Opinion of the Scientific Committee on Plants on the evaluation of sulfosulfuron in the context of Council Directive 91/414/EEC concerning the placing of plant protection products on the market (opinion adopted by the Scientific Committee on Plants on 30 November 2000)

1. TITLE

OPINION OF THE SCIENTIFIC COMMITTEE ON PLANTS ON THE EVALUATION OF SULFOSULFURON IN THE CONTEXT OF COUNCIL DIRECTIVE 91/414/EEC FOR PLACING PLANT PROTECTION PRODUCTS ON THE MARKET.

(Opinion adopted by the Scientific Committee on Plants on 30 November 2000)

2. 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 give its opinion on the occurrence of bladder tumours in the 18 months mouse study?
- **2)** Can the SCP consider whether it would be appropriate to establish an acute reference dose (ARfD) for sulfosulfuron?
- **3)** Can the SCP confirm that a sub-lethal study for earthworms is unnecessary, notwithstanding the persistence of the soil metabolites?
  In addition the SCP expressed an opinion on two specific issues of concern identified in the course of the evaluation.
- **4)** The relevance of three unidentified metabolites "M5", "M6" and "M8".
- **5)** The assessment of a risk for sensitive aquatic plants.

3. BACKGROUND

Sulfosulfuron is a new active substance in the context of Directive 91/414/EEC [1]. The draft Commission Directive for inclusion of this substance in Annex I to Directive 91/414/EEC concerning the placing of plant protection products on the market was submitted to the Committee for opinion. The Committee had been supplied with a draft evaluation report (monograph) prepared by the Rapporteur Member State (Ireland) on the basis of a dossier submitted by the notifier (Monsanto), a review report prepared by the Commission and the Recommendations of the ECCO [2] Peer Review Programme.

Sulfosulfuron is a member of the herbicide family of the sulfonylureas. It is intended for use as spring post-emergence treatment in wheat to control a number of grass weeds as well as various broad-leaved weeds. The supported rate of use is 20 g a.s./ha. Sulfosulfuron acts by contact and residual action.

4. OPINION

4.1 Question 1

"Can the Committee give its opinion on the occurrence of bladder tumours in the 18 months mouse study?"

Opinion of the Commitee

The Committee concluded that as sulfosulfuron did not show genotoxic potential, the increased frequency of submucosal bladder lesions/tumours observed in treated mice in association with urinary calculi was produced by a non-genotoxic mechanism that is not fully characterised. The lesions/tumours of microscopic size that were observed in mice were of mesenchymal origin, were benign, and were of a kind that may occur naturally in mice of certain strains. Such lesions/tumours are not known to occur in humans. The Committee considered that these lesions/tumours do not predict carcinogenic hazard to humans.

Scientific background on which the opinion is based

4.1.2 Assessment of the data

The Committee took note of the responses of the notifier to its requests for further information. The notifier's responses included the following: (a) histopathologic details of the submucosal lesions/tumours of the mouse urinary bladder; (b) documentation that these lesions also occur in untreated mice of certain genetic lineages; and (c) an hypothesis regarding the role of urinary calculi and chronic obstructive uropathy in their development.

Sulfosulfuron has been adequately tested for its potential genotoxicity and was not genotoxic *in vivo*. Chromosome aberration induction was demonstrated *in vitro* in Chinese hamster lung fibroblast cultures at cytotoxic concentrations and at the limit of compound solubility. In contrast, negative results were obtained in human lymphocyte cultures. The Chinese hamster *in vitro* positive result was not considered relevant to intact animals (including humans) as the cytotoxic concentrations could not be achieved *in vivo*.

Sulfosulfuron produced bladder calculi in both rats and mice. Sulfosulfuron caused urinary calculi and urothelial hyperplasia in rats to which were administered diets containing 5,000 and 20,000 ppm sulfosulfuron but only 1 benign and 1 malignant epithelial bladder tumour were observed, both in females of the 5,000 ppm group [3]. These tumours were plausibly related to the presence of urinary calculi.

