EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate B - Scientific Health Opinions
Unit B2 – Management of scientific committees I

SCIENTIFIC COMMITTEE ON PLANTS

SCP/VINCLO/021-Final
31 March 2000

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

(Opinion adopted by the Scientific Committee on Plants on 17 March 2000)

Rue de la Loi 200, B-1049 Bruxelles/Wetstraat 200, B-1049 Brussel - Belgium - Office: B232 6/25.
Telephone: direct line (+32-2)295.77.05, switchboard 299.11.11. Fax: 295.73.32.
Internet: Michael.Walsh@cec.eu.int
P:\walshmi\scp\docs-scp\opinions-Final\VINC21F.doc

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

TERMS OF REFERENCE

The Scientific Committee on Plants (SCP) is requested to consider the following questions:

(1) What is the appropriate NOEC[1] of vinclozolin in birds?

(2) On the basis of the available data, the SCP is requested to comment on the acceptability of the risk to birds and wild mammals which could arise from the intended uses. The Committee is also asked to comment on the appropriate approach for a refined risk assessment for birds and small mammals?

BACKGROUND

Vinclozolin is an existing active substance in the context of Directive 91/414/EEC[2] concerning the placing of plant protection products on the market and is one of the active substances covered by the first stage of the work programme provided for under the Directive. The Committee has been supplied with a dossier provided by the notifier BASF, a monograph from the French Authorities acting as Rapporteur Member State, the results of the "Peer Review" report involving several Member States. The Committee issued an earlier opinion on the establishment of MRLs[3] for vinclozolin pursuant to the relevant Community legislation which is available as SCP/VINCLO/019- FINAL[4].

Vinclozolin belongs to the dicarboximides group of fungicides and is fungitoxic against spore germination and mycelial growth. It is a contact fungicide with local systemic properties and controls infections caused by *Botrytis, Monilinia and Sclerotinia* in a wide range of fruit, vegetables and ornamentals.

[1] No Observed Effect Concentration

[2] OJ NO L230, 19.08.91 p.1

[3] Maximum pesticide Residue Limits

[4] http://europa.eu.int/comm/dg24/health/sc/scp/out52_en.html

OPINION OF THE COMMITTEE

Question 1.

What is the appropriate NOEC of vinclozolin in birds?

Opinion

On the basis of the data provided, the SCP is of the opinion that 125 mg/kg food is the appropriate NOEC of vinclozolin for birds.

Scientific and Technical Background on which the Opinion is Based

The results of the bird reproduction tests submitted to assessors are presented in Table 1. In two studies (A and C), it was noted mallard ducks engaged in fighting, which was probably encouraged by the sexual mix in replicate groups (2 males and 5 females). This fighting may have caused some of the mortality, and probably increased variation in some of the other parameters, but this does not render the studies invalid *per se*.

Table 1 *Results of the studies conducted to assess the effects of vinclozolin on bird reproduction.*

Reference	*Concentrations*	*Measurements*	*Results*	*NOEC (1 mg/kg = 1ppm)*
IIA 8.1.3 **Study A:** **Bobwhite study 1** **Mallard study 1**	5, 50 mg/kg	Body weight, Food consumption, Mortality, egg production, Embryo viability (etc)	No significant adverse effects on quail Mallard in 50 mg/kg groups had significantly lower food consumption, Higher proportions of infertile eggs, Higher embryonic mortality and body weight of ducklings was lower	**No effect at highest tested concentration** (quail) **5 mg/kg** (mallard)
IIA 8.1.3 i (follow up) **Study B:** **Mallard study 2**	2.5, 10, 50 mg/kg	Body weight, Food consumption, Mortality, egg production, Embryo viability (etc)	No significant adverse treatment effects	**No effect at highest tested concentration** (mallard)
IIA 8.1.3 ii (follow up) **Study C:** **Bobwhite study 2** **Mallard study 3**	25, 50, 125, 250 mg/kg	Body weight, Food consumption, Mortality, egg production, Embryo viability (etc)	Number of eggs per female quail and 14-day old survival quail chicks significantly Decreased and embryonic mortality increased in 250 mg/kg Hatchability and production of viable eggs of mallard decreased at 250 mg/kg	**125 mg/kg** (quail) **125 mg/kg** (mallard)

Both the Notifier and RMS appear to agree that the strict minimum NOEC value of 5 mg/kg (derived from study A) was too low, on the basis of follow up studies. The Notifier originally proposed that, on the basis of these further studies, a value of 125 mg/kg be therefore taken as the NOEC. However, the RMS proposed that 50 mg/kg should be considered the relevant NOEC.

