Opinion of the Scientific Committee on Plants regarding the inclusion of Kresoxim-methyl in annex I to Directive 91/414/EEC concerning the placing of plant protection products on the market (Opinion expressed by SCP on 14 July 1998)

1. Terms of reference

The draft proposed inclusion of kresoxim-methyl in Annex I of Directive 91/414/EEC had been referred to the Scientific Committee on Plants for consultation without specific questions being addressed to the Committee.

2. Background

The draft Commission Directive for the inclusion of kresoxim-methyl in Annex I of Directive 91/414/EEC (OJ No L230, 19. 8. 91 p.1) concerning the placing of plant protection products on the market was submitted to the Committee for consultation. The Committee had been supplied with a dossier provided by BASF Aktiengesellschaft, the monograph prepared by the Belgian authorities, the results of the 'Peer review' report involving several Member States and the draft Commission Directive.

Kresoxim-methyl is a fungicide which belongs to the strobilurin family of chemical active substance, it acts as a mitochondrial inhibitor, has slight systemic action and possesses both curative and eradicative properties. Information available to the Committee at the time of writing of this report indicated that the maximum rates and number of applications were as follows:

Pome fruit	A maximum of 4 applications per season with a maximum of 100 g ai/ha per application, i.e. 400 g ai/season.
Grapes	A maximum of 3 applications per season with a maximum of 150 g ai/ha per application, i.e. 450 g/season.
Cereals	Depending on the particular crop a maximum of 2 applications per season with a maximum of 150 g ai/ha per application, i.e. 300 g/season.

The Committee was aware that it is the intention of the registrant to seek in due course further authorisations for new uses of preparations containing kresoxim-methyl but these have not been taken into account in the present opinion.

3. Opinion of the committee

Following a review of the data supplied, the Committee decided to deal with the following aspects:

1. Assessment of the carcinogenic potential of kresoxim-methyl and its relevance for man due to the evidence of rat liver carcinomas in a long-term carcinogenicity study.

2. The soil leaching behaviour of the main metabolite (BF 490-1) in soil which must be considered very mobile on the basis of its very low Koc value measurements.

1. Assessment of the carcinogenic potential of kresoxim-methyl and its relevance for man due to the evidence of rat lever carcinomas in a long-term and carcinogenicity study.

Introduction

When judging the adequacy of experimental carcinogenicity data it is necessary that sufficient evidence of carcinogenicity in animals is provided when a causal relationship has been established between the agent under test and an increased incidence of malignant neoplasms, or of an appropriate combination of benign and malignant neoplasms, in two or more species of animals or in two or more independent studies in one species, carried out at different times or in different laboratories or under different protocols.

In proceeding from a qualitative identification of carcinogenic hazard to a quantitative assessment of carcinogenic risk, the physiologic mechanism(s) of action of a carcinogenic substance must be considered. Carcinogenic agents that can chemically change the structure of chromosomes or of DNA, or that can be biotransformed into metabolites that have such effects, are designated genotoxic carcinogens, and are generally considered to be hazardous at any level of exposure. Non-genotoxic mechanisms of carcinogenic action also exist, some of which are specific to certain tissues or to certain mammalian species or both, and agents that act through such mechanisms may present a hazard to humans only at exposure levels above a necessary minimum. To characterise a carcinogenic agent as non-genotoxic it is necessary to demonstrate both a credible non-genotoxic mechanism to account for carcinogenicity in animals, and the absence of activity in appropriate tests for genotoxicity.

Synopsis of carcinogenicity data (1)

In two successive studies in rats, kresoxim-methyl was carcinogenic to the liver when administered in the diet, producing hepatocellular neoplasms including hepatocellular carcinoma. No tumours at other sites were associated with exposure to this agent. A carcinogenicity study in mice was negative. Tests for mutagenicity in bacteria and in mammalian cells, and for other forms of genotoxicity in both rodents and in human lymphocytes, were negative. A promoting effect on hepatocarcinogenesis, associated with stimulation of cell proliferation in the liver, was seen at carcinogenic doses only. It was concluded that kresoxim-methyl is a non-genotoxic hepatocellular tumour promoter and hepatocarcinogen in rats.

Interpretation of carcinogenicity data on kresoxim-methyl by the Scientific Committee on Plants.

