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SCIENTIFIC COMMITTEE ON PLANTS

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

(Opinion adopted on 31 January 2002)

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A. TITLE

OPINION OF THE SCIENTIFIC COMMITTE ON PLANTS ON SPECIFIC QUESTIONS FROM THE COMMISSION CONCERNING THE EVALUATION OF IPRODIONE IN THE CONTEXT OF COUNCIL DIRECTIVE 91/414/EEC
(Opinion expressed by the Scientific Committee on Plants, 31 January 2002)

B. TERMS OF REFERENCE

The Scientific Committee on Plants (SCP) is requested to respond to the following questions in the context of the Commission's work on the implementation of Council Directive 91/414/EEC concerning the placing of plant protection products on the market:

1. After a thorough assessment of all available information the Rapporteur Member State (RMS) concluded that sufficient information is available to reliably estimate the Predicted Environmental Concentrations in soil (PEC_s) and groundwater (PEC_{gw}). Can the Committee comment on this assessment?

2. Can the Committee comment on the Acceptable Operator Exposure Level (AOEL) selected?

C. OPINION OF THE COMMITTEE

Opinion on question 1:

The Committee agrees that sufficient information is available to reliably estimate the Predicted Environmental Concentrations in soil (PEC_s) for the parent compound. However, the Committee does not agree that sufficient information is available to estimate the PEC_s for the metabolite RP 30228 for soils with pH below 6. The Committee does not agree with the half-lives of iprodione and the metabolite RP 30228 that were used in the calculation of the PEC_s presented by the RMS and therefore made its own assessment based on realistic worst-case assumptions for iprodione and on worst-case assumptions for RP 30228. This resulted in a PEC_s of iprodione in topsoil of 43 mg/kg for soils with pH below 6 and in a PEC_s of 28 mg/kg for soils with pH above 6. The PEC_s of the metabolite RP 30228 was 58 mg/kg for soils with pH below 6. These PEC_s values as estimated by the Committee are higher than the PEC_s values estimated by the RMS.

The Committee does agree that sufficient information is available to reliably estimate the Predicted Environmental Concentrations in ground water (PEC_{gw}) for soils with pH above 6 but assessment of leaching for soils with pH values below 6 deserves further attention. The Committee does not agree with the half-life of iprodione used in the calculation of PEC_{gw} of the parent compound presented by the RMS and therefore made its own assessment as well for leaching. There is negligible risk of groundwater contamination for the parent compound for soils with pH above 6. However, for soils with pH below 6, leaching at concentration levels exceeding 0.1 μg/l may occur in some

realistic vulnerable situations. The risk of leaching of the metabolites 3,5-dichloroaniline and RP 30228 is negligible.

The Committee notes that the revision of PEC values may in turn require the respective risk assessments to be amended.

Opinion on question 2:

Available studies indicate that liver, the haematopoietic system, sex organs and adrenal glands are the target organs of iprodione toxicity in rodents and dogs. At high doses, iprodione also induces Leydig cell tumours in rats, through a non-genotoxic mechanism of carcinogenesis via inhibition of steroid/testosterone synthesis or secretion. In mice, iprodione induces hepatocellular carcinoma at very high doses. The overall examination of scientific evidence and toxicological data, leads to the conclusion that the observed tumours in rats and mice are not relevant for risk assessment in humans.

For the establishment of the Acceptable Operator Exposure Level (AOEL), the SCP believes that there is no need for a correction factor for oral absorption, nor for the use of an additional safety factor. The Committee is of the opinion that the No Observed Adverse Effect Level (NOAEL) for AOEL setting should be selected from the short-term study in rats, which is supported by data from the studies in dogs.

A. TITLE

REPORT OF THE SCIENTIFIC COMMITTEE ON PLANTS ON SPECIFIC QUESTIONS FROM THE COMMISSION CONCERNING THE EVALUATION OF IPRODIONE IN THE CONTEXT OF COUNCIL DIRECTIVE 91/414/EEC
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B. TABLE OF CONTENTS

C. BACKGROUND

Iprodione is an existing active substance in the context of Directive 91/414/EEC[1] 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[2].

In order to prepare its opinion, the Scientific Committee on Plants had access to documentation listed below, including a draft assessment report (monograph) prepared by

[1] OJ N° L 230, 19. 8.1991, p.1.
[2] Commission Regulation N° 933/94 of 27 April 1994 (OJ N° L 107 – 28. 4.1994, p. 8).

France as a Rapporteur Member State (RMS) on the basis of a dossier submitted by the notifier (Rhône-Poulenc Agrochimie now Aventis CropSciences).

Iprodione is a fungicide which is effective against a broad range of fungi (*Botrytis, Alternaria, Monilia, Helminthosporium, Rhizoctonia,* etc.). It is used to control diseases in a wide range of crops such as vineyard, fruit, vegetable, cereals, ornamentals, etc.

