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SCIENTIFIC COMMITTEE ON PLANTS

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OPINION ON SPECIFIC QUESTIONS FROM THE COMMISSION CONCERNING THE EVALUATION OF FOSTHIAZATE [IKKI-1145 / TO-1145] IN THE CONTEXT OF COUNCIL DIRECTIVE 91/414/EEC

(Opinion adopted by the Scientific Committee on Plants, 20 December 2001)

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A. TITLE

OPINION OF THE SCIENTIFIC COMMITTEE ON PLANTS ON SPECIFIC QUESTIONS FROM THE COMMISSION CONCERNING THE EVALUATION OF FOSTHIAZATE [IKKI-1145 / TO-1145] IN THE CONTEXT OF COUNCIL DIRECTIVE 91/414/EEC.

(Opinion adopted by the Scientific Committee on Plants, 20 December 2001)

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:

Concerning the evaluation of fosthiazate (IKKI-1145 / TO-1145) in the context of Directive 91/414/EEC:

1. Can it be confirmed that use scenarios exist which pose no unacceptable risk to groundwater?

2. Can the Committee confirm that the risk to soil dwelling organisms has been adequately addressed?

3. Can the Committee confirm that a safe use with respect to birds and mammals exists?

4. Does the Committee consider that there is sufficient information available on the risk of organophosphate-induced delayed polyneuropathy (OPIDP) in humans following severe intoxication incidents to confirm that there is a safe use, or whether further *in vitro* tests of relative inhibitory potency of the individual isomers of fosthiazate for acetylcholinesterase (AChE) and neuropathy target esterase (NTE) in hen and human tissues are required?

C. OPINION OF THE COMMITTEE

Opinion on question 1

The Committee cannot confirm that use scenarios exist which pose no unacceptable risk to groundwater.

Considering only the parent compound, calculations for FOCUS[1] scenarios showed a concentration below 0.1 μg/L for one out of nine scenarios.

Soil metabolism and field persistence studies have demonstrated the formation of five soil metabolites (BSA, MBSo, TZO, DTTO and DETO). There is insufficient information on the leaching characteristics of these metabolites to determine their contribution to groundwater risk.

[1] Forum for the Co-ordination of pesticide fate models and their Use.

Therefore, based on the available information, the Committee cannot confirm a safe use for any scenario. It is possible that lysimeter studies might demonstrate lack of leaching for one or more scenarios but none are reported.

Opinion on question 2

The majority of observed effects on soil-dwelling organisms were well within the Annex VI criteria, and the Committee concludes that the risk to soil-dwelling organisms following exposure to fosthiazate will in general be low. However, the single study that was conducted to evaluate effects of the active substance on the breeding performance of beetles, reported a significant reduction in the number of surviving offspring. The SCP therefore recommends that the implications of this result are evaluated in more details.

No direct tests of the effects of soil metabolites on soil-dwelling organisms were conducted and no reasoned argument was provided. The Committee considers therefore that the risk posed by the different metabolites to soil organisms has not been addressed.

Opinion on question 3

The Committee concludes that the available information is not sufficient for a definitive assessment of risk to birds and wild mammals from the use of Nemathorin 10G on potatoes or outdoor tomatoes. This is due to the relatively high toxicity of Nemathorin 10G combined with substantial uncertainty about the degree of exposure.

Ingestion of a single granule of Nemathorin 10G would be sufficient to achieve an acute avian TER of less than 10 for small birds.

Birds may ingest pesticide granules when seeking grit. The Committee estimated exposure by this route and concluded that, even when incorporation of granules into the soil is consistently high (above 98%), the acute avian TER could be either above or below 10. Other factors also influence exposure by this route but their effect is uncertain.

The Committee considered it possible that birds may mistake granules for seeds when searching for food, but it is unknown whether this actually occurs. If birds do ingest Nemathorin 10G granules in mistake for seeds, acute TER's below 10 are expected.

The Committee also obtained TER's below the Annex VI thresholds for acute exposure of birds to fosthiazate through consuming earthworms, and for short-term and long-term exposure of mammals consuming vegetation.

The Committee therefore considers that the potential for exposure of birds and wild mammals by all the routes mentioned above requires further consideration. In addition, the exposure of birds via drinking water should be considered.

The Committee agrees with the Rapporteur Member State that the use of Nemathorin 10G on *indoor* tomatoes is unlikely to pose a significant risk to birds and wild mammals due to the limited opportunity for exposure from indoor use. Risks from the use of Nemathorin 10G on potatoes and outdoor tomatoes require further consideration.

Opinion on question 4

The Committee is of the opinion that there is not sufficient information available on the risk of organophosphate-induced delayed polyneuropathy (OPIDP) in humans following severe intoxication incidents with fosthiazate. The Committee is of the opinion that NTE (neuropathy target esterase) inhibition by fosthiazate and its isomers has not been adequately assessed. A repeated *in vitro* study with metabolic activation of the racemate and its isomers with hen and human liver microsomes by comparing NTE inhibition with that of acetylcholinesterase (AChE) is necessary. Useful information will be obtained only if the range of AChE inhibitions includes values >90%. Depending on the *in vitro* results, an *in vivo* study after a dose well above the LD_{50} with proper protection of the animal and measurements of NTE and AChE inhibitions might also be necessary.

A. TITLE

REPORT OF THE SCIENTIFIC COMMITTEE ON PLANTS ON SPECIFIC QUESTIONS FROM THE COMMISSION CONCERNING THE EVALUATION OF FOSTHIAZATE [IKKI-1145 / TO-1145] IN THE CONTEXT OF COUNCIL DIRECTIVE 91/414/EEC.

B. TABLE OF CONTENTS

C. BACKGROUND

Fosthiazate is a new active substance (a.s.) in the context of Council Directive 91/414/EEC[2]. A draft assessment report (monograph) has been prepared by the Rapporteur Member States (RMS, the United Kingdom) on the basis of a dossier submitted by the notifier (ISK Biotech). The evaluation was peer reviewed by the

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[2] OJ N° L 230, 19. 8.1991, p. 1.

ECCO[3] programme. For its assessment, the Committee had been supplied with the source documents listed below.

Fosthiazate is a soil applied nematicide intended for use in potato and tomato crops for the control of potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*) and the root-knot nematodes (Meloidogyne spp.). It is claimed to act by contact in soil and to have systemic activity in plants. It is intended for use at a maximum rate of 3000 g a.s./ha at planting with only one application per year.

<u>Source documents made available to the Committee:</u>

1. Terms of Reference - Evaluation of fosthiazate in the context of Council Directive 91/414/EEC concerning plant protection products on the market (Submitted by DG Health and Consumer Protection, 23 January 2001) (SCP/FOSTHIAZ/001).

2. Fosthiazate - Evaluation table doc 7466/VI/98-rev9 (18.08.2000) (Submitted by DG Health and Consumer Protection, 23 January 2001) (SCP/FOSTHIAZ/003).

3. Fosthiazate - End Points - Physical and chemical properties (Submitted by DG Health and Consumer Protection, 23 January 2001) (SCP/FOSTHIAZ/004).

4. Fosthiazate - End Points - Ecotoxicology - August 2000 (Submitted by DG Health and Consumer Protection, 23 January 2001) (SCP/FOSTHIAZ/005).

5. Fosthiazate - End Points Residues - August 2000 (Submitted by DG Health and Consumer Protection, 23 January 2001) (SCP/FOSTHIAZ/006).

6. Fosthiazate - End Points Residues September 2000 (Submitted by DG Health and Consumer Protection, 23 January 2001) (SCP/FOSTHIAZ/006-Rev. 1).

7. Fosthiazate - End points Toxicology - August 1999 (Submitted by DG Health and Consumer Protection, 23 January 2001) (SCP/FOSTHIAZ/007).

8. Fosthiazate - End Points - Fate and behaviour - August 2000 - (Submitted by DG Health and Consumer Protection, 23 January 2001) (SCP/FOSTHIAZ/008)

9. Fosthiazate - Addendum to the monograph - BSA metabolite - March 2000 - (Submitted by DG Health and Consumer Protection, 23 January 2001) (SCP/FOSTHIAZ/009).

10. Fosthiazate - Danish comments (February 2000) - Operator exposure (TOX) - (Submitted by DG Health and Consumer Protection, 23 January 2001) (SCP/FOSTHIAZ/010).

11. Fosthiazate - UK reply to Danish comment - 17 March 2000 - (Submitted by DG Health and Consumer Protection, 23 January 2001) (SCP/FOSTHIAZ/011).

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[3] European Commission Co-ordination.

12. Fosthiazate - Danish comments: Proposed questions to the SCP - 3 November 2000 (Submitted by DG Health and Consumer Protection, 23 January 2001) (SCP/FOSTHIAZ/012).

13. Fosthiazate - Report of the evaluation of additional data by the United Kingdom, made to the European Commission under Article 8(1) of Council Directive 91/414/EEC - Draft: August 2000 (Revision 1) - Addendum to the draft assessment report (Submitted by DG Health and Consumer Protection, 23 January 2001) (SCP/FOSTHIAZ/013).

14. Fosthiazate – Draft assessment report, prepared by the United Kingdom (volumes 1 to 3), December 1997.

15. Counting of Nemathorin 10 G and soil particles which have same size as Nemathorin 10 G in soil top layer under field conditions, Kay, 1997 – study n° DP53120, report n° 9702 (property of ISK), submitted by notifier, October 2001.

