a Spray Solution Huntingdon Life Sciences Ltd, Eye, Suffolk, UK DWC891/972958, October 1997 | GHE-T-828 | Yes | Unpublished Protected |
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| IIA 5.2.3 | Clements, CM, Cieszlak, FS, XDE-570: Acute Aerosol Inhalation Toxicity Study with Fischer 344 Rats The Dow Chemical Company, Midland, Michigan, USA DECO-HET DR-0312-6565-015, March 1995 | DECO-HET DR-0312-6565-015 | Yes | Unpublished Protected |
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| IIA 5.2.6 | Gilbert, KS, XDE-570: Dermal Sensitisation Potential in the Hartley Albino Guinea Pig | DR-0312-6565-012E | Yes | Unpublished Protected |

| Annex point(s) 91/414/EEC | Author, Title, Test institute, Report number/Study ID, Date of report For publications: reference | Dow AgroSciences Report No. | GLP GEP | Published Protected |
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| IIA 5.6.1 | Liberacki, AB, Carney, EW, Kociba, RJ, XDE-570: Two Generation Dietary Reproduction Study in CD rats<br>The Dow Chemical Company, Midland, Michigan, USA | DR-0312-6565-028 | Yes | Unpublished Protected |

| Annex point(s) 91/414/EEC | Author, Title, Test institute, Report number/Study ID, Date of report For publications: reference | Dow AgroSciences Report No. | GLP GEP | Published Protected |
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| IIA 5.3.2.1 | Redmond, JM, Johnson, KA, XDE-570: 13-Week Dietary Toxicity and 4-Week Recovery Study in F344 Rats<br>The Dow Chemical Company, Midland, Michigan, USA<br>DR-0312-6565-011, January 1996a | DR-0312-6565-011 | Yes | Unpublished Protected |
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| Annex point(s) 91/414/EEC | Author, Title, Test institute, Report number/Study ID, Date of report For publications: reference | Dow AgroSciences Report No. | GLP GEP | Published Protected |
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| IIA 5.3.3.3 | Scortichini, BH, Kociba, RJ, XDE-570: 28-Day Repeated Dose Dermal Toxicity Study in Fischer 344 Rats<br>The Dow Chemical Company, Midland, Michigan, USA<br>970142, July 1997 | DR-0312-6565-033 | Yes | Unpublished Protected |
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| IIA 5.3.1.3 | Sullivan, JM, Singleton, NC, XDE-570: Exploratory Four-Week Dietary Toxicity Study in Beagles<br>The Dow Chemical Company, Indianapolis, Indiana, USA<br>DR-0312-6565-018, June 1995a | DR-0312-6565-018 | Yes | Unpublished Protected |
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| IIA 5.6.2.2 | Zablotny, CL, Quast JF, XDE-570: Oral Gavage Teratology Probe Study in New Zealand White | DR-0312-6565-023 | Yes | Unpublished Protected |

| Annex point(s) 91/414/EEC | Author, Title, Test institute, Report number/Study ID, Date of report For publications: reference | Dow AgroSciences Report No. | GLP GEP | Published Protected |
|---|---|---|---|---|
| | Rabbits<br>The Dow Chemical Company, Midland, Michigan, USA<br>DR-0312-6565-023, November 1996 | | | |