Following the peer review process, two questions remained unresolved. The first one deals with the estimation of the predicted environmental concentration in soil and ground water (in particular PEC_s estimated from US field studies). The other concerns the setting of the AOEL (choice of the NOAEL and the use of a correction factor for absorption as well as the use of an additional safety factor).

Source documents made available to the Committee:

1. Iprodione: Terms of reference, submitted by DG Health and Consumer Protection, 14 November 2000 (SCP/IPRODIO/001).

2. Iprodione: Evaluation table Doc. 5037/VI/98 rev. 6 (18.05.00), submitted by DG Health and Consumer Protection, 14 November 2000 (SCP/IPRODIO/003).

3. Iprodione: Addendum to the Monograph Ecotoxicology submitted by DG Health and Consumer Protection, 14 November 2000 (SCP/IPRODIO/004).

4. Iprodione: Addendum to the Monograph Fate and Behaviour Metabolite 3,5-dichloroaniline (RP32596), submitted by DG Health and Consumer Protection, 14 November 2000 (SCP/IPRODIO/005).

5. Iprodione: Addendum to the Monograph Fate and Behaviour, submitted by DG Health and Consumer Protection, 14 November 2000 (SCP/IPRODIO/006).

6. Iprodione: List of intended uses, submitted by DG Health and Consumer Protection, 14 November 2000 (SCP/IPRODIO/007).

7. Iprodione: Belgium comments on Mammalian toxicology, submitted by DG Health and Consumer Protection, 14 November 2000 (SCP/IPRODIO/008).

8. Iprodione: German comments on Mammalian Toxicology, submitted by DG Health and Consumer Protection, 14 November 2000 (SCP/IPRODIO/009).

9. Iprodione: Danish comments on AOEL, submitted by DG Health and Consumer Protection, 14 November 2000 (SCP/IPRODIO/010).

10. Iprodione: UK comments on field dissipation submitted by DG Health and Consumer Protection, 14 November 2000 (SCP/IPRODIO/011).

11. Iprodione: France response to UK comments (field dissipation), submitted by DG Health and Consumer Protection, 14 November 2000 (SCP/IPRODIO/012).

12. Iprodione: Draft assessment report (Monograph) prepared by France as Rapporteur Member State, (Volumes 1 to 3), June 1996.

13. Iprodione: Updating of Draft assessment report (Monograph) prepared by France as Rapporteur Member State, February 1998.

14. Evaluation table Doc. 5037/VI/98-rev0, prepared by the RMS (21 May 1998) (SCP/IPRODIO/013).

15. Aventis position paper: Definition of the Short Term Systemic Acceptable Operator Exposure Level (AOEL), A Percy & P Fisher, 11 October 2001 (SCP/IPRODIO/014).

16. Broadmeadow A. *et al.*, 1985: Iprodione: 52-week toxicity study in dietary administrition to beagle dogs – LSR Report N° RNP 346/RHO 022/24 (Feb. 1985). Property of Aventis CropSciences.

17. Coquet C., 1973: 3-month study of toxicity of 26019 RP orally in the dog – IFFA CREDO Report N° DREB-R 731008 (Oct. 1973). Property of Aventis CropSciences.

18. Kangas L., 1991: A 52-week dietary study of iprodione in the Beagle dog. Y Bio Research Laboratories. Report N° 84296 (Dec. 1991). Property of Aventis CropSciences.

D. SCIENTIFIC BACKGROUND ON WHICH THE OPINION IS BASED

I. Question 1

After a thorough assessment of all available information the Rapporteur Member State (RMS) concluded that sufficient information is available to reliably estimate the Predicted Environmental Concentrations in soil (PEC_s) and groundwater (PEC_{gw}). Can the Committee comment on this assessment?

Opinion of the Committee:

The Committee agrees that sufficient information is available to reliably estimate the PEC_s for the parent compound. However, the Committee does not agree that sufficient information is available to estimate the PEC_s for the metabolite RP 30228 for soils with pH below 6. The Committee does not agree with the half-lives of iprodione and the metabolite RP 30228 that were used in the calculation of the PEC_s presented by the RMS and therefore made its own assessment based on realistic worst-case assumptions for iprodione and on worst-case assumptions for RP 30228.

This resulted in a PEC_s of iprodione in topsoil of 43 mg/kg for soils with pH below 6, and in a PEC_s of 28 mg/kg for soils with pH above 6. The PEC_s of the metabolite RP 30228 was 58 mg/kg for soils with pH below 6 (assuming a worst case scenario of no transformation in soil). These PEC_s values as estimated by the Committee are higher than the PEC_s values estimated by the RMS.