16. A study to quantify the numbers of dummy fosthiazate granules left on the soil surface after normal methods of application and incorporation, Imai 1997 – study n° DP53122, report n° 342-97-ISK-FOS (property of ISK), submitted by notifier, October 2001.

17. Fosthiazate and its isomer: *In vitro* enzyme inhibition assay and *in vitro* metabolic study, Kosei Inui, October 19, 2001 – study number AN-2081 (property of ISK), submitted by notifier, October 2001.

D. SCIENTIFIC BACKGROUND ON WHICH THE OPINION IS BASED

I. Question 1

Can it be confirmed that use scenarios exist which pose no unacceptable risk to groundwater?

<u>Opinion of the Committee:</u>

The Committee cannot confirm that use scenarios exist which pose no unacceptable risk to groundwater.

Considering only the parent compound, calculations for FOCUS[4] scenarios showed a concentration below 0.1 µg/L for one out of nine scenarios.

Soil metabolism and field persistence studies have demonstrated the formation of five soil metabolites (BSA, MBSo, TZO, DTTO and DETO). There is insufficient information on the leaching characteristics of these metabolites to determine their contribution to groundwater risk.

Therefore, based on the available information, the Committee cannot confirm a safe use for any scenario. It is possible that lysimeter studies might demonstrate lack of leaching for one or more scenarios but none are reported.

[4] Forum for the Co-ordination of pesticide fate models and their Use.

Scientific background on which the opinion is based:

I.1 Soil metabolism and degradation rates in laboratory studies

The transformation rate of fosthiazate was measured in seven soils as shown in the table hereafter. All half-lives were calculated back to the standard temperature of 20°C and to the standard moisture content corresponding with a suction of -10 kPa as recommended by FOCUS (2000, see p. 90). For the correction of soil temperature, a Q_{10} factor of 2.2 was assumed (FOCUS, 2000, p. 92).

Table 1 *Measured and estimated half-life of fosthiazate*

Soil type	**temperature (°C)**	**moisture condition**	**measured half-life (d)**	**estimated half-life (d) at 20°C and -10 kPa**
Sandy loam	25	75% of -33 kPa	44	45
Sandy clay loam	30	-33 kPa	23	44
Sandy loam	30	-33 kPa	21	39
Loam	20	40% of MWHC[5]	11	7
Sandy loam	20	40% of MWHC	27	18
Sandy loam	20	40% of MWHC	60	40
Sandy loam	10 and 20	40% of MWHC	53 and 22	15

Aerobic soil metabolism studies were carried out for the first three incubation systems of the table. In the study with the sandy loam at 25 °C, the metabolites MBSo and BSA were detected at maximum levels of 9.5 and 16%, respectively. In the studies with the sandy clay loam and sandy loam at 30°C, BSA was detected at maximum levels of 28% and 4%, respectively, and the metabolites TZO, DBTO, DTTO, DETO were all detected but accounted for less than 10% of the applied radioactivity. No information is given in these two studies about observations of MBSo.

The transformation rate of BSA was studied via incubation of BSA in the first soil of the table (sandy loam at 25°C and 75% of -33 kPa) and a half-life of 22 days was found.

I.2 Sorption and mobility

The sorption of fosthiazate was studied via experiments with soil slurries using six soil types with soil textures ranging from loamy sand to sandy clay loam. The pH of the soils was not reported in the monograph. The Committee rejected one of the studies because the decrease in the concentration in the liquid phase was too low to be measured with sufficient accuracy. The remaining five K_{OC}[6] values were 43, 97, 58, 96 and 25 L/kg and the corresponding Freundlich exponents were 0.92, 0.89, 0.88, 0.87, 0.94, respectively.

The sorption of BSA was studied via experiments with soil slurries using four soil types with soil textures ranging from sandy loam to clay loam. The pH of the soils ranged from 5 to 7. No sorption could be detected so the Committee estimated the K_{OC} of BSA to be 0.

[5] MWHC is abbreviation for maximum water holding capacity
[6] Organic carbon absorption coefficient.

There are no sorption or mobility studies available for the other soil metabolites.

I.3 Field persistence studies

In two field studies with sandy loam soils the DT_{50}[7] of fosthiazate was found to be 10 and 15 days. The monthly averages of the maximum air temperatures ranged from 25 to 34^{o}C in the first three months after application and monthly rainfall ranged between 90 and 270 mm. The application rate of fosthiazate in these studies ranged from 4.4 to 5.1 kg/ha and it was incorporated to 15 cm immediately after application. In the first study, the metabolites BSA and MBSo were found only in the 0-15 cm layer at levels up to 0.1 mg/kg. This corresponded with 10% of the applied amount of substance[8] of fosthiazate both for BSA and MBSo (assuming a dry bulk density of 1500 kg/L for the top 15 cm). In the second study BSA and MBSo were found in the 0-15 cm layer at levels up to 0.1 mg/kg and at a level of 0.02 mg/kg in the 15-30 cm layer. Assuming again a dry bulk density of 1500 kg/L resulted in maximum levels of 12% of the applied amount of substance of fosthiazate for both BSA and MBSo.

In a third field study in North Carolina, a DT_{50} of fosthiazate of 17 days was found after application of 3.1 kg of a.s./ha. The metabolites TZO, DTTO and DETO were detected at levels up to 0.05 mg/kg in the 0-15 cm layer which corresponds with 10, 5 and 4 % respectively of the applied amount of substance of fosthiazate (assuming again a dry bulk density of 1500 kg/L). The difference in these percentages is caused by the difference in molar mass of the metabolites.

I.4 Leaching to groundwater

The notifier assessed the leaching of fosthiazate via scenario calculations with the PELMO 2.01 model and a German scenario (Borstel soil with 1.5% organic carbon in top 30 cm and weather from Hamburg with 870-1000 mm rainfall per year). It was assumed that fosthiazate was incorporated at a rate of 3 kg/ha to 7.5 cm depth at 5 April each year over a 10-year period. The crop was sugar beet (as a surrogate for potatoes). The half-life was assumed to be 27 days and the K_{OC} 55 L/kg. Maximum monthly concentrations in the percolating water at 1.1 m depth never exceeded 0.02 μg/L. In a second scenario study the notifier calculated leaching with PELMO 2.01 using the same soil and same Hamburg weather station but now using 870 mm rainfall per year. A crop rotation of sugar beet, potato, spring wheat and maize was modelled. It was assumed 3 kg/ha of fosthiazate was incorporated to 7.5 cm depth on 5 April once every 4 years (in years 2 and 6, simulating application to potato crop). The model was run for 10 years (using repeatedly the same weather data). The leaching was characterised by the average concentration leaching past 1.1 m depth over a period of 4 years (years 3 to 6). Calculations were carried out for a range of combinations of half-life (11-44 days) and K_{OC} (43-97 L/kg). Amongst these combinations, the Committee considers the combination of 27 days and 68 L/kg most appropriate because it is closest to the average of the values reported in Sections I.1 and I.2. For this combination of half-life and K_{OC} the average concentration was found to be 0.06 μg/L. In a third scenario study the notifier calculated leaching with PELMO 2.01 and another German scenario (soil with 1.7% organic carbon in top 25 cm and weather from Bad Kreuznach with 320-760 mm

[7] Period required for 50% dissipation.
[8] With amount of substance, here the base physical quantity is indicated which has the unit "mole". This implies that the calculated percentages for the metabolites are mole fractions (and not mass fractions).

rainfall per year). The crop was beans (as a surrogate for tomatoes). It was assumed that fosthiazate was incorporated at a rate of 3 kg/ha to 7.5 cm depth on 5 April each year over a 10-year period. Now the leaching was characterised by the maximum annual average concentration in soil water at 1.2 m depth. Calculations were carried out for a range of combinations of half-life (11-27 days) and K_{OC} (43-97 L/kg). Amongst the combinations used by the notifier, the Committee considers the combination of 27 days and 68 L/kg most appropriate because it is the closest to the average of the values reported in Sections I.1 and I.2. For this combination of half-life and K_{OC} the concentration was found to be 0.001 μg/L. This third scenario was claimed to be representative for tomatoes in Southern Europe.

The Committee considers the incorporation depth of 7.5 cm for the potato scenarios too shallow because the monograph reports incorporation to 10-15 cm depth. Furthermore the Committee considers the third scenario not vulnerable enough for tomatoes in Southern Europe because irrigation was not considered: according to FOCUS (2000, p.29) irrigation should be included in scenarios for tomatoes. In general the Committee cannot fully assess the vulnerability of the scenario because not enough information was provided in the monograph (admittedly it is practically impossible to provide enough information for an assessment of scenario vulnerability because many input parameters may have a large effect on the outcome).

Therefore the Committee conducted PEC[9] groundwater calculations for the FOCUS scenarios (FOCUS 2000) with the PEARL model version 1.1.1. The calculations were based on the following model input:

- fosthiazate was incorporated into the top 15 cm of soil at 14 days before emergence of potatoes at a rate of 3 kg/ha;
- fosthiazate was applied once every three years (assuming a three year rotation for potato crops);
- the K_{OM}[10] of fosthiazate was 37 L/kg which was based on the average K_{OC} of 64 L/kg;
- the Freundlich exponent of fosthiazate was 0.90 which is the average of the values reported in Section I.2;
- the half-life of fosthiazate in top soil at 20°C and matric pressure of -10 kPa was 30 days which is the average of the values in the last column of the table in Section I.1.

