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SCIENTIFIC COMMITTEE ON PLANTS

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

(Opinion of the SCP adopted on 7 March 2001)

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1. TITLE

OPINION OF THE SCIENTIFIC COMMITTEE ON PLANTS ON THE EVALUATION OF IPROVALICARB IN THE CONTEXT OF COUNCIL DIRECTIVE 91/414/EEC FOR PLACING 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 comment on the acceptability of the risk of metabolite PMPA on earthworms?

2 Can the Committee comment on the relevance to human of the occurrence of rare tumours in rats at high doses?

3. BACKGROUND

Iprovalicarb is a new active substance in the context of Council Directive 91/414/EEC[1]. The draft Commission Directive for inclusion of iprovalicarb 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 dossier prepared by the notifier (Bayer AG), a draft assessment report (monograph) prepared by the Rapporteur Member State (Ireland), under the co-Rapporteur system (co-Rapporteur Member State: Germany).

Iprovalicarb is a new systemic fungicide and member of a new class of compounds termed amino acid amide carbamates, for use in vine and potatoes for the control of downy mildew and potato blight respectively. The proposed rate of use ranges from 0.12 kg a.s./ha to 0.48 kg a.s./ha in grapevine and 0.18 kg a.s./ha in potatoes, applied up to five time per season with 7-10 days between applications. Iprovalicarb is chemically composed of two (S,R and S,S) diastereoisomers.

4. OPINION

4.1 Question 1

"Can the Committee comment on the acceptability of the risk of metabolite PMPA on earthworms?"

OPINION OF THE COMMITTEE:

The protocol for evaluating the effects of chronic exposure of earthworms to the parent compound did not include a sufficiently high test concentration to allow for a margin of safety, and therefore these tests are inconclusive. No direct tests of the long-term effects of the metabolite PMPA on earthworms were conducted, and the concentrations of PMPA were not estimated in the available chronic test on the parent. Given the uncertainties, the SCP recommends that additional chronic tests of the parent compound and metabolite PMPA are needed to enable a reliable evaluation of the acceptability of the risk to earthworms.

[1] OJ N° L 230, 19. 8.1991, p.1.

SCIENTIFIC BACKGROUND ON WHICH THE OPINION IS BASED:

4.1.1 Fate, exposure and ecotoxicity data

4.1.1.1 Degradation of the parent compound and metabolite

The estimated half-life of the parent compound in soil ranged from 2.0-29.6 days (DT_{90}[2] 6.7-98.3 days) under a variety of laboratory and field conditions. In aerobic soils (4 at 20°C, 1 at 10°C) a total of 9 metabolites were observed in the soil extracts but only one of these reached > 6% of applied radioactivity, namely PMPA. PMPA, which is likely to be formed from cleavage of the amide bond, reached a maximum of 31.2%, 50.7%, 30.3%, 47.8% and 32.7% applied radioactivity after a recorded 31, 100, 31, 7 and 59 days respectively. The peak of 50.7% recorded after 100 days may be an artefact because an earlier peak of 47.5% was recorded after 31 days. The estimated half-life of PMPA (first order reaction kinetics) ranged from 41-118 days (n = 3) with median 52 days.

4.1.1.2 Estimated maximum concentration of the parent in soil

Estimates of the initial PEC_{soil}[3] of the parent in the top 5 cm of soil varied from 0.32 mg a.s./kg dry weight soil (single application at 0.48 kg/ha) to 1.0 mg a.s./kg dry weight soil (multiple application of a total 1.5 kg a.s./ha)). The predicted initial PEC_{soil} derived from modelling studies (PELMO) was 0.64 mg a.s./kg following multiple application. The maximum soil residue concentration recorded for the parent following a single application of 0.48 kg a.s./ha was 0.147 mg/kg soil (0-10 cm), after 14 days.