Sulfosulfuron also caused urinary calculi and urothelial hyperplasia in treated mice, but no epithelial tumours were observed. Sulfosulfuron increased the incidence of only an unusual submucosal lesion/tumour of the urinary bladder in mice. Such lesions/tumours occurred mostly at the higher dose (7000 ppm) and mostly in males, but one such tumour did occur in an untreated female mouse. The submucosal lesions/tumours in mice were invariably microscopic in size, were of mesenchymal origin, and were histologically benign. The pathogenesis of these rare lesions/tumours is not well established. While a hypothesis has been presented that contraction and distension of the murine urinary bladder consequent to chronic or intermittent obstruction of the urinary tract by calculi may play a mechanical role

in the development of these lesions/tumours, this is not well validated. There is no clear evidence that the presence of calculi in the urinary bladder is causally related to the increased incidence of mesenchymal lesions/tumours in treated mice over controls. Bladder calculi and other persistent foreign bodies are known to be associated with epithelial neoplasms of the urinary bladder in rodents, especially rats, but a causal association with any other kind of true neoplasm in the urinary bladder in rodents is not established. There are some indications that urinary calculi are associated with a slightly increased risk of epithelial neoplasms of the urinary tract in humans. However, mesenchymal lesions/tumours of the kind seen in mice do not occur in humans.

4.1.2 Conclusion

The Committee concluded, on the basis of previously supplied information and the notifier's responses including additional data, that:

- - Sulfosulfuron did not show genotoxic potential *in vivo*;
- - Sulfosulfuron caused urinary calculi and urothelial hyperplasia in rats at doses of 5,000 and 20,000 ppm but treatment was associated with only 1 benign and 1 malignant epithelial bladder tumour, both in low-dose females. These were plausibly related to the presence of calculi;
- - Sulfosulfuron also caused urinary calculi and urothelial hyperplasia in treated mice, but increased the incidence of only an unusual submucosal lesion/tumour of the urinary bladder, mostly at the highest dose (7,000 ppm). The submucosal lesions/tumours observed in mice after chronic treatment were invariably microscopic in size, were of mesenchymal origin, and were histologically benign and of a kind that may occur naturally in mice of certain strains. Such lesions/tumours do not occur in humans;
- - There is no clear evidence that the presence of calculi in the urinary bladder is causally related to the increased incidence of mesenchymal lesions/tumours in treated mice over controls;
- - Bladder calculi are known to be associated with epithelial neoplasms of the urinary bladder in rodents, especially rats, and are associated with increased risk of cancer of the urinary tract in humans, but a causal association with any other kind of true neoplasm in the urinary bladder either in rodents or in humans is not established;
- - The submucosal lesions/tumours in mice treated with sulfosulfuron do not predict a cancer hazard to humans.

4.2 Question 2

"Can the Scientific Committee on Plants consider whether it would be appropriate to establish an acute reference dose (ARfD) for sulfosulfuron?"

Opinion of the Committee:

The Committee concludes that it is not necessary to establish an acute reference dose for sulfosulfuron. The acute toxicity of sulfosulforon is low, and repeated dose studies did not identify relevant toxic endpoints.

Scientific background on which the opinion is based

4.2.1 General consideration on ARfD setting

The toxicological information provided by the Annex II to Directive 91/414/EEC represents the basis for deriving the ARfD. The FAO/WHO 1997 consultation defined acute reference dose as: " *An estimate of the amount of substance in food or drinking water, expressed on a body-weight basis, that can be ingested over a short period of time, usually during one meal or one day, without appreciable risk to the consumer on the basis of all the known facts at the time of evaluation. It is usually expressed in milligrams of the chemical per kilogram body weight.*" [4]

The SCP has concluded that the setting of ARfD should be considered for all plant protection products, though in many instances it will be unnecessary to set one. If a plant protection product is not acutely toxic and an ARfD is considered unnecessary, the reasons supporting this conclusion must be described in detail. Furthermore, the SCP also indicated that the entire toxicity database should be considered in determining the most appropriate species and end-point for deriving an ARfD. The critical end-point should be one relevant to a single exposure in humans.

