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OPINION OF THE SCIENTIFIC COMMITTEE ON PLANTS REGARDING THE EVALUATION OF FLUMIOXAZIN [S-53482, V-53482] 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 25 April 2001)

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1. TITLE

OPINION OF THE SCIENTIFIC COMMITTEE ON PLANTS REGARDING THE EVALUATION OF FLUMIOXAZIN [S-53482, V-53482] IN THE CONTEXT OF COUNCIL DIRECTIVE 91/414/EEC CONCERNING THE PLACING OF PLANT PROTECTION PRODUCTS ON THE MARKET

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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 confirm that the test protocol used to assess the effect on Lemna is adequate in view of the proposed uses?

2. Can the Committee confirm that the test protocol used to assess the effect on earthworms is adequate in view of the proposed uses?

3. Are the development effects seen in animal studies of relevance to human?

3. BACKGROUND

Flumioxazin [S-53482, V-53482] is a new active substance (a.s.) in the context of Council Directive 91/414/EEC[1]. The draft Commission Directive for the inclusion of flumioxazin 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 (RMS, France) based on a dossier submitted by the notifier (Sumitomo), a review report prepared by the Commission and the Recommendations of the ECCO[2] Peer Review Programme.

Flumioxazin is a new pre-emergence herbicide for use in vineyards and orchards at a rate of 600 g a.s/ha. It acts by contact and penetrates at the level of aerial parts of the plants (mainly first shoot of seedlings). It is recommended to use it once a year before the emergence of weeds and the bud burst of the crop. It is not systemic.

[1] OJ N° L 230, 19. 8.1991, p. 1.
[2] European Commission Co-ordination.

4. OPINION

4.1 Question 1

Can the Committee confirm that the test protocol used to assess the effect on Lemna is adequate in view of the proposed uses?

Opinion of the Committee:

The Committee considers that the test protocol is not adequate to assess the effect on Lemna because it does not simulate the main expected exposure scenario. In view of the mode of uptake (contact followed by absorption by first shoots) and the main route of exposure (drift), a foliar application (spray) of the test substance directly onto the fronds is considered to be the most appropriate way to carry out the test.

The use of a sediment layer in the test lowered the concentration in the water layer and the test organisms were therefore exposed to lower concentrations. The measurements carried out during the test to estimate the concentration in the water layer did not quantify the concentration of flumioxazin. The provided test does not give insight into the exposure/effect relationship for either surface runoff or drift.

Scientific background on which opinion is based:

4.1.1 Suitability of test method for exposure scenario

For a first tier risk assessment the starting point is a realistic or reasonable worst case scenario. Exposure is defined as a ditch directly adjacent to the treated field with a water layer of 30 cm depth. For sprayed products the three main routes of exposure for organisms in the ditch are drift, run-off and drainage. The objective of a Lemna Growth Inhibition Test (e.g. the proposal for a new OECD guideline 221) is to quantify substance-related effects on vegetative growth. The test can be carried out under flow-through or semi-static conditions or without renewal (static) of the test solution. But, the semi-static and flow-through methods are recommended for substances that are rapidly lost from solution as a result of e.g. volatilisation, photodegradation, precipitation or biodegradation. With regard to the use of plant protection products it is stated in paragraph 32 of the Draft OECD guideline 221: “For some substances, e.g. pesticides, a foliar application (spray) of the test substance directly onto the fronds may be applicable if this is considered to be the most likely exposure scenario and/or if it is required by regulation.” Sediment is not included in the design of the new test.

The effect of flumioxazin on *Lemna gibba* was tested in a static system. Each test vessel, containing a 2 cm deep sediment layer, received 0.4 L of test water (six concentrations) which resulted in a 5 cm deep water layer. Five plants with 3 fronds were introduced in each vessel after 35 minutes.

The appropriate standard test would have been a test without sediment and either a semi-static or a flow-through test, because the degradation of the compound is relatively quick, less than 2 days (SCP/FLUMIO/009).

The exposure method used in the test (adding the test substance to the culture medium approximately 35 minutes prior to the introduction of the duckweed plants) would be appropriate for assessing the potential exposure due to drainage and/or run-off. However, in view of the intended use of flumioxazin as spray treatment in viticulture and

arboriculture exposure by drift is one of the main two routes of exposure (surface run-off being considered the other, especially from vineyards on slopes). Both scenarios must be covered by the risk assessment. In view of the mode of uptake and action of the compound (flumioxazin is a non systemic herbicide, absorbed by foliage and germinating seedlings; symptoms are necrosis of the stem, inhibition of root growth, bleaching followed by tissue necrosis on the leaves), a foliar application (spray) of the test substance directly onto the fronds is considered to be the most appropriate worst case exposure scenario.

