Opinion of the Scientific Committee on Plants on the evaluation of cyclanilide 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 CYCLANILIDE 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)

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) Does the SCP consider the effects on the mouse immune system to be of any relevance for man?

2) Does the SCP consider the teratological effects observed in the rabbit study to be of any relevance to man?

In addition, in the course of the evaluation of the documentation submitted to it, the Committee raised a specific environmental issue relating to the setting of the half-life for the soil metabolite 2,4-DCA.

3) The opinion of the Committee on this issue is expressed in Section 4.3.

3. BACKGROUND

The draft Commission Directive for inclusion of cyclanilide 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 documentation comprising a draft evaluation report (monograph) prepared by the Rapporteur Member State (Greece) on the basis of a dossier prepared by the notifier (Rhône-Poulenc Agrochimie now Aventis CropScience), a review report prepared by the Commission and the Recommendations of the ECCO [1] Peer Review Programme.

Cyclanilide (RPA-90946) is a new active substance, intended for use in cotton field in combination with the plant growth regulator ethephon. The mixture of the two substances promotes boll opening, leaf abscission and inhibits growth following defoliation. The intended rate of use of cyclanilide ranges from 0.12 to 0.18kg a.s./ha.

4. OPINION

4.1 Question 1:

"Does the SCP consider the effects on the mouse immune system to be of any relevance for man?"

Opinion of the Committee:

The SCP does not consider the effects of cyclanilide on the thymus and spleen of mice relevant for the assessment of the risk in humans. Thymus involution and spleen depletion/atrophy were detected in mouse only at very high doses causing severe systemic effects and lethality. Thus, there is no sufficient evidence to conclude that the detected effects are related to a specific immunotoxic activity of cyclanilide. These effects should be considered to represent an unspecific response to stress following exposure to very high levels of the active substance. Further argument for the absence of specific immunotoxicity by cyclanilide is provided by the lack of any evidence of immunotoxicity in other short- and long-term toxicity studies.

Scientific background on which the opinion is based:

4.1.1 Toxicity profile

Cyclanilide is moderately toxic to rats, minimally irritant to the skin, and irritant to the eye. The toxicity profile from short-term and long-term studies in different species is characterised by macroscopic and microscopic pathology in liver, kidney, thymus, spleen, and mesenteric lymph nodes. Effects on reproduction are discussed in section 4.2. Behavioural neurotoxicity was reported in subchronic studies, with no evidence of neuropathological changes. An ADI [2] of 0.0075 mg/kg bw [3] was based on the NOAEL [4] of 1.5 mg/kg bw in the reproductive study in rats, and a safety factor of 200.

The concerns raised on the potential immunotoxicity of cyclanilide were based on the findings observed in the 90-day mouse study and also on studies in other species (1-year dog study and 2-year rat study).

4.1.2 Thymus involution and mortality in the 90-day mouse study

Groups of 10 mice/sex/dose were administered 0, 40, 200, 2000, and 4000 ppm of cyclanilide for 3 months. Mortality up to 50% occurred at the two highest doses (2000 and 4000 ppm), mainly during the first month. At 4000 ppm, decreased body weight and food consumption were observed at the beginning of the study. Macroscopic post-mortem examination did not reveal any specific cause of death. Toxic signs like lethargy, hypothermia, pallor and/or yellow staining of the anogenital area were observed in several animals prior to death.

Haematology and clinical chemistry findings were: decrease in platelet count at 2000 (not significant) and 4000 ppm (significant); significant elevation of serum AP activity at the two top doses; significant decrease in serum globulin and total protein levels in males at 4000 ppm. No effects were observed on total and differential WBC [5] counts.

The main microscopic findings in the animals which died were liver, lung and thyroid congestion, and gastric changes. Moderate to severe thymus involution and slight to severe spleen depletion/atrophy were observed. Malignant lymphoma of the lymphoreticular tissue was the cause of death in one female at 2000 ppm. There were no substance-related effects on other lymphoid organs. Mortality was attributed to acute stress. Animals surviving up to study termination did not show any alterations of thymus, spleen or other lymphoid organs.

The NOAEL was established at 200 ppm, equivalent to 34 mg/kg bw for males (37 mg/kg bw for females).

The observed changes in the thymus and spleen cannot be interpreted as primary "immunotoxic effects" because they were only observed in animals with severe or lethal systemic effects.