I. Assessment of tumour induction by kresoxim-methyl.

Kresoxim-methyl was evaluated for its potential carcinogenicity in three studies:

1) A chronic toxicity study for 24 months in Wistar rats at dietary concentrations of 200, 800, 8 000, and 16 000 ppm, at 20 animals of each sex per dose (2). Increased incidence of hepatocellular carcinomas was observed in males and females at 8 000 and 16 000 ppm.

2) A carcinogenicity study for 24 months in Wistar rats at dietary concentrations of 200, 800, 8 000 and 16 000 ppm, at 50 animals of each sex per dose (3). Increased incidence of hepatocellular carcinomas was again observed in males and females at 8 000 and 16 000 ppm.

In both studies, no hepatocellular carcinomas were seen at 800 ppm, but the increased incidence relative to control rats was significant at 8 000 ppm and above. There was also a trend to increased incidence of hepatocellular adenomas in female rats in the carcinogenicity study at 800 ppm, but this was not significant at the $p < 0.01$ level. No tumours were described at any other site.

3) A carcinogenicity study for 18 months in mice (strain C57BL) at dietary concentrations of 400, 2 000, and 8 000 ppm, at 50 animals per group (4). No increase in tumours at any site was observed in this study. However, the advisory group expressed reservations about the strain of mouse selected and the length of the treatment period. Specifically, strain C57BL and substrains derived from it are genetically resistant to hepatocellular tumour promoters; strains such as DBA/2 or F1 hybrids of C57BL with a susceptible strain, such as B6C3F1, would have been more suitable.

Conclusion: Kresoxim-methyl is a carcinogen in rat liver at doses of 8 000 ppm or higher. No carcinogenic effect was observed at 200 ppm. The marginal effect seen at 800 ppm cannot be interpreted with certainty. On the basis of these data alone it could be concluded that the active substance is a possible carcinogen for humans.

II. Analysis of the mechanisms which may account for the induction of rat liver tumours following exposure to kresoxim-methyl.

1) **In vitro** genotoxicity studies **.**

The potential of kresoxim-methyl to induce mutagenic changes was evaluated in a series of comprehensive genotoxicity studies. These studies included the measurement of induced point mutation in the presence and absence of S9 mix in 5 strains of **Salmonella typhimurium** and one strain of **E. coli** (5, 6 **)** and in the Chinese hamster cell line CHO (7). Chromosome damage was measured in human lymphocytes (8). In none of these studies was there evidence that kresoxim-methyl induced point mutations or chromosome damage **in vitro.**

2) **In vivo** genotoxicity studies.

To evaluate the potential of kresoxim-methyl to induce genetic damage **in vivo** its ability to induce chromosome damage and repairable DNA lesions was measured in the bone marrow of mice (9) and in rat hepatocytes respectively (10, 11). None of the studies provided evidence that kresoxim-methyl induced chromosome damage in mouse bone marrow or repairable DNA damage in rat hepatocytes.

In the mouse bone marrow study there was no evidence of bone marrow toxicity. In contrast, hepatocytes derived from rat liver exposed to kresoxim-methyl showed an increase in the proportion of cells in the DNA synthetic phase of division. These **in vivo** data indicate that

kresoxim-methyl is not a DNA damaging agent. However, the increase in hepatocytes in S phase indicates that kresoxim-methyl is potentially capable of inducing cell proliferation in rat liver.

3) Evaluation of the ability of kresoxim-methyl to induce rat liver foci.

Rats were subjected to 2/3 partial hepatectomy and received a single oral dose of

2388 mg kresoxim-methyl/kg body weight before cells of the regenerating liver entered the S phase of the mitotic cycle. This treatment, with and without subsequent promotion by phenobarbital, produced no more GST-P positive liver cell foci than treatment with the solvent alone, while a positive control study using N-nitrosomorpholine in place of kresoxim-methyl produced numerous foci. This study provided no evidence of potential for kresoxim-methyl to initiate carcinogenesis in rat hepatocytes (12).

4) Evaluation of the ability of kresoxim-methyl to induce cell proliferation in rat liver.

The ability of kresoxim-methyl to increase the frequency of rat liver cells in the S phase of the mitotic cycle was evaluated in a number of studies:

i) Single dose oral exposure at doses of 0, 20, 200, and 1 000 mg/kg body weight (10).

ii) Dietary exposure for 3 weeks at 0, 200 and 16 000 ppm (13).

iii) Dietary exposure of aged (16 month old) animals for 3 weeks at 0, 200 and 16 000 ppm (14).

iv) Dietary exposure at 0 and 16 000 ppm for periods of 1, 6 and 13 weeks followed by 0, 2 and 5 weeks recovery (15).

v) Dietary exposure at 800 and 8 000 ppm for 3 weeks (16).