In Table 2 all the relevant data are assembled. Clear effects were seen in the highest test level of 250 mg/kg food for both species. At all lower test levels no effects were recorded except for the mallard duck in the first study at the 50 mg/kg food level.

Table 2 *Summary of data from reproduction studies with Mallard duck and Bobwhite quail.*

Study	Dose levels mg/kg						
	2.5	**5**	**10**	**25**	**50**	**125**	**250**
Mallard Study 1	--	No effects	--	--	Significant effects	--	--
Mallard Study 2	No effects	--	No effects	--	No effects	--	--
Mallard Study 3	--	--	--	No effects	No effects	No effects	Significant effects
Bobwhite Study 1	--	No effects	--	--	No effects	--	--
Bobwhite Study 2	--	--	--	No effects	No effects	No effects	Significant effects

If the first mallard study (Study 1) is acceptable the overall NOEL[5] would be 5mg/kg food.
However, if this first mallard study is discounted then the overall NOEC would be 125 mg/kg food.

Arguments for including the mallard Study 1 in the derivation of an overall NOEC are:

1. The majority of birds were in good health throughout the study and the few clinical signs of ill health which were observed were not considered to be treatment-related.
2. Despite the high variability observed between replicates (a higher variability is expected when wild trapped birds are used) the proportion of infertile eggs was significantly higher in the 50 mg/kg food level.

Argument for excluding the mallard Study 1 in the derivation of an overall NOEC is:
Comparing the data from single replicates the fertility rate seems to be related to male birds with small or underdeveloped testes in the replicates.

In order to establish if the findings in testes of the mallard duck are due to unrelated (circumstantial) effects two other studies with the same species were carried out.

The first additional study (Mallard Study 2) was focused particularly on the testes effects. No substance related effects were detected up to the highest tested concentration (50 mg/kg food).

[5] No Observed Effect Level

The second additional study (Mallard Study 3) with higher test concentrations (25, 50, 125 and 250 mg/kg food) did not reveal any deviations in the size of the testes in the macroscopic *post-mortem* examination.

Conclusion

The two additional studies supported the interpretation of the results in the first mallard study that the effects seen at the 50 mg/kg food level are probably not due to the compound vinclozolin. This means that the appropriate NOEC for birds exposed to vinclozolin is 125 mg/kg food.

Question 2

On the basis of the available data, the SCP is requested to comment on the acceptability of the risk to birds and wild mammals which could arise from the intended uses. The Committee is also asked to comment on the appropriate approach for a refined risk assessment for birds and small mammals?

Opinion

On the basis of the data provided, the SCP is of the opinion that the intended uses of vinclozolin (maximum application rates of vinclozolin in the EU of 0.75 kg as/ha in orchards, 1 kg as/ha in vineyards, strawberries, vegetables and rape seed) will not pose an unacceptable risk to wild mammals. While short-term effects on birds and wild mammals are not to be expected, possible long -term effects of vinclozolin on birds cannot be excluded. Accordingly, a refined risk assessment under the conditions of individual Member States is required.

Scientific Background on Which the Opinion is Based

The risk from the use of an active substance for birds and mammals is normally based on a risk quotient: the toxicity over exposure ratio (TER). The first step in the risk assessment is based on realistic worst case assumptions. When the TERs are above 10 (acute and short term risk assessment based on LD50[6] values or LC50[7] values) and/or above 5 (long term risk assessment based on NOEC values) the use of the active substance is considered to be safe. When the TERs are lower than 10 or 5 a refined risk assessment should be carried out.

