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SCIENTIFIC COMMITTEE ON PLANTS

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

(Opinion adopted by the Committee on 7 March 2001)

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1. TITLE

OPINION OF THE SCIENTIFIC COMMITTEE ON PLANTS REGARDING THE EVALUATION OF QUINOXYFEN 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 properties of quinoxyfen in soil raised some concerns about its potential to accumulate in soils. However, 5 year accumulation studies in various locations (south and north of France and Germany and UK) showed no trend for accumulation in soil and only a slight accumulation to a plateau of the metabolite 3-hydroxyquinoxyfen.

Therefore, the Scientific Committee on Plants (SCP) is requested to respond to the following question 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:

"Is there any scientific reason to suspect that the use of quinoxyfen under the proposed conditions of use would lead to accumulation in soil at such levels that an unacceptable impact on the environment would occur?"

3. BACKGROUND

The draft Commission Directive for inclusion of quinoxyfen in Annex I to Directive 91/414/EEC[1] 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 (the United Kingdom) based on a dossier submitted by the notifier (Dow Elanco now Dow AgroSciences), a review report prepared by the Commission and the Recommendations of the ECCO[2] Peer Review Programme.

Quinoxyfen is a systemic fungicide of the phenoxyquinoleine group. It is absorbed by the foliar parts of the plants. Its intended use is to control powdery mildew on wheat and barley. The intended use conditions are 1 or 2 applications per year of 75 to 250 g a.s./ha each, to a maximum annual total dose of 400 g a.s./ha.

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

4. OPINION

4.1 Question

"Is there any scientific reason to suspect that the use of quinoxyfen under the proposed conditions of use would lead to accumulation in soil at such levels that an unacceptable impact on the environment would occur?"

The question required the Committee to assess a) if accumulation in soil will occur, b) if a plateau would be reached, and at which concentrations, c) which effects such concentrations in soil would have on soil organisms, and d) on organic matter breakdown in particular. The following opinion and scientific background is structured accordingly.

4.2 Opinion of the Committee

Assuming a worst-case scenario with application to bare soil, plateau levels of quinoxyfen of about 0.3 mg/kg in the top 30 cm can be expected. Three accumulation studies under more realistic conditions in which quinoxyfen was applied to the crop over a period of 5 years showed plateau levels of 0.08 to 0.18 mg/kg when averaged over the top 20 cm.

The likely long-term effects on the soil ecosystem were assessed using single-species tests, field monitoring of several taxa, and tests on ecosystem functions (microbial activity; organic matter decomposition). Given the complex picture of the results and a number of problems for the evaluation (partly as a consequence of the study design), the Committee is of the opinion that the available studies on quinoxyfen and the field study in particular do not convincingly demonstrate acceptable impact on the environment. Those effects which occurred do raise concern, considering the persistence of quinoxyfen and the intended use in large-scale crops which are grown in short crop rotation in many EU countries.

The Committee further noted that in the order of 10% of the dose of quinoxyfen may volatilise after application to a crop. In view of the uncertainty in the estimated atmospheric half-life of quinoxyfen of 1.9 days, the Committee expects that measurements of this half-life will be necessary after appropriate schemes have been developed for assessing the environmental risks of atmospheric transport of plant protection products.

4.3 Scientific background on which the opinion is based

4.3.1 Fate

Laboratory studies with four topsoils at 20°C resulted in half-lives ranging from 224 to 448 days (average of 359 days). In these studies two metabolites were identified: 3-hydroxyquinoxyfen and 5,7-dichloro-4-hydroxyquinoline (DCHQ). 3-Hydroxy-quinoxyfen reached maximum levels of 27% in one soil and of 0-8% in the other three soils. DCHQ reached maximum levels of 4-6% in two soils but was not detectable in the other two soils. Soil bound residues reached levels of 15-25% after 200 days and CO_2 evolution was less than 2% over 200 days.