The Committee does agree that sufficient information is available to reliably estimate the PEC_{gw} for soils with pH above 6 but assessment of leaching for soils with pH values below 6 deserves further attention. The Committee does not agree with the half-life of iprodione used in the calculation of PEC_{gw} of the parent compound presented by the RMS and therefore made its own assessment as well for leaching. There is negligible risk of groundwater contamination for the parent compound for soils with pH above 6. However, for soils with pH below 6, leaching at concentration levels exceeding 0.1 µg/l may occur in some realistic vulnerable situations. The risk of leaching of the metabolites 3,5-dichloroaniline and RP 30228 is negligible.

The Committee notes that the revision of PEC values may in turn require the respective risk assessments to be amended.

Scientific background on which the opinion is based:

General comment: the Committee notes that, in a number of cases, information relevant for the interpretation of the data was not reported in the monograph (see below for details).

I.1 Soil metabolism and degradation rates in aerobic laboratory studies

Half-lives of iprodione in aerobic soil laboratory studies at 25°C ranged from 7 to more than 70 days. At low pH the degradation rate was much slower than at high pH. In soils with pH values of 5.7 and higher, repeated addition of the test substance enhanced the degradation rate. Repeated addition did not enhance the degradation rate in two soils with pH values of around 5. Degradation studies showed in total 12 soil metabolites. Four of these were found in amounts above 4% of applied radioactivity for at least one of the soils that were studied:

- RP 30228 (an isomer of iprodione) at 6, 31 and 54% in three soils;
- RP 36221 up to 17%;
- 3,5-dichloroaniline up to 9% in soil metabolism studies but probably close to 100% if enhanced degradation occurs;
- RP 25040 up to 8% in one soil.

In aged-leaching studies with three top soils (30 days incubation and 8-10 days leaching), 3,5-dichloroaniline was found in soil at a maximum level of 22% of applied radioactivity and RP 30228 at 19%. In an aged-leaching study with a sand with only 0.2% organic matter, 27.1% of RP 35606 was found. Significant amounts of non-extractable residues

were formed (24 and 76% for ^{14}C-phenyl labelled iprodione in two soils). Formation of CO_2 from the phenyl ring was in one study 5% after 276 days.

The transformation rate of 3,5-dichloroaniline was studied in aerobic laboratory studies with two top soils (a silt loam and a sandy loam, pH values not reported in the addendum to the monograph) at 25°C. The half-lives were 6 and 17 days. After three and nine months of incubation, soil bound residues (^{14}C) ranged between 61 and 77%.

The transformation rate of RP 30228 was studied in aerobic laboratory studies with three soils (a clay loam, a sandy loam and a sand, pH values not reported in the addendum to the monograph) at 25°C. After 100 days more than 90% of the applied amount of RP 30228 was recovered as RP 30228. So the half-life was much longer than 100 days in these studies.

I.2 Rate of hydrolysis in water

Hydrolysis studies in aqueous buffers at 25°C showed that the half-life of iprodione decreased from 149 days at pH=5 to 3 days at pH=7. The transformation products were RP 35606 (maximum of 35% after 2 days at pH=7) and RP 30228 (about 95% after 32 days at pH=7). Hydrolysis studies at 50°C with RP 30228 showed that this compound was stable at pH=4 and pH=7 and was rapidly transformed at pH=8 (half-life of 1.8 day).

I.3 Sorption and mobility

Sorption studies with iprodione and three arable top soils with pH values of 6.0-6.1 resulted in K_{OC}[3] values of 223 to 543 L/kg. For the metabolite RP 30228 K_{OM}[4] values of 3800 to 30 000 l/kg were found for three soils with pH values of 5.2-7.5. Sorption of the metabolite 3,5-dichloroaniline was studied in four arable soils (pH values not reported the in addendum to the monograph) and K_{OC} values of 380 to 930 L/kg were found.

Aged-residue leaching studies with four agricultural top soils showed that the average penetration depth of the metabolites 3,5-dichloroaniline, RP 36221 and RP 30228 was less than 6 cm after percolation of 51 cm of water. However, these results cannot be interpreted in terms of sorption coefficients because the distribution of the radioactivity across these metabolites at the start of the leaching period was not reported in the monograph.

In two field studies from the USA, contents of iprodione and the soil metabolites RP 30228 and RP 32490 were always below 0.01 mg/kg at depths greater than 30 cm. The Committee cannot interpret these results because the following items were not reported in the monograph: 1) total rainfall plus irrigation (the monograph states “heavy irrigation” and “above average rainfall”), 2) estimates of potential evapotranspiration, 3) average air temperature, 4) organic matter contents of the soils, 5) pH of the soils.

[3] Organic carbon adsorption coefficient.
[4] Organic matter adsorption coefficient.