Calculated 80th percentile groundwater concentrations of fosthiazate for the nine FOCUS groundwater scenarios were 0.004, 0.2, 0.8, 0.9, 2.0, 2.4, 2.5, 3.2 and 5.6 μg/L. Because one of the nine scenarios does not exceed 0.1 μg/L, the Committee concludes that safe use scenarios exist if only the leaching of the parent compound is considered. However, there is insufficient information on the leaching characteristics of all potentially relevant metabolites to determine their contribution to groundwater risk. Therefore the Committee cannot confirm a safe use for any scenario.

FOCUS (2000) has shown that calculations with PELMO result in lower groundwater concentration than PEARL at concentration levels close to 0.1 μg/L. Therefore, repeating the calculation with PELMO would probably result in more than one scenario below 0.1 μg/L. Both models are equally valid and the difference of results reflects the uncertainty of science behind each of them.

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[9] Predicted Environmental Concentration.
[10] Organic matter adsorption coefficient.

II Question 2

Can the Committee confirm that the risk to soil dwelling organisms has been adequately addressed?

Opinion of the Committee:

The majority of observed effects on soil-dwelling organisms were well within the Annex VI criteria, and the Committee concludes that the risk to soil-dwelling organisms following exposure to fosthiazate will in general be low. However, the single study that was conducted to evaluate effects of the active substance on the breeding performance of beetles, reported a significant reduction in the number of surviving offspring. The SCP therefore recommends that the implications of this result are evaluated in more details.

No direct tests of the effects of soil metabolites on soil-dwelling organisms were conducted and no reasoned argument was provided. The Committee considers therefore that the risk posed by the different metabolites to soil organisms has not been addressed.

Scientific background on which the opinion is based:

II.1 Risk to soil-dwelling organisms following exposure to the parent substance

The maximum application rate of the parent substance is 3 kg a.s./ha while the maximum initial PEC_{soil} of the parent has been estimated as 1.12 mg a.s./ kg soil from field dissipation studies (Monograph Vol. 3, page 226) and 1.0 mg a.s. kg from models with first-order kinetics (worst case laboratory DT_{50} 27 days, SCP/FOSTHIAZ/008, page 3). The relevant laboratory ecotoxicological data that relate to the effects of the parent substance on soil-dwelling organisms (with the exception of non-target plants) are summarised in table 2 below.

Overall a satisfactory range of soil-dwelling arthropods was tested, although tests on other taxonomic groups associated with soil such as Collembola would have been helpful in evaluating risk. The majority of observed effects were well within Annex VI trigger criteria, so in general the Committee concludes that the risk to soil-dwelling organisms following exposure to fosthiazate is likely to be low. Nevertheless, the effect of the active substance on the breeding performance of *A. bilineata* raises some cause for concern, especially since this was the only study that used reproduction as an endpoint. The SCP accepts that surface dwelling arthropods are unlikely to experience prolonged exposure to the compound following a single application of the parent substance (field DT_{50} of 9-15 days, lab DT_{50} 11-53 days) and that breeding (in this case parasitism) may not coincide with the timing of application. However, given the observed reproductive effects, the SCP feels that the notifier should further evaluate the implications of their results for beetle population dynamics, either through further experiments or through reasoned argument.

***Table 2** Effects of the fosthiazate on soil-dwelling organisms – lab. data*

	Species	Application rate(s) [Maximum application rate 3 kg/ha. Maximum initial PEC_s 1.12 mg a.s. / kg soil]	End point(s)	Annex VI Trigger
Surface dwelling	*Poecilius cupreus* (Carabidae)	4 kg a.s. / ha	3.4 % adjusted mortality	30%
	Poecilius cupreus (Carabidae)	3 kg a.s. / ha	6.7 % adjusted mortality	30%
	Pardosa spp (Lycosidae)	4 kg a.s. / ha	5.3% adjusted mortality	30%
	Pardosa spp. (Lycosidae)	3 kg a.s. / ha	6.7% mortality vs 3.3% control mortality	30%
	Aleochara bilineata (Staphylinidae)	3 kg a.s. / ha	83% reduction in surviving offspring	30%
Soil dwelling	*Eisenia foetida*	0, 6.25, 12.5, 25, 50, 100, 125,250, 500, 1000 mg a.s. / kg soil	14 d LC_{50}[11] 209 mg a.s. / kg soil 14 d NOEC[12] 100 mg a.s. / kg soil (body weight) 14 day TER[13] Acute 209 14 day TER Chronic 100	 (<) 10 (<) 5
	Eisenia foetida	2 mg a.s. / kg soil	Mean levels of fosthiazate in individual earthworms after 14 days was maximum 0.79 mg / kg	
	Micro-organisms	5.33, 53.3 mg a.s./kg	21% reduction in CO_2 evolution after 60 days in Speyer soil following application at 53.3 mg a.s./kg. Nitrification formation initially depressed, but no effects after 60 days at 53.3 mg a.s./kg.	No agreed trigger

II.2 Risk to soil-dwelling organisms following exposure to metabolites

The rates at which the various metabolites form following degradation of the parent substance in soil have been reviewed in the response to question 1. As the SCP has previously suggested (SCP, 2000), there are practical limitations to testing all metabolites, but the risks posed by each of them should at the very least be considered by reasoned argument. The molecular structure of the metabolite BSA suggests that it is unlikely to have effects on soil-dwelling organisms (since it lacks the OP[14] moiety of the parent compound. No direct tests of the effects of soil metabolites on soil-dwelling organisms were conducted and no reasoned argument was provided. The Committee considers therefore that the risk posed by the different metabolites to soil organisms have not been addressed.

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[11] Lethal concentration, median.
[12] No Observed Effect Concentration.
[13] Toxicity Exposure Ratio
[14] Organo-phosphate.

III Question 3

Can the Committee confirm that a safe use with respect to birds and mammals exists?

<u>Opinion of the Committee:</u>

The Committee concludes that the available information is not sufficient for a definitive assessment of risk to birds and wild mammals from the use of Nemathorin 10G on potatoes or outdoor tomatoes. This is due to the relatively high toxicity of Nemathorin 10G combined with substantial uncertainty about the degree of exposure.

Ingestion of a single granule of Nemathorin 10G would be sufficient to achieve an acute avian TER of less than 10 for small birds.

Birds may ingest pesticide granules when seeking grit. The Committee estimated exposure by this route and concluded that, even when incorporation of granules into the soil is consistently high (above 98%), the acute avian TER could be either above or below 10. Other factors also influence exposure by this route but their effect is uncertain.

The Committee considered it possible that birds may mistake granules for seeds when searching for food, but it is unknown whether this actually occurs. If birds do ingest Nemathorin 10G granules in mistake for seeds, acute TER's below 10 are expected.

The Committee also obtained TER's below the Annex VI thresholds for acute exposure of birds to fosthiazate through consuming earthworms, and for short-term and long-term exposure of mammals consuming vegetation.

The Committee therefore considers that the potential for exposure of birds and wild mammals by all the routes mentioned above requires further consideration. In addition, the exposure of birds via drinking water should be considered.

The Committee agrees with the Rapporteur Member State that the use of Nemathorin 10G on *indoor* tomatoes is unlikely to pose a significant risk to birds and wild mammals due to the limited opportunity for exposure from indoor use. Risks from the use of Nemathorin 10G on potatoes and *outdoor* tomatoes require further consideration.

<u>Scientific background on which the opinion is based:</u>

III.1 Origin of the question

Under the heading of 'birds and mammals', one Member State observed that the risk assessment for birds in particular requires a soil incorporation of granules of 99% to even approach a safe use, and questioned the general applicability of such restrictions or requirements. The question actually posed to the Committee is whether a safe use exists with respect to birds and mammals. The Committee considered both aspects: the general applicability of the degree of incorporation assumed in the RMS's assessment, and the risks associated with varying degrees of incorporation. In addressing these questions, the Committee concentrated on acute risks to birds as, based on the available information, these appear to exceed the risks to wild mammals and long-term risks to birds.

The Committee notes that assessing the exposure of birds to granules presents special difficulties, and that scientific knowledge in this area has continued to develop since the Committee's previous opinion on avian risks of a granular pesticide (SCP, 1999)[15]. However, significant uncertainties remain and there is currently no generally-accepted approach for this issue. The Committee therefore developed this opinion taking account of the available data, the approach employed by the RMS and recent research findings. The Committee also considered the effect of remaining uncertainties on the outcome of its assessment.

III.2 Description of granular formulation

Nemathorin is formulated as a fine granule (carrier: Taiwanese clay) of light beige colour, containing 10% w/w fosthiazate. 92% w/w of the formulation containes particles greater than 0.5 mm and 100% are smaller than 0.85 mm (Fosthiazate monograph, volume 3, Annex B, page 16). The average weight of one granule is 0.1724 mg (Fosthiazate monograph, volume 3, Annex B, page 260), so the average granule contains approximately 0.017 mg fosthiazate.

III.3 General effectiveness of incorporating granules in the soil

The notifier submitted two studies on the incorporation of Nemathorin 10G when used on potatoes, where application was followed by rotavation and ridging. In those studies, incorporation rates in excess of 99.8% were achieved (see Appendix for details).

