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SCIENTIFIC COMMITTEE ON PLANTS

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OPINION OF THE SCIENTIFIC COMMITTEE ON PLANTS ON SPECIFIC QUESTIONS FROM THE COMMISSION CONCERNING THE EVALUATION OF FLORASULAM [EF-1343] IN THE CONTEXT OF COUNCIL DIRECTIVE 91/414/EEC

(Opinion adopted by the Committee on 27 September 2001)

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A. TITLE

OPINION OF THE SCIENTIFIC COMMITTEE ON PLANTS ON SPECIFIC QUESTIONS FROM THE COMMISSION CONCERNING THE EVALUATION OF FLORASULAM [EF-1343] IN THE CONTEXT OF COUNCIL DIRECTIVE 91/414/EEC.
(Opinion adopted by the Committee on 27 September 2001).

B. TERMS OF REFERENCE

The Scientific Committee on Plants (SCP) is requested to respond to the following questions in the context of the Commission's work on the implementation of Council Directive 91/414/EEC concerning the placing of plant protection products on the market:

1. Can the Committee comment on the relevance of metabolites ASTCA and DFP-ASTCA?

2. Is it correct to establish an acute reference dose (ARfD) based on the neurotoxicity?

C. OPINION OF THE COMMITTEE

Question 1:

Can the Committee comment on the relevance of metabolites ASTCA and DFP-ASTCA?

Opinion of the Committee:

The Committee concludes that exposure resulting from the concentrations of the ASTCA and DFP-ASTCA metabolites predicted for groundwater will not exceed the threshold of toxicological concern, although the Committee notes that in some agricultural scenarios the groundwater concentration might be higher than that used for the present assessment. Neither metabolite appears to pose an unacceptable risk to non-target aquatic organisms. Data reportedly showing no toxicity to soil and aquatic organisms were not available to the Committee, and would need to be assessed by the RMS.

Question 2:

Is it correct to establish an acute reference dose (ARfD) based on the neurotoxicity?

Opinion of the Committee:

The Committee is of the opinion that the allocation of an acute reference dose based on neurotoxicity is not needed since no unequivocal acute neurotoxic end-point has been identified.

A. TITLE

REPORT OF THE SCIENTIFIC COMMITTEE ON PLANTS ON SPECIFIC QUESTIONS FROM THE COMMISSION CONCERNINGING THE EVALUATION OF FLORASULAM [EF-1343] IN THE CONTEXT OF COUNCIL DIRECTIVE 91/414/EEC.

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C. BACKGROUND

Florasulam is a new active substance (a.s.) in the context of Council Directive 91/414/EEC[1]. A draft assessment report (monograph) has been prepared by the Rapporteur Member State (RMS, Belgium) and the co-Rapporteur (UK) on the basis of a dossier presented by the notifier (Dow AgroSciences).

Florasulam is a broad-leaved weed herbicide. It is intended for use in winter cereals at a rate ranging from 0.5 to 7.5 g a.s./ha. One or two applications per season can be considered, with a total rate of 7.5 g a.s./ha and a spray interval of six to eight weeks. Florasulam is an inhibitor of the acetolactase synthase enzyme (ALS).

Source documents made available to the Committee:

1. Terms of Reference - Evaluation of florasulam in the context of Council Directive 91/414/EEC concerning the placing of plant protection products on the market - Submitted by DG Health and Consumer Protection, 6 December 2000, (SCP/FLORAS/001).

2. Florasulam - Evaluation table - Doc. SANCO/2274/2000 rev. 3 (15.11.00) - Submitted by DG Health and Consumer Protection, 6 December 2000, (SCP/FLORAS/003).

[1] OJ N° L 230, 19. 8.1991, p. 1.

3. Florasulam - Listing of end points - August 2000 - Submitted by DG Health and Consumer Protection, 6 December 2000, (SCP/FLORAS/004).

4. Florasulam - Danish comments - Neurotoxicity and Dermal absorption - 30 November 2000 - Submitted by DG Health and Consumer Protection, 6 December 2000, (SCP/FLORAS/005).