4.2.2 Chemical and toxicological properties of sulfosulfuron

Sulfosulfuron is a sulphonylurea herbicide with a high water-solubility. It is readily absorbed from the gastrointestinal tract after single administration of a low dose while at high doses the absorbed proportion of the dose decreases in rats. At low single oral doses most of the compound is excreted in the urine whereas at high oral doses (1000 mg/kg) excretion in the faeces predominate excretion in the urine being less important suggesting less complete absorption from the gastrointestinal tract.

The acute toxicity of sulfosulfuron is low both orally and dermally in rats; in both cases the LD_{50} value exceed 5000 mg/kg. In short-term inhalation studies a concentration of 3 mg/l did not induce any observable acute toxicity. The compound does not turn out not to be a primary skin irritant or a dermal sensitizer in studies with rabbits. In a 28-day oral toxicity study the NOAEL [5] for males was 186 and for females 987 mg/kg/day. The toxic end points, transient body-weight loss, a single ocular opacity, and induction of palmitoyl CoA are not relevant for setting an ARfD. In another 28-day study with rats, a NOAEL of 136 mg/kg/day for males and of 154 mg/kg/day for females was based on a decrease in thromboplastin time and a slight kidney effect. In an oral 90-day dose-finding study in dogs a NOAEL of 100 mg/kg/day was established for females and of 300 mg/kg/day in males due to increased thyroid and heart weights. The NOAEL defined on a decrease in serum alkaline phosphatase level in a 90-day oral feeding mouse study was 7000 ppm (350 mg/kg/day). These findings are irrelevant for setting an ARfD for sulfosulfuron.

Sulfosulfuron induced in a 90-day oral feeding study in dogs urolithiasis in males sacrificed *in extremis*. These findings were considered secondary to urinary calculi found in high-dose groups of dogs both in males and females. Similar changes occurred in a one-year feeding study with dogs both in females and males with NOAELs of 100 mg/kg/day for both genders.

Sulfosulfuron is not genotoxic in tests exploring both gene mutations and chromosomal aberrations *in vivo*. Positive *in vitro* results in Chinese hamster were not considered relevant in intact animals or man.

In a chronic two-year feeding study in rats, there was increased mortality among the exposed high-dose rats (20 000 ppm, i.e. 1000 mg/kg/day). This was most likely a result of formation of renal calculi and related abnormalities present in the kidney, ureters, and urinary bladder. Body weights were decreased in the high-dose groups (5000 or 20 000 ppm, 250 or 1000 mg/kg/day), and blood urea nitrogen (BUN) was elevated in the high dose groups. There was one benign and one malignant epithelial bladder tumour in the 5000 ppm dose, but such tumours did not occur at the high-dose group (20 000 ppm). The NOEL [6] for tumorigenicity was 500 ppm in rats.

Sulfosulforon also caused urinary calculi and urothelial hyperplasia in treated mice, but no epithelial tumours occurred. Sulfosulforon increased the incidence of only an unusual submucosal lesion/tumour of the urinary bladder in mice. These tumours occurred mostly at the higher dose (7000 ppm) and mostly in males, but one such tumour occurred in an untreated female mouse. Bladder calculi and other persistent foreign bodies are known to be associated with epithelial neoplasms of the urinary bladder in rodents, especially in rats, but a causal association with any other kind of true neoplasm in the urinary bladder in rodents has not been established. In a long-term mouse study, BUN was also elevated both in the male and female mice in the high-dose group (7000 ppm).

In reproductive studies in rats the NOEL was 5000 ppm (312-378 mg/kg/day). Weight was reduced at the high dose, but reproductive parameters were not adversely affected. In a developmental toxicity study with rabbits, no maternal toxicity or toxic effects in pups were noted up to a dose of 1000 mg/kg/day of sulfosulfuron from days 7-19 of gestation. The NOEL was 1000 mg/kg/day.

In additional studies exploring acute (single dose) or subchronic (14 weeks) neurotoxicity, no signs of behavioural alterations or morphological changes in peripheral nerves were observed.