One Member State has queried whether the high levels of incorporation in those studies are generally achievable. The Committee has identified several reasons why these studies may over-estimate the degree of incorporation to be expected in general farming practice (see Section E Appendix, page 24 for more details):

- a higher degree of care and control may be expected in small-scale research studies than in practical use,
- poorer incorporation may be expected in headlands near the field edge, due to partially-overlapping operations and some previously-incorporated granules being brought back to the surface,
- soil types, application methods and machinery are expected to vary widely in different parts of the EU.

Incorporation rates between 60 and 100% have been reported in other studies with various types of granules and application methods, and varying soil conditions (e.g. Maze *et al.* 1991, Fischer and Best 1995, and other studies listed in Table C-1 in Appendix C of the ECOFRAM report, ECOFRAM 2000).

The Committee is therefore uncertain whether incorporation rates for Nemathorin 10G will consistently and reliably achieve the levels reported by the notifier. In addition, the Committee is uncertain whether application and incorporation will always be conducted in a single operation or, if not, how much time might elapse between them (which would

[15] http://europa.eu.int/comm/food/fs/sc/scp/out27_en.html

provide foraging time for birds which are frequently attracted by freshly tilled fields). In order to form a more definitive judgement about the availability of granules to birds and mammals, data would be needed on (a) the types of methods and machinery likely to be used by farmers (taking account of any conditions or recommendations which will be issued), (b) the actual levels of incorporation achieved by farmers in practical use, in both the centre and headland area of fields, and (c) the range of time intervals that might occur between application and incorporation.

The RMS considered that, for use on outdoor tomatoes, high levels of incorporation could not be assumed because ridging was unlikely to be carried out. The Committee agrees that the available information is inadequate to form a conclusion regarding incorporation in tomatoes.

As the degree of incorporation to be expected in normal use is uncertain, where relevant the effect of varying degrees of incorporation is examined in the assessment of exposure (see below).

III.4 Routes of avian exposure

The Committee identified the following routes by which birds might be exposed to fosthiazate:
- ingestion of granules by animals seeking grit,
- ingestion of granules by animals seeking seeds as food,
- ingestion of residues in other food items such as earthworms and plant seedlings,
- ingestion of granules with soil, unintentionally ingested with food/water,
- ingestion of residues in drinking water.

It is not usual to assess all these routes in detail. However, the Committee noted that ingestion of very few granules would be sufficient to achieve an acute TER of less than 10. For example, based on the lowest available LD_{50} (8.3 mg a.s./kg bw), a 15 g seed-eating bird such as a linnet (*Acanthis cannabina*) would require just one granule to achieve a TER<10. This implies that some small species, of above-average sensitivity to fosthiazate, might require only one or a few granules to obtain a lethal dose.

Because such a small exposure is potentially significant, it is appropriate to give particular consideration to whether granule ingestion is likely to occur in the field. This question cannot be answered with certainty, due to the variability of conditions in the field (e.g. soil types) and insufficient knowledge about many factors involved in the assessment (e.g. grit and seed densities on soil surfaces, selection of grit and seeds by birds). The Committee therefore conducted a rudimentary analysis of uncertainty in the assessment, by considering a range of values for some factors.

III.5 Acute risks to birds

III.5.1 Acute avian risks due to ingestion of granules when seeking grit

Granivorous species of birds may intentionally seek out and ingest grit particles, to assist with the breakdown of ingested seeds (Best & Gionfriddo, 1991). Such birds may ingest pesticide granules when seeking grit. This is often considered the primary route of exposure for granular formulations, but there is no generally accepted approach for assessing it. In order to make clear the basis of this opinion, a detailed description of the approach adopted by the Committee on this occasion is presented in the Appendix (page 24).

The Committee considered that there is a high level of uncertainty about the acute risk to birds consuming granules as grit. Major contributors to this uncertainty include lack of data on grit densities on relevant soils, limited data and understanding of mechanisms determining grit ingestion, uncertainty regarding the level of granule incorporation generally achievable, uncertainty and variability in the extent to which birds use treated fields, and natural variation in the sensitivity of different species to the active substance.

Calculations exploring three of these factors (grit density, granule density, daily particle intake) are described in the Appendix. The results are summarised in Figure 1.

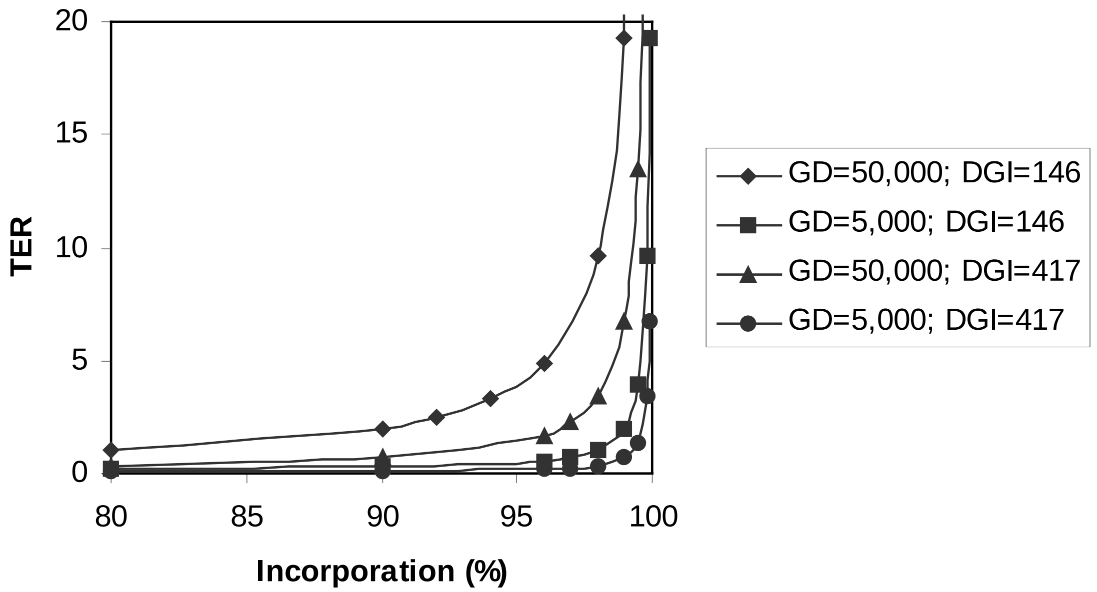

Figure 1. *Effect of varying assumptions on toxicity-exposure ratios (TER) for birds ingesting granules when seeking grit. GD = grit density on soil surface (particles/m^2), DGI = daily grit intake of birds (number of particles/day), incorporation = overall percentage of granules incorporated into soil after application, rotavation and ridging. See Appendix for details of calculations and assumptions.*

Figure 1 shows that the TER is highly sensitive to the efficiency with which granules are incorporated, particularly for incorporation rates between 95% and 100%. Most importantly, when incorporation rate was below 98%, TERs below the Annex VI threshold of 10 were obtained for all four combinations of the other two variables (grit density and daily grit intake).

The calculations were conducted using the geometric mean of the available toxicity data. Therefore, a TER of 5.7 on the graph can be interpreted as predicting 50% or higher mortality for 5% of species (see Appendix for explanation and confidence limit). A TER of 1 can be interpreted as predicting 50% or higher mortality for over half the species. In fact, the proportions of species and individuals actually affected are expected to be lower, because the calculations imply worst-case assumptions about two other important factors. They assume that birds obtain a whole day's grit intake from potato fields freshly-treated with Nemathorin 10G, and that they show no preference or avoidance for granules in comparison to natural grit. These factors are discussed in more detail in the Appendix. In summary, they would tend to reduce the risk (i.e. increase the TER's shown in Figure 1), but the extent of the reduction is unknown. Furthermore, these factors are unlikely to protect the most sensitive species, for which only one or a few granules may be lethal. In the absence of firmer information about these factors, and those varied in Figure 1, the Committee considers the results in Figure 1 as providing a reasonable worst-case assessment.

The Committee's assessment shows that the efficiency of granule incorporation is a key factor determining risk. Figure 1 shows that if incorporation is below about 98%, TER<10 is expected in reasonable worst case conditions. If incorporation exceeds 98%, then the TER may be above or below 10, depending on the true values for grit density and daily grit intake. As mentioned above, the notifier's studies report incorporation in the region of 99.8%, but the Committee is uncertain whether this would be achieved generally in normal farm practice. Even if incorporation rates were generally between 98% and 99.8%, uncertainty about the other factors in the assessment (grit density and daily grit ingestion rate) would prevent a definitive conclusion on the risk.

One of three acceptance (palatability) studies reported in the monograph demonstrates a low risk for the particular case of Japanese quail (*Coturnix coturnix japonica)*, even for applications well in excess of the maximum proposed rate and without incorporation. However, these studies do not rule out the possibility of mortality for other species under realistic conditions (see Appendix).