4.1.1.3 Estimated maximum concentration of the metabolite PMPA in soil

In field dissipation studies, the metabolite PMPA was found at a maximum of 0.0413 mg/kg soil 30-33 days after a single application of 0.48 kg a.s./ha. PELMO estimates for the PEC_{soil} based on "pseudo" cumulative application rate of the metabolite of 0.184 kg PMPA /ha was 0.16 mg/kg soil (0-5 cm).

4.1.1.4 Ecotoxicological data

The available ecotoxicological data relating to earthworms are summarised below.

Test substance	**Test type**	**Concentrations** mg/kg d.wt soil {1 mg a.s./kg d.wt soil ≈ 750 g a.s./ha }	**Lowest lethal concentration** mg/kg d.wt soil	**NOEC**[4] mg/kg d.wt soil
Parent	14d acute	0,32,100,316,1000	> 1000	32 (weight loss)
Metabolite PMPA	14d acute	3.2,10,32,100,316,1000	1000	316 (weight loss)
Parent	28+28d chronic	0.2, 0.4, 1.0	> 1	> 1

[2] Period required for 90% dissipation.
[3] Predicted Environmental Concentration in soil.
[4] No Observed Effect Concentration.

4.1.2 Evaluation

4.1.2.1 *Evaluation of acute and chronic tests of parent on earthworms*

On the basis of these data the worst case acute TER[5] (LC_{50}/PEC_{soil}) for the parent substance is estimated as > 1000 (> 1563 if we use PELMO). The worst case long-term sub-lethal TER for the parent substance ($NOEC/PEC_{soil}$) is estimated as greater than 1 (> 1.56 if we use PELMO).

As the long-term sub-lethal trigger for the parent is 5, this raises concern. The current Guidance document on Terrestrial Ecotoxicology (2021/VI/98 rev 7) proposes that "W*hen planning the* [sublethal] *test, the upper concentration level must be chosen high enough in order to find out whether the long-term TER is above the trigger of 5*". Yet in this case the maximum concentration tested was 5 times less than the critical NOEC that would establish a TER of 5 or above.

We acknowledge that recent research findings (Barber *et al.* 1998, Heimbach 1998) indicate that earthworm reproduction tests are considerably more sensitive than field tests, and agree that such data should form the basis for future discussions (Bembridge 1998). However, at present the relationships between chronic laboratory and field responses are not well understood and questions remain as to the extent to which laboratory tests on one earthworm species can reliably predict the field responses of other earthworm species, or even field responses of the same species in different soils (Jones & Hart 1998). Of particular relevance is that even if Barber *et al.* (1998) recommended lower trigger TER of 2 was adopted, then further testing of the parent substance would be required.

We note that the parent substance degrades relatively rapidly (DT_{50}[6] 2.0-29.6 days), and that no significant sub-lethal effects were observed over the 56 day trial at field-relevant concentrations (up to 1 mg/kg). However, given that the product is to be used 1-5 times per season and the maximum field residue level of the parent recorded after a single application was 0.48 mg/kg (estimated 1.0 mg/kg following multiple applications), the SCP feels that current data do not incorporate a sufficient margin of safety for us to conclude that the parent substance is unlikely to have long-term effects on earthworms.

4.1.2.2 *Evaluation of acute tests of metabolite PMPA on earthworms*

The worst case acute TER for the metabolite is > 6250, based on the PELMO-derived PEC_{soil} with multiple applications. Furthermore, we note that the metabolite had no sub-lethal effect at concentrations of 316 mg/kg soil or less in the 14 day acute test.

Despite the persistence of the metabolite, and the fact that the parent substance is likely to be applied several times per year, no long-term test for PMPA was provided. PMPA is normally formed relatively rapidly, so that it is possible that it reached a peak during, and perhaps early in, the 56 day chronic test on the parent. Unfortunately however, no estimates of the concentration of PMPA were provided in the test on the parent compound, and it is not known whether the degradation kinetics of the parent are the same in artificial soils as they are in more natural soils. Furthermore, even if 100% of the parent were immediately converted to PMPA, then the TER would only just exceed 5 (= 6.25).