5. Florasulam - Notifier position (12 October 2000) - Definition of the relevant residue in soil and water compartments – the case for considering the 5-OH metabolite not to be relevant - Submitted by DG Health and Consumer Protection, 6 December 2000, (SCP/FLORAS/006).

6. Florasulam – Draft Assessment Report prepared by Belgium in the context of the inclusion in Annex I to Council Directive 91/414/EEC, Volumes 1 to 3, November 1999.

7. Florasulam – Position paper of the notifier on the relevance of 5-hydroxy, ASTCA and DFP-ASTCA metabolites (SCP/FLORAS/007), submitted by the notifier, 24 April 2001.

D. SCIENTIFIC BACKGROUND ON WHICH THE OPINION IS BASED

I. Question 1

Can the Committee comment on the relevance of metabolites ASTCA and DFP-ASTCA?

Opinion of the Committee:

The Committee concludes that exposure resulting from the concentrations of the ASTCA and DFP-ASTCA metabolites predicted for groundwater will not exceed the threshold of toxicological concern, although the Committee notes that in some agricultural scenarios the groundwater concentration might be higher than that used for the present assessment. Neither metabolite appears to pose an unacceptable risk to non-target aquatic organisms. Data reportedly showing no toxicity to soil and aquatic organisms were not available to the Committee, and would need to be assessed by the RMS.

Scientific background on which the opinion is based:

I.1 Formation of metabolites

Under aerobic conditions the active substance, florasulam, is degraded into the major metabolite 5-hydroxy and then into the metabolite DFP-ASTCA. ASTCA is formed via the cleavage of the sulphonamide bridge of DFP-ASTCA. Florasulam degrades rapidly in soil at 20°C (DT_{50}[2] of 0.7 – 4.5 days). The metabolite 5-hydroxy has an aerobic DT_{50} of 7-31 days. (In anaerobic situations the 5-hydroxy metabolite underwent almost no degradation at all.) Aerobic laboratory soil degradation studies at 20°C of the DFP-ASTCA and ASTCA

[2] Period required for 50% dissipation.

metabolites were carried out in a sandy clay loam and a sand (Table 1). The main route of dissipation was through the formation of bound residues (29.6-57.1% after 100 days).

Table 1. Laboratory DT_{50} (days) of metabolites DFP-ASTCA & ASTCA

	DFP-ASTCA	ASTCA
Sand	25	502
Sandy clay loam	8	158

In field soil florasulam had a DT_{50} of 2-18 days and the 5-hydroxy a DT_{50} of 9-95 days. Both florasulam and the 5-hydroxy metabolite were detected in deeper soil layers (40-50cm). No measurements of the DFP-ASTCA and ASTCA metabolites were made.

The DFP-ASTCA metabolite peaked in laboratory studies at 59 days at 17% and the Monograph suggests that DFP-ASTCA is a transient metabolite. The maximum % of radioactivity as ASTCA was 40% at 59 days but in another study the % was still increasing at 100 days at 19.7%.

Some of the reported Koc[3] values in the Monograph were based on a measured decrease in the concentration in the liquid phase of less than 10%. Only those considered to be reliable are included here. The Koc_{ads} data indicates that both the active substance, florasulam, and the metabolites are likely to be very mobile in soil.

Table 2 Adsorption data for the florasulam and metabolites

Active substance and metabolites	Koc_{ads}
Florasulam	13-38
5-hydroxy	10-32
DFP-ASTCA	28-110
ASTCA	49-159

Predictive modelling, by the notifier, using PELMO 3 was used to calculate the PEC_{gw}[4] over a five year period, following one application of florasulam to winter wheat at the maximum rate of 7.5g a.s./ha (with 50% plant intercept) on 15 April. Two soil types were modelled using the Hamburg average climate scenario:

– Parabraunerde, a silty soil with topsoil organic carbon of 1.7%,
– Borstel, a sandy soil with a topsoil organic carbon of 1.5%.

