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SCIENTIFIC COMMITTEE ON PLANTS **SCP/FLUSILAZOLE/002-Final**

OPINION OF THE SCIENTIFIC COMMITTEE ON PLANTS ON SPECIFIC QUESTIONS FROM THE COMMISSION CONCERNING THE EVALUATION OF FLUSILAZOLE IN THE CONTEXT OF COUNCIL DIRECTIVE 91/414/EEC

(Opinion adopted by the Scientific Committee on Plants, 18 July 2002)

A. TITLE

OPINION OF THE SCIENTIFIC COMMITTEE ON PLANTS ON SPECIFIC QUESTIONS FROM THE COMMISSION CONCERNING THE EVALUATION OF FLUSILAZOLE IN THE CONTEXT OF COUNCIL DIRECTIVE 91/414/EEC
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B. 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.

Flusilazole has slight inhibitory effects on the aromatase and therefore may interfere with the reproductive functions in aquatic organisms. The Rapporteur Member State concluded that an early life stage study in fish is a sufficiently sensitive study in this case to predict reproductive effects of flusilazole.

1. Can the Committee comment on this conclusion of the Rapporteur, whether in the specific case of flusilazole the proposed NOEC[1] for long term effects on fish is adequate to ensure a sufficient protection of fish from adverse effects on reproduction. The Commission would further appreciate a general comment on the comparative sensitivity of the early life stage test vs. the full fish life cycle study.

Flusilazole is also persistent in soil having a DT^2_{50} > 60 days and DT_{90} > 365 days (soil dissipation studies with German soils)**.** Nevertheless it does not accumulate and its concentration following recommended treatment rate will reach a plateau concentration around 0.1 mg/kg.

2. Can the Committee comment on the potential impact of flusilazole on organic matter decomposition under the intended use conditions?

C. OPINION OF THE COMMITTEE

Question 1:

Although fish early life-stage tests provide useful information on sensitive life stages of fish, for flusilazole in particular the risk assessment has explicitly identified fish and other aquatic species to be at risk from agricultural use of this a.s., and there is evidence that flusilazole may have specific effects on the reproductive process. Therefore the SCP cannot conclude that a NOEC based on a fish early life-stage test for a single species is necessarily adequate in this particular case to ensure sufficient protection of fish populations from adverse effects on reproduction.

[1] No Observed Effect Concentration

[2] Time required for 50% dissipation.

In general, it is the SCP's view that fish early life-stage tests are a useful tool for assessing subchronic aquatic toxicity and include more endpoints than the standard 28 day tests. However, early life-stage tests are not designed to detect potential effects on reproduction (including mating behaviour, time to sexual maturity, reproductive output and timing, fertilisation success, and sex ratio of offspring) and if there are reasons to expect such processes to be adversely affected (e.g., because the substance has shown endocrine-disrupting effects), a test that incorporates such endpoints should be conducted.

<u>Question 2:</u>

For flusilazole, no data are available to assess the impact on organic matter decomposition. Except earthworms and soil microflora, no soil-dwelling organisms have been tested. Given the persistence of flusilazole in soil and the environmental and agronomical importance of the organic matter breakdown for soil fertility, the Committee considers a risk assessment based solely on the existing data as not adequate.

A. TITLE

REPORT OF THE SCIENTIFIC COMMITTEE ON PLANTS ON SPECIFIC QUESTIONS FROM THE COMMISSION CONCERNING THE EVALUATION OF FLUSILAZOLE IN THE CONTEXT OF COUNCIL DIRECTIVE 91/414/EEC

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C. BACKGROUND

Flusilazole is an existing active substance (a.s.) in the context of Directive 91/414/EEC[3]. A draft evaluation report (monograph) has been prepared by the Rapporteur Member State (RMS, Ireland) on the basis of a dossier presented by the notifier (DuPont.).

Flusilazole has inhibitory effects on aromatase and may therefore interfere with the reproductive functions in aquatic organisms. A 90-day study with rainbow trout was carried out and the RMS proposed an overall NOEC of 3.3 μg/L as the most sensitive end-point for long term toxicity to fish. Based on literature available in the public domain, the Rapporteur Member State (RMS) and the notifier consider that the early life cycle test is more sensitive than the full life cycle to predict reproductive effects of chemicals in general, and of flusilazole in particular and therefore a full life cycle study will not provide for a more accurate NOEC for long term fish toxicity. The Commission would like to have the opinion of the SCP on such a conclusion in the specific case of flusilazole and in general.