1. BASIC DATA

1.1. Toxicity data:

LD50birds	> 2510 mg/kg body weight
LC50birds	> 5620 mg/kg food
NOECbirds	= 125 mg/kg food

LD50mammals	> 5620 (>5620, > 10000, >15000 and >15000) mg/kg BW
NOED[8]mammals	= 23 mg/kg BW per day (lowest value of 23 studies, see appendix)
	= 287.5 mg/kg food (rat BW = 250g and DFI = 20g)

[6] Lethal Dose, median
[7] Lethal Concentration, median
[8] No Observed Effect Dose

1.2 Application rate

Maximum application rates of vinclozolin are:
0.75 kg as/ha in orchards,
1.0 kg as/ha in vineyards, strawberries, vegetables and rape seed.

The highest residues values can be expected with an application rate of 1 kg as/ha in vineyards, strawberries, vegetables and rape seed.

1.3 Exposure data

Normally the Kenaga nomogram data (Hoerger and Kenaga, 1972) are used for the first step in the risk assessment. There are two different data sets, one for the maximum residues (realistic worst case) and one for "typical" residues (mean values). In Table 1 these data are provided for an application rate of 1 kg as/ha. Later it was proposed to use the data for small seed as surrogate for small insects (Kenaga, 1973). Recently data have become available indicating that such high concentrations on insects are over estimations of real values (Dave Fisher personal communication).

Table 1. Residues in mg as/kg food (ww[9]) that can be expected after an application of 1 kg as/ha.

Food items	Typical	Maximum
Short grass	112	214
Long grass	82	98
Leaves and leafy crops	31	112
Small seeds	29	52
Pods	2.7	11
Grains	2.7	8.9

1.4 Daily food intake

It can be assumed that small animals (under 100 g) may eat daily 30% of their body weight and larger animals may eat 10% of their body weight (both percentages are based on dw[10]).
It is also possible to use the Nagy relationships (Nagy, 1987) between daily food intake (DFI on dw base) and body weight (see EPPO vertebrate scheme which is more scientific - EPPO, 1994). The water content of some food items are presented in Table 2.

[9] wet weight
[10] dry weight

Table 2 Water contents (in percentages) of some food items (after Jongbloed et al. (1996))

Food items	Mean	Minimum	Maximum
Leaves	91.5	85	95.5
Seeds	24.4	6.3	87.6
Insects	71.1	51	87
Insect larvae	79.8	75	87

2. INITIAL RISK ASSESSMENT

2.1 Definition of a realistic worst case

A realistic worst case scenario for a bird would be a small bird (e.g.10 gram) consuming 30% (dw base) of its body weight as leaves or leafy crops. On ww base this corresponds to 120% of its body weight, taking into account a dw/ww conversion of 75%.
A realistic worst case scenario for a mammal would be a small mammal (e.g. 10 gram) consuming 30% (dw base) of its body weight as short grass. On ww base this corresponds to 300% of its body weight, taking in account a dw/ww conversion of 90%.
In practise (see vinclozolin monograph volume 3 annex b page 8-9) very often the typical values of Kenaga are used for the worst case and 30% of the body weight as the amount of food (not taking into account the dw and ww differences).

LD50(10 gram bird) = 2510/100 = 25.1 mg a.s.
LD50(10 gram mammal) = 5620/100 = 56.2 mg a.s.

DFI[11](10 gram bird) = 12 gram per day
DFI(10 gram mammal) = 30 gram per day

2.2. Risk assessment based on a realistic worst case (rwc)

TER (10 gram bird acute rwc) = 25.1 / (112 * 12 / 1000) = 18.6
TER(10 gram mammal acute rwc) = 56.2 / (214 * 30 / 1000) = 8.7

Based on relationships of Nagy the outcome of the TERs would be:
TER(10 gram bird acute rwc) = 25.1 / (112 * 11.2 / 1000) = 20
TER(10 gram mammal acute rwc) = 56.2 / (214 * 15.6 / 1000) = 16.8

TER(birds short term rwc) = 5620 / 112 = 50.2

The TERs for acute exposure (based on Nagy relationships) and short term exposure are above 10 and therefore no refined risk assessment is necessary.