The metabolite DFP-ASTCA reached a maximum annual average concentration of 0.019 µg/L in year 2 in the Parabraunerde soil. The metabolite ASTCA reached an annual average maximum concentration of 0.224 µg/L in year 3 in the Borstel soil and 0.273 µg/L in the Parabraunerde soil. Average annual concentrations in all other years were <0.1 µg/L. The Koc/DT_{50} input values used for the DFP-ASTCA and ASTCA metabolites were approximate averages of the two studies in Table 1 and were the same for both leaching scenarios, regardless of soil pH.

[3] Organic carbon absorption coefficient.
[4] Predicted Environmental Concentration in groundwater.

Table 3 DT_{50} and Koc_{ads} input parameters used in PELMO modelling (SCP/FLORAS/007)

	DT_{50} (days)	Koc_{ads}
DFP-ASTCA	10.5	53
ASTCA	33.5	83

The SCP notes that FOCUS[5] (2000) recommends, that where there is pH dependency, combinations of DT_{50}/Koc values which consider the pH profiles of the chosen scenarios should be used. The SCP considers that the use of corrected Koc_{ads} (Table 2) and the use of appropriate revised input data (recognising the influence of pH dependency) for the DFP-ASTCA metabolite is likely to predict slightly greater PEC_{gw} values than those stated above. The summary data provided do not indicate the full range of concentrations monitored, nor the range of soil conditions to which florasulam may be applied and therefore may underestimate the worst case PEC_{gw} concentrations. Insufficient information is available to determine with certainty the range of PEC_{gw} values which are likely to occur. Predictive modelling using the FOCUS groundwater scenarios would reduce this uncertainty as a range of climates and soil types are represented (FOCUS, 2000).

Lysimeter studies were carried out in a sand and a loam soil. Experimental conditions varied to include early (February) or mid April application, an exaggerated application rate (25 g a.s./ha) or applications of 5 g a.s./ha in two successive years. Monitoring continued for up to 3 years. The pH of the soil in the sand lysimeter ranged from 5.4 in the subsoil to 6.2 in the topsoil. In the loam topsoil the pH was 6.5 and 8.5 in the deepest subsoil. All metabolites were seen in leachate but at a combined total of < 0.1 µg/L for the 5 g/ha applications (single and two successive years) and 0.27 µg/l maximum in year one of the 25 g/ha application. The SCP notes that the total leachate volumes for the different replicates in the first two years of study were particularly low (between 137-213 litres; precipitation in year 1 was 1005 mm and 773 mm in year 2) and not quoted for the third year when annual rainfall/irrigation total was only 509 mm. It would follow that PEC_{gw} concentrations of metabolites (particularly ASTCA due to its persistence in soil) might exceed 0.1 µg/L where wetter scenarios exist.

Water sediment study

Florasulam was degraded in the water sediment with a DT_{50} of 8.7-18 days. The 5-hydroxy metabolite had a DT_{50} of up to 244 days. An unknown metabolite accounted for a maximum of 39% at 82 days and a further unknown for 15% at 100 days in one system. No further explanations are provided concerning these unknown metabolites. The DFP-ASTCA or ASTCA metabolites were not identified in this study.

Summary of environmental concentrations

Surface water: The maximum $PEC_{sw,}$ occurring from spray drift, for both florasulam and the 5-hydroxy metabolite was calculated by the notifier to be 0.1µg/L. No surface water PECs were calculated for DFP-ASTCA or ASTCA but it is likely that their concentration (as breakdown products of the 5-hydroxy metabolite) from spray drift will not exceed 0.1 µg/L. No assessment has been made for the potential for the DFP-ASTCA or ASTCA metabolites to reach surface water via run-off or drainflow.

[5] Forum for the Co-ordination of pesticide fate models and their Use.