The observed changes in the thymus and spleen of animals which died during the first weeks of high-dose treatment may be interpreted as a response to acute stress occurring at the beginning of the study, when significant decreases in food consumption and body weight were observed. The evidence that stress can influence immunity and onset of diseases has been reported by several authors [6].

A single occurrence of lymphoreticular tissue malignant lymphoma cannot be related to the test substance administration. Even though its occurrence is not frequent in young animals, malignant lymphoma is the most common haematopoietic tumour in the CD-1 mice. Moreover, in the mice long-term study (18 months) neither an increase of lymphoreticular tissue tumours nor treatment-related effects on the lymphoid organs were seen.

4.1.3 Evidence from other studies

The short-term and long-term toxicity studies conducted with cyclanilide in different species were reviewed with the purpose of identifying possible effects on the immune system.

Chronic active inflammation in the liver was reported in the 1-year dog study, only in the top dose group (640 ppm), with central lobular hepatocellular degeneration and necrosis. This effect should be interpreted as a direct consequence of the extensive destruction of hepatic tissue, rather than as an immune effect.

Lung purulent inflammation/abscesses and sub-acute and chronic inflammation and fibrosis of the visceral pleura/capsule were reported in the 2-year rat study, at the end of study, but not at interim sacrifice. These findings were restricted to the high dose animals (1000 ppm). No effects on lymphoid organs were observed. This effect should be considered as a response to acute stress due to the high doses administered to aged rats.

Markers of possible effects on the immune system (total and differential leukocyte counts, serum globulin levels, weight changes and histology of lymphoid organs) were not affected in any of the short- and long-term studies.

4.1.4 Conclusions

Since thymus involution and spleen depletion/atrophy were detected in the mouse only at very high doses causing severe systemic effects and lethality, there is no sufficient evidence to

conclude that these effects are related to a specific immunotoxic activity of cyclanilide; these effects should be considered to represent an unspecific response to stress following exposure to high levels of cyclanilide.

Further argument for the absence of specific immunotoxicity by cyclanilide is provided by the observation that no consistent evidence of immunotoxicity of cyclanilide was observed in any of the other short- and long-term toxicity studies.

4.2 Question 2

Does the SCP consider the teratological effects observed in the rabbit study to be of any relevance to human?

Opinion of the Committee:

The SCP does not consider the results of the rabbit study indicative of reproductive toxicity potential of cyclanilide to humans. The reported findings in the rabbit study are considered substance related, however they were only observed in the top-dose animals showing salient signs of systemic toxic effects. The types of effects on reproduction were all seen also in control rabbits either in the present study or in control groups from previous studies in the performing laboratory (historical controls). In addition no similar effects of cyclanilide on reproduction were seen in other studies for reproductive toxicity i.e. teratology study in rats or two generation reproductive toxicity study in rats.

Scientific background on which the opinion is based:

4.2.1 Toxicological evaluation

No specific effects of cyclanilide on reproduction were seen in studies for reproductive toxicity in rats, i.e. teratology study and two generation reproductive toxicity study.

The Rapporteur Member State has established an ADI of 0.0075 mg for the active substance using the NOAEL of 1.5 mg/kg bw/ day in the reproductive toxicity study in rats based upon renal lesions (microscopic mineralization foci in the renal papilla and microscopic calculi in the renal pelvis) observed in the F_1 females and applying an uncertainty factor of 200 (the additional safety factor of 2 is applied as NOAEL is considered as a LOEL [7]) .

4.2.2 The teratology study in rabbits [8]

The teratology study in rabbits is a well reported study performed in compliance with GLP. Cyclanilide was administered to pregnant rabbits (artificial insemination) during the period of major organogenesis, gestation day 6 through gestation day 18. The 20 females per group received 0 (controls), 3, 10 and 30 mg/kg bw/ day in 0.5 ml corn oil.

The animals dosed 30 mg/kg bw/day showed overt signs of toxicity including wobbly gait, partial hind limb paralysis, decreased activity and partial hair loss. In addition body weight gain and food consumption were markedly reduced during the treatment period.