In these studies, increases in cell proliferation (as indicated by increases in the proportion of S phase cells) was detected in the livers of rats exposed to 200 and 1 000 mg/kg body weight by gavage and 8 000 and 16 000 ppm by dietary administration. No changes in S phase response were detected at 20 mg/kg body weight by gavage, or at 200 and 800 ppm by dietary administration.

In study (iv) data were presented which indicated that the frequency of S phase cells was reduced to below the level of controls if animals were given a 5 week recovery period following 13 weeks of kresoxim-methyl exposure. This is an indication that cell proliferation induced by kresoxim-methyl may be reversible.

5) Evaluation of the ability of kresoxim-methyl to promote hepatic preneoplastic foci (17).

Following exposure to the genotoxic carcinogen, N-nitrosodiethylamine, given as an initiator of carcinogenesis, rats were fed kresoxim-methyl for a period of 6 weeks, at doses of 0 (control), 200, 800, 8 000, and 16 000 ppm. Following sacrifice, the livers of the animals were evaluated for GST-P positive hepatocellular foci, which were quantified. The numbers and

size of foci were increased by all dietary concentrations of kresoxim-methyl; the increases achieved statistical significance at 8 000 and 16 000 ppm.

III. Interpretation of the data

Kresoxim-methyl at dosage levels of 8 000 and 16 000 ppm induced increases in the frequencies of hepatocellular carcinomas in the rat. There was also a trend toward increased hepatocellular adenomas at 800 ppm in female rats. The compound displays no evidence of mutagenicity in bacteria or in mammalian cells **in vitro,** is not clastogenic to mouse bone marrow **in vivo** or to human lymphocytes **in vitro**, and does not cause increased DNA-repair (USD) in cultured rat hepatocytes, indicating that there is no DNA damage. At carcinogenic concentrations in the diet, it induces an increase in hepatocellular proliferation and promotes the development of preneoplastic hepatocellular foci. Carcinogenic dietary concentrations of kresoxim-methyl are hepatotoxic.

On the basis of the data evaluated by the Committee, it was concluded that kresoxim-methyl shows no mutagenic or other genotoxic activity and thus could be classified as a non-genotoxic carcinogen. It did not induce the formation of GST-P positive liver foci. The data presented indicate that kresoxim-methyl induces cell proliferation in the rat liver and promotes the development of phenotypically altered hepatocellular foci. On the basis of the above data it was concluded that kresoxim-methyl is a non-genotoxic carcinogen whose mechanism of action most likely involves the promotion of spontaneously initiated hepatocytes. The activity of kresoxim-methyl as a hepatocellular tumour promoter may be due at least in part to induction of hepatocellular proliferation **in vivo**. Data concerning the mechanism by which kresoxim-methyl induces cell proliferation in the rat liver were not available to the Committee.

Carcinogenicity for the rat liver (and frequently for the mouse liver as well) that resembles the effects reported for kresoxim-methyl is well documented for many other chemicals, including certain pharmaceutical drugs that are prescribed for human beings, often at high dosage levels and for long periods of time. These include the anticonvulsant, phenobarbital. Phenobarbital is classified by IARC in Group 2B--possibly carcinogenic to humans. Humans who are prescribed phenobarbital ingest 1-5 mg/kg body weight daily. Epidemiological studies of epileptics who had received phenobarbital for long periods have found no evidence of increased incidence of cancer in the liver or elsewhere that can be attributed to phenobarbital.

Additional useful information could be provided by research designed to clarify the mechanism by which kresoxim-methyl induces cell proliferation in the rat liver. Such data might include studies of the induced transcription of specific genes such as P450 2B1, that are characteristically activated by tumour promoters such as phenobarbital.

On the basis of the data evaluated by the Committee there is no reason to believe that the promotional activity of kresoxim-methyl in the rat liver is confined to the rat. Accordingly, the compound must be considered a potential human tumour promoter.

Kresoxim-methyl showed promotional activity at concentrations which induced other effects on the rat liver, including enhanced DNA synthesis. Thus, kresoxim-methyl is unlikely to represent a carcinogenic hazard to humans at levels of exposure below those which produce similar effects in rat liver.