Furthermore, flusilazole is a persistent compound having a DT_{50} > 60 days and DT_{90} > 365 days**.** The RMS found that nevertheless it does not accumulate and its concentration following the recommended treatment rate will reach a plateau concentration. The toxicity to soil organisms has been studied in a range of assays. The RMS proposed a TER[4] for earthworms > 22. The RMS considered that the inherent earthworm population is the major contributor to the decomposition process and from the finding of the chronic earthworm toxicity test, it can be considered that flusilazole under the intended use will not have any detrimental effects on organic matter decomposition.

[3] OJ N° L 230, 19. 8.1991, P. 1.
[4] Toxicity exposure ratio

The Committee is requested to comment on the RMS conclusion, in the absence of a litter bag study.

In order to prepare its opinion the Scientific Committee on Plants had access the documents listed below.

Source documents made available to the Committee:

1. Flusilazole: Terms of reference, submitted by Health and Consumer DG, 22 January 2002 (SCP/FLUSILAZOLE/001).

2. Flusilazole: Evaluation table Doc 7571/VI/97 rev. 15.1 (28.03.2001), submitted by Health and Consumer DG, 22 January 2002 (SCP/FLUSILAZOLE/003).

3. Flusilazole: Danish comments, 24 Sept 2001, submitted by Health and Consumer DG, 22 January 2002 (SCP/FLUSILAZOLE/004).

4. Flusilazole: RMS comments on ecotoxicology evaluation, submitted by Health and Consumer DG, 22 January 2002 (SCP/FLUSILAZOLE/005).

5. Flusilazole: RMS comment on Full life cycle Vs Partial life cycle (general information), submitted by Health and Consumer DG, 22 January 2002 (SCP/FLUSILAZOLE/006).

6. Flusilazole: Finnish comments, 22 Nov 2000, submitted by Health and Consumer DG, 22 January 2002 (SCP/FLUSILAZOLE/007.).

7. Draft assessment report, prepared by Ireland as Rapporteur Member State (volumes 1 to 3), July 996.

8. Addendum to the Draft assessment report, prepared by Ireland as Rapporteur Member State (volumes 1 to 3), April 1999.

9. Addendum to Draft assessment report, prepared by Ireland as Rapporteur Member State (volumes 1 to 3), October 2000.

10. Kristensen,P.[1990]Evaluation of the sensitivity of short term fish early life stage tests in relation to other FELS test methods. Final Report. Water Quality Institute, Contract No. 136614-43-89. VKI File No.305019.

11. Macek, K.J. and Sleight, B.H. (1977) Utility of toxicity tests with embryos and fry of fish in evaluating hazard associated with the chronic toxicity of chemicals to fishes. Eds. F.L. Meyer and J.L. Hamelink, pp.137 – 146. ASTM STP 634. Philadelphia: ASTM 1977.

12. Mc Kim, J.M. (1977) Evaluation of tests with early life stages of fish for predicting long-term toxicity. J. Fish Res. Bd. Can. 34: 1148 – 1154.

D. SCIENTIFIC BACKGROUND ON WHICH THE OPINION IS BASED

Question 1:

Can the Committee comment on the conclusion of the Rapporteur, whether in the specific case of flusilazole the proposed NOEC for long term effects on fish is adequate to ensure a sufficient protection of fish from adverse effects on reproduction. The Commission would further appreciate a general comment on the comparative sensitivity of the early life stage test vs. the full fish life cycle study.

Opinion:

Although fish early life-stage tests provide useful information on sensitive life stages of fish, for flusilazole in particular the risk assessment has explicitly identified fish and other aquatic species to be at risk from agricultural use of this a.s., and there is evidence that flusilazole may have specific effects on the reproductive process. Therefore the SCP cannot conclude that a NOEC based on a fish early life-stage test for a single species is necessarily adequate in this particular case to ensure sufficient protection of fish populations from adverse effects on reproduction.