Given these uncertainties and low test concentration of the parent, the SCP proposes that a more detailed assessment of the risks to earthworms following chronic exposure to metabolite PMPA would be helpful in evaluating risk.

[5] Toxicity over Exposure Ratio.
[6] Period required for 50% dissipation.

4.2 Question 2

Can the Committee comment on the relevance to human of the occurrence of rare tumours in rats at high doses?

OPINION OF THE COMMITTEE:

The Committee concluded that the weight of evidence does not exclude that the tumours in rats are compound-related. Even if their incidence is low and they occur only in rats and mostly at the highest dose, the Committee is concerned about the multiple sites where tumours were observed and the occurrence of some tumours that are normally rare. The data set presented does not rule out the relevance to humans of the tumours observed in rats after iprovalicarb treatment.

SCIENTIFIC BACKGROUND ON WHICH THE OPINION IS BASED:

4.2.1 Assessment of toxicological data

Iprovalicarb is readily absorbed from the gastrointestinal tract (>91%), is widely distributed and is rapidly excreted (>98% within 48 hours). The main route of excretion is faecal. It is extensively metabolised and shows no evidence for accumulation. The main metabolite is iprovalicarb carboxylic acid, in the form of a pair of diastereomers.

Iprovalicarb has a very low acute toxicity when tested in rodents orally, dermally or by inhalation. It is not irritating to the skin or eyes, and has no skin sensitisation potential.

Iprovalicarb was administered to beagle dogs over a period of 53 weeks (at levels of 0, 80, 800 or 8000 ppm, four animals/dose/sex). The liver was the only affected organ. While no adverse effects were observed in females at the lowest dose, the effects observed in males included slight increased liver weight, hepatic microsomal enzyme induction (N-DEM, O-DEM and slight increase of P-450 activities), minimal cytoplasmatic alterations and hypertrophy and corresponded to the much more severe effects than were observed at higher dose levels where animals had a marked reduction of general health.

In order to establish a clear NOEL[7] a supplementary four-week study was performed (0, 10, 20,40 or 80 ppm). The 80 ppm data of the 53-week study reflects the 80 ppm data of the supplementary study (enzymes induction, no cumulative effects and reversibility). A NOEL of 20 ppm equal to 0.77 mg/kg b.w./day for males and females was established based on the increase of N-demethylase activity and slight increase of cytochrome P-450 activity at the next highest dose (40 ppm).

4.2.1.1 *Genotoxicity testing*

Iprovalicarb has been tested in bacteria and mammalian cells for potential genotoxicity *in vitro*. None of the tests revealed any clear evidence of mutagenic or genotoxic activity. Negative results were obtained in the V79/HGPRT assay with and without metabolic activation, in four *Salmonella typhimurium* LT2 mutants, in Chinese hamster ovary cells and in a test on unscheduled DNA synthesis in rat liver primary cell culture.

Iprovalicarb was not genotoxic *in vivo* in the micronucleus test in mice. Iprovalicarb was tested in an organ specific ^{32}P-post labelling assay *in vivo* in the uterus and whole urinary bladder/urinary

[7] No Observed Effect Level.

bladder epithelium of female rats to investigate DNA-adduct formation. No indication of DNA adduct formation by iprovalicarb was found in uterus, whole urinary bladder or urinary bladder epithelium. The data in this study are presented only in a qualitative form.

4.2.1.2 *Long term study in mice*

Iprovalicarb was administered through the diet to $B6C3F_1$ mice over a period of 105 weeks (at levels of 0, 280, 1400 or 7000 ppm, 50 animals/dose/sex).

Mortality and body weights, and haematology parameters were unaffected by treatment. Differences in some clinical chemistry parameters were evident, dose dependence was lacking, and they were not considered compound related. In males at 1400 ppm and above, absolute and relative kidney weights were lower, which correlates with decreased incidence of tubular vacuolisation, which was recorded during histopathological examination in males. At 7000 ppm, a significant increase in the incidence of "fatty changes" in the liver were described in males. There was no evidence that the tumours, which were recorded throughout the study, were treatment related. A NOEL of 280 ppm (equal to 58.5 mg/kg b.w.) was established based on slightly increased blood urea concentrations and decreased kidney weights at 1400 ppm and above in both sexes, and on higher water intake at 7000 ppm in males. There is no evidence of any tumourigenic potential associated with the administration of iprovalicarb to mice over a 105 weeks period.