Each test vessel contained a sediment layer of 2 cm and a water layer of 5 cm. The test may have been targeted to address the run-off scenario because the use of a sediment layer in the test will lower the concentration in the water layer (as would adsorption to suspended particles in the run-off slurry), and the test organisms will therefore be exposed to lower concentrations. The concentration of flumioxazin was not measured at the time of introduction of the fronds (measurement was carried out about half an hour before the introduction). Further measurements were made at day 1 and at test termination (day 14) but could not quantify flumioxazin (concentrations were below the LOQ). The current result therefore does not give insight into the exposure/effect relationship.

4.1.2 Conclusion

In view of the mode of uptake (contact followed by absorption by first shoots) and the likely route of exposure (drift; in addition to surface run-off), a foliar application (spray) of the test substance directly onto the fronds is considered to be the most appropriate way to carry out the test in this case, in order to cover the expected worst case scenario. The test does not give insight into the exposure/effect relationship of flumioxazin on duckweed.

4.2 Question 2

Can the Committee confirm that the test protocol used to assess the effect on earthworms is adequate in view of the proposed uses?

Opinion of the Committee:

The Committee concludes that the test protocol used to assess the effect on earthworms is adequate in view of the proposed uses. The use of a natural clay/clay loam soil to replace the kaolin clay is considered to be an acceptable deviation from the described protocol.

Scientific background on which opinion is based:

4.2.1 OECD guideline 207

The important part of the guideline with respect to question 2 is the following section:

> "Artificial soil test substrate, for example, as follows:
>
> 10 per cent sphagnum peat (as close to pH 5.5 to 6.0 as possible, no visible plant remains, finely ground, dried to measured moisture content)
>
> 20 per cent kaolin clay (kaolinite content preferably above 30 per cent)"

This section means that an artificial soil test substrate should be used but the example given is not mandatory.

The guideline does not mention a positive control. In SCP/FLUMIO/007, it is stated that according to ISO guideline 11268-1:1993 the LC_{50}[3] for chloracetamide should be between 20 and 80 mg/kg (usually between 15 and 35 mg/kg), but is not explicitly taken as a validation criterion.

4.2.2 Test method used for assessing the toxicity of flumioxazin to *Eisenia foetida*

The effect of flumioxazin on *Eisenia foetida* was tested in a system comparable to OECD 207 with one exception, instead of kaolin clay a clay/clay loam soil was used. The percentage amount of clay was identical to the amount suggested by the guideline (SCP/FLUMIO/003).

4 replicates of 10 earthworms each were exposed for 14 days to flumioxazin at concentrations ranging from 61 to 982 mg/kg soil at 19-21.5°C (pH at the start of the test was 6.7 and at the end of the test 7.6 to 7.8). Water content was at the start of the test about 34.3% and 27.9 to 41.8% at the end of the test. There were no mortalities in the control group. The mortality in the treated groups ranged from 0 to 7.5%. No significant differences in body weight between the treated and control worms were found and no abnormal symptoms of toxicity were detected in the surviving worms.

Chloracetamide was used as a positive control and a 7 day and 14 day LC_{50} values of 96 and 84 mg/kg respectively were found (draft assessment report, Volume 3 Annex B, p 203).

It is noted that:

- the chloracetamide results are unusually high,
- the pH at the start of the experiment and at the end is higher than usual (OECD guideline; pH at start 6±0.5), and
- the water content shows an unusually high variation at the end of the test (experience: most of the time not more than 3 % variation).

But in view of the results found for the control group (no mortality and no toxicological effects) and the large safety factor (TER[4] > 1228) the Committee considers that these variations do not alter the conclusion of the risk assessment.

4.2.3 Conclusion

The Committee concludes that the test protocol used to assess the effect on earthworms is adequate in view of the proposed uses. The guideline does not give a binding prescription for an artificial soil test substrate, but only an example. Therefore, the guideline does not give indications whether the deviation from the protocol is acceptable or not. The use of a natural clay/clay loam soil to replace the kaolin clay is considered to be an acceptable deviation from the described protocol in this particular case. The Committee noted the observed variations in the test. But in view of the results found for the control group (no mortality and no toxicological effects) and the large safety factor (TER > 1228) the Committee considers that in this case, these variations do not alter the conclusion.

[3] Lethal concentration, median.
[4] Toxicity Exposure Ratio.

4.3 Question 3

"Are the developmental effects seen in animal studies of relevance to humans?"