In supervised field trials according to good agricultural practices, residues of sulfosulfuron were below 0.01 mg/kg cereal grain. This would mean a potential short-term dietary intake (assuming large portion consumption data for the most sensitive population, i.e. young children) of less than 0.0005 mg/kg body-weight.

4.2.3 Conclusion:

The SCP concluded that it is not necessary to establish an acute reference dose for sulfosulfuron because acute toxicity is low and repeated dose studies did not identify relevant toxic endpoints.

4.3 Question 3

Can the SCP confirm that a sub-lethal study for earthworms is unnecessary, notwithstanding the persistence of the soil metabolites?

Opinion of the Committee:

A newly submitted sub-lethal study for earthworms allowed the SCP to conclude that no significant long term risks to earthworms are likely to arise from use of sulfosulfuron at the recommended treatment rate (20 g a.s./ha, once per year), despite the persistence of metabolites in the soil.

Scientific background on which the opinion is based

4.3.1 Fate

The persistence of the parent compound varies considerably with soil type and conditions, with estimates of the field DT_{50} [7] in European studies varying from 11-47 days (DT_{90} [8] 131-358 days), and estimates of field DT_{50} in US studies varying from 13-52 days (DT_{90} 370-1190 days). The major routes of degradation of sulfosulfuron in soil are through: (a) cleavage of the sulfonyl-urea bond leading to the formation of aminopyrimidine and sulphonamide and (b) oxidative demethylation of sulfosulfuron to form desmethyl. Sulphonomide was reported at a maximum of 15% (after 225 days, the duration of the study) and 53% (after 360 days, the duration of the study) of applied radioactivity in EU and US soils respectively. Desmethyl was reported at a maximum of 29% applied radioactivity after 100 days in EU soils, but was consistently < 5% applied radioactivity in US soils. Aminopyrimidine was recorded at a maximum of 10.6 % applied radioactivity (after 100 days, the duration of the study) and 39.4% (after 272 days) in EU and US soils respectively.

The steady-state concentrations of the parent and its metabolites have been estimated by evaluators assuming a 50% crop interception and an annual application rate of 20 g a.s./ha [9]. Based on the expected peak concentrations in soil and extreme estimates of DT50 values, it has been estimated that the parent compound will reach a steady state of approximately 3.5 mg/kg after 5 years; sulfonomide will reach a steady state of approximately 4.7 mg/kg after 8 years; desmethyl will reach a steady state of approximately 5.1 mg/kg after 9 years, while aminopyrimidine will reach a steady state of approximately 5.9 mg / kg after 12 years.

4.3.2 Ecotoxicity

Originally, only the results of a 14-day earthworm (*Eisenia fetida*) study were submitted. In this study, earthworms were exposed to sulfosulfuron at a test concentration of 1000 mg a.s./kg dry soil (mean measured concentration 848 mg a.s./kg) for 14 days. Despite the unrealistically high test concentration (maximum initial PEC [10] for worst case exposure to bare soil 0.0267 mg/kg soil in 0-5cm), no significant effects on earthworm mortality, appearance, behaviour or body weight were observed over the exposure period.

Annex II criteria propose that an extended test for sub-lethal effects of earthworms is required if the field DT90 is above 365 days, independent of the number of times it is applied. Furthermore, metabolites, which reach a concentration greater than 10% of the dose applied should also be tested for long-term sublethal effects, unless they are formed so rapidly that potential effects are covered by the available earthworm tests for the parent compound. Given these recommendations, a further sub-lethal study was appropriate [11]. Indeed, one was about to be called for by the SCP.

In the recently submitted sub-lethal study, adult earthworms (*Eisenia fetida*) were exposed to two concentrations of sulfosulfuron in artificial soils at rates equivalent to 1x and 5x times the maximum proposed application of 20 g a.s./ha, i.e. 0.026 and 0.13 mg a.s./kg dry soil. After 28 days exposure the adult worms were counted and weighed. After a further 28 days the soil was examined for juveniles and cocoons. No mortalities were observed in the study, and the body weights of earthworms did not differ significantly from the controls. Similarly, the number of juveniles produced by the end of the study did not differ significantly (5.5 to 14.2 per adult worm in control replicates, 4.7 to 13.4 per adult in 5x treatment replicates).