4.2.1.3 *Long term study in rats*

Groups of Wistar (Hsd/WIN:WU) rats (50/animals/dose/sex), were administered iprovalicarb in their diet at concentration of 0, 500, 5000 or 20000 ppm for 24 months. There was no evidence of a substance-related effect on mortality and hematology parameters. Treatment-related effects developed in females at 5000 ppm and above, and in males at 20000 ppm. Body weights of females were lower compared to the controls (by 12% at 20000 ppm; by 5% at 5000 ppm) at study termination. Altered liver parameters were mainly observed in females at 20000 ppm (depressed AST, ALT and GLDH activities in plasma as well hepatocellular hypertrophy that correlates with elevated liver weights and reduced kidney weights).

In 6/50 (12%) females at 20000 ppm uterine *adenocarcinomas* were observed. No statistically significant trend was observed (2/50, 3/49, 3/48 and 6/50) and the incidence was within the historical control range (0 – 14/20%). There was an increase in malignant *mixed Mullerian tumours* in the uteri of females treated at 5000 (1/48, 2%) and 20000 (2/50, 4%) ppm. The historical control values vary between 0 and 2%. The incidence of such tumours appears to be slightly above the historical. Iprovalicarb gave no evidence of a DNA-adduct forming potential in a ^{32}P-postlabeling assay performed with uterine tissue.

Benign neoplastic lesions of the urinary bladder 2/50 (4%) (*transitional cell papilloma*) were observed at 20000 ppm in females.

There was a statistically significant positive trend in the incidence of thyroid *follicular cell adenomas* (at 5000 and 20000 ppm doses) in female rats (0/50, 0/50, 1/50 and 2/50) and thyroid *follicular cell carcinomas* were observed (at 5000 and 20000 ppm doses) in female rats (0/50, 0/50, 1/50 and 1/50). No effect was seen in the thyroid of male rats. The incidence of thyroid gland neoplasms is within the historical (adenomas 6% and carcinomas 5%).

In two females at 20000 ppm (2/50, 4%), *squamous cell carcinoma* of the clitoral gland was observed.

Malignant tumours of the skeletal system were seen in 20000 ppm males. There were three *osteosarcomas* (two of the femur, 2/50, 4% and one of the lower jaw, 1/50, 2%) and one *chondrosarcoma* of the nasal cavity, 1/50, 2%. Historical data showed incidences of spontaneously occurring osteosarcomas in the range of 0 - 2%. No data are available on chondrosarcoma.

It should be noted that the animals concerned were subjected to high doses (20000 ppm equal to 1109.6 mg/kg b.w./day for males and 1397.7 mg/kg b.w./day for females), as the test substances was observed to be of very low general toxicity in subchronic studies.

4.2.2 Conclusions

Daily administration of iprovalicarb in mice, at dietary exposure of up to 280 ppm in males, and 7000 ppm in females for a period of 105 weeks did not produce any adverse effects, and similarly doses of up to 7000 ppm did not produce any evidence of carcinogenic effect in either sex.

The administration of iprovalicarb is associated with the incidence, only in rats, of certain tumours especially at the dose of 20000 ppm (equal to 1109.6 mg/kg b.w./day for males and 1397.7 mg/kg b.w./day for females). Some of the observed tumour types were not seen in control animals, and have rarely been observed in long-term studies. The increased incidence of each kind of tumours as such was only minimal and close to the historical control range.

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

- Although some *in vitro* studies showed some limitations, the overall results do not provide evidence of genotoxic activity for iprovalicarb.