Two females in the top dose group and one in the control group aborted on gestation days 18, 20 and 28 respectively. No remarkable internal findings were observed at necropsy. A slight

but not statistically significant increase was noted in the number of early resorptions at the 30 mg/kg bw/day level when compared to the control group. This finding was due to a slightly lower than normal group value in the controls and two females with total litter resorption. A correspondent slight increase in mean post implantation loss was recorded. Embryo-foetal death is known to be a consequence of salient toxicity in dam as observed in the present study [9]. Death of an embryo or foetus is usually followed by resorption of the tissue or abortion in rabbits [10].

No statistically significant increase in the incidence of treatment-induced malformations nor foetal weight effects were noted. Some scattered foetal findings were observed, some of which only occurred in the dosed animals. The type of effects (e.g. heart and/or great vessel anomaly, right-sided retro-oesophageal aortic arch, extra site of ossification anterior to sternebra 1, 8th sternal costal cartilage, 25 presacral vertebrae, 7th sternebrae, and reduced ossification of the skull) have however all been seen in control animals either in the present study or in control groups from previous studies in the performing laboratory (historical controls). The incidence of the individual findings was close to or within the range of the historical controls of this rabbit strain. It is a common observation that treatment at very high maternally toxic dose levels can provoke small increase (2-3 fold) in the number of foetus with abnormalities [11].

4.2.3 Conclusion

The SCP considers the findings in the study substance related and as such relevant for human risk assessment. However, the SCP does not consider the data indicative of a reproductive toxicity potential of cyclanilide to humans for the following reasons:

- - The effect of cyclanilide on reproduction is only observed in the top-dose animals showing salient signs of systemic toxicity.
- - The types of effects on reproduction are all seen in control animals either in the present study or in control groups from previous studies in the performing laboratory (historical controls).
- - The slight but not statistically significant increase in embryolethality noted in the high dose group, can be considered to be a consequence of salient toxicity in dams.
- - No similar effects of cyclanilide on reproduction were seen in other studies for reproductive toxicity i.e. teratology study in rats or two generation reproductive toxicity study in rats.

4.3 Specific issue raised by the SCP

The Committee has noticed that the DT $_{50}$ value of 2.5 days (as calculated by "Model Manager" version 1.1 [12]) for the soil metabolite 2,4-dichloroaniline (as reported in Appendix II: Endpoints and related information) is not consistent with the experimental results as summarised by the Rapporteur Member State: "2,4-DCA increased from 3% at day 1 to 10% at 14 days and decreased to 5% at the end of the study (180 days)". Therefore the Committee recommends that the estimation procedure for this half-life is re-evaluated.

Opinion of the Committee:

The Committee recommends that the estimation procedure for the half-life for the soil metabolite 2,4-dichloroaniline is re-evaluated.

5. REFERENCES

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- 7. Rodwell, D.E. Teratology study in rabbits with RPA 090946. Springborn Laboratories, INC. Submitted by Rhône-Poulenc Agrochimie. Report/ file N°: SLS 3147.73 Date: January 3, 1991.
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- 9. Tyl, R.W., Myers, C.B., Marr, M.C. Two generations reproductive study of RPA 090946 administered in the feed to CD (Sprague - Dawley) rats. Centre for Life Sciences and Toxicology. Submitted by: Rhône-Poulenc Agrochimie. Report/ file N°: RTI 65C-5250 Date: December 7, 1994.
- 10. Khera, K.S. Maternal Toxicity: A possible etiological factor in embryo-foetal deaths and foetal malformations of rodents-rabbit species. Teratology 31, 129-153, 1985.
- 11. Barlow, Susan M. and Frank M. Sullivan. Reproductive toxicity testing in animals in reproductive hazards of industrial chemicals. Academic Press, 1982.
- 12. Debruyne E. and Semino G. Cyclanilide, response document to the ECCO 73 Peer review meeting: request of mechanistic study on the immune system. Rhône-Poulenc Toxicology Department, March 1999.

6. DOCUMENTATION MADE AVAILABLE TO THE COMMITTEE

- 1. Evaluation of cyclanilide in the context of Council Directive 91/414/EEC concerning the placing of plant protection products on the market (Doc. SCP/CYCLAN/001), submitted by DG SANCO, 3 April 2000.
- 2. Cyclanilide: Evaluation table 7462/VI/98rev. 9 (Doc. SCP/CYCLAN/003-Rev.1), submitted by DG SANCO, 31 May 2000.