In eight field dissipation studies (in the UK, France and Germany) quinoxyfen was applied to bare soil. The DT50[3] values estimated with best-fit kinetics according to Timme-Frehse equation (see Timme *et al.*, 1986) range between 11 and 454 days whereas the DT50 values estimated with first-order kinetics range between 174 and 587 days. The estimated DT50 values depended strongly on the calculation procedure in five of the eight experiments: for these five experiments first-order kinetics resulted in DT50 values that were 2 to 21 times longer than DT50 values estimated using best-fit kinetics according to Timme-Frehse. This difference was in most of the cases caused by the apparent biphasic nature of the dissipation: an initial rapid decline followed by a slower decline. The initial rapid decline may have been caused by loss processes at the soil surface whereas the slower decline may reflect transformation in the soil matrix. The Committee considers DT50 of 11 days to be very unreliable because it is based on initial recovered amounts of more than two times the dose. The DT90[4] values ranged from 416 to 970 days for four of the eight experiments and were not reached at the end of the study for the other four experiments, which lasted for 540 to 732 days. The Committee concludes that the field persistence of the fraction of quinoxyfen that penetrates into soil is consistent with the transformation rates measured in the laboratory studies.

Three field studies were conducted with annual applications of quinoxyfen over five years. The first year quinoxyfen was applied to bare soil and in all subsequent years it was applied to winter wheat crops at growth stages leading to significant crop interception (BBCH 32 and 49). In all years the annual dose was 0.4 kg a.s./ha. No significant accumulation was observed in these experiments. At the end of the five-year period contents of quinoxyfen averaged over the top 20 cm ranged from 0.08 to 0.18 mg/kg whereas a cumulative dose of 2 kg a.s./ha would have corresponded with about 0.8 mg/kg in the top 20 cm. The contents of the metabolite 3-hydroxy-quinoxyfen averaged over the 0-20 cm layer ranged from 0.03 to 0.04 mg/kg at the end of the five-year period. These contents imply that 4-5% of the cumulative dose of quinoxyfen was present as 3-hydroxyquinoxyfen at the end of these three field experiments.

Application of quinoxyfen to bean leaves resulted in about 5% volatilisation in the first day after application. In efficacy studies it was also observed that quinoxyfen has some action via the vapour phase (fungicidal action was observed at untreated plants placed at 14 cm distance of treated plants). It can also not be excluded that significant photochemical transformation takes place on leaves (photochemical transformation in water is rapid but there are no data on photochemical transformation on leaves).

Available field and laboratory data thus indicate that the transformation rate of quinoxyfen is very slow after it has entered the soil (corresponding with a half-life in the order of a few hundred days) but that it is less persistent on the crop. Model calculations assuming a realistic worst case scenario for accumulation in soil based on a half-life at 20°C of 359 days and an annual application to soil of 0.4 kg a.s./ha, showed plateau levels for the top 30 cm of about 0.3 mg/kg.

[3] Period required for 50% dissipation.
[4] Period required for 90% dissipation.

Given the result of the plant volatilisation experiment, it cannot be excluded that in the order of 10% of the dose of quinoxyfen may volatilise into the atmosphere after application to crops. The ratio of the concentration in the gas phase divided by the concentration in the liquid phase (i.e. the dimensionless Henry constant) of quinoxyfen is about 2 10^{-5} at pH=7 (for the neutral molecule). This value indicates also that measurable volatilisation cannot be excluded when quinoxyfen would be present in water droplets on leaves. Volatilisation from soil was measured to be 0.6% in the first day after spraying to bare soil. Given the extremely strong sorption to soil (the organic-carbon/water distribution coefficient, K_{OC}[5], is about 20000 l/kg), volatilisation from soil will probably decrease strongly with time after application and will be much less than volatilisation from leaves.

The atmospheric half-life of quinoxyfen was estimated to be 1.9 days using the Atkinson method (Atkinson *et al*, 1999). This method calculates the half-life from the rate of reaction with OH radicals and the concentration of these OH radicals. The uncertainty in the estimated rate of reaction with OH radicals of molecules like quinoxyfen may be about a factor five (Atkinson *et al.*, p. 226) and the uncertainty in the concentration of OH radicals is about a factor two (Atkinson *et al.*, p. 237). Consequently, the uncertainty in the estimated atmosperic half-life of 1.9 days is considerable and this half-life is not short given the comparatively high transport velocities in the atmosphere. The Committee recognises that so far no appropriate schemes are available for assessing the environmental risks of atmospheric transport of plant protection products (PPPs) and that there are no agreed protocols for measuring transformation rates in the air (Guicherit *et al.*, 1999). Nevertheless the Committee expects that measurements of this half-life will be necessary after appropriate schemes have been developed[6] for assessing the environmental risks of atmospheric transport of PPPs.