III.5.2 Acute risk to birds consuming granules when seeking seeds as food

Granules of 'Nemathorin 10G' are smaller than most seeds taken by birds but are of comparable size to some of the smaller seeds of arable weeds e.g. *Stellaria media*, *Capsella bursa-pastoris*, *Veronica arvensis* and *Urtica dioica*. Some of these (e.g. *Stellaria*, *Capsella*) are among the plant species most commonly taken by birds. Plant groups known to be important in the diet of the seed-eating linnet include Polygonaceae, Chenopodiaceae, Caryophyllaceae, Cruciferae, Compositae and Gramineae. It is therefore possible that granules of ‘Nemathorin 10G’ may be ingested by birds searching for seeds as food. This possibility has generally been discounted in previous assessments of risks from granular pesticides but, having examined samples of some relevant seeds, the Committee considers that exposure by this route is plausible and should be considered.

The Committee is unaware of any published information on the ability of birds to discriminate between seeds and other small particles. A worst-case scenario would occur if birds showed perfect discrimination between seeds and natural grit, but no discrimination between seeds and granules. In this case, the potential risk can be illustrated by estimating a TER in a manner analogous to that used in the Appendix for considering granules taken in mistake for grit, i.e. by assuming that granules and seeds are taken in proportion to their availability. Recent studies on UK arable fields show varying densities of crop and weed seeds up to about 20,000/m^2, based on soil cores to a depth of 20 cm (Jones 1998, Jones & Maulden 1999, Jones *et al*. 1997). The Committee assumed that seeds taken by small birds average about 1 mm diameter and are therefore visible to birds only if they are contained in the top 1 mm of soil. Ploughing is intended to invert the soil and has been shown to bury over 90% of new seeds from the surface to a depth of 5 cm or more, but additional ploughing in successive years tends to redistribute surviving seeds more evenly (Moss, 1998). The Committee therefore assumed a uniform distribution of seeds in the top 20 cm, such that 20,000/m^2 in the top 20 cm would correspond to about 100 seeds/m^2 in the top 1 mm. Assuming a granule density of 20/m^2 (based on the notifier’s studies, see Appendix), the ratio of granules to seeds would be 1:5. Based on Nagy’s equation for dry food intake (EPPO 1994, Note 8) and an estimated moisture content of 13%, a 15.3 g linnet would require 4.65 grams/day or about 660 seeds/day (based on an average weight for canary seeds, 7 mg). If a bird took 660 items/day in the ratio 1 granule : 5 seeds it would ingest about 60 granules/day. Taking the lowest available LD_{50}, this would lead to a TER for the linnet of about 0.1.

This result is less sensitive to incorporation efficiency of the granules than the TERs in Figure 1, because the estimated density of seeds is much lower than the estimated density of natural grit. Even with a density of 1 granule per square metre (an incorporation rate of over 99.99%), the TER is only increased to 2.2 and is still well under the threshold of 10.

The above calculations assume that birds ingest seeds and granules without preference, in proportion to their availability. It is often suggested that granules based on mineral substrates have no nutritional value, and would therefore not be consumed as food. However, the Committee is doubtful whether birds would be able to detect the lack of nutritional value in the granules if they were ingested along with real seeds. Also, the Committee was uncertain whether Nemathorin 10G contains any components that might give it nutritional value, e.g. oils. In any case, given that only a single granule would be required to achieve a TER of <10, the Committee considers that a sensitive species would be unlikely to learn to discriminate before ingesting a lethal dose. Other mechanisms which could protect birds are cessation of feeding either as a result of repellency of the active substance (e.g. unpleasant taste) or sublethal intoxication. The Committee considers that these mechanisms are likely to provide only partial protection, again because of the very low number of granules required to provide a lethal dose to a sensitive species.

These considerations indicate a potential risk but are affected by significant uncertainties, including the following.

– Some natural grit presumably also resembles seeds. If birds also mistake this for seeds then it will dilute the proportion of seed-like items that are granules, and hence reduce the probability of ingesting a granule.

– It is known that birds take small weed seeds while they are still on the plant in pods or seed capsules when these are available, e.g. in summer and autumn. The extent to which they also seek such seeds on bare soil later in the season is unknown, although as mentioned in the Appendix, surveys in the UK have recorded linnets present on bare fields.
– The proportion of individuals that forage on potato fields newly-treated with Nemathorin is unknown but likely to be small (see Appendix for a more detailed discussion of this factor). However, even if an individual linnet obtains only a fraction of its daily seed intake from freshly-treated black fen soils, over a sufficient period of time it will eventually ingest a granule, which would be lethal for a sensitive species. How frequently this occurs will depend on all the factors discussed above, and cannot be estimated with any precision using the information currently available.

Acceptance studies, discussed in the Appendix, are relevant. One of these studies (referred to in the Monograph as Aoki 1997a) presented groups of Japanese quail with very high densities of Nemathorin granules on a soil background, in some cases mixed with food. Mild and transient sublethal effects were recorded, but no severe effects or mortality. This negates the potential risk for Japanese quail but it cannot be assumed that other species will respond in the same way, especially smaller and more sensitive species (see Appendix for more discussion of these studies).

The Committee concludes that there is considerable uncertainty about the potential risk due to birds mistaking granules for seeds, but that it is possible that it may exceed the risk from the route that is usually considered (mistaking granules for grit). If birds do mistake granules for seeds, TER<10 may occur even when granule incorporation is extremely high.

III.5.3 Acute risk to birds consuming residues in other foods

The RMS considered risks to birds exposed to residues in their food by assessing 3 separate scenarios relating to the consumption of earthworms, vegetation and insects. The Committee agrees with the general approach taken in these assessments, but some of the specific assumptions may require further attention.

Birds eating worms

When evaluating the risk to songthrush (*Turdus philomelos*) eating earthworms, the RMS assumed a residue of fosthiazate on earthworms of 1.02 mg a.s./kg (based on a laboratory study with *Eisenia foetida*), and arrived at an acute TER of 40, above the Annex VI threshold. The Committee used the same residue estimate but made different assumptions for other elements of the assessment, based on information from the literature (see table 3 below). These assumptions lead to a TER of 6, below the Annex VI threshold, suggesting that further consideration of the risk by this route is required. Several areas of uncertainty could be examined further, including variation among individual birds in the proportion of food obtained from freshly-treated fields and the residues expected in field conditions (taking into account the effect of realistic levels of granule incorporation).

Table 3 *Assumption for the assessment of risk to birds eating worms*

Variable	**Assumption in monograph**	**Alternative assumption**	**Reference**
Songthrush body weight	89g	67.7g	Average for males and

			females, Dunning (1993)
Daily food requirement (dry weight)	8.8g	14.3g	Based on Nagy's equation for songbirds, EPPO (1994)
Moisture content of earthworms	60%	84%	Traas *et al.* (1996)
Avian acute LD_{50}	10 mg a.s./kg	8.3 mg a.s./kg	Monograph (lowest value for formulation).

Birds eating vegetation

The Committee agrees with the RMS's approach to this scenario and with the conclusion that the risk to herbivorous birds is low.

Birds eating insects

The Committee agrees with the RMS's approach, although to estimate food intake it would have used Nagy's equation for songbirds rather than 'all birds' (Note 8 in EPPO, 1994). This leads to a small decrease in the TER from 55 to 44, which is still above the Annex VI threshold of 10.

III.5.4 Acute risk to birds consuming granules as part of soil intake

Soil particles are found in many species that are not thought to require grit to grind their food. It is thought that these species ingest soil incidentally, mixed with their food (de Leeuw *et al.* 1995).

One approach to estimating exposure of these species to pesticides in soil is to use estimates of the amount of soil ingested as a proportion of the total food intake, based on estimates published by Beyer *et al.* (1994). These estimates were calculated from data on the ash contents of food and faeces, the digestibility of food and the mineral content of soil. Their relation to actual soil intakes is therefore subject to several sources of uncertainty. The species in Beyer *et al.*'s study (4 species of duck (Anas, Aythya & Aix spp.), Canada goose (*Branta canadensis*) four species of sandpiper (Calidris & Micropalama spp.), American woodcock (*Scolopax minor*) and wild turkey (*Meleagris gallopavo*)) include some which are relevant to European agricultural habitats or may be similar to European species, but do not include any passerines. The three highest estimates (17, 18 and 30% soil in food, dry weight/dry weight) relate to sandpipers, which probe or peck for invertebrates in mud or shallow water. Using the highest of these figures and assuming it applies to a 67.7g songthrush eating 14.3 g dry weight of earthworms/day (based on Nagy's equation, Note 8 of EPPO 1994), and that the soil contains 2.14 mg a.s./kg (based on 3 kg a.s./ha evenly incorporated to a depth of 10 cm in soil of bulk density 1.4 kg/l), the Committee obtained an acute TER of 43. This TER is much higher than that obtained above by an alternative method of estimation for songthrush eating earthworms, based on residues measured in earthworms. The Committee considers that further consideration of the scenario of songthrush eating earthworms remains desirable given the firmer basis of the earlier estimate, but concludes that risk from other scenarios involving soil ingestion (e.g. herbivores) is probably low given that the other species examined by Beyer *et al.* all had lower proportions of soil in their diets.

III.5.5 Risks to birds exposed via drinking water

This route of exposure is not routinely considered in regulatory assessment. However, some bird poisoning incidents have occurred in the UK and USA, which are believed to have been caused by birds drinking water contaminated with pesticide granules (e.g. Fletcher, 1994). Although the incident reported by Fletcher related to unusual circumstances (large pools formed by excessive irrigation after granule application), similar exposure might be possible after rainfall. The Committee therefore considers it would be advisable to consider exposure by this route in the case of granular formulations. This could be based on estimates of drinking water intake (Note 8 in EPPO, 1994) and estimated concentrations of the active substance in puddles on treated fields under realistic worst-case conditions, taking account of the likely degree of granule incorporation. In principle, exposure via drinking water should be added to that occurring by other oral routes.