TER(birds long term rwc) = 125 / 112 = 1.1
TER(mammals long term rwc) = 287.5 / 214 = 1.34

TER values are smaller than 5, a refined risk assessment is necessary

[11] Daily food intake

3. REFINED RISK ASSESSMENT

In the realistic worst case the maximum residue values of Kenaga were used for estimating the concentration on a certain food item and it was assumed that no dissipation of the active substance was taking place, that the animals were eating only one food type and that the they were always foraging at the same location. In a refined risk assessment one should reconsider all these assumptions.

3.1 Residues

Typical values according to Hoerger & Kenaga (1972) and Kenaga (1973) for small insects, small leaves/leafy crops and for short grass are respectively 29, 31 and 112 mg/kg food after an application of 1 kg active substance per hectare.
Recent literature (Fletcher et al., 1994; Pfleeger et al., 1996 and Brewer et al., 1997) proposes to use in the typical (mean) case for short grass and leafs/small seeds the values of 76, and 40, respectively, for an application rate of 1 kg as/ha.
Mean residue values provided by the applicant are 0.25 to 2.5 mg/kg food after application of 0.75 kg a.s./ha (equivalent to 0.33 to 3.3 for 1 kg a.s./ha), source SCP/VINCLO/018 and SCP/VINCLO/016. Other data show values in the range 0.3-11.4 mg/kg food (at day 0) and 0.2-6.2 (at day 7) and 0.2 (day 120 after application), source SCP/VINCLO/016.

3.2 Dissipation

- From the data in SCP/VINCLO/016 a DT50[12] of approximately 8-9 days can be derived.
- Residues on strawberries Annex B B.6 page 6-10
  80% after 10 days, 60% after 20 days, 46% after 24 days and 31% after 31 days.
  DT50 of ± 22 days
- Residues on/in peaches Annex B B.6 page 6-13 after application of 0.75 kg a.s./ha.
  After 2 hours 7.7 mg/kg, 3.1 mg/kg after 7 days and 2.4 mg/kg after 14 days.
  DT50 of ± 9 days
- Residues on/in lettuce Annex B B.6 page 6-15 after application of 1.12 kg a.s./ha.
  After 1 hour 3.87 mg/kg, 6 days 0.87 mg/kg, 12 days 0.09 mg/kg and 21 days 0.01 mg/kg.
  DT50 of ± 4.3 days

3.3 Food source

Most animals do not eat from a single source (e.g. only short grass). e.g. voles mainly eat short grass but also seeds and roots and sometimes insects. The diet of the skylark, for instance, at a certain time of the year consists of 20% insects, 20% seeds, 50% leaves and 10% earthworms.
Under realistic conditions however most animals (e.g. highly mobile birds) will certainly not exclusively feed on a contaminated diet only.

Therefore, it seems logical to use a less conservative value for the long term risk assessment, a mean exposure level of 4.7 mg/kg food seems more appropriate. This time weighted average value is derived by using the highest measured residue value in the field (leafy crop) of 11.2 mg/kg, a mean DT50 value of 9 days and an exposure period of 28 days.

In addition, it is assumed that for birds only half of their daily food is contaminated by vinclozolin. For mammals this assumption is not made because most of the mice and voles have small home ranges.

[12] Disappearance time for first 50% of compound

3.4 Risk assessment based on refined exposure estimates

For the typical case, a skylark of 40 gram is used for the risk assessment and a common vole of 30 g.
The DFI of the skylark is according to Nagy 9.15 g/day dw which is equivalent to 36.6 g/day ww (75% water content).
The DFI of the common vole is according to Nagy 3.85 g/day dw which is equivalent to 34.6 g/day ww (90% water content).