4.3.2 Ecotoxicological assessment of the concentrations in soil

Standard effects testing with soil organisms includes earthworms (in a single-species test) and soil micro-organisms (in a test on activity of the microbial community). Further, in the case of substances which are likely to persist in soil, testing of organic matter breakdown (as a function of the soil community) is required (Annex III 10.6.2). A wide variety of 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). Several different sets of data allow the Committee to assess the likely affects of quinoxyfen on soil organisms and organic matter breakdown: laboratory tests on surface dwelling arthropods, laboratory tests on earthworms, laboratory tests on soil microflora and field tests involving the monitoring of soil invertebrates and the decomposition of buried leaves (litter bags).

[5] Organic carbon adsorption coefficient.
[6] The Committee is aware of such work under EPPO.

4.3.2.1 Laboratory tests on surface/plant-dwelling arthropods

Quinoxyfen had very little effect on the carabid beetle *Poecilius cupreus* in laboratory studies (13.9% mortality, no effect on behaviour) when exposed at concentrations equivalent to 250 g a.s./ha. However, in two separate studies, quinoxyfen caused 93.3% reduction and 100% reduction in beneficial capacity of the predatory mite *Typhlodromus pyri* when exposed at 100 g a.s./ha and 30-60 g a.s./ha respectively (the intended use rates are 75 - 250 g a.s./ha).

4.3.2.2 Laboratory tests on earthworms

Two 14 day acute tests were submitted, one with the active substance and one with the formulation EF-1186. The LC_{50}[7] and NOEC[8] from the first trial were estimated as > 923 mg a.s./kg and 923 mg a.s./kg, while the LC_{50} and NOEC from the second trial were estimated as > 413 mg a.s./kg and 413 mg a.s./kg respectively. In both cases the TER was well above 1000. A long-term (28+28d) sub-lethal study was also submitted during the review process, yielding (after suitable conversion) a NOEC equivalent to the top dose, namely 2.67 mg a.s./kg. Given the high Kow[9], EPPO guidelines (ref. 4) suggest that a factor of 2 be introduced to account for differences in test soil and typical agricultural soil, yielding a long-term TER[10] of 4.9, which is just below the recommended critical threshold of 5. Despite this result, field trials (see below) provide some re-assurance that this borderline TER is not indicative of significant risk.

4.3.2.3 Laboratory tests on soil microflora

Data have been submitted that indicate that there was no statistically significant effect on microbial respiration and on nitrogen turnover in two soil types at concentrations of 0.53 to 5.3 mg a.s./kg soil, equivalent to approximately two and twenty times the maximum calculated PEC[11]. Therefore, the risk to soil microbial processes is considered to be acceptable.

4.3.2.4 Field trials

A field study was conducted from March 1994-November 1996 at a farm in Devon, UK. Although the intended use of quinoxyfen is in wheat and barley, experimental plots on this farm consisted of grazing pasture, as they provide greater invertebrate diversity. Quinoxyfen was applied to 3 plots in the spring at 250 g a.s./ha and summer at 150 g a.s./ha, while 3 plots received a toxic reference (Hostathion®; a.s. triazophos). Three control plots were left untreated, and the same treatments were allocated to the same plots throughout the study. Arthropods were collected by pitfall traps which were left open for 1 week periods at various times through the study. Similarly, earthworms were sampled by applying formalin within two quadrats per plot at various times through the study period. Organic matter decomposition was monitored by comparing the extent of breakdown of a known weight of sweet chestnut (*Castanea sativa*) leaves in treated areas compared to controls (see results in table below). These leaves were placed within

7 Lethal concentration, median.
8 No observed effect concentration.
9 Octanol-water partition coefficient.
10 Toxicity over exposure ratio.
11 Predicted environmental concentration.

course, medium and fine mesh bags (3.36 mm, 0.5 mm and 0.025 mm), 3 bags per plot (9 per treatment), and buried to a depth of 10 cm for approximately 3 months. There was no analytical confirmation of exposure.

In brief, the data on arthropods exhibited high variance and seasonality typical of field trials. The majority of taxa (carabid beetles, staphylinid beetles, linypiid spiders, soil mites) were not consistently affected by quinoxyfen. However, lycosid spiders (Pardosa sp.) and collembola (superfamily Entomobryoidea) were reduced in treated plots compared to controls, possibly as a result of indirect rather than direct effects. Entomobryoidea were the most abundant prey species found in the pitfall traps in all three years and they tended to show an approximate decrease of 20-60% in population size in the one to two months following exposure to quinoxyfen, before recovering to control levels. These organisms are largely fungivores and are common in leaf litter, and therefore they may play some role in organic matter decomposition.