In general, it is the SCP's view that fish early life-stage tests are a useful tool for assessing subchronic aquatic toxicity and include more endpoints than the standard 28 day tests. However, early life- stage tests are not designed to detect potential effects on reproduction (including mating behaviour, time to sexual maturity, reproductive output and timing, fertilisation success, and sex ratio of offspring) and if there are reasons to expect such processes to be adversely affected (e.g., because the substance has shown endocrine-disrupting effects), a test that incorporates such endpoints should be conducted.

Scientific background on which the opinion is based:

The monograph (volume 3, pp. 230-233) identified aquatic species, and fish in particular, as possibly at risk from flusilazole. In addition, a dose-dependent decrease in serum estradiol levels by flusilazole, considered to be indicative of aromatase inhibition, was observed in studies with rats (volume 3, p. 73). Aromatase inhibition is significant for reproduction since aromatization of testosterone is the process by which oestrogen is formed in vertebrates (Trant et al. 1997). This reaction is mediated by the cytochrome P450 aromatase. It has been shown that oestrogen (i.e., oestradiol) plays a major role in the reproductive physiology of all vertebrates, including gamete development and maturation, and induces the hepatic synthesis of the yolk precursor, vitellogenin. Studies in which fish have been exposed to aromatase inhibitors suggest that aromatase activity, specificity or expression levels vary with maturation stage and among species (Blázquez et al. 2001, Zerulla et al. 2002).

The 90-day fish early life-stage test with Rainbow trout (*Oncorhynchus mykiss*) was initiated with 'freshly fertilized' eggs that were 22 h old. The SCP notes that the OECD defines 'freshly fertilized' as within 8 hours, and it is unclear why the test was not conducted according to this protocol. The endpoints measured were: number of dead

eggs at hatching, first and last day of hatching, first day of swim-up, survival and abnormalities from hatching to thinning (i.e., when surviving fish were reduced to a manageable number), survival and abnormalities from the thinning to test end, length and wet weight of surviving fingerlings at test end. The most sensitive endpoints were abnormalities from thinning to test end, and length and weight of fingerlings at test end, all of which had a NOEC of 3.3 µg/l (measured). This NOEC can be compared to the result of a 60-day ELS test with the same species (though initiated with older, 'eyed' eggs) that was presented in the monograph (p. 226). The most sensitive endpoints in this test were also length and weight of fish at the end of the test, and these had a NOEC of 30 µg/l. Thus there is approximately a factor of 10 difference in the NOECs between these two tests for the same species and endpoints, which might be explained by the longer exposure period and/or the younger age of eggs at test initiation in the 90-day test.

Neither of the above tests was designed to investigate possible effects on reproductive output or mating behaviour of adult fish. Given that there is evidence that flusilazole is an aromatase inhibitor, there are specific concerns that reproduction could be adversely affected by this substance. Therefore potential effects on mating behaviour, time to sexual maturity, reproductive output and timing, fertilisation success, and sex ratio of offspring are also of concern and should be explicitly addressed by a test designed for this purpose. For example, fish full life-cycle tests typically begin with embryos or newly hatched young fish less than 8 days old, continue through maturation and reproduction and end not less than 28 days (60 days for salmonids) after hatching of the next generation. Alternatively, a partial life-cycle test, that begins with immature juveniles at least two months before active gonad development, continues through maturation and reproduction and ends not less than 30 days (60 days for salmonids) after the hatching of the next generation, could similarly address effects on reproductive processes.

Although fish early life-stage tests provide useful information on sensitive life stages of fish, for flusilazole in particular, the risk assessment has identified fish and other aquatic species to be at risk from agricultural use of this a.i., and there is evidence that flusilazole may have specific effects on the reproductive process. Therefore the SCP cannot conclude that a NOEC based on a fish early life-stage test for a single species is necessarily adequate in this specific case to ensure sufficient protection of fish populations from adverse effects on reproduction.

In general, it is the SCP's view that fish early life-stage tests are a useful tool for assessing subchronic aquatic toxicity and include more endpoints than the standard 28 day test. However, early life-stage tests are not designed to detect potential effects on reproduction (including mating behaviour, time to sexual maturity, reproductive output and timing, fertilisation success, and sex ratio of offspring) and if there are reasons to expect such processes to be adversely affected (e.g., because the substance has shown endocrine-disrupting effects), a test that incorporates such endpoints should be conducted.