Opinion of the Committee:

The developmental effects observed in animals consist of increases in the incidence of ventricular septal defects, wavy ribs, curvature of the scapula and decreased ossification of sacrococcygeal vertebral bodies. All of these occur in rats, but not in rabbits at a 100 fold higher dose. The Committee is of the opinion that only the ventricular septal defects are important and considered them to be relevant for humans. However, the data are sufficient to support the establishment of a NOEL[5] for the developmental effects. The proposed mechanism by which flumioxazin causes developmental toxicity in rats but not in rabbits is the inhibition of the enzyme protoporphyrinogen oxidase, thereby interfering with normal heme synthesis and resulting in anaemia. The rat enzyme is about 2.5 fold more sensitive to flumioxazin than the human enzyme which is about 8 fold more sensitive than the rabbit enzyme.

Scientific background on which the opinion is based:

4.3.1 Assessment

Flumioxazin is a herbicide belonging to the N-phenylimides family. Studies in rats showed that the substance is readily absorbed (about 83%) and widely distributed in the organism. Radio labelled/radioactive residue is highest in blood and to some extent in liver and kidney. Flumioxazin is mainly excreted via faeces and to a lesser degree via urine within 7 days. The substance is extensively metabolised and 35 metabolites have been detected and quantified. The major metabolites are sulfonate derivatives in faeces and sulfonate alcohol and acetanilide derivatives in urine.

The critical systemic toxic effect in rats is anaemia, the lowest NOEL being 50 ppm (1.8 mg/ kg body weight in males and 2.2 mg/ kg body weight in females) found in combined long term carcinogenicity study. Liver is the main target organ in the other species tested e.g. dog and mouse.

Flumioxazin showed no carcinogenic potential in rats and mice. Apart from a positive result in an *in vitro* test for chromosome aberration in presence of metabolic activation (CHO cells in presence of S9), flumioxazin did not show mutagenic effects in other *in vitro* tests as well as in *in vivo* tests.

In a two-generation reproduction toxicity study in rats dietary concentrations higher than 200 ppm, flumioxazin produced toxic effects in adults i.e. effects on clinical appearance, reduced body weight and reduced weight of testes and epididymidis, brain and prostate. The NOEL for offspring was 100 ppm. Higher dietary concentrations caused effects like reduced pup weight, stillbirths and consequently reduction in live born pups.

No developmental toxicity was found in a teratogenicity study in pregnant rabbits in doses up to 3000 mg flumioxazin/ kg body weight. In a corresponding test in pregnant rats, oral doses of 1, 2, 10 or 30 mg flumioxazin/ kg body weight on days 6-15 of

[5] No Observed Effect Level.

gestation were administered. The incidence of foetuses with cardiovascular abnormalities, primarily ventricular septal defects (VSD), was increased in the 30 mg/ kg body weight group together with increased incidence of wavy ribs and curvature of the scapula and a decrease in the number of ossified sacrococcygeal vertebral bodies. The incidence of cardiovascular abnormalities in the 10 mg/ kg body weight group was not significantly different from that of the controls, was nearly within the historical control range and was considered to be within the range of spontaneous occurrence. Thus, NOAEL[6] for the developmental effects was 10 mg / kg body weight. The maternal NOEL was greater than 30 mg/ kg body weight, the highest tested dose.

Defect of the closure of the interventricular septum is not an unusual finding in rats. Closure of this interventricular septum completes on about day 15 in rats (Nishimura and. Tanimura, 1976). VSD is reported one of the most frequent congenital anomalies in humans and in rats. The most sensitive period in rats is around 10-11 days of the gestation period (Yamakita, O *et al*, 1994). VSD occurred in control rats in the teratogenicity study with flumioxazin. It has been reported that foetuses from pregnant rats exposed to an atmosphere with a low oxygen concentration develop cardiovascular malformations, mainly VSD (Nakatsu, *et al.*, 1993). The same reference reports a study with aniline hydrochloride, a methemoglobin-inducing compound administered subcutaneously to pregnant rats at different days of gestation. VSD was induced in the foetuses, the most sensitive period being day 12 to 14 of the gestation. Change in feeding regimen has also been shown to influence the incidence of VSD. Thus, food restriction in pregnant rats to 40% of that of controls during day 6 to 16 of the gestation period caused foetal growth retardation with slight delay in ossification and increase in cardiovascular malformations, mainly VSD (Ikemi, *et al*, 1993).