There was no indication of adverse effects of quinoxyfen on earthworms, since they appeared at approximately equal mean abundance in treated and control plots over each of the three study years.

The data on the litter decomposition part of the study are clearly of most relevance to the assessment of the effects of quinoxyfen on organic matter breakdown, and these results have attracted considerable attention (document SCP/QUINOX/007). A summary is provided in the following table:

<u>Litter decomposition:</u>

		% of control breakdown					
		1994		1995		1996	
mesh size	**substance**	**spring**	**autumn**	**spring**	**autumn**	**spring**	**autumn**
Coarse (3.36 mm)	Quinoxyfen			100	117	**74**	**67**
	Triazophos *			100	121	92	102
Medium (0.5 mm)	Quinoxyfen	92		107	102	91	100
	Triazophos *	130		103	86	86	103
Fine (0.025 mm)	Quinoxyfen	74		100	110	92	98
	Triazophos *	91		100	106	92	102

* intended as toxic reference

Although differences in decomposition rates between quinoxyfen treated plots and control plots were not statistically significant for any mesh size at any time, the high differences between treatment and control observed in 1996 with coarse mesh bags raise cause for concern. Lack of statistical effects clearly do not mean no effect. Compared to other studies the number of 3 bags/replicate is low, and the power of the study to detect effects was further reduced in autumn 1996 because some of the bags (6 from a total of 27) could not be located. Since the effect observed at study termination was high compared to effects seen in other similar studies with different active substances, it should not be dismissed lightly on the basis of weak statistics. Compared to other available tests of this type an effect of this magnitude has not been observed before (the

Committee is aware that in the studies with other substances which are available to regulatory authorities, there were either no effects on decomposition at study termination, or observed deviations from the control were at maximum 10%).

Additionally the effect was only observed in the third year of consecutive applications of the active substance, also a deviation from the existing experience.

The fact that the toxic reference triazophos did not show a consistent effect adds further uncertainty to the interpretation of the study.

One possibility raised by the study author is that the apparent reduction in decomposition rates observed in large-mesh bags in 1996 may have been due to a cumulative effect of quinoxyfen applications on some of the larger soil invertebrates. If correct, this interpretation would provide particular cause for concern, given that quinoxyfen is a persistent compound which is to be applied in large-scale crops. Another possibility is that this difference arose through some interaction with weather: decomposition rates are strongly influenced by weather conditions and the first year (1994) was relatively wet, while1995 and 1996 were relatively dry. However, the study does not allow to conclude with confidence one way or the other.

If there is an important cumulative effect of quinoxyfen on organic matter decomposition then one might expect to see a trend in decomposition rates over time, which is not apparent. However, apart from the low statistical power by which such trend might be hidden, this would require that the bags were equally exposed to quinoxyfen during the different phases/years of exposure. But, as the application was done on pasture (with dense grass cover and no tilling), and as quinoxfen is of little mobility in soil, it is unclear when and at which levels quinoxyfen really reached the bags in 10 cm depth. Given the low mobility, the effect in year 3 (occurring consecutively for both sampling dates) could be the start of the effect because the substance might require 1 or 2 years to reach the bags in 10 cm depth.

From the surface dwelling species activity evaluated by pitfalls traps no clear picture can be drawn from the different years. The lycosid spiders appeared to be adversely affected in all three years. Some collembolean and carabid spiders also showed a decrease in numbers. Recovery was observed in all cases in the latter part of each field season. Although recovery was observed, a temporary decline of the population could be the reason for a reduced decomposition. Therefore it is important to know whether the effect observed in 1996 in litter decomposition has arisen through sample variation, through variation in weather conditions, or through a cumulative effect of quinoxyfen on organisms.

As with pitfall traps only the surface dwelling species are subject to the evaluation, there is a considerable uncertainty concerning species living in deeper soil layers like eudaphic mites.

4.3.2.5 *Drawbacks/Problems for the evaluation*

In reviewing the laboratory and field data, the SCP observed that bags were buried at 10 cm rather than the recommended 5 cm, which would have allowed greater access by surface dwelling invertebrates. Hence, organic matter breakdown was relatively slow even in the control, thus making it more difficult to detect differences.