The mechanism by which kresoxim-methyl induces carcinogenesis in the rat liver can be predicted to involve a threshold of activity below which the events which lead to liver cancer would not occur. No-effect levels of exposure can be calculated below which kresoxim-methyl would be predicted to have no tumour promoting activity in susceptible species of animals and, by inference, also in humans. At no-effect levels and below, exposure to kresoxim-methyl would be predicted not to represent a potential human liver carcinogen.

2. The soil leaching behaviour of the main metabolite (BF 490-1).

The information supplied to the Committee indicates that only the parent compound and the metabolite BF 490-1 need to be considered in the context of possible soil leaching.

Mobility of the metabolite BF 490-1 in soil

The Committee agrees that under aerobic conditions kresoxim-methyl is very rapidly degraded by micro-organisms to the metabolite BF 490-1, which is the corresponding acid of kresoxim-methyl. Due to this rapid degradation, entry of the active substance into ground water at concentrations exceeding 0.1 µg/l is not to be expected. This finding is substantiated by the results from lysimeter and field studies.

BF 490-1: The documentation provided to the Committee establishes that the metabolite BF 490-1 should be considered to be very mobile in soil. Appropriately, the leaching potential of BF 490-1 was addressed in the monograph.

In the case of cereals, lysimeter studies carried out at the intended use rate showed that the entry of BF 490-1 into ground water at concentrations exceeding 0.1 µg/l is not expected. With respect to the use in viticulture and orchards, it has been necessary to rely on model calculations because the intended use rates have not been tested in lysimeter studies. Model calculations under realistic worst case conditions show that in viticulture contamination of ground water by BF 490-1 at concentrations > 0.1 µg/l is not expected.

However, model calculations under realistic worst case conditions for orchards have shown that the entry of BF 490-1 into ground water at concentrations > 0.1 µg/l cannot be fully excluded, e.g. under unfavourable soil (light soils of low organic carbon content) and climatic conditions. This situation can only apply to regions with cold climates and high precipitation rates, e.g. in limited areas in Northern European countries.

Conclusion of the SCP regarding mobility in soil: According to the information supplied to the Committee on the intended use pattern of kresoxim-methyl, contamination of groundwater exceeding the 0.1 µg/l limit is unlikely for either the parent or the metabolite BF 490-1 in cereals and grapes. In orchards, contamination of ground water by BF 490-1 is unlikely in Southern and Central European countries but cannot be fully excluded in Northern European countries. It is therefore recommended that in these countries an authorisation for use in orchards should be connected with the requirement of ground water monitoring studies in the regions described above.

Higher application rates or frequencies would require a re-assessment.

The Committee recommends that, in the course of an authorisation, Member States should take into account appropriate conditions of use for risk minimisation in viticulture and particularly in orchards.

4. Conclusion

The Scientific Committee on Plants is satisfied that it had been provided with adequate documentation comprising the dossier from the notifier BASF, the monograph prepared by the Rapporteur Member State (Belgium) and the documentation from the Peer Review Meetings involving national experts of Member States and the draft proposal from the Commission services of DG VI for inclusion of the active substance in Annex 1 to Directive 91/414/EEC. The Committee decided that in the light of the documentation it overviewed, the carcinogenic potential and the soil leaching behaviour of kresoxim-methyl merited detailed examination. Following such an examination, the Committee is of the opinion that kresoxim-methyl can be included in Annex 1 to Directive 91/414/EEC after the Acceptable Daily Intake (ADI) and the Acceptable Operator Exposure Limit (AOEL) have been reviewed in the light of the Committee's opinion on the carcinogenic potential of kresoxim-methyl. In addition, the Committee wishes to draw attention to the necessity that appropriate measures are taken to protect groundwater when granting national authorisations involving specific risk management pursuant to Annex 6 (Uniform Principles) of Directive 91/414/EEC.

Acknowledgements

The Committee wished to acknowledge the contribution of the following two working groups that prepared the initial draft opinions:

Toxicology: Professor M. Maroni (Chairman), and Committee Members Dr M. P. Delcour and Professor A Silva Fernandes and invited experts Professors F. Schulte-Hermann, J. Parry and Dr G. Rice.

Environmental Fate: Dr H-G Nolting (Chairman), Mr H. Koepp and Professor A. Silva Fernandes

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