I.4 Field persistence studies

In two field studies in the USA, the time needed for 50% disappearance of iprodione from soil was less than a month. At one site, the maximum amount of RP 30228 recovered from soil corresponded with 14% of the cumulative iprodione dose. The half-life of RP 30228 in soil was estimated to be 215 and 319 days at the two sites. These are the same studies as reported at the end of the previous section. The Committee cannot assess the relevance of these studies for EU agriculture mainly because the pH of the soils was not reported in the monograph.

I.5 Assessment of leaching to groundwater (PEC_{gw})

In an addendum to the monograph, a modelling study for iprodione was reported based on PELMO[5] and the German Borstel scenario. The half-life of iprodione in soil was 12 days which was considered to be a realistic worst case under multiple application conditions. The Committee does not accept this half-life because much longer half-lives have been reported for soils with pH values around 5 and because enhanced transformation resulting from repeated application has only been shown to occur in soils with pH values of 5.7 and higher.

The Committee performed PEC-groundwater calculations for the FOCUS[6] scenarios (FOCUS 2000) using the FOCUS PELMO v1.1.1 and FOCUS PEARL[7] v1.1.1 models considering only the parent compound. The calculations were based on the following input parameters:

– The K_{OC} of iprodione was 399 L/kg and the Freundlich exponent was 0.885 (both average from studies with three arable top soils).

– The half-life of iprodione in top soil at 20°C and matric pressure of -10 kPa was assumed to depend on the pH of the top soil of the corresponding FOCUS scenario. For scenarios with pH-H_2O below 6 (Okehampton and Porto), it is assumed that the transformation rate is slow as found for two soils with pH-values close to 5.0 by Walker *et al.* (1986) and Walker (1987). For these soils a half-life of 100 days was assumed (half-lives of >70 and >80 days were reported). For scenarios with pH-H_2O values above 6 (all remaining seven FOCUS scenarios), the half-life was derived from studies with soils with pH values above 6 (7 and 23 days at 25°C and 30 days at 20°C). Correcting for soil moisture and temperature resulted in half-lives of 9, 29 and 28 days. These values were averaged (22 days) and this was used as the half-life for soils with pH-H_2O values above 6. The effect of enhanced degradation was ignored.

– Iprodione was applied to soil at a rate of 7.5 kg/ha on 1st of May. The crop was turf and it was assumed that the crop parameters derived for grass by FOCUS (2000) could be used. This is the same crop-application scenario as used in the addendum to the

[5] Pesticide Leaching Model.
[6] Forum for the Co-ordination of pesticide models and their Use.
[7] Pesticide Emission Assessment at Regional and Local scales.

monograph with the only difference that one application was assumed per year to be consistent with the ignorance of enhanced degradation for the estimation of the half-lives.

Calculated 80th percentile groundwater concentrations of iprodione for all scenarios except Okehampton were less than 0.001 μg/l (both for PELMO and PEARL). For Okehampton, the PELMO calculation resulted in a concentration of 0.85 μg/L and the PEARL calculation resulted in 2.1 μg/L. These results indicate that the risk of iprodione leaching to groundwater is negligible for soils with pH values above 6 but that assessment of leaching to groundwater for soils with pH values below 6 deserves further attention.

The Committee did not include the 3,5-dichloroaniline and RP 30228 metabolites in the calculations because any leaching of these compounds is very unlikely in view of their strong sorption and of the comparatively fast transformation rate of 3,5-dichloroaniline.

I.6 Assessment of persistence in top soil (PEC_s)

As described before the persistence of iprodione depends strongly on the pH of the soil. In the calculation of the PEC_s of iprodione as reported in the monograph (p. 315) this pH dependency is ignored and the calculations are based on a half-life of 30 days for a single application and of 12 days for repeated application. The Committee made calculations for two situations: soils with pH below and above 6. It was assumed (as in the monograph) that iprodione was mixed over the top 5 cm of soil (with dry bulk density of 1.3 kg/L) and that it only disappeared from this layer via transformation. The realistic worst-case scenario for application was derived from an addendum of the monograph: four applications per year of 7.5 kg/ha at 14 days intervals in spring. A period of only one year was considered to be consistent with mixing over the top 5 cm. For longer periods mixing over a thicker layer can be expected which leads to lower contents. Therefore the one-year period is a better assumption for a realistic worst-case scenario. For soils with pH below 6 a half-life of 100 days at 20^{o}C was used and for soils with pH above 6 a half-life of 11 days at 20^{o}C was assumed (50% of the half-life calculated in Section I.5 to account for the effect of repeated applications). The persistence was evaluated assuming an average soil temperature of 10^{o}C so the half-lives were multiplied by 2.2 to account for the effect of temperature. These assumptions resulted in a maximum content of iprodione in the top 5 cm of 43 mg/kg for soils with pH below 6 and of 28 mg/kg for soils with pH above 6.