Given the data provided in the two separate studies, it is highly unlikely that a single use of sulfosulfuron at the recommended application rate will pose a significant risk to earthworms. However, to address the possible effects of the parent compound and its metabolites accumulated over several seasons, it is necessary to consider the steady-state concentrations. If we treat the NOEC [12] of > 0.13 mg as/kg for the parent as toxicity endpoints for all metabolites, and assume the steady state concentrations previously calculated, then the minimum possible estimate of the long-term TER [13] for the main metabolites is 22. Given that this estimated figure is well above the standard trigger value, the SCP is of the opinion that no significant long-term risks to earthworms are likely to arise from the use of sulfosulfuron at the recommended rate.

4.4 Relevance of unidentified metabolites [M5, M6 and M8]

The Committee has noticed that three unidentified metabolites ("M5", "M6" and "M8") were detected in the leachate of a sandy soil lysimeter at annual average concentrations of 0.1 mg/l.

Opinion of the Committee:

The SCP notes that the three metabolites ("M5", "M6" and "M8") have not been chemically identified and therefore the non necessity for further testing should be scientifically assessed.

Scientific background on which the opinion is based:

The Committee has noticed that three unidentified metabolites ("M5", "M6" and "M8") were detected in the leachate of a sandy soil lysimeter at annual average concentrations of 0.1 mg/l (parent equivalent) or higher (after two treatments of the soil with sulfosulfuron rates of 30 g/ha). The metabolites were not identified in the soil degradation study.

The SCP agrees with the Rapporteur Member State that it is unlikely that the concentration of the metabolites will exceed 0.1µg/l when the a.s. is applied in spring at a rate of 20g/ha. However, the SCP notes that these metabolites have not been chemically identified and therefore the need for further testing should be scientifically assessed (see Opinion of the Scientific Committee on Plants regarding the draft guidance document on relevant metabolites).

4.4 Drain flow calculation

The Committee has noticed that drain flow calculations for a UK clay loam soil with a leaching model that includes preferential flow (MACRO [14]) , have indicated risk for sensitive aquatic plants in surface water.

Opinion of the Committee:

This risk does not appear to have been addressed by the Rapporteur Member State.

5. REFERENCES

1. IARC. IARC monograph vol. 73, 1999 Capen et *al* 1999, IARC

2. Opinion of the Scientific Committee on Plants regarding the draft guidance document on relevant metabolites (Document SANCO/221/2000-rev2) adopted on 30 November 2000.