Groundwater: The PELMO model predicted a maximum annual average DFP-ASTCA concentration of 0.019 µg/L and a maximum ASTCA concentration of predicted 0.273 µg/L. These values may not represent a worst case scenario when uncertainty in the model parameters, scenario selection, and the fact that these are summary results (maximum annual averages), are considered. The concentrations detected in lysimeter leachate may be underestimates for scenarios where wetter climates occur. The values predicted by the modelling can be used for toxicological assessments but it should be recognised that they may be exceeded in some scenarios.

Soil: No estimates of the concentration of DFP-ASTCA or ASTCA metabolites were made for soil.

I.2 Ecotoxicological relevance of the metabolites

I.2.1 *Aquatic organisms*

The (main) 5-hydroxy metabolite has been tested for aquatic ecotoxicity using the standard short-term studies with fish, Daphnia and planktonic algae. Results showed very low toxicity to fish and Daphnia (above 91 mg/L = highest concentrations tested). Herbicidal activity on algae was decreased by a factor of > 2000.

No long-term tests were supplied for the 5-hydroxy metabolite. However, given the very low surface water PEC's of florasulam and the 5-hydroxy metabolite (0.1 µg/L) and very high TER's[6] for the active substance for most species (>100000), chronic toxicity endpoint values would have to be more than 10^6-fold lower than the acute ones, which the Committee considers of low probability.

For *Lemna*, the TER for the active substance is borderline, and an assessment for the rather persistent metabolite 5-hydroxy is required (see below).

Table 4. Aquatic toxicity data of active substance and metabolites

Group/species	Time-scale	Endpoint	a.s. (mg/L)	TER for a.s	5-hydroxy (mg/L)
Oncorhynchos mykiss	96 h	NOEC[7]	> 100	1000000	> 91
Daphnia magna	48 h	NOEC	174	2920000	>96.7
Algae (*Selenastrum capricornutum*)	72 h	EC_{50}[8]	0.00894 (ErC_{50}[9])	89	21.32 (EbC_{50}[10])
Oncorhynchos mykiss	28 d	NOEC	119	1190000	-
Daphnia magna	21 d	NOEC	38.9	389000	-
Chironomus riparius	28 d	NOEC	> 10	> 100000 (used PECsw of a.s. as worst-case surrogate)	-
Lemna gibba	14 d	EC_{50}	0.00118	12	-see table 5

[6] Toxicity Exposure Ratios.
[7] No Observed Effect Concentration.
[8] Median Effective Concentration.
[9] Concentration affective 50% of the population (based on growth rate).
[10] Concentration affective 50% of the population (based on biomass)

The detoxification via degradation which was indicated by the algae toxicity data reported above could also be shown by assays on herbicidal activity with 2 plant species and on the activity of the target enzyme, as listed in table 5 below.

Table 5: Herbicidal activity of active substance and metabolites

Group/species	Time-scale	Endpoint	a.s. (mg/L)	5-hydroxy (mg/L)	DFP-ASTCA (mg/L)	ASTCA (mg/L)
Lemna gibba *	9 days	EC_{50}	0.00001	0.75	83	> 100
Agrostis palustris (bentgrass) *	9 days	EC_{50}	0.6	> 100	> 100	> 100
Acetolactate synthetase activity	imme-diate	EC_{50}	0.0004	15.5	> 27	13.5

***** hydroponic assays with whole plants; visual rating of effects 0 - 100 %

The 5-hydroxy metabolite as well as the mobile soil metabolites DFP-ASTCA and ASTCA are far less active than the parent substance for the target enzyme and both plant species, the monocotyledonous Agrostis and the dicotyledonous Lemna. No data were provided for animal species but given the low PEC's an unlikely increase of toxicity of app. 10^6 or higher between the a.s./the main metabolite and the other metabolites would be necessary to cause concern. Data not yet submitted to the RMS (or the SCP) are reported to have shown no toxicity to Daphnia, Lemna and algae for both metabolites. The SCP recommends that the RMS carries out an appropriate risk assessment when data are available.