**Toxicology and metabolism of the formulation EF-1343 (Annex IIIA 7)**

| Annex point(s) 91/414/EEC | Author, Title, Test institute, Report number/Study ID, Date of report For publications: reference | Dow AgroSciences Report No. | GLP GEP | Published Protected |
|---|---|---|---|---|
| IIIA 7.3 (see IIA 5.1.2) | Bounds, SVJ, XDE-570: Dermal Absorption of [$^{14}$C] XDE-570 in Male Fischer 344 Rats Following Exposure to Undiluted EF-1343 and a Spray Solution<br>Huntingdon Life Sciences Ltd, Eye, Suffolk, UK<br>DWC891/972958, October 1997 | GHE-T-828 | Yes | Unpublished Protected |
| IIIA 7.1.1 | Haut, KT, Brooks, KJ, DE-570 50 g/l SC Herbicide: Acute Oral Toxicity Study in Fischer 344 Rats<br>The Dow Chemical Company, Midland, Michigan, USA<br>971064, June 1997a | DR-0355-9486-001 | Yes | Unpublished Protected |
| IIIA 7.1.1 | Haut, KT, Brooks, KJ, DE-570 50 g/l SC Herbicide: Acute Oral Toxicity Study in CD-1 Mice<br>The Dow Chemical Company, Midland, Michigan, USA<br>971063, June 1997b | DR-0355-9486-001 | Yes | Unpublished Protected |
| IIIA 7.1.5 | Haut, KT, Brooks, KJ, DE-570 50 g/l SC Herbicide: Acute Primary Eye Irritation Study in New Zealand White Rabbits<br>The Dow Chemical Company, Midland, Michigan, USA<br>971065, June 1997c | DR-0355-9486-002 | Yes | Unpublished Protected |
| IIIA 7.1.1 | Johnson, IR, EF-1343 (XDE-570 50 SC): Acute Oral Toxicity Study in the Rat<br>Huntingdon Life Sciences Ltd, Eye, Suffolk, UK<br>95/DES312/0908, February 1996a | GHE-T-609 | Yes | Unpublished Protected |
| IIIA 7.1.2<br>IIIA 7.1.4 | Johnson, IR, EF-1343 (XDE-570 50 SC): Acute Percutaneous Toxicity Study in the Rat<br>Huntingdon Life Sciences Ltd, Eye, Suffolk, UK<br>95/DES313/0906, February 1996b | GHE-T-610 | Yes | Unpublished Protected |
| IIIA 7.1.5 | Johnson, IR, EF-1343 (XDE-570 50 SC): Acute Eye Irritation Test in the Rabbit<br>Huntingdon Life Sciences Ltd, Eye, Suffolk, UK<br>95/DES315/0909, February 1996c | GHE-T-611 | Yes | Unpublished Protected |
| IIIA 7.1.6 | Johnson, IR, EF-1343 (XDE-570 50 SC): Delayed Contact Hypersensitivity Study in the Guinea-Pig<br>Huntingdon Life Sciences Ltd, Eye, Suffolk, UK<br>95/DES316/1400, February 1996d | GHE-T-612 | Yes | Unpublished Protected |

# ANNEX B

# Florasulam

# Appendix B : Estimation of the operator exposure

| 1.UK model for the determination of the operator exposure : tractor mounted boom with hydraulic nozzles | | | |
|---|---|---|---|
| PRODUCT DATA | | | |
| Product | Primus SC | | |
| Active substance | florasulam | | |
| Concentration | 50 mg/ml | | |
| Formulation type | SC | | |
| Maximum in use rate concentration | 0.075 mg/ml | | |
| EXPOSURE DURING MIXING AND LOADING | | | |
| Container size | 1L | | |
| Hand contamination/operation | 0.01 ml | | |
| Application dose | 0.15 L product/ha | | |
| Work rate | 50 ha/day | | |
| Number of operations | 8/day | | |
| Hand contamination | 0.08 ml/day | | |
| Protective clothing | none | | |
| Transmission to the skin | 100% | | |
| Dermal exposure to formulation | 0.08 ml/day | | |
| EXPOSURE DURING SPRAY APPLICATION | | | |
| Tractor, hydraulic boom and nozzles | | | |
| Application volume | 100 l spray/ ha | | |
| Volume of surface contamination | 10 ml/h | | |
| Distribution | Hands | Trunk | Legs |
| | 65 | 10 | 25 |
| Clothing | none | permeable | permeable |
| Penetration | 100% | 5% | 15% |
| Dermal exposure | 6.5 | 0.05 | 0.375 ml/h |
| Duration of exposure | 6 h | | |
| Total dermal exposure to spray | 41.55 ml/day | | |

| ABOSRBED DOSE | Mixing/loading | Application |
|---|---|---|
| Dermal exposure | 0.08ml/day | 41.55 ml/day |
| Concentration of a.s. | 50 mg/ml | 0.075 mg/ml |
| Dermal exposure to a.s. | 4 mg/day | 3.116 mg/day |
| Percent abosrbed | 12% | 12% |
| Absorbed dose | 0.48 mg/day | 0.373 mg/day |

| INHALATION EXPOSURE DURING SPRAYING | |
|---|---|
| Inhalation exposure | 0.01 ml/h |
| Duration of exposure | 6 h |
| Concentration of a.s. | 0.075 mg/ml |
| Inhalation exposure to a.s. | 0.045 mg/day |
| Percent absorbed | 100% |
| Absorbed dose | 0.0045 mg/day |

| PREDICTED EXPOSURE | |
|---|---|
| Total absorbed dose | 0.857 mg/day |
| Operator body weight | 60 kg |
| Operator exposure | 0.0142 mg/kg bw/d |
| % AOEL | 28% |