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6. DOCUMENTS MADE AVAILABLE TO THE COMMITTEE

- 1. Evaluation of sulfosulfuron in the context of Council Directive 91/414/EEC concerning the placing of plant protection products on the market: Terms of Reference (Doc. SCP/SULFO/001-rev.1), submitted by DG SANCO, 3 April 2000.
- 2. Sulfosulfuron: Evaluation table 7458/VI/98-rev.8 (Doc. SCP/SULFO/003-rev.1), submitted by DG SANCO, 3 April 2000.
- 3. "Spontaneous neoplastic lesions in the CRL/CD-1 â Mouse" P. L. Lang, Charles River laboratories, March 1995, (Doc. SCP/SULFO/004), submitted by Monsanto, 5 January 2000.
- 4. "Expert report on renal hispathologic changes in a mouse study with MON 37500 (sulfosulfuron)", G.C. Hard, August 1999, (Doc. SCP/SULFO/005), submitted by Monsanto, 16 January 2000.
- 5. Sulfosulfuron: Danish comments (Doc. SCP/SULFO/006), submitted by DG SANCO, 31 January 2000.
- 6. Sulfosulfuron: Appendix III: Listing of end points (Doc. SCP/SULFO/007), submitted by DG SANCO, 3 April 2000.
- 7. Sulfosulfuron: German comments (Doc. SCP/SULFO/009), submitted by DG SANCO, 3 April 2000.
- 8. Sulfosulfuron: Appendix I and Appendix II (Doc. SCP/SULFO/010), submitted by DG SANCO, 4 April 2000.
- 9. Sulfosulfuron: question from the SCP to the notifier regarding the evaluation of sulfosulfuron (Doc. SCP/SULFO/011), submitted by the SCP, 23 May 2000.
- 10. Preliminary response from the notifier to the question raised by the SCP (Doc. SCP/SULFO/012), submitted by Monsanto, 26 May 2000.
- 11. Sulfosulfuron: Draft review report 7459/VI/98-rev.1 (Doc. SCP/SULFO/013), submitted by DG SANCO, 31 May 2000.
- 12. Sulfosulfuron: Danish comments (Doc. SCP/SULFO/014), submitted by DG SANCO, 9 June 2000.
- 13. Sulfosulfuron: Comments from France (Doc. SCP/SULFO/015), submitted by DG SANCO, 9 June 2000.
- 14. Sulfosulfuron: Danish comments (Doc. SCP/SULFO/016), submitted by DG SANCO, 9 June 2000.
- 15. Sulfosulfuron: Response from RMS to Danish comments [Doc. SCP/SULFO/006], (Doc. SCP/SULFO/017), submitted by DG SANCO, 9 June 2000.
- 16. Sulfosulfuron: Volume 3 Addendum to Annex B to the Draft report and proposed decision (Doc. SCP/SULFO/019), submitted by DG SANCO, 23 June 2000.
- 17. Sulfosulfuron: Dutch assessment of the lysimeter study (Doc. SCP/SULFO/021), submitted by DG SANCO 24 July 2000.
- 18. Sulfosulfuron: UK questions on sulfosulfuron to the SCP (Doc. SCP/SULFO/022), submitted by DG SANCO, 24 July 2000.
- 19. Further response from the notifier to questions (SCP/SULFO/011) raised by the SCP (Doc. SCP/SULFO/023), submitted by Monsanto, 23 August 2000.
- 20. RMS response to the Dutch comments [SCP/SULFO/021] on the lysimeter study (Doc. SCP/SULFO/025), submitted by DG SANCO, 26 September 2000.

- 20. European Commission: Peer review programme: Sulfosulfuron, monograph prepared by Ireland, March 1998 (Volumes 1 to 4).

7 ACKNOWLEDGEMENTS

The Committee wishes to acknowledge the contributions of the working groups that prepared the initial draft opinion.

Carcinogenicity WG: Prof. Maroni (Chairman) and Committee members: Dr. Delcour-Firquet, Dr. Meyer, Dr. Moretto, Prof. Savolainen, Prof. Silva Fernandes, Dr. Speijers and invited experts: Prof. Galli, Prof. Parry, Dr. Rice, Prof. Schulte-Hermann and Dr. Wester.

Toxicology: Prof. Maroni (Chairman) and Committee members: Dr. Delcour-Firquet, Dr. Meyer, Dr Moretto, Prof. Savolainen, Prof. Silva Fernandes, Dr. Speijers, invited expert Dr. Fait.

Environmental assessment WG: Prof. Hardy (Chairman) and Committee members: Mr. Koepp, Dr. Nolting, Dr. Sherratt, Prof. Silva Fernandes, and invited experts: Dr. Boesten, Dr. Carter, Dr. Forbes, Dr. Luttik.

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[1] OJ N° L 230 of 19. 8.1991, p. 1.
[2] European Community Co-ordination.
[3] In the oral study in rats, the concentrations tested were of 0, 50, 500, 5,000 and 20,000 ppm.
[4] See Opinion of the SCP on the general criteria for setting acute reference doses for plant protection products. click here
[5] No observed adverse effect level.
[6] No observed effect level.
[7] Period required for 50 % dissipation.
[8] Period required for 90 % dissipation.
[9] see UK questions Doc. SCP/SULFO/022, and RMS Addendum to the Annex B of the Monograph.
[10] Predicted environmental concentration.
[11] See Opinion of the Scientific Committee on Plants regarding the draft guidance document on relevant metabolites
[12] No observed effect concentration.
[13] Toxicity exposure ration.
[14] Simulation model of plant protection product fate (Jarvis, 1991) see FOCUS report "Soil persistence models and EU registration",