The Committee therefore concludes that risks to aquatic organisms from the metabolites are very low. This conclusion is based on:

– the loss of the specific herbicidal activity shown for the target enzyme and several plant/algal species,
– the lack of toxicity for fish and Daphnia of both the a.s. and the main metabolite which would require an increase in metabolite toxicity of 10^6 or higher to cause concern,
– data reportedly showing no toxicity to Daphnia, Lemna and algae for both metabolites (pending confirmation by the RMS),
– the very low PEC's to be expected for the metabolites, and
– the overall experience with toxicity of active substances and their metabolites, with in most cases lower and unspecific metabolite toxicity (SCP, 1999).

The Committee notes that two unknown metabolites identified in the water sediment study were not evaluated by the notifier.

I.2.2 *Terrestrial organisms*

No separate assessments or studies were reported by the RMS to determine whether the DFP-ASTCA or ASTCA metabolites impacted on non-target terrestrial species. However, in a notifier position paper dated April 2001 (SCP/FLORAS/007), studies not yet submitted to the RMS (or the SCP) are reported to have shown no toxicity to earthworms for both metabolites. The SCP recommends that the RMS carries out an appropriate risk assessment when data are available.

The SCP's opinions on the draft guidance document on relevance metabolites (SCP, 2000) and on imazosulfuron (SCP, 2001) provide models for guidance on the interpretation of the

risk of metabolites to non-target organisms. Such assessment should be part of any ecotoxicological assessment for a given compartment.

I.3 Toxicological relevance of the metabolites

After oral administration in rats, absorption of florasulam was rapid and extensive, reaching 85-91% of the dose. Metabolism of the parent compound was minor and limited to hydroxylation of the phenyl-ring without affecting the sulfonamide bond. Excretion reached 96–99% of the dose within seven days, mainly as unchanged florasulam. In laying hens and lactating goats florasulam was also rapidly and extensively excreted. Metabolism in these two species and in plants does not occur at a high rate and does not differ from rats (Monograph, Annex B, pp. 103). Some minor unknown metabolites were observed in rats, goats and in hens skin but were not further characterised. The four metabolites found in soil were not observed in animals.

From the values found with the predictive PELMO 3 modelling calculation and in the lysimeter studies, ASTCA appears to be the only metabolite that may reach groundwater at levels above 0.1 µg/L on some occasions.

Since concentrations in lysimeter studies may be underestimates, the Committee used values from PELMO modelling to assess the toxicological relevance of the two metabolites (DFP-ASTCA 0.019 µg/L; ASTCA 0.273 µg/L). Assuming a daily intake of two litres of drinking water, the amount of the metabolites ingested would be well below the threshold of toxicological concern[11] (1.5 µg/ person/day, SCP (2000)) using the annual average values predicted from the PELMO modelling provided by the notifier.

The Committee considers therefore that no significant health risk is likely to arise from the metabolites DFP-ASTCA and ASTCA in groundwater at the levels reported in the studies submitted to the Committee.

I.4 Conclusion

The metabolites DFP-ASTCA and ASTCA are mobile and were detected in lysimeter leachate at a concentration of the total radioactivity for combined residues <0.1µg/L. Since this concentration may be exceeded in regions where groundwater recharge is greater or where soil properties accentuate the mobility of the metabolites, the Committee used for the toxicological assessment the values from the PELMO modelling which showed to be well below the threshold of toxicological concern.

The most sensitive species to the active substance was *Lemna gibba.* Studies with *Lemna minor,* the target enzyme, and other species indicated that the DFP-ASTCA and ASTCA metabolites are less active than the parent compound and, in combination with very low PEC's, no unacceptable risk to aquatic organisms is anticipated.

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[11] Dose of a chemical in the diet that is consumed at levels below this threshold (value) poses no appreciable risk. - ILSI Europe Report Series. August 2000. Threshold of Toxicological Concern for Chemical Substances Present in the Diet. Report of a workshop held on 5-6 October 1999 in Paris, France. Organised by ILSI Europe Threshold of Toxicological Concern Task Force.

No terrestrial ecotoxicology data is presented for the DFP-ASTCA or ASTCA metabolites but according to notifier statements, their activity appears to be negligible to soil and aquatic organisms. The SCP recommends that the RMS carries out an appropriate risk assessment when data are available.