In an addendum to the monograph, the PEC_s of the metabolite RP 30228 was also estimated (this is relevant because of the long half-life of this compound). In this calculation a DT_{50}[8] of 319 days was assumed (taken from the USA field studies described in Section I.4). Given the limited information provided about the USA field studies and the discrepancy between this DT_{50} and the results of laboratory studies with three soils (see Section I.1), it is not defensible to extrapolate this DT_{50} to European field conditions.

[8] Period required for 50% dissipation.

For instance no pH was provided whereas hydrolysis studies at 50°C have shown that the half-life in water decreases strongly between pH 7 and 8 (very long at pH 7 and only 1.8 day at pH 8). The interpretation of the USA field results may therefore depend on whether the pH was far below 7 or not. The Committee made PEC_s calculations for RP 30228 considering only soils with pH below 6. At higher pH values, enhanced transformation is expected to occur with probably close to 100% formation of 3,5-dichloroaniline which has a short half-life. It was assumed that 50% of the iprodione molecules transform directly into RP 30228 and that RP 30228 does not disappear from the top 20 cm of soil which are worst-case assumptions. It was assumed that iprodione was applied each year over a period of 10 years. The scenario was the same as the one used for the PEC_s calculation of iprodione with the exception that a soil layer of 20 cm was considered instead of 5 cm in view of the period of 10 years. These assumptions resulted in a maximum content of 58 mg/kg of RP 30228 for the top 20 cm layer.

I.7 Conclusions

The Committee agrees that sufficient information is available to reliably estimate the PEC_s for the parent compound. However, the Committee does not agree that sufficient information is available to estimate the PEC_s for the metabolite RP 30228. The Committee does not agree with the half-lives of iprodione and the metabolite RP 30228 that were used in the calculation of the PEC_s presented by the RMS and therefore made its own assessment based on realistic worst-case assumptions for iprodione and on worst-case assumptions for RP 30228. This resulted in a PEC_s of iprodione in topsoil of 43 mg/kg for soils with pH below 6 and in a PEC_s of 28 mg/kg for soils with pH above 6. The PEC_s of the metabolite RP 30228 was 58 mg/kg for soils with pH below 6. These PEC_s values as estimated by the Committee are higher than the PEC_s values estimated by the RMS.

The Committee does agree that sufficient information is available to reliably estimate the PEC_{gw} for soils with pH above 6 but assessment of leaching for soils with pH values below 6 deserves further attention. The Committee does not agree with the half-life of iprodione used in the calculation of PEC_{gw} presented by the RMS and therefore made its own assessment as well for leaching. There is negligible risk of groundwater contamination for the parent compound for soils with pH above 6. However, for soils with pH below 6, leaching at concentration levels exceeding 0.1 µg/l may occur in some realistic vulnerable situations. The risk of leaching of the metabolites 3,5-dichloroaniline and RP 30228 is negligible.

The Committee notes that the revision of PEC values may in turn require the respective risk assessments to be amended.

II. Question 2:

Can the Committee comment on the Acceptable Operator Exposure Level (AOEL) selected?

Opinion of the Committee:

Available studies indicate that liver, the haematopoietic system, sex organs and adrenal glands are the target organs of iprodione toxicity in rodents and dogs. At high doses, iprodione also induces Leydig cell tumours in rats, through a non-genotoxic mechanism of carcinogenesis via inhibition of steroid/testosterone synthesis or secretion. In mice, iprodione induces hepatocellular carcinoma at very high doses. The overall examination of scientific evidence and toxicological data, leads to the conclusion that the observed tumours in rats and mice are not relevant for risk assessment in humans.

For the establishment of the AOEL, the SCP believes that there is no need for a correction factor for oral absorption, nor for the use of an additional safety factor. The Committee is of the opinion that the NOAEL for AOEL setting should be selected from the short-term study in rats, which is supported by data from the studies in dogs.

Scientific background on which the opinion is based:

II. 1 Assessment of toxicological data

II.1.1 *Toxicokinetics, metabolism, and correction for bioavailability*

A correction factor of 60% for oral absorption was applied by the RMS (SCP/IPRODIO/013, p. 2) to the NOAEL[9] used for AOEL setting, on the basis of data on urinary excretion from three oral toxicokinetics studies in rats.