III.6 Short and long term risks to birds

The RMS obtained a higher TER for short-term risk (based on 5-day dietary LC_{50} as the measure of toxicity) than the corresponding acute TER (based on acute 24 hours LD_{50}) for each of the scenarios they considered. Furthermore, the RMS estimated the long-term TER to be 24.5 (based on NOEC for reproductive effects), and argued that due to short persistence this was likely to over-estimate risk. The Committee supports this conclusion and considers that any further refinement of the avian assessment should concentrate on acute risks as indicated above.

III.7 Risks to wild mammals

Acute risks to wild mammals are likely to be significantly lower than those to birds in this case because:

- Acute toxicity to mammals is lower (oral LD_{50} of 57 mg a.s./kg for rat compared to 8.3 mg a.s./kg for birds),
- Wild mammals are not thought to deliberately ingest grit to grind their food,
- Wild mammals make more use of smell than birds and may be less likely to mistake granules for food,
- Soil ingestion rates reported for wild mammals are lower than those for birds (Beyer *et al.*).

The RMS presented an assessment of acute and chronic risks to wild mammals and obtained TER values well above the Annex VI thresholds for acute risks but close to the thresholds for short-term and chronic risks to wild mammals consuming vegetation such as volunteer cereal shoots contaminated with fosthiazate. The RMS argued that the latter results over-estimate the risks because:

- the estimate of initial residues in cereal shoots was an over-estimate,
- studies in tomatoes show a rapid decline in residues,
- there is unlikely to be any significant foraging of newly germinating weed seeds in the first 2-3 weeks after incorporation of Nemathorin.

However, the TERs for short-term and chronic risks to wild mammals consuming vegetation were supplied by the notifier and are based on a 2.5 kg rabbit (probably rather high for a wild rabbit) eating 125 g food/day and a 250 g ‘mammal’ consuming 20 g food/day. Applying Nagy’s equation for dry food intake (Note 8 in EPPO, 1994) and assuming 70% moisture content in cereal shoots (estimate for monocotyledenous plants and young grasses in US EPA, 1993) would indicate consumption rates of 486 g/day and 73 g/day respectively. Using these estimates would reduce the short-term and chronic

TER’s by a factor of over 3.5 and place them well below the Annex VI thresholds. This in turn places more stress on the arguments regarding limitation of exposure, listed above. The Committee therefore considers that the short-term and chronic risks for wild mammals consuming vegetation require further examination.

III.8 Conclusions

The Committee noted that ingestion of a single granule of Nemathorin 10G would be sufficient to achieve an acute avian TER of less than 10 for small birds. Because such a small exposure is potentially significant, the Committee considered the various possible routes of exposure in some detail.

Estimation of some routes of exposure is very uncertain. The Committee assessed these as far as possible given the information available, and has attempted to indicate how the uncertainties affect the outcome of the assessment.

Birds may ingest pesticide granules when seeking grit. This may or may not lead to acute TER’s below 10, depending on the incorporation efficiency of granules in normal practice and other uncertain factors including the densities of natural grit on worst-case soils and the daily grit intake of relevant species of seed-eating bird.

The possibility that birds may mistake granules for seeds when searching for food has generally been discounted in previous assessments of risks from granular pesticides. However, having examined samples of some relevant seeds, the Committee considers that exposure by this route is plausible and should be considered in assessments of granular pesticides. In this case, if birds ingest Nemathorin 10G granules in mistake for seeds, then acute TERs below 10 are expected.

The Committee estimated an acute TER of 6 for birds exposed to fosthiazate residues by consuming earthworms, and considers that risk from this route of exposure requires further examination. The Committee estimated short-term and long-term TERs below the Annex VI thresholds for mammals exposed to fosthiazate residues by consuming vegetation, and considers that risk from this route of exposure requires further examination. The Committee also considers that the potential for exposure of birds and wild mammals via drinking water should be assessed.

The Committee’s calculations relate to the use of Nemathorin 10G on potatoes. The Committee agrees with the RMS that use of Nemathorin 10G on *indoor* tomatoes is unlikely to pose a significant risk to birds and wild mammals due to the limited opportunity for exposure from indoor use. Risks for use of Nemathorin 10G on *outdoor* tomatoes may be similar to the risks for use on potatoes but are more uncertain, because no information is available on the incorporation of granules applied to tomatoes.

The Committee concludes that the available information is not sufficient for a definitive assessment of risk to birds and wild mammals from the use of Nemathorin 10G on potatoes or outdoor tomatoes. This is due partly to uncertainty about the degree of granule incorporation that is generally achievable, and partly to uncertainties about other factors affecting exposure.

IV. Question 4

Does the Committee consider that there is sufficient information available on the risk of organophosphate-induced delayed polyneuropathy (OPIDP) in humans

following severe intoxication incidents to confirm that there is a safe use, or whether further *in vitro* tests of relative inhibitory potency of the individual isomers of fosthiazate for acetylcholinesterase (AChE) and neuropathy target esterase (NTE) in hen and human tissues are required?

Opinion of the Committee:

The Committee is of the opinion that there is not sufficient information available on the risk of organophosphate-induced delayed polyneuropathy (OPIDP) in humans following severe intoxication incidents with fosthiazate. The Committee is of the opinion that NTE (neuropathy target esterase) inhibition by fosthiazate and its isomers has not been adequately assessed. A repeated *in vitro* study with metabolic activation of the racemate and its isomers with hen and human liver microsomes by comparing NTE inhibition with that of acetylcholinesterase (AChE) is necessary. Useful information will be obtained only if the range of AChE inhibitions includes values >90%. Depending on the *in vitro* results, an *in vivo* study after a dose well above the LD_{50} with proper protection of the animal and measurements of NTE and AChE inhibitions might also be necessary.

Scientific background on which the opinion is based:

An acute neurotoxicity study to assess the potential of fosthiazate to cause delayed polyneuropathy (OPIDP) was performed in hens (the animal model for OPIDP studies) which were administered by gavage 20 mg/kg bw of fosthiazate on days 1 and 23 of the test. Surviving animals were sacrificed on day 24. The dose selection was based on a range-finding study showing 3/3 mortality at 20 and 1/3 mortality at 10 mg/kg bw in non-protected animals. Mortality was 7/18 despite atropine and oxime treatment and 2/18 hens were killed while in a moribund condition. Neither clinical nor morphological signs of OPIDP were observed. Positive controls (tri-ortho-cresyl phosphatetreated) showed both clinical and morphological signs of OPIDP. In this study, measurements NTE and of AChE activities in the nervous system after dosing were not performed**.** NTE is believed to be target of OPIDP whereas AChE is the target acute toxicity.

The Notifier has provided a recent *in vitro* study with racemic fosthiazate and the (-) and (+) isomers (Inui, 2001[16]). The report of the study lacks of important experimental details. However, based on reported results it appears that at the given conditions (not specified):

a) hen brain AChE activity, but not NTE activity, was slightly inhibited by the racemate and the isomers activated after incubation with hen liver microsomes;
b) after incubation with monkey liver microsomes monkey brain AChE activity was inhibited by the racemate (by about 40%) and the (-) isomer (by about 50%) and less so by the (+) isomer (by about 20%); NTE activity was slightly inhibited (by about 20% by all compounds);
c) the rate of metabolism of the racemate and its isomers did not differ when incubated with liver microsome from any of the species tested; however, the metabolic rate was highest in monkeys (crab eating monkey), intermediate in humans and lowest in hens.

The slight AChE inhibition in hen tissues might be explained by the slower hen metabolism and, therefore, the use of a different timing or other experimental conditions

[16] Document 17 submitted to the SCP.

might demonstrate AChE inhibition in hen brain. Taken together, these results do not exclude the possibility that NTE might be inhibited by concentrations of fosthiazate not much higher than those that inhibit AChE.

Higher than 70% inhibition of NTE 24-48 hours (or even later, depending on compound kinetics) after dosing correlates with the development of OPIDP 2-3 weeks later (Lotti, 2000). Repeated dosing causes OPIDP only if this threshold of inhibition is reached (Lotti and Johnson, 1980). NTE and AChE show different sensitivities to inhibition by organophosphates and structure-activity relationships have been identified (Johnson, 1975). On this basis, fosthiazate is unlikely to be a strong NTE inhibitor, as indirectly suggested by the negative results of the acute neurotoxicity study. Animals treated with atropine and oxime can survive doses of organophosphates well above the LD_{50} and, therefore, the possibility exists that, in these conditions, NTE might be critically inhibited by compounds which are weak (relatively to AChE) NTE inhibitors. Consequently OPIDP might develop after the severe cholinergic syndrome has recovered. *In vitro* comparison between IC_{50} for AChE and NTE of a given compound are helpful for extrapolation of such possibilities (Lotti and Johnson, 1978). Alternatively, NTE activity can be measured after administration of doses of the organophosphate well above the LD_{50} (i.e. a dose which requires high and repeated administration of atropine and oxime, and, possibly, pretreatment with eserine) (Moretto, 1999). In this respect, it should be born in mind that the therapy of organophosphate poisoning in humans could be more effective than the conventional therapy in hens. In fact, in humans mechanical ventilation is available and continuous infusion of atropine and oxime keeps these drugs at optimal blood levels (Moretto and Lotti, 1998). Consequently, humans are likely to survive comparatively higher doses than hens, if properly treated. For these reasons information on NTE inhibition (or potential for inhibition) is important for risk assessment of acute exposures.