The NOECmallard = 125 mg a.s. / kg food
The NOEDmallard = 21.4 mg a.s. / bird per day (DFI = 171 g/bird per day; bw[13] is 1133 g)
The NOEDskylark = 0.75 mg a.s. / bird per day.
The NOECquail = 125 mg a.s. / kg food
The NOEDquail = 1.94 mg a.s. / bird per day (DFI = 15.5 g/bird per day; bw is 189 g)
The NOEDskylark = 0.41 mg a.s. / bird per day.

The NOEDrat = 23 mg a.s. / kg bw per day
The NOEDvole = 23 * 30 / 1000 = 0.69 mg a.s. / mammal per day

The skylark is exposed to 4.7 mg/kg food ww.
PEC[14]skylark = 36.6 * 4.7 / 1000 = 0.172 mg a.s. per day.

The common vole is exposed to 4.7 mg/kg food ww.
PECvole = 34.6 * 4.7 / 1000 = 0.163 mg a.s. per day

TER(birds long term rwc) = 0.41 / (0.172 / 2) = 4.77
TER(mammals long term rwc) = 0.69 / 0.163 = 4.2

The TER for birds is less than (but close to) 5. Due to the uncertainty for the toxicity (2 species tested) there is no justification to lower the uncertainty factor of 5. Therefore, possible long-term effects cannot be excluded.

The TER for mammals is below 5. In this case the data for the toxicity data are more extensive (23 tests, 4 species; see Table 3 of the appendix) which makes an uncertainty factor in the context of Annex VI of the Uniform Principles redundant. Therefore, long term effects for mammals are not expected.
In addition information from two field studies (see below) did not reveal biological significant effects on voles at an application rate of 5.04 kg a.s. per ha (5 times the application rate which is believed to be the appropriate one for this risk assessment).

3.5 Field study with mammals
In an article published by Caslin and Wolff (1999) two field studies with voles were described with an application rate of 12.2 l Curalan per ha (which is equivalent to 5.04 kg a.s./ha, data on application rate provided by Tracie Caslin (personal communication)). Their results revealed no biological significant effects of vinclozolin at the population level, but they do suggest that multiple applications or a higher application rate may have negative effects on male reproductive development and demography in wild populations.

[13] body weight
[14] Predicted Environmental Concentration

4. CONCLUSION

The TERs for acute and short term exposure in birds and mammals for realistic worst case scenarios are above 10, therefore no refined risk assessment is necessary. The TERs for chronic exposure (rwc) in birds and mammals are both smaller than 5 and therefore a refined risk assessment should be carried out. After a refined risk assessment for chronic exposure in birds, the TER is still below 5 and therefore possible long-term effects cannot be excluded. Further refined risk assessments (e.g. at a Member State level) are necessary. Options for such further refinement include:
(i) using exposure estimates under regional conditions (e.g. where application rates and residues may be different from those used here);
(ii) applying probabilistic risk assessment methods, once accepted methodology is available (e.g., results of the upcoming SETAC workshop on higher-tier risk assessment for birds and mammals).
The chronic TER after a refined risk assessment for mammals is above 1 and no long-term effects are expected. The outcome of the refined risk assessment for mammals is confirmed by the field studies with voles (no effects when applied at a rate of 5.04 kg a.s./ha.).

REFERENCES

Brewer, L.W., J.P. Sullivan, J.M. Akins, L.K. Kamiri and E.M. Mihaich (1997) Measured pesticide residues on insects in relation to standard EPA estimates. Platform presentation to the Society of Environmental Toxicology and Chemistry 18th annual meeting, San Francisco, USA

Caslin, T.M. and J.O. Wolff (1999) Individual and demographic responses of the gray-tailed vole to vinclozolin. Environ. Toxicol. Chem. 18: 1529-1533.

EPPO (1994) Decision-making scheme for the environmental risk assessment of plant protection products, Chapter 11 Terrestrial vertebrates. European and Mediterranean Plant Protection Organization (EPPO) Bulletin 24: 37-87.

Fletcher, J.S., J.E. Nellessen and T.G. Pfleeger (1994) Literature review and evaluation of the EPA food-chain (Kenaga) nomogram, an instrument for estimating pesticide residues on plants. Environmental Toxicology and Chemistry, vol. 13, no. 9, pp. 1383-1391.

