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SCIENTIFIC COMMITTEE ON PLANTS **SCP/IPROVA-BIS/002-Final**
28 November 2001

OPINION OF THE SCIENTIFIC COMMITTEE ON PLANTS ON AN ADDITIONAL QUESTION FROM THE COMMISSION ON THE EVALUATION OF IPROVALICARB [SZX 0722] IN THE CONTEXT OF COUNCIL DIRECTIVE 91/414/EEC

(Opinion adopted by the Scientific Committee on Plants on 8 November 2001)

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A. TITLE

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TERMS OF REFERENCE

"Given that the SCP cannot rule out the relevance to humans of the tumours observed in rats following iprovalicarb treatment, does the Committee consider that a sufficient Margin of Safety exists having regard to the human exposure likely to arise from the intended use?"

C. OPINION OF THE COMMITTEE

In its initial evaluation of iprovalicarb, the Committee concluded that the data set presented could not rule out the relevance to humans of the tumours observed in rats. Iprovalicarb acts by an unknown, but non-genotoxic mechanism that has no counterpart in mice, therefore toxicity has a threshold dose and there is a level of exposure below which the probability of adverse effects is negligible. The Committee considers that a sufficient Margin of Safety exists having regard to the consumers' exposure likely to arise from the intended use of iprovalicarb. In the case of operators, a sufficient Margin of Safety only exists when operators wear gloves.

Because the term "Margin of Safety" is used in the literature with various meanings, the Committee is of the opinion that the European Commission should establish a formal definition of Margin of Safety.

A. TITLE

REPORT OF THE SCIENTIFIC COMMITTEE ON PLANTS ON AN ADDITIONAL QUESTION FROM THE COMMISSION ON THE EVALUATION OF IPROVALICARB [SZX 0722] IN THE CONTEXT OF COUNCIL DIRECTIVE 91/414/EEC

B. TABLE OF CONTENTS

C. BACKGROUND

Iprovalicarb is a new active substance in the context of Council Directive 91/414/EEC[1]. On 7 March 2001, the SCP delivered an opinion on the evaluation of the new active substance iprovalicarb (SCP, 2001). In relation to the opinion of the Committee on the issue relating to carcinogenicity, the Committee is now requested to respond the following supplementary question:

"Given that the SCP cannot rule out the relevance to humans of the tumours observed in rats following iprovalicarb treatment, does the Committee consider that a sufficient Margin of Safety exists having regard to the human exposure likely to arise from the intended uses?"

A draft assessment report has been prepared by the Rapporteur Member State (RMS, Ireland) on the basis of a dossier submitted by the notifier (Bayer AG). In order to prepare its opinion, the Committee has been supplied with the additional documentation listed below.

Source documents made available to the Committee:

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

[1] OJ N° L 230, 19. 8.1991, p.1.

2. Iprovalicarb: Consequence on the Opinion of the Scientific Committee on Plants (SCP/IPROVA/002-Final, March 7, 2001) – submitted by the notifier on 25 April 2001 (doc. SCP/IPROVA-BIS/003).

3. Comments from the Co-Rapporteur Member-State, Germany, regarding the opinion of the Scientific Committee on Plants on iprovalicarb (SCP/IPROVA/002-Final), adopted on 7 March 2001 (SCP/IPROVA-BIS/004), submitted 14 May 2001.

4. Historical control data. RITA rats database (version 13 June 2000) Report created 13 June 2001, submitted by Germany (coRapporteur), 18 June 2001 (SCP/IPROVA-BIS/005).

5. SZX 0722 Salmonella microsome test Plate incorporation and preincubation method using Salmonella thyphimurium TA 102, Bayer AG, Report n° PH 31331, 10 September 2001, submitted by Bayer AG 20 September 2001 (Property of Bayer AG).

6. SZX 0722 *In Vitro* Chromosome aberration test with Chinese Hamster V79 cells using 18 hours treatment without S9 MIX, Bayer AG, Report n° PH 31333, 10 September 2001, submitted by Bayer AG 20 September 2001 (Property of Bayer AG).

D. SCIENTIFIC BACKGROUND ON WHICH THE OPINION IS BASED

Question:

"Given that the SCP cannot rule out the relevance to humans of the tumours observed in rats following iprovalicarb treatment, does the Committee consider that a sufficient Margin of Safety exists having regard to the human exposure likely to arise from the intended use?"

Opinion of the Committee:

In its initial evaluation of iprovalicarb, the Committee concluded that the data set presented could not rule out the relevance to humans of the tumours observed in rats. Iprovalicarb acts by an unknown, but non-genotoxic mechanism that has no counterpart in mice, therefore toxicity has a threshold dose and there is a level of exposure below which the probability of adverse effects is negligible. The Committee considers that a sufficient Margin of Safety exists having regard to the consumers' exposure likely to arise from the intended use of iprovalicarb. In the case of operators, a sufficient Margin of Safety only exists when operators wear gloves.

Because the term "Margin of Safety" is used in the literature with various meanings, the Committee is of the opinion that the European Commission should establish a formal definition of Margin of Safety.

Scientific background on which the opinion is based:

The basis for concluding that the SCP cannot rule out the relevance to humans of the tumours observed in rats following iprovalicarb treatment was supplied in a previous opinion adopted on 22 March 2001 (SCP, 2001)**.** It is as a consequence of that opinion that this follow-up question was submitted.