The Notifier presents several arguments for not conducting a fish full life-cycle test (Revised Addendum to Annex B, October 2000, p. 96) to which the SCP would like to respond directly.

1. **Notifier Response:** No OECD or European guideline exists for performing a full life cycle Fathead minnow study, hence criteria for evaluating whether the study is

acceptable are unavailable. **SCP Comment:** This is not sufficient justification for not performing a test and such criteria can be handled on a case-by-case basis.

2. **Notifier Response:** Analyses of data for the Fathead minnow full life cycle test under US EPA (72.5) guideline indicate that there is not sufficient statistical power to detect small decreases in reproduction. **SCP Comment:** The SCP agrees that this is a serious issue that needs to be addressed. As long as the effects assessment is based on statistical significance rather than effect size such variability will limit the usefulness of egg production as a test endpoint. However, there are other endpoints included in the test that are not as variable (and therefore overall the test provides a variety of useful information), it may be possible to employ other species for which reproductive output is not as variable as in the Fathead minnow, and it is possible to base test interpretation on a predetermined effect size rather than a statistical significance criterium.

3. **Notifier Response:** The early life portion of a full fish life cycle study has been found to be the most sensitive study phase. A fish early-life stage test (ELS) is, therefore, as sensitive (within a factor of two) as the early life stage portion of a full fish life cycle study in predicting reproductive effects. **SCP Comment:** The reviews to which the Notifier refers are two decades old (Macek and Sleight 1977; McKim 1977). Although this does not mean that the test results on which they are based are invalid, it is not certain that all potentially relevant reproductive endpoints were measured in the older tests. Further, more recent reviews (Nagel and Isbener 1998, Peter and Heger 1997, Chorus 1987) argue that the early life stages of the F2 – generation have been shown to be more sensitive than those of the F1 – generation, and that therefore ELSs can not substitute for truly chronic tests. When there are specific concerns that reproductive processes may be impaired then appropriately targeted tests should be performed that directly assess these processes.

4. **Notifier Response:** The Rainbow trout is typically more sensitive to chemical toxicity than is the Fathead minnow. A review of aquatic toxicity data for 40 chemicals has shown either daphnids or Rainbow trout as the most sensitive organisms 73% of the time (World Wildlife Fund 1992). **SCP Comment:** This would argue for conducting a full or partial life-cycle study (that covers reproduction) in Rainbow trout, or another appropriate species, rather than Fathead minnow.

5. **Notifier Response:** It is the applicants contention that a new early life-stage study with Rainbow trout (i.e., the 90 day test that was conducted and discussed above) will meet harmonized guidelines and provide sufficient data to evaluate potential chronic flusilazole toxicity and address concerns from long term exposure. **SCP Response**: As indicated in detail above, the early life stage study does not investigate effects on a number of important reproductive traits and therefore cannot fully assess potential effects of chronic exposure to flusilazole on reproduction.

Question 2:

Can the Committee comment on the potential impact of flusilazole on organic matter decomposition under the intended use conditions?

Opinion:

For flusilazole, no data are available to assess the impact on organic matter decomposition. Except earthworms and soil microflora, no soil-dwelling organisms have been tested. Given the persistence of flusilazole in soil and the environmental and agronomical importance of the organic matter breakdown for soil fertility, the committee considers a risk assessment based solely on the existing data as not adequate.

Scientific background on which the opinion is based:

Currently, standard effects testing with soil organisms includes earthworms (in single-pecies tests) and soil micro-organisms (in a test on activity of the microbial community). However, a wide variety of other organisms are involved in the ecologically and agronomically important process of decomposition of organic matter. These range from micro-flora and -fauna (e.g. bacteria, fungi, nematodes, protozoa), to mesofauna (e.g. mites and springtails,) and macrofauna (e.g. isopods, millipedes, harvestmen, molluscs), none of which are routinely tested (and as in this case). The different species perform different functions in the overall process (e.g. shredding the litter into smaller particles which are then more easily edible by smaller organisms or can then be colonised by fungi and bacteria). Thus, effects on key species may hamper the whole process, while effects on other species may be buffered within the system (redundancy). The decomposition of organic matter is therefore one of the most integrating processes within the soil ecosystem, i.e., a functional endpoint of the ecosystem that can be measured and used in the ecotoxicological risk assessment.