In the first study, radioactivity was rapidly excreted via urine (62% of dosed radioactivity), and faeces (36%) (Laurent M. *et al*. 1976 – monograph Annex III p. 80). More than 20 metabolites were detected in urine, and also in faeces, thus indicating that the compound is extensively metabolised after absorption. Only 10% of dosed radioactivity was excreted in faeces as unchanged iprodione, more than 12% being detected in 26 metabolites. In the second study, following repeated dosing at 50 mg/kg, 71.1% of the administered dose was considered as absorbed, based on the sum of radioactivity in urine, cage wash and tissues (Hallifax, D. *et al*. 1989 – monograph Annex III p. 80). This amount does not take into account the radioactivity eliminated via faeces (24% of dose), both as unchanged iprodione and a number of metabolites (about 12.5% of dosed radioactivity). Therefore, both studies would indicate an oral absorption of 80-90%.

[9] No Observed Adverse Effect Level.

A different picture was reported in the third study (Souza, G.A.D. 1993 – monograph Annex III p. 80), where only 40% of the administered dose (50 mg/kg) was excreted in urine, and 53% in faeces, a high proportion of this being unchanged iprodione. The low oral absorption observed in this study, was reported to be explained by the larger particle size in the dose suspension. Therefore the Committee is of the opinion that this study should not be taken into consideration for the estimation of the oral absorption rate.

The observation that iprodione metabolites in faeces are the results of absorption and metabolism is also supported by a percutaneous study (Laurent M. *et al*. 1983 – monograph Annex III p. 81). In this study, the absorbed dose (only 0.65% of the applied dose) was excreted mainly via urine (0.2%), but also in faeces (0.1%), where two metabolites were detected in addition to iprodione.

Due to the lack of data on biliary excretion in the metabolism studies, the absorption rate cannot be accurately established. In addition, metabolism studies were not performed in the dog. The Committee concluded that 80-90% of the orally administered iprodione is likely to be absorbed in the rat and there is no reason for applying a correction factor for absorption, when setting the AOEL.

II. 1.2 *Short-term toxicity*

Short-term oral toxicity was studied in 90-day experiments in rats and dogs. Two further studies were performed in mice, but will not be considered since they provide the highest NOAELs.

The effects on the adrenals (adrenal enlargement, vacuolisation of zona glomerulosa and fasciculata) are the critical effects in the 90-day rat study, with a NOAEL at 500 ppm, equivalent to 30.8 mg/kg bw for males, and 35.8 mg/kg bw for females (Bigot, 1997 – Addendum to the monograph, Feb 1998 p. 4). Other effects of iprodione toxicity appear also in liver (increased liver weight, hepatocyte enlargement) and sex organs (decreased uterus and ovary weights, atrophic changes of uterus and reduced number of corpora lutea, prostate atrophy, Leydig cell hyperplasia) at higher doses (2000 and 3000 ppm).

In the 13-week dog study, groups of 4 Beagle dogs were given diets containing iprodione at 0, 800, 2400, 7200 ppm (Coquet, 1973 - monograph Annex III p. 97). Small islands of hypervacuolated spongiocytes in parts of the zona glomerulosa of the adrenals were observed in one dog per treated group. Testicular and prostatic hypoplasia and atrophy were reported only in one dog at 800 ppm, and one dog at 7200 ppm. Biliary pigments in urine were reported in one dog per treated group. Congestion of mesenteric lymph nodes was observed in 2 animals at 2400 ppm. Other effects reported at 7200 ppm were: slight increase in liver weight, increased plasma alkaline phosphatase activity, transient decrease in alanine aminotransferase and aspartate activities, slight anaemia (2 dogs). The notifier proposed a NOAEL of 67.8 mg/kg bw (2400 ppm), but a lower NOAEL of 18 mg/kg bw (800 ppm), was established during the ECCO[10] peer review.

[10] European Commission Co-ordination.

The establishment of this NOAEL (18 mg/kg bw) is questionable because the NOAEL was based on the observations of biliary pigments in urine in one dog and congestion of mesenteric lymph nodes in 2 animals, at 2400 ppm. The toxicological significance of these findings, as well as those on testes and prostate, are equivocal given also the results of the more recent long-term dog studies described below.

II.1.3 *Long-term toxicity and carcinogenicity*

The chronic toxicity of iprodione has been studied in dietary experiments in mice (1 two-year test), rats (2 two-year tests) and dogs (2 one-year tests).

In the first dog study (Broadmeadow A., 1984/85 – monograph Annex III p. 104), Beagle dogs (6/sex/group) were given diets containing iprodione for 1 year (0, 100, 600, and 3600 ppm). Administration of 600 ppm, was associated with slight adverse effects (slight and transient increase in Heinz bodies, lower prostate weight, slight microscopic changes of adrenals and kidneys, slight and occasional retinal hyper-reflection). Microscopic changes in the adrenals were also reported in some dogs in the control group. The highest dose (3600 ppm) induced haematological changes (decreased RBC[11], Hb[12], Ht[13], increased platelet count and partial thromboplastin time, and transient increase of Heinz bodies). Effects on liver, prostate and the adrenal glands were also observed at the highest dose (reduced prostate weight, increased liver and adrenals weight, and microscopic changes of the adrenal glands). It was concluded that 600 ppm (25 mg/kg bw) represented a minimal toxic effect level, while the NOAEL was established at 100 ppm (4.2 mg/kg bw).