Hoerger, F.D. and E.E. Kenaga (1972) Pesticides residues on plants, correlation of representative data as a basis for estimation of their magnitude in the environment. Environmental Quality. Academic Press, New York, I: 9-28.

Jongbloed, R.H., T.P. Traas, and R. Luttik (1996) A probabilistic model for deriving soil quality criteria based on secondary poisoning of top predators. II Calculations for Dichlorodiphenyltrichlorethane (DDT) and Cadmium. Ecotox. Environ. Saf. 34, 279-306.

Kenaga, E.E. (1973) Factors to be considered in the evaluation of toxicity of pesticides to birds in their environment. Environmental Quality and Safety. Academic Press, New York, II:166-181.

Nagy, K.A. (1987) Field metabolic rate and food requirement scaling in mammals and birds. Ecological Monographs, 57(2): 111-128.

Pfleeger, T.G., A. Fong, R. Hayes, H. Ratsch and C. Wickliff (1996) Field evaluation of the EPA (Kenaga) nomogram, a method for estimating wildlife exposure to pesticide residues on plants. Environmental Toxicology and Chemistry, vol. 15, no. 4, pp. 535-543.

Appendix

Mammalian toxicity data for vinclozolin.

Table 3 summarises the toxicity data of 23 studies with 4 different species.

Table 3 Mammalian toxicity; effects on growth, reproduction, survival of vinclozolin

Study type	route	species	NOAEL[15]	LOAEL[16]
Subchronic study	oral	rat	> 210 mg/kg bw	--
Subchronic study	oral	rat	>4 mg/kg bw.	--
Subchronic study	oral	rat	> 44.4 mg /kg bw.	--
Subchronic study	oral	rat	33.3 mg/kg bw.	100 mg/kg bw.
Subchronic study	oral	rat	390 mg/kg bw.	770 mg/kg bw.
Subchronic study	oral	rat	> 940 mg/kg bw.	--
3-6 month dog	oral	dog	> app. 80 mg/kg bw.	--
12 month dog	oral	dog	> 48.7 mg/kg bw.	--
long-term tox/carc	oral	rat	23 mg/kg bw.	71 mg/kg bw.
long-term tox/carc	oral	rat	> 23 mg/kg bw.	--
long-term tox/carc	oral	mouse	24.6 mg/kg bw.	492 mg/kg bw.
Reproduction	oral	rat	> 150 mg/kg bw.	--
Reproduction	oral	rat	> 200 mg/kg bw.	--
Reproduction	oral	rat	> 400 mg/kg bw.	--
Reproduction	oral	rat	< 600 mg/kg bw.	--
Reproduct/teratol.	oral	rabbit	300 mg/kg bw.	--
Reproduct/teratol.	oral	rabbit	300 mg/kg bw.	900 mg/kg bw.
Reproduct/teratol.	oral	rabbit	200 mg/kg bw.	800 mg/kg bw.
Reproduct/teratol.	oral	rabbit	< 400 mg/kg bw.	--
Reproduct/teratol.	oral	rat	104 mg/kg bw.	--
Reproduct/teratol.	oral	rat	29 mg/kg bw.	290 mg/kg bw.
Reproduct/teratol.	oral	rat	> 4.1 mg/kg bw.	--
Reproduct/teratol.	oral	mouse	100 mg/kg bw.	1000 mg/kg bw.

ACKNOWLEDGEMENTS

The Committee wishes to acknowledge the contribution of the following working groups that prepared the initial draft opinion:

Environmental Assessment: Professor A Hardy (Chairman), and Committee Members Mr H. Koepp, Dr. H. G. Nolting, Professor A. Silva Fernandes and Dr. T. Sherratt and invited experts Dr. V. Forbes, Dr. J. Boesten, Dr. A. Carter, Dr R. Luttik.

[15] No Observed Adverse Effect Level
[16] Lowest Observable Adverse Effect Level