- The weight of evidence does not exclude that the tumours in rats are compound-related. Even if their incidence is low and they only occur in rats and mostly at the highest dose, the Committee is concerned about the multiple sites where tumours were observed and the occurrence of some tumours that are normally rare. The data set presented does not allow ruling out the relevance to humans of the tumours observed in rats after iprovalicarb treatment.

5. REFERENCES

Barber, I., Bembridge, J., Dohmen P., Edwards, P. Heimbach, F., Heusel, R., Romijn, K. & Rufli, H. (1998). Development and evaluation of triggers for earthworm toxicity testing with plant protection products. In: *Advances in Earthworm Ecotoxicology* (S.C. Sheppard, J.D. Bembridge, M. Holmstrup & L. Posthuma Eds), pp 269-278. SETAC Press.

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Heimbach, F. (1998). Comparison of the sensitivities of an earthworm reproduction test and a standardized field test on grassland. In: *Advances in Earthworm Ecotoxicology* (S.C. Sheppard, J.D. Bembridge, M. Holmstrup & L. Posthuma Eds), pp 235-245. SETAC Press.

Jones, A. & Hart, A.D.M. (1998). Comparison of laboratory toxicity tests for pesticides with field effects on earthworm populations: a review. In: *Advances in Earthworm Ecotoxicology* (S.C. Sheppard, J.D. Bembridge, M. Holmstrup & L. Posthuma Eds), pp 247-267. SETAC Press.

6. DOCUMENTS MADE AVAILABLE TO THE COMMITTEE

1. Evaluation of Iprovalicarb in the context of Direcitve 91/414/EEC: Terms of reference, submitted by DG SANCO 7 August 2000 (Doc. SCP/IPROVA/001).

2. Iprovalicarb: Evaluation table doc SANCO/1508/2000 REV. 0 (04.07.00), submitted by DG SANCO 7 August 2000 (Doc. SCP/IPROVA/003).

3. Iprovalicarb: Danish comments on the monograph on Iprovalicarb prepared by Ireland, submitted by DG SANCO 16 Nov. 2000 (Doc. SCP/IPROVA/004).

4. Iprovalicarb: UK response to Danish comments on End Points Document submitted by DG SANCO 16 Nov. 2000 (Doc. SCP/IPROVA/005).

5. Iprovalicarb: Mutagenicity study for the detection of induced forward mutation in the V79/HGPRT assay in vitro – submitted by Bayer 16 Nov. 2000 (Doc. SCP/IPROVA/006).

6. Iprovalicarb: Assessment of toxicologic properties with particular consideration of the histopathologic findings in the chronic rat study - submitted by Bayer 16 Nov. 2000 (Doc. SCP/IPROVA/007).

7. Iprovalicarb: Salmonella microsome test - submitted by Bayer 16 Nov. 2000 (Doc. SCP/IPROVA/008).

8. Iprovalicarb: In vitro mammalian chromosome aberration test with Chinese hamster ovary - submitted by Bayer 16 Nov. 2000 (Doc. SCP/IPROVA/009).

9. Iprovalicard: Test on unscheduled DNA synthesis in rat liver primary cell culture in vitro - submitted by Bayer 16 Nov. 2000 (Doc. SCP/IPROVA/010).

10 Iprovalicarb: Micronucleus test on the mouse - submitted by Bayer 16 Nov. 2000 (Doc. SCP/IPROVA/011).

11. Iprovalicarb: 32P- Postlabelling assay in female rat uterus and urinary bladder epithelium in vivo - - submitted by Bayer 16 Nov. 2000 (Doc. SCP/IPROVA/012).

12. Iprovalicarb: Evaluation of the genotoxicity data on iprovalicarb – submitted by Prof. Parry 8 Dec. 2000 (Doc. SCP/IPROVA/013).

13. Iprovalicarb: Draft assessment report (monograph) prepared in the context of the possible inclusion of the following active substance in Annex I of Council Directive 91/414/EEC: Iprovalicarb (Volumes 1 to 4) – Rapporteur Member State, Ireland – Co-rapporteur, Germany – May 2000.

7. ACKNOWLEDGEMENTS

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

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