It is also a very important process. During the last four decades this process was intensively investigated in agronomy and soil ecology in general (e.g. Eijsackers and Zehnder 1990). In the scientific literature effects on degradation in various ecosystems (mainly forests) and soil types have been described (Römbke 1994, Schönborn and Dumpert 1990). Based on the existing knowledge and data, it is generally accepted that disturbance of this complex process might disturb nutrient cycling and, in the long run, soil fertility which is not only of environmental but also of agronomical relevance.

However, when deciding on the need for and design of studies on organic matter decomposition, it must be considered that natural factors may strongly influence organic matter decomposition especially on a short-term time scale. For example a dry period in summer may reduce the activity of the soil community to such an extent that hardly any decomposition occurs over several weeks. Therefore, given the high short-term variability of this process, a study on organic matter breakdown should be of a long-term design, and should be triggered by long-term exposure to the active substance, in order to be interpretable in a meaningful way. Thus, persistence of the active ingredient is an

appropriate trigger for such study. Flusilazole, with a $DT90^{5}_{field}$ in soil of far more than one year, clearly meets this trigger as defined in Annex III, point 10.6.2, of directive 91/414/EEC. More recently, the EU guidance document on terrestrial ecotoxicology (08.07.2000 rev. 7) also lists studies for testing effects of persistent substances (DT90 > 365 days) on soil organisms. Included are tests on species level like reproduction of collembola, as well as tests on the functional level include organic matter breakdown. The SCP supported this approach already in its opinion on the Draft guidance document on Terrestrial Ecotoxicology (SCP, 1999**)**.

Earthworms also contribute to organic matter decomposition in soils. The RMS considered that the inherent earthworm population is the major contributor to the decomposition process and from the finding of the chronic earthworm toxicity test, it can be considered that flusilazole under the intended use will not have any detrimental effects on organic matter decomposition. Consequently, the earthworm tests alone are supposedly sufficient to assess the impact of flusilazole on the soil compartment.
The Committee does not share this view. Existing litter bag studies (submitted for the registration of plant protection products) with different mesh sizes typically show that fine mesh sizes (e.g. 0.025 mm, where no access of earthworms to the litter is possible) yield decomposition rates of e.g. 50 % after 2 months. Access of bigger organisms like earthworms (mesh size of about 3.5 mm) enhanced the degradation, but only by ca. another 20%, i.e. a smaller share as compared to the decomposition without earthworms. In addition, it must be considered that there is no information on how much the earthworm population would have achieved alone, in the case of a severe impact on the other contributing organisms.
As another example, the study of Beck et al. (1988) showed an increase of the litter layer on the soil surface after the application of high concentrations of the biocide pentachlorophenol (PCP) in a beech wood forest. In this study, the accumulation of litter was not caused by effects on the rarely present earthworms in the acid soil, but was due to effects on various taxonomic groups of the mesofauna and microflora (Schönborn and Dumpert, 1990; Römbke, 1994). Hence, especially when earthworms numbers are low (e.g. on sites with special soil types, or when earthworm populations have been reduced by other factors such as other PPP's or tilling), other soil organisms other than earthworms can dominate the decomposition process. This may in fact be the case for a large proportion of agricultural soils (arable fields), since regulatory fieldstudies with earthworms are mostly done on pastures because of the generally low earthworm populations in many arable fields.In summary, no data are available to assess the impact of flusilazole on organic matter decomposition (ref. revised addendum to Annex B to the Draft Report and Proposed Decision, October 2000). Except earthworms and soil microflora (see background section for summary of the results) no soil organisms have been tested (the beetle species *Poecilus cupreus* and *Aleochara bilineata, for which data are available,* are more surface-dwelling species rather than "real" soil-dwelling species and therefore no adequate surrogates). Given its persistence in soil and the environmental and agronomical importance of the organic matter breakdown, the committee considers a risk assessment based solely on the existing data as not adequate.

[5] Time required for 90% dissipation

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