The test design had rather low statistical power which was even lower when a number of the bags could not be found after the exposure.

The exposure situation for the leaf litter bags and for the surrounding soil is entirely unclear. No analytical measurements were made. The lack of a consistent effect in the toxic reference raises further doubts about the exposure situation and possibly about suitability of the study design. In the absence of exposure data, it can be assumed from the fate data, that the substance needed considerable time to reach the layer where the bags where buried, so that there might not have been exposure during the first one or two years. It is therefore important to ensure that the duration of the study and the exposure is appropriate for the properties of the active substance concerned.

The plots consisted of grassland, whereas the intended use is on cereal fields. The soil community in undisturbed grassland may differ in terms of species diversity, composition and abundance from that in regularly disturbed and treated arable field. It is therefore uncertain to which extent results from grassland soil communities can be extrapolated to arable soils. Hence, tests like this field test should be done on arable fields with the crop intended to be treated.

The organic matter consisted of sweet chestnut leaves which are more easily decomposed than cereal straw. The crop to be treated and ploughed under should preferably be chosen for the organic matter breakdown test.

Concern was raised about the laboratory results on the predatory mite *Typhlodromus pyri*, although we note that Acari (mainly Trombidiidae) were monitored in the field surveys of 1995 and 1996 and did not differ between treatments and controls.

4.4 Conclusion

Given the variety of organisms from different taxonomic and functional groups that contribute to the decomposition process, and the influence of prevailing environmental conditions, ascertaining the likely effects of a plant protection product on ecosystem functions such as organic matter breakdown is complex. The issue began to receive attention several years ago, and has been recognised by OECD as a priority area for test guideline development. Although there are no internationally agreed guidelines yet, there is growing experience with different active substances, and a developing consensus on a number of key issues of such tests.

Given the complex picture of results and the problems for the evaluation (partly as a consequence of the study design), the Committee is of the opinion that the available studies on quinoxyfen and the field study in particular do not convincingly demonstrate acceptable impact on the environment. Those effects which occurred do raise concern, considering the persistence of quinoxyfen and the intended use in large-scale crops which are grown in short crop rotation in many EU countries.

5. REFERENCES

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6. DOCUMENTS MADE AVAILABLE TO THE COMMITTEE

1. Evaluation of quinoxyfen in the context of Council Directive 91/414/EEC concerning the placing of plant protections products on the market: Terms of reference (Doc. SCP/QUINOX/001) submitted 3 April 2000.

2. Evaluation of quinoxyfen in the context of Council Directive 91/414/EEC concerning the placing of plant protections products on the market: Evaluation table Doc. 5725/VI/97-rev.8 (Doc. SCP/QUINOX/003-Rev1) submitted 13 June 2000.

3. Evaluation of quinoxyfen in the context of Council Directive 91/414/EEC concerning the placing of plant protections products on the market: Draft review report Doc. 6781/VI/97-rev.8 (Doc. SCP/QUINOX/004-Rev.1) submitted 13 June 2000.

4. Finnish comments on quinoxyfen (Doc. SCP/QUINOX/005) submitted 21 April 2000.

5. Swedish comments on quinoxyfen (Doc. SCP/QUINOX/006) submitted 26 April 2000.

6. Evaluation of quinoxyfen in the context of Council Directive 91/414/EEC concerning the placing of plant protections products on the market: Appendices (25/05/00), (Doc. SCP/QUINOX/007) submitted 13 June 2000.

7. Quinoxyfen, comments of Germany concerning the evaluation of data on litter decomposition [Appendix III to the Monograph] (Doc. SCP/QUINOX/008) submitted 4 September 2000.

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17. The dissipation of quinoxyfen and its 3-hydroxy metabolite in soil at intervals following a single application of EF-1186, Germany - 1993. A Gambie (1996). Report GHE-P-5135, Dow AgroSciences, UK.

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7. ACKNOWLEDGEMENTS

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Environmental assessment WG: Prof A. Hardy (Chairman) and Committee members: Mr. H. Koepp, Dr. T. Sherratt, Prof. A. Silva Fernandes, invited experts: Dr. J. Boesten, Dr. A. Carter, Dr. V. Forbes, Dr. R. Luttik and Dr. C. Kula.