In light of these considerations, fosthiazate has been properly assessed for acute exposures deriving from normal use. However, the possibility that very high accidental exposures might result in OPIDP, following recovery from the cholinergic syndrome recovered, cannot be excluded. The Committee is of the opinion that a repeated *in vitro* study in which high (>90%) AChE inhibition by the racemate and its isomers is obtained and compared with that of NTE must be performed. Depending on the results of the *in vitro* study, an *in vivo* study in hens with very high doses (well above the LD_{50}) of fosthiazate (racemate) and biochemical measurements (NTE and AChE) might also be necessary.

E. APPENDIX - RISK TO BIRDS

Evaluation of Notifier's studies on density of granules on soil surface

The nominal application rate of Nemathorin 10G is 30 kg product/ha, i.e. 17400 granules/m^2 (based on average granule weight).

In the UK the density of dummy (untreated) Nemathorin 10G granules was assessed in 3 trials on different soils, each with two replicate plots of 6m x 12m (Kay, 1997). Prior to application, the plots had been rotavated to a depth of 10-15cm and the soil quality was described as 'medium/fine' (2 sites) or 'fine'. Granules were applied with a hand-held Fischer airflow granule applicator with fishtail nozzles. Incorporation of the granules was done with a tractor-mounted rotavator to a depth of 10 to 15 cm. Subsequently, half of each plot was formed into potato ridges with a tractor-mounted Massey Ferguson ridger. Granules remaining on the surface were counted in 10 x 10cm quadrats, randomly placed.

The proportion of granules found on the soil surface after application (based on the nominal application rate of 17400/m^2) was between 44 and 81% (7610 to 14120/m^2). The proportion found at the end of the operations was less than 1% (20 to 52/m^2) on the unridged areas, and less than 0.2% (4 to 14.1/m^2) on the ridged areas. This represents an overall incorporation rate (after application, rotavation and ridging) of greater than 99.8%.

The UK site with black fen soil showed the highest densities of granules at every stage of the operation. The mean recovery of granules on this soil before rotavation was 71% and 81% in the two plots, so the Committee assumes that the numbers of granules recorded after rotavation and ridging represent approximately 71% and 81% respectively of those actually present in the surface layer of soil. Allowing for this, the Committee estimates the densities present after ridging in the two plots as 15.7 and 19.8/m^2. After allowing for recovery during sampling in the manner described above, the incorporation rates for the UK study plots range from 99.89% to 99.96%.

Note that these figures are averages: higher and lower densities occurred in different areas within the study plots. Also, higher and lower average densities would be expected if more than two plots had been measured. Furthermore, in a real field, higher densities of exposed granules may possibly occur near the edge of the field. This is because it is common for farmers to treat first the centre of the field and then the outer edge (headlands). When treating the headlands, they are likely to overlap small areas of the previously-treated central area and may bring some of the previously-incorporated granules up to the surface. This might result in surface densities closer to those found after rotavation (see above) in restricted areas.

In a separate study in Japan, with the same dosage of 30 kg product/ha, the number of granules remaining on the surface after incorporation ranged from 17 – 31 per m^2, mean number 26 granules/m^2 (based on ten 1 m^2 plots arranged in two rows within a single 10m x 6m area; Imai, 1997). The soil in this study was stated in the Monograph to be a sandy loam (Fosthiazate, volume 3, Annex B, page 227), but no details of the method of application are reported. The results represent a mean incorporation efficiency of 99.85%.

Both the available studies reported incorporation efficiencies over 99.8% after broadcast application, rotavation and ridging. It is difficult to assess whether incorporation was enhanced in these studies, compared to farmer applications, due to the small size of the plots and the degree of care likely to be taken in research studies. It is also difficult to assess how consistently this level of incorporation could be achieved in different countries and conditions, including different soils, different machinery and different application practices. It is therefore conceivable that, in actual practice, granule densities in Europe might frequently exceed the highest density estimated from the UK study (20/m^2).

Assessment of acute risk to birds consuming granules when seeking grit

Choice of indicator species

Granivorous species of birds may intentionally seek out and ingest grit particles, to assist with the breakdown of ingested seeds (Best & Gionfriddo 1991). The linnet (*Carduelis cannabina*) is an example of a small granivorous bird that might be expected to make some use of potato fields. Linnets were recorded in 5 of 41 survey visits to ploughed and harrowed fields in the UK (J. Crocker, pers. comm.), which are similar to potato fields immediately after sowing. Smaller species like the linnet are at increased risk compared to larger species, because they require fewer granules to obtain a LD_{50} and because the LD_{50} itself tends to scale positively with weight (i.e. smaller species have lower LD_{50}s, Mineau *et al.* 2001).

Daily grit intake

de Leeuw *et al.* (1995) examined grit in the gizzards of birds collected in the Netherlands. They reported an average of 100 grit particles greater than 0.5 mm in the gizzards of two linnets. Of seven granivorous species they examined, two had higher numbers of grit (122 and 188 for single individuals of twite and brambling respectively).

Fischer and Best (1995) carried out an experiment to determine the daily turnover of granules fed to captive birds, and found that the number present in the gizzard at the end of the experiment represented 24% of the total number ingested during the day. In the absence of better information the Committee assumes that a similar turnover applies to linnets. This implies that the 100 grit particles found in the crops of linnets represent an intake rate of 100/0.24 = 417 grit particles per day.

About 35% of the grit particles found in linnets by de Leeuw *et al*. (1995) were in the size range 0.5-1 mm, which roughly corresponds to the size of fosthiazate granules. If it is assumed that particles of all sizes have the same turnover rate, then the ingestion rate for grit particles in the size range 0.5-1 mm can be estimated as 35/0.24 = 146 particles/day.

A number of uncertainties affect the use of this estimate:
- The average of 100 particles in the gizzard based on just two individual linnets. The true average could therefore be substantially lower or higher, due to sampling variation. Furthermore, individual linnets will vary above and below the average. Similarly, other granivorous species may have higher and lower grit contents.
- Turnover/retention rates may differ between particles of different types and sizes, either due to differential breakdown in the gizzard or differential transfer to the intestines
- Birds in areas where soils contain less large grit than in de Leeuw *et al*.'s study may maintain their intake by taking more small particles. If they did so, it is unknown whether they would take an equivalent number of small particles (i.e. a total of 417 per day) or an equivalent weight or volume (i.e. many more than 417, because more small particles are required to achieve the same weight or volume).

To explore in a rudimentary way the possible effect of these uncertainties, the assessment is repeated with both the estimates given above (146 and 417).

Incorporation of granules

Studies provided by the notifier demonstrated incorporation rates in excess of 99.8% under experimental conditions on 4 soils in the UK and Japan. The RMS used this study as the basis of their assessment. It is recognised that this level of incorporation is potentially achievable, although there are several factors that could lead to lower incorporation rates in practice (see first section of this Appendix). One Member State has questioned whether this level of incorporation is generally achievable. Therefore, the

Committee explored the effects of a range of possible values for the incorporation rate. The plausibility of the lower rates is discussed in the main part of this opinion.

Availability of natural grit

In order to assess how many granules may be ingested by birds, it is necessary to consider their density in relation to the density of natural grit particles of similar size, which are also on the soil surface. There are no direct measurements of the densities of natural grit particles on the surface of black fen soils, though they are thought to be very few and mostly bound up in organic matter (J Hollis, pers. comm). The Committee considered two alternative approaches to estimating the surface density for grit particles in the main size range of Nemathorin 10G granules (0.5-0.85 mm).

(a) Luttik and de Snoo (R Luttik, pers. comm.) examined samples of three soils, selected to be typical of three major agricultural areas in the Netherlands. They separated each sample into a series of size ranges using sieves, and found that the proportion (by weight) of particles over 0.5mm was 4.7%, 6.7% and 1.6% for the three soils. Based on their measurements, they estimated the number of particles 0.5-0.75mm present on the surface as 631,388 and 673,324 and 1,627,029/m^2 respectively for the three soils. However, all of their samples were from soil types with relatively high grit contents: a clay and two sands. Black fen soil in the UK generally has less than 1% of 'coarse sand' (between 0.6 and 2mm, J Hollis pers. comm.). Luttik and de Snoo's soils therefore do not represent a realistic worst case.

(b) The ECOFRAM report (ECOFRAM, 2000) describes a simple arithmetic approach in which the density of grit particles is estimated by calculating the number which can be contained in a surface layer of a defined depth. For example, the volume of a layer 0.6 mm deep by 1 metre square is equal to the volume of 5,305,165 spherical particles of diameter 0.6 mm. If only 1% of the volume is comprised of such particles (approximately representing a black fen soil with 1% coarse grit by weight), then the number of particles reduces to 53,052. However, only about half this number of spheres can fit in a 0.6 mm monolayer, due to the empty spaces around them. Furthermore, grit is much more dense (about 2.6 g/cm^3) than the rest of the black fen soil (about 1 g/cm^3), so a grit content of 1% by weight implies about 0.4% by volume. Finally, as mentioned above, black fen soils often contain less than 1% coarse sand by weight, part of this comprises larger particles (up to 2 mm), and many of the particles are likely to be bound in the peat (J Hollis, pers. comm.). Taking these considerations together, the Committee estimates that the density of 0.5-0.85 mm grit particles on the surface of black fen soils is in the range 5,000-50,000/m^2 or even lower.