II. Question 2

Is it correct to establish an acute reference dose (ARfD) based on the neurotoxicity?

Opinion of the Committee:

The Committee is of the opinion that the allocation of an acute reference dose based on neurotoxicity is not needed since no unequivocal acute neurotoxic end-point has been identified.

Scientific background on which the opinion is based:

Florasulam was found to be of very low acute toxicity since the oral rat LD_{50}[12] was > 5000 mg/kg bw, some deaths occurring at 6000 mg/kg bw. Florasulam was not found to be a developmental toxicant. It was considered not to be genotoxic or carcinogenic. Kidney (dogs and rodents) and red blood cells (anaemia in dogs) were the relevant target organs in short and long term studies. The rat was particularly sensitive to renal lesions which were observed at 800-1000 mg/kg bw per day in a 28- and two 90-day dietary studies, at 500 mg/kg bw per day in parental groups in the two-generation reproduction study, and at 125 mg /kg bw per day and above in the two-year study. Kidney histopathology consisted of degeneration of renal tubules, multifocal necrosis of proximal tubule epithelial cells, and collecting ducts hypertrophy. These effects were generally associated with perineal staining, and slight reductions, though mostly within normal values, in urine pH and specific gravity. In particular, in a recovery study (90 days of treatment followed by a 4-week recovery period) renal lesion almost completely recovered and perineal soiling was not observed any longer.

An oral acute neurotoxicity study was conducted in rats given up to 2000 mg florasulam/kg bw by gavage. Animals were observed for functional observational battery (FOB) and motor activity prior to treatment and on day 1, day 8 and day 15 after treatment. The level of motor activity as well as responsiveness to sharp noise were slightly reduced in high dose males on day 1 and motor activity was slightly increased in high dose females on day 8. Increased urination was observed in high dose males on day 8. All these changes, although statistically significant, were minor, did not show a clear dose-response curve and involved only one sex. Perineal staining was observed on days 2 and 3 in some males treated with the two top doses (1000 and 2000 mg/kg bw) (no dose-response seen). In the 1-year neurotoxicity study in rats only perineal soiling on months 6, 9, and 12 and, possibly, increased urination were observed at and above 250 mg/kg bw per day. Perineal soiling and the possible effects on urination seem likely to be associated with kidney toxicity in this study as well as in both the short- and long-term studies discussed above. In the acute neuroxicity study they occurred at doses not far from the minimal lethal dose (1000 vs 6000 mg/kg bw).

[12] Lethal dose median.

The SCP considers that no unequivocal neurotoxicity was observed in rats and concluded that neither the data from the neurotoxicity study nor those from any other available study indicate the necessity of allocating an acute reference dose for florasulam.

E. REFERENCES

FOCUS (2000). FOCUS groundwater scenarios in the EU review of active substances. EC Document Sanco/321/2000 rev.2, 197 pp.

SCP, (1999): Opinion of the Scientific Committee on Plants regarding the Draft guidance document on Aquatic Ecotoxicology (DG VI –8075/VI/97-rev4 of 18 December 1998) adopted by the SCP on 24 September 1999. http://europa.eu.int/comm/food/fs/sc/scp/out47_en.pdf

SCP, (2000): Opinion of the Scientific Committee on Plants regarding the Draft guidance document on relevant metabolites (Document SANCO/221/2000-Rev.2 of October 1999) (Opinion adopted by the SCP on 30 November 2000), http://europa.eu.int/comm/food/fs/sc/scp/out82_ppp_en.html

SCP, (2001): Opinion of the Scientific Committee on Plants regarding the Evaluation of Imazosulfuron [TH-913] in the Context of Council Directive 91/414/EEC Concerning the Placing of Plant Protection Products on the Market (Opinion expressed by the Scientific Committee on Plants, 25 June 2001) http://europa.eu.int/comm/food/fs/sc/scp/out103_ppp_en.pdf

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