- 3. Cyclanilide: Review report for the active substance 7463/VI/98-rev. 1 (Doc. SCP/CYCLAN/004), submitted by DG SANCO, 31 May 2000.
- 4. Evaluation of cyclanilide in the context of Council Directive 91/414/EEC concerning the placing of plant protection products on the market: Appendices (Doc. SCP/CYCLAN/005-Rev. 1), submitted by DG SANCO, 13 June 2000.
- 5. Reconsideration of effect on reproduction of the current LOEL, safety factor and derived ADI and AOEL (Doc. SCP/CYCLAN/009), submitted by DG SANCO, 5 June 2000.
- 6. Cyclanilide: Volume 3 Annex B ADDENDUM to the draft Assessment Report prepared by Greece, June 2000 (Doc. SCP/CYCLAN/020), submitted by DG SANCO, 15 June 2000.
- 7. Attachment to the monograph of cyclanilide: volume 3, Annex B 7: Environmental fate and behaviour - March 2000 (Doc. SCP/CYCLAN/021), submitted by DG SANCO, 15 June 2000.
- 8. Cyclanilide: Evaluation of additional data by the RMS, Section: Fate and behaviour in the environment - 14 March 2000 (Doc. SCP/CYCLAN/022), submitted by DG SANCO 15 June 2000.
- 9. Letter from Greek authority (Doc. SCP/CYCLAN/023), submitted by DG SANCO, 15 June 2000.
- 10. Danish comments on the full report and the evaluation table (Doc. SCP/CYCLAN/006), submitted by DG SANCO, 15 June 2000.
- 11. Comment from Denmark to RMS concerning aquatic ecotoxicology (Doc. SCP/CYCLAN/007), submitted by DG SANCO, 15 June 2000.
- 12. Response from Greece on Danish comments (Doc. SCP/CYCLAN/008), submitted by DG SANCO, 5 June 2000.
- 13. Comments from Austria relating to Lemna study with the formulation (Doc. SCP/CYCLAN/010), submitted by DG SANCO, 15 June 2000.
- 14. Comments from Austria relating to Lemna study (Doc. SCP/CYCLAN/011).
- 15. Comments from Greece (Doc. SCP/CYCLAN/012), submitted by DG SANCO, 15 June 2000.
- 16 Comments from The Netherlands (Doc. SCP/CYCLAN/013), submitted by DG SANCO, 15 June 2000.
- 17. Comments from the United Kingdom (Doc. SCP/CYCLAN/014), submitted by DG SANCO, 15 June 2000.
- 18. Comments from France (Doc. SCP/CYCLAN/015), submitted by DG SANCO, 15 June 2000.
- 19 Response from RMS to Austrian comments on the need for a Lemna study with the formulation (Doc. SCP/CYCLAN/016), submitted by DG SANCO, 15 June 2000.
- 20. Response from RMS to Danish comments on the need for a Lemna study with the formulation (Doc. SCP/CYCLAN/017), submitted by DG SANCO, 15 June 2000.
- 21. Response from RMS to comments from Belgium on the evaluation table 74/VI/98 rev.3 (Doc. SCP/CYCLAN/018), submitted by DG SANCO, 15 June 2000.
- 22. Response from RMS to comments from France on the evaluation table 74/VI/98 rev.6 (Doc. SCP/CYCLAN/019), submitted by DG SANCO, 15 June 2000.
- 23. Danish comments to the list of end points (Doc. SCP/CYCLAN/024), submitted by DG SANCO, 25 May 2000..
- 24. Draft evaluation report (Monograph) prepared in the context of inclusion of cyclanilide in Annex I of Council Directive 91/414/EEC - Ministry of Agriculture, Greece (Volumes 1 to 4 - January 1998).

- 25 Cyclanilide/evaluation of additional data. S. Vizantinopolous (2000). Letter from NAGREF to Ministry of Agriculture of Greece, 14 March 2000.

7. ACKNOWLEDGEMENTS

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

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

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[1] European Community Co-ordination.

[2] Acceptable daily intake.

[3] Body weight.

[4] No observed adverse effect level.

[5] White blood cell.

[6] See ref. 2 Dean et al.

[7] Lowest observable effect level.

[8] See Rodwell, D.E. Teratology study in rabbits with RPA 090946, January 3, 1991.

[9] See Barlow and Sullivan, 1982, Khera, 1985

[10] See Barlow and Sullivan 1982.

[11] See Barlow and Sullivan 1982.

[12] Modelling software package from Cherwell Scientific Ltd, Oxford, UK.