Because of the uncertainty in these considerations, the Committee used a range of 5,000 – 50,000/m^2 in its evaluation, although it is recognised that the true values might possibly be still lower.

Estimation of daily granule intake

The information reviewed above is used to estimate daily intake of granules by linnets on black fen soils as follows.

Proportion of particles that are granules = granule density/(granule density + grit density).

Average number of granules expected to be ingested/day = proportion of particles that are granules x daily intake of particles.

For example, assuming a grit density of 5000/m^2, a granule density of 20/m^2 (based on the UK field study) and a particle ingestion rate of 146 particles/day leads to a daily intake of 0.58 granules/day. In practice, birds would ingest a whole number of granules, i.e. 0, 1, 2, etc. The calculation provides an average; some birds would ingest more and others less. As the averages are so low it would be more informative to model the distribution of ingestion rates and hence the proportions of individuals ingesting 0, 1, 2 granules etc. However, estimating the average is simpler and is adequate for the purposes of this assessment.

Estimation of daily dose of a.s.

The loading on granules = granule weight x formulation rate. The Committee assumed an average granule weight of 0.1724 mg and a formulation rate of 10%, based on the Monograph.

Dose per kg bw per day = granule intake per day x granule loading / body weight. The Committee assumed an average body weight for linnet of 15.3g (Dunning, 1993).

For example, an average granule intake per day of 0.58 would lead to an average dose of 0.655 mg a.s./kg bw/day for the linnet. Note that this is again an average; some birds would experience higher doses, others lower doses.

Toxicity

The toxicity data provided were as follows:

Technical fosthiazate	Mallard duck	20 mg a.s./kg bw
Technical fosthiazate	Bobwhite quail	10 mg a.s./kg bw
Nemathorin 10G	Japanese quail	83 mg product/kg bw (8.3 mg a.s./kg bw)
Nemathorin 10G	Mallard duck	129 mg product/kg bw (12.9 mg a.s./kg bw)

These limited data suggest that the intrinsic toxicity of the active substance is not reduced by the formulation.

For the purposes of its assessment, the Committee used the geometric mean of the three species, having first taken the geometric mean of the two results (technical and formulation) for the mallard. This was done in order to enable the Committee to relate the resulting TERs to the full range of species variation in toxicity, as estimated by the method of Luttik and Aldenberg (1997). Obtaining a TER of precisely 5.7 when using the geometric mean LD_{50} implies that the proportion of species incurring 50% or higher mortality would have a median expected value of 5%. As the mean LD_{50} is based on 3 species, a TER of over 15.6 would be required to be 95% confident that less than 5% of species experience 50% mortality (Luttik and Aldenberg 1997, Table 1). Obtaining a TER of precisely 1 would imply that a species of average sensitivity would incur 50% mortality.

Note that this approach does not take account of the tendency for increased sensitivity in smaller avian species, mentioned earlier (Mineau *et al.* 2001). If the toxicities were adjusted to allow for the small size of the linnet, the resulting TER's would be lower.

Calculation of acute toxicity exposure ratios

The acute toxicity exposure ratio (TER_a) = LD_{50} / dose per kg bw per day.

For example, based on a grit density of 5,000/m^2, granule density of 20/m^2, total intake of 146 particles/day, granule weight of 0.1724 mg, formulation rate of 10%, body weight of 15.3 g and LD_{50} of 11 mg a.s./kg bw, the resulting TER is 16.8.

Three of the factors in this calculation (grit density, granule density, daily particle intake) are subject to considerable uncertainty, as discussed in preceding subsections. The Committee therefore conducted a series of TER calculations using the upper and lower ends of the ranges defined above for grit density (5,000-50,000) and daily particle intake (146-417), and a number of alternative values for incorporation efficiency (in the range 80% to 99.8%). The results are shown in Figure 1 (in Section III.5.1 p. 16).

Figure 1 shows that the TER is highly sensitive to the efficiency with which granules are incorporated. When incorporation rate was below 98%, TER's below the Annex VI threshold of 10 were obtained for all four combinations of the other two variables (grit density and daily grit intake).

Slightly lower TER's would have been obtained had the Committee made an allowance for the scaling of LD_{50} with body weight, as mentioned earlier.

Studies conducted in the UK and Japan demonstrate incorporation efficiencies in excess of 99.8%. If this level of incorporation were generally achieved, then Figure 1 shows that the TER would exceed 10 even for worst case assumptions. The likelihood of this level of incorporation being generally achieved is therefore critical.

Consideration of other factors that influence risk

The Committee's calculations imply worst-case assumptions about two other important factors: it is assumed that birds obtain a whole day's grit intake from potato fields freshly-treated with Nemathorin 10G, and that they show no preference or avoidance for granules in comparison to natural grit.

It is unknown whether linnets would discriminate between granules and natural grit. Nemathorin 10G is formulated on clay. In experimental studies, captive birds prefer silica-type grit to clay granules, which should reduce their intake of Nemathorin 10G and increase the TER's shown in Figure 1 (p. 16). However, this result is based on experiments where birds are able to develop a preference over a period of hours, during which they may sample a number of the less-preferred granules (Best and Gionfriddo, 1994). It is not known how quickly this preference will develop after a bird encounters a new type of clay particle. Unless the preference develops very quickly, it will not be effective for the more sensitive species, for which a single Nemathorin granule could potentially represent a lethal dose. Therefore it is not possible to estimate how much the preference for silica-type grit would reduce the risk, given current knowledge. Birds may also reduce their ingestion of grit in response to the pesticide itself – an avoidance reaction. This could be caused by repellency and/or sublethal intoxication. Again, such a response would need to develop very quickly to protect the more sensitive species and it is not possible to estimate this from the available data, although some relevant information is provided by acceptance studies (see below). In summary, there is reason to expect some degree of avoidance or discrimination against granules, but it is unlikely to be effective in protecting species which are sensitive enough for a small number of granules to constitute a lethal dose (expected to apply to about 10% of species, based on the available information).

Data quoted earlier show that Nemathorin is likely to be used in black fen soils and that linnets do visit bare fields, so those aspects of the exposure scenario are realistic. However, the calculations refer to birds that obtain all their grit from black fen soils freshly-treated with Nemathorin. In fact, only a proportion of fields will be treated. Furthermore, individual birds may obtain a proportion of their grit from other sources such as roads, especially as the black fen soil is a poor source of grit. Also, decay of the active substance and breakdown of the granules limit the period during which a treated field remains hazardous. These factors will all increase the average acute TER's. However, all these factors are insufficient to prevent mortality entirely, because a sensitive species requires only one or a few granules to obtain a lethal dose. Even if an individual linnet obtains only a fraction of its daily particle intake from freshly-treated fields on black fen soils, over a sufficient period of time it will eventually ingest a granule. How frequently this occurs will depend on the factors discussed in this paragraph and those varied in Figure 1, and cannot be estimated with any precision using the information currently available.

In summary, the factors considered in this subsection would tend to reduce the risk (i.e. increase the TER's shown in Figure 1), but the extent of this reduction is unknown. In the absence of firmer information about these factors, and those varied in Figure 1, the Committee considers the results in Figure 1 as providing a reasonable worst-case assessment.

Evaluation of acceptance/palatability studies

Acceptance studies conducted with Japanese quail, summarised in the monograph, are relevant to the potential risks identified above. However, the available LD_{50} for Japanese quail shows that individuals of this species would require about 50-70 granules (depending on body weight) to obtain the equivalent of one LD_{50}. One of the 3 studies presented too few granules in each pen to provide even one LD_{50} – so that mortality would not be expected even if all the granules were eaten by one individual. A second study presented enough granules for about 1.7 LD_{50}s in a pen housing six birds. Therefore, the lack of mortality in these studies does not demonstrate safety to Japanese quail.

However, the third study (referred to in the Monograph as Aoki 1997a) presented groups of 6 birds with a range of treatments providing between 25 and 8,500 LD_{50}s. In this study, mild and transient sublethal effects were recorded in the higher treatments but no severe effects or mortality. It is possible that the lack of mortality was partly or wholly due to avoidance/repellency responses, but this cannot be determined by the methods used in this study.

This result indicates that mortality of this species was absent in conditions which can be regarded as an extreme worst case (because of the very high granule densities). It is conceivable that the mechanisms that prevented mortality for Japanese quail in this study would also prevent mortality in other species. However, it is not possible to draw conclusions for other species from this study. This is because (a) some species will have lower LD_{50} than Japanese quail, (b) smaller species require fewer granules to achieve the same LD_{50}, (c) other species may have higher (or lower) daily grit intakes, (d) smaller species are likely to ingest smaller particles, whether as grit or seeds, and (e) an avoidance response (if present) may vary in intensity between species. Even if a strong avoidance response exists, species for which very few granules (e.g. <5) are lethal, may not develop an avoidance response quickly enough to avoid ingesting a lethal dose.

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