Mechanism of action:

The postulated mechanism by which flumioxazin causes developmental toxicity in rats is the inhibition of the enzyme protoporphyrinogen oxidase (PPO) interfering with normal heme synthesis and resulting in anaemia. This condition leads to hypoxia in foetal tissues followed by impairment of other liver functions including protein synthesis and ultimately foetal oedema and anaemia. Concurrently, the foetus would compensate for the anaemia by pumping a greater volume of blood leading to the observed enlargement of the heart. The VSD is believed to be a consequence of a mechanical distortion of the heart. The experimental support for part of the postulated mechanisms is as follows:

– The data from repeated dose toxicity studies show that rats appear to be the most sensitive species regarding anaemia.

– Dietary administration to female rats at doses of 3000 or 10000 ppm for up to 5 weeks showed that flumioxazin interferes with normal heme biosynthesis resulting in sideroblastic anaemia and porphyria in adult rats.

– ^{14}C flumioxazin administered to pregnant rats on day 12 of gestation reaches at the maximum level of ^{14}C parent flumioxazin in rat foetuses 4 hours later. No clear pattern of absorption, distribution, metabolism or excretion was evident which could account for the species specific developmental toxicity in rats.

[6] No Observed Adverse Effect Level.

– A histological examination of rat foetuses from pregnant rats administered 1000 mg flumioxazin/ kg body weight on day 12 indicated signs of foetal anaemia within 6 hours after dosing but no histological changes in the foetal heart were observed until 36 or 48 hours after treatment. No effects were observed in rabbit embryos treated the same manner as rats.

– Flumioxazin, 400 mg/ kg body weight was administered to pregnant rats on day 12 of gestation and the embryos/foetuses were removed from uterus on days 13 through 20 of gestation. Enlarged heart, oedema and anaemia (decreased red blood cell count and haemoglobin) were observed on days 15 and 16 of gestation. Delayed closure of the interventricular foramen was observed as well. Increased foetal deaths occurring before day 15 of gestation were also reported. These deaths are considered to be a consequence of the observed effects (enlargement of the heart, oedema and anaemia).

  In a separate study, it was determined that the VSD did not resolve during the postnatal period and remained a permanent malformation. Serum protein concentration was reduced on days 15 and 16 but returned to control values by day 17. Evidence of incomplete chondrification of the ribs was observed on day 16, delayed ossification was observed on day 17 and on day 20 wavy ribs and other skeletal abnormalities were observed.

– To determine species difference in accumulation of protoporphyrin IX (PPIX) as an indicator of inhibition of the porphyrin biosynthesis and consequently hematoxicity, pregnant rats and rabbits were administered a single oral dose of 1000 mg/ kg body weight, and the concentration of PPIX was measured in embryos and maternal liver at various hours thereafter. The concentration in maternal rabbit liver and rabbit embryos was low at all time point whereas that of rats was increased with a peak concentration at 12 hours for embryos and 6-12 hours for maternal liver. These findings demonstrate a difference in the response to exposure to flumioxazin between the two species.

– The PPIX accumulation in rat embryos was measured after dosing pregnant rats 1000 mg/ kg body weight on day 12 of gestation of either flumioxazin or one of two chemically related compounds one of which did not cause developmental toxicity in neither rats nor rabbits. The results showed a strong correlation between PPIX accumulation in embryos and the chemicals which were identified as developmental toxicants.

– Pregnant rats and rabbits received 400 and 1000 mg/ kg body weight respectively as a single oral dose on one of day of gestation beginning on day 10 through day 15. The PPIX concentration in embryos 14 hours later was increased in the rat embryos only. Peak concentrations occurred after dosing on days 11 and 12 which coincide with the critical period for developmental toxicity in rat embryos exposed to flumioxazin.

– The results from three *in vitro* studies measuring inhibition of PPO further support the hypothesis that the developmental toxicity in rats dosed with flumioxazin is related to disruption of normal heme synthesis. Based upon the inhibition of PPO activity in mitochondria from livers of adult female rats, rabbits and humans in one of the three studies species differences were demonstrated, the relative sensitivity of the species

being rat > human > rabbit (IC_{50}[7] values: rat, 0.00715 μM; human, 0.0173 μM; rabbit, 0.138 μM).

4.3.2 Conclusion

The developmental effects observed in animals consist of increases in the incidence of ventricular septal defects, wavy ribs, curvature of the scapula and decreased ossification of sacrococcygeal vertebral bodies. All of these occur in rats, but not in rabbits. Only the ventricular septal defects are important and considered to be relevant for humans. However, the data are sufficient to support the establishment of a NOEL for the developmental effects. The proposed mechanism by which flumioxazin causes developmental toxicity in rats but not in rabbits is the inhibition of the enzyme protoporphyrinogen oxidase, thereby interfering with normal heme synthesis and resulting in anaemia. The rat enzyme is about 2.5 fold more sensitive than human which is about 8 fold more sensitive than the rabbit enzyme.