In order to establish a clear NOAEL between 100 and 600 ppm, a second 1-year study was performed (0, 200, 300, 400, 600; 6 dogs/sex/dose) (Kangas L., 1991 – monograph Annex III p. 106). Administration of 600 ppm was only associated with slight and occasionally significant reductions in RBC count, Hb, and Ht. However, since the haematological values recorded before treatment initiation in these animals were very similar to those observed during the study, these changes were not considered as treatment-related. No histopathological findings were reported, with the exception of mild multifocal cortical vacuolisation in the adrenal glands observed at the highest dose only in one animal, which was considered incidental and unrelated to the treatment. No effects on prostate weight, nor ocular effects were noted at any time during the study. A NOAEL was established at 400 ppm (17.5 mg/kg bw in males, and 18.4 in females), taking into account the results of the two 1-year studies.

The Committee is of the opinion that this NOAEL is rather conservative. In fact, the transient haematological changes observed by Broadmeadow *et al*. at 600 ppm (LOAEL[14]=25 mg/kg), were not confirmed by Kangas in the second study. Therefore, the true NOAEL in dog could be higher than 25 mg/kg bw (600 ppm).

[11] Red Blood Cells.
[12] Haemoglobin.
[13] Hematocrite.
[14] Lowest Observable Effect Level.

In Sprague Dawley rats, non-neoplastic findings were reported in testes, adrenal glands, prostate, spleen, and thyroid (increased testes, thyroid and adrenal weights, interstitial cell hyperplasia and atrophy of seminiferous tubules, etc.). Statistically significant neoplastic changes found in the rat study consisted of an increased incidence of testicular interstitial cell tumours at 1600 ppm (29/60) compared with the control (3/60) (Chambers P.R., 1992 – monograph Annex III p. 108). It was also shown that the incidence of Leydig cell tumours in the 150 ppm and 300 ppm falls in the range of historical control group. The NOAEL for carcinogenic effects was established at 300 ppm (14.5 mg/kg bw), and the NOAEL for toxicity at 150 ppm (7.25 mg/kg bw).

In CD-1 mice, non neoplastic changes were reported in testes, ovaries, liver, stomach, spleen and kidney at both 800 and 4000 ppm (Chambers P.R., 1993 - monograph Annex III p. 112, and SCP/IPRODIO/014). Statistically significant increases in benign and malignant hepatocellular neoplasms were observed in both sexes in the 4000 ppm group (females 21/50, males 26/50) compared with the controls (females 2/50, males 7/50). In addition, there was an increased incidence of benign luteomas in the ovaries in the same group (5/50) compared with the controls (0/50). At this dose level there was a significant decrease in body weight gain in both sexes. The NOAEL for carcinogenic effects was considered to be 800 ppm (125 mg/kg bw), while the NOAEL for toxicity was established at 160 ppm (25 mg/kg bw).

II.1.4 *Genotoxicity*

Iprodione has been tested for genotoxic activity in a suitable range of *in vivo* and *in vitro* assays that have produced mainly negative results. A positive result was obtained in a single bacterial assay for DNA damage (*Bacillus subtilis* spot test, Felkner, 1985 – monograph Annex III p. 102), while all other tests, including the DNA damage assay in mammalian cells were negative.

II.1.5 *Reproductive toxicity and teratogenicity*

Results of a two-generation reproductive study in rats indicate that iprodione is not toxic to reproduction. No effects on reproductive performance were observed at dietary concentrations up to 3000 ppm. Evidence of parental toxicity was observed at 1000 ppm. Effects on pup viability and pup weight were noted at 2000 and 3000 ppm. Results of two teratogenicity studies in rat and rabbit indicate that iprodione has no teratogenic properties at dose levels up to 200 mg/kg bw.

II.1.6 *Mechanistic studies*

Since iprodione is not genotoxic, a possible non-genotoxic mechanism of Leydig cell carcinogenesis was investigated. It was suggested that iprodione could possibly interfere with the regulation of sex hormones, since it had already been demonstrated that some structural analogues possess anti-androgenic potential (the fungicides vinclozolin, procymidone, and the antiandrogenic drug flutamide).

Two modes of action have been suggested:

1) inhibition of androgen biosynthesis by an inhibitory effect on Leydig cell enzymes, resulting in a reduced concentration of circulating hormones, or

2) competitive inhibition of the androgen-binding sites at the receptor level of the target organs (as with the structurally similar fungicide vinclozolin), which would prevent the physiological effect of the androgen.