| 2.UK model for the determination of the operator exposure :tractor mounted boom with hydraulic nozzles | | | |
|---|---|---|---|
| PRODUCT DATA | | | |
| Product | Primus SC | | |
| Active substance | florasulam | | |
| Concentration | 50 mg/ml | | |
| Formulation type | SC | | |
| Maximum in use rate concentration | 0.25 mg/ml | | |
| EXPOSURE DURING MIXING AND LOADING | | | |
| Container size | 1L | | |
| Hand contamination/operation | 0.01 ml | | |
| Application dose | 0.15 L product/ha | | |
| Work rate | 50 ha/day | | |
| Number of operations | 8/day | | |
| Hand contamination | 0.08 ml/day | | |
| Protective clothing | none | | |
| Transmission to the skin | 100% | | |
| Dermal exposure to formulation | 0.08 ml/day | | |
| EXPOSURE DURING SPRAY APPLICATION | | | |
| Tractor, hydraulic boom and nozzles | | | |
| Application volume | 30 L spray/ ha | | |
| Volume of surface contamination | 10 ml/h | | |
| Distribution | Hands | Trunk | Legs |
| | 65 | 10 | 25 |
| Clothing | none | permeable | permeable |
| Penetration | 100% | 5% | 15% |
| Dermal exposure | 6.5 | 0.05 | 0.375 ml/h |
| Duration of exposure | 6 h | | |
| Total dermal exposure to spray | 41.55 ml/day | | |

| ABOSRBED DOSE | Mixing/loading | Application |
|---|---|---|
| Dermal exposure | 0.08ml/day | 41.55 ml/day |
| Concentration of a.s. | 50 mg/ml | 0.25 mg/ml |
| Dermal exposure to a.s. | 4 mg/day | 10.3875 mg/day |
| Percent absorbed | 12% | 12% |
| Absorbed dose | 0.48 mg/day | 1.246 mg/day |

| INHALATION EXPOSURE DURING SPRAYING | |
|---|---|
| Inhalation exposure | 0.01 ml/h |
| Duration of exposure | 6 h |
| Concentration of a.s. | 0.25 mg/ml |
| Inhalation exposure to a.s. | 0.015 mg/day |
| Percent absorbed | 100% |
| Absorbed dose | 0.015 mg/day |

| PREDICTED EXPOSURE | |
|---|---|
| Total absorbed dose | 1.741 mg/day |
| Operator body weight | 60 kg |
| Operator exposure | 0.0290 mg/kg bw/d |

| | |
|---|---|
| % AOEL | 58% |

| German model for the determination of operator exposure : field crop tractor mounted | | | |
|---|---|---|---|
| PRODUCT DATA | | | |
| Product | PRIMUS SC | | |
| Active ingredient | florasulam | | |
| Concentration of the product (g/kg) | 50 g/l | | |
| Formulation type | SC | | |
| Application technique | field crop tractor mounted | | |
| Use rate (kg as/ha) | 0.008 kg/ha | | |
| Area treated per day (ha) | 20 | | |
| Absorption factor (%) | 100% | | |
| | | | |
| AOEL CALCULATION | AOEL oral | | |
| NOAEL(mammal)(mg/kg bw/d) | 5 | | |
| Body weight (kg) | 70 | | |
| Safety factor | 100 | | |
| AOEL (human)(mg/kg bw/d) | 0.05 | | |
| | Otol | Dtol | Itol |
| Tolerable exposure (human)(mg/person/d) | 3.5 | 42 | 3.5 |
| SPECIFIC VALUES OF EXPOSURE FOR THE TYPE OF APPLICATION | | | |
| D*M(H)(mg/person* kg a.s.) | 2.4 | | |
| D*A(H)(mg/person* kg a.s.) | 0.38 | | |
| D*A(B)(mg/person* kg a.s.) | 1.6 | | |
| D*A(C) (mg/person* kg a.s.) | 0.06 | | |
| I*M (mg/person* kg a.s.) | 0.0006 | | |
| I*A (mg/person* kg a.s.) | 0.001 | | |
| ESTIMATION OF OPERATOR EXPOSURE | | | |
| | No protection | | |
| MIXING | | | |
| DM (H) | 0.36 | | |
| IM | 0.00009 | | |
| Application | | | |
| DA (H) | 0.057 | | |
| DA (B) | 0.24 | | |
| DA (C) | 0.009 | | |
| IA | 0.00015 | | |
| RISK ASSESSMENT | | | |
| DM(H)/Dtol | 0.0571 | | |
| DA(H)/Dtol | 0.00904 | | |
| DA(B)/Dtol | 0.00380 | | |
| DA(C)/Dtol | 0.00142 | | |
| IM/Itol | 0.0001714 | | |
| IA/Itol | 0.000285 | | |
| **Degree of exposure (E)** | **0.106** | | |