5. REFERENCES

OECD Guideline 207, OECD Guideline for testing of chemicals "Earthworm, Acute Toxicity Testing", Adopted 4 April 1984, Paris, France, pp 9.

OECD Draft guideline 221, OECD guideline for testing of chemicals, Proposal for a new guideline 221 "Lemna sp. Growth Inhibition Test, revised draft document October 2000, Paris, France, pp. 24.

Nishimura, H & T. Tanimura. Clinical Aspects of the Teratogenicity of Drugs, 1976, Excerpta Medica.

Yamakita, O *et al.* The relationship between ventricular septal defect and intrauterine growth retardation, and their postnatal recovery in rats. J. Toxicological Science. Nov 1994; 19(4): 364.

Nakatsu, T *et al.* Cardiovascular malformations in rat fetuses induced byadministration of aniline hydrochloride. Teratology November 1993, 48: 5, 506, abstract from Japanese Teratology Society.

Ikemi, N *et al.* Effects of food restriction on fetal development during major organogenesis in rats. Teratology May 1993, 47: 5, 423, abstract from Teratology Society.

6. DOCUMENTS MADE AVAILABLE TO THE COMMITTEE

1. Flumioxazin: Terms of reference (SCP/FLUMIO/001 submitted by DG Health and Consumer Protection, 7 August 2000).
2. Flumioxazin: Evaluation table Doc. 7470/VI/98 rev. 7 (12-07-00) (SCP/FLUMIO/003 submitted by DG Health and Consumer Protection, 7 August 2000).

[7] Inhibitory concentration, median.

3. Flumioxazin: List of end points (based on doc 1654/VI/94, Rev. 6.4, 31 Oct 1997) Effects on Non-target Species (SCP/FLUMIO/004 submitted DG Health and Consumer Protection, 7 August 2000).

4. Flumioxazin: Volume 3–- Addendum to Monograph B2 à B4 (SCP/FLUMIO/005 submitted by DG Health and Consumer Protection, 7 August 2000).

5. Flumioxazin: Addendum to the Monograph 27-07-2000 – Ecotoxicology (SCP/FLUMIO/006 submitted by DG Health and Consumer Protection, 7 August 2000)

6. Flumioxazin: Austrian comments on evaluation table "earthworms toxicity study" (SCP/FLUMIO/007 submitted by DG Health and Consumer Protection, 7 August 2000).

7. Flumioxazin: Addendum to the monograph of Flumioxazin - Environmental fate (SCP/FLUMIO/008 submitted by DG Health and Consumer Protection, 7 August 2000).

8. Flumioxazin: France reply to S, DK and UK comments 7 September 2000 (SCP/FLUMIO/009 submitted by DG Health and Consumer Protection, 13 December 2000).

9. Flumioxazin: response from France to DK comments on fate and ecotoxicology, 28 July 1999 (SCP/FLUMIO/010 submitted by DG Health and Consumer Protection, 7 August 2000).

10. Flumioxazin: Swedish comments on mammalian toxicology, 19 June 2000 (SCP/FLUMIO/011 submitted by DG Health and Consumer Protection, 7 August 2000).

11. Flumioxazin: Appendices I to III to draft report, 30 August 2000 (SCP/FLUMIO/012 submitted by DG Health and Consumer Protection, 13 December 2000).

12. Flumioxazin: Addendum to the monograph, studies submitted after the peer review programme and not included in the monograph (SCP/FLUMIO/013, submitted by DG Health and Consumer Protection, 30 October 2000).

13. Flumioxazin: draft evaluation report (monograph), volumes 1 to 3 prepared by France as Rapporteur Member State, December 1997.

14. S-53482 50WP – Toxicity to Duckweed, *Lemna gibba*, under static water/sediment conditions, J.R. Hoberg, Springborn Study N° 13048.6201, 10 March 2000 - submitted by Sumitomo Chemical Agro Europe, 26 February 2001.

7. ACKNOWLEDGEMENTS

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

Toxicology: Prof. Maroni (Chairman) and Committee Members: Dr. Delcour-Firquet, Prof. Leszkowicz, Dr. Meyer, Dr Moretto, Prof. Petzinger, Prof. Savolainen, Prof. Silva Fernandes, Dr. Speijers, and invited experts Dr. Fait, Dr. McGregor.