Both modes of action act through the hypothalamus-pituitary axis to increase the synthesis and release of LH, which in turn stimulates the Leydig cells to produce testosterone. The sustained stimulation of these cells results in compensatory hyperplasia. Tumours may develop in sensitive species, particularly in rats, due to the persistent hyperplasia.

An *in vitro* receptor-binding study (Fail *et al.,* 1994 – monograph Annex III p. 121) revealed that neither iprodione nor its major metabolites bind to the androgen receptor, whereas three minor metabolites (RP25040, RP36112 and RP36115) did show such activity, although less so than the reference compound, flutamide. Two other *in vitro* studies (Benhamed, 1995 – monograph Annex III p 121; Benhamed, 1996 – Addendum to the monograph, Feb 1998, p. 13) showed that iprodione and two minor metabolites (RP36112 and RP36115) inhibit testosterone secretion in cultured Leydig cells, by interfering at the level of cholesterol transport (parent compound and RP36115) and/or with certain steroidogenic enzyme activity (RP36112). This inhibitory effect is reversible. Increases in LH and FSH levels following 14/15 days of iprodione treatment were reported in rat *in vivo* studies (Fail *et al.,* 1994 – monograph, Annex III, p. 121). The involvement of the adrenal glands, evidenced by the results of *in vivo* studies in rats, supports the assumption that iprodione interferes with the biosynthesis of steroid hormones. Data from subchronic and chronic toxicity studies in rodents and dogs show that several target organs (adrenal glands, testes, and ovaries) are tissues which secrete steroid hormones. The effects observed in these organs are likely to be related to an inhibition of steroidogenesis.

The available evidence leads to the conclusion that iprodione and its minor metabolites RP36112 and RP36115 act essentially as inhibitors of steroid/testosterone secretion. This proves consistent with the moderate and transient change in hormone levels, since a similar transient effect was also described in the literature for other inhibitors of testosterone secretion, such as ketoconazole (Heckman W.R., 1992). The absence of effects of iprodione on reproduction appears to be consistent with the fact that plasma concentrations of testosterone do not change following iprodione treatment, and this is likely to result from the hormonal homeostasis.

II.1.7 *Relevance of carcinogenic effects to humans*

A number of reviews on LCTs[15], in rodents have been published in recent years, to assess the relevance of this type of tumours in humans (Cook *et al*., 1999; Clegg *et al*., 1997; Prentice *et al.*, 1995).

Prentice *et al*., in 1995, concluded that drug-induced LCTs in rats are most probably not predictive for man, and their occurrence has little relevance in human safety assessment.

Cook *et al.*, in 1999, highlighted different plausible mechanisms for chemical induction of LCTs, among them the inhibition of testosterone synthesis and disruption of the hypothalamo-pituitary-testes axis. The incidence of LCTs in humans is much lower than in rodents, and particularly the rat has a much greater susceptibility to both spontaneous and chemical-induced LCTs. Moreover, human epidemiological studies are available for a number of chemicals that induce LCTs in rats (ethanol, cadmium, lead, nicotine, lactose and trichloroethylene) showing no association with induction of Leydig cell hyperplasia or adenomas in man (Cook *et al*., 1999).

Given this background, little if any significance for human risk assessment can be given to an excess of LCTs in rats alone at a very high dose.

Iprodione administration elicited hepatocellular tumours in mice. Since these tumours occurred at a dose level at which the MTD[16] was exceeded (body weight gain was severely reduced) and appeared to be secondary to hepatic toxicity, it is concluded that these tumours in mice are not relevant for risk assessment in humans.

II.2. Conclusions for the establishment of the AOEL

During the ECCO peer review, a systemic AOEL of 0.18 mg/kg bw/day was set, based on the NOAEL of 30.8 mg/kg bw in the 90-day study in rat (corrected for 60% bioavailability), and a safety factor of 100.

The Committee recommends to consider the following elements for establishing the AOEL:

– The overall evidence of toxicokinetics data indicate that iprodione absorption rate is likely to be 80-90% following oral administration, and that there is no basis for applying a correction factor for oral absorption.

– Leydig cell tumours were only observed at high doses in rats and hepatocellular tumours at high doses in mice.

  The use of an additional safety factor to account for irreversible effects occurring in the carcinogenicity studies is not justified, because the overall scientific evidence

[15] Leydig Cell Tumours.
[16] Maximum Tolerated Dose.

supports the conclusion that the tumours observed in rats and mice are not relevant for risk assessment in humans.

- For the establishment of the AOEL, the NOAEL should be selected from the short-term rat study (30.8 mg/kg bw) which is supported by data in dogs indicating a NOAEL likely to be between 25 mg/kg bw and 67.8 mg/kg bw.

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