1. Margin of Safety

The Committee noted that no definition of Margin of Safety has been established. It can be defined in at least three different ways (see General considerations on Margin of Safety in the annex to this opinion). For substances that are not neoplastic and for neoplastic substances that are not so because of DNA-reactivity, it is generally assumed that toxicity has a threshold dose and there is a level of exposure below which the probability of an adverse effect is negligible. Therefore, to determine a Margin of Safety, two characteristics must be applicable to a substance. These are:

1. a confidently established level of exposure for the target of interest;
2. a NOAEL for toxicity and for tumours.

2. Exposure assessment

The derivation of expected exposure levels is made in the draft assessment report for both the consumer and the operator.

In the case of the consumer, calculations of total exposure, based upon the standard WHO European diet, indicate a Theoretical Maximum Daily Intake (TMDI) of 0.004736 mg/kg bw.

In the case of the operator, calculations of occupational and bystander exposure were made according to both the German and UK (POEM[2]) models. In the latter model, calculations were made for exposure during mixing and loading operations and during spray application assuming that the operator either did or did not wear gloves. The predicted absorbed dose was 0.3754 mg/kg bw/day when not wearing gloves and 0.0103 mg/kg bw/day when gloves are worn during all operations.

3. Hazard identification and characterisation

3.1 *The NOAEL for tumour findings*

In preparing its response to the question, the Committee defined the NOAEL[3] for tumour endpoint based on the two-year dietary study in which Wistar (Hsd/WIN:WU) rats were exposed at levels of 0, 500, 5000 or 20000 ppm (equal to 0, 26.0, 262.5 & 1109.6 mg/kg bw for males and 0, 31.7, 326.3 & 1379.7 mg/kg bw for females).

The proportion of tumour bearing rats was not significantly increased in either male or female rats. Nevertheless, in 6/50 (12%) females at 20000 ppm uterine *adenocarcinomas* were observed, although no statistically significant trend was observed (2/50, 3/49, 3/48 and 6/50) and the incidence was within the historical control range (0 – 14/20%). Malignant *mixed Müllerian tumours* of the uterus were found at 5000 (1/48, 2%) and 20000 (2/50, 4%) ppm, but not at 0 and 500 ppm. The historical control values vary between 0 and 2%. This tumour, which is derived from pluripotent mesodermal cells of the Müllerian duct, seems to have been described in rats (Lewis strain) for the first time in 1990 (Rittinghausen J, Deerberg F., 1990). S*quamous cell carcinoma* of the clitoral gland was found in two females at 20000 ppm (2/50, 4%). This is another rare tumour, although the clitoris is seldom examined histologically unless some gross anomaly is noticed.

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[2] Pesticide Operator Exposure Model.
[3] No Observed Adverse Effect Level.

In addition, in female rats, benign *transitional cell papilloma* of the urinary bladder was found in 2 animals at 20000 ppm (2/50, 4%) and there was a statistically significant positive trend in the incidence of thyroid *follicular cell adenomas* at 5000 and 20000 ppm doses (0/50, 0/50, 1/50 and 2/50); thyroid *follicular cell carcinomas* were observed at 5000 and 20000 ppm doses in 2 animals (0/50, 0/50, 1/50 and 1/50). The incidence of thyroid gland neoplasms is within the historical (adenomas 6% and carcinomas 5%) and no other indications of an effect upon the thyroid emerge from this or other studies with iprovalicarb. No effect was seen in the thyroid of male rats.

In male rats, the only neoplasms of note were malignant tumours of the skeletal system in animals of the 20000 ppm group. There were three *osteosarcomas* (two of the femur, 2/50, 4% and one of the lower jaw, 1/50, 2%) and one *chondrosarcoma* in the nasal cavity (1/50, 2%). Historical data showed incidences of spontaneously occurring osteosarcomas in the range of 0 - 2%. No data are available on chondrosarcoma.

It is noteworthy that in this study there was no increased incidences of any neoplasm more commonly found in rats of any strain and that there were slightly decreased incidences of non-neoplastic and/or neoplastic lesions in the mammary glands of females (decreased diffuse hyperplasia, adenocarcinoma) and for the pituitary gland (decreased hyperplasia of the pars distalis) of females.

The NOAEL for the tumour findings is 500 ppm, equal to 31.7 mg/kg bw for female rats, the LOAEL[4] being 5000 ppm (equal to 326.3 mg/kg bw).

3.2 *The NOAEL for toxicity other than tumour toxicity*

The 53-week dog dietary study is the basis for the lowest NOAEL for general toxicity. The NOAEL for liver toxicity was 80 ppm for females (equal to 2.62 mg/kg bw), dose at which an hepatotoxic effect occurred in male rats. Given the absence of histological changes in the females in this study and the reversibility of liver enzyme induction in a supplementary 4-week study in rats, it appears likely that the dose level of 80 ppm for males, which is a LOAEL, would be rather close to a NOAEL.

3.3 *Genotoxicity*

Iprovalicarb has been tested in bacteria and mammalian cells for potential genotoxicity *in vitro*. None of the tests revealed any clear evidence of mutagenic or genotoxic activity. Increases in the frequency of a particular effect in some tests were limited to observations at particular concentrations that showed no dose-dependency. Shortcomings in two of the original assays submitted (a bacterial mutation test and a mammalian cell chromosomal aberrations test *in vitro*) have been corrected in more recent studies reported to the Committee.

Iprovalicarb was not genotoxic *in vivo* in the micronucleus test in mice. Iprovalicarb was tested in an organ specific ^{32}P-post labelling assay *in vivo* in the uterus and whole urinary bladder/urinary bladder epithelium of female rats to investigate DNA-adduct formation. No

[4] Lowest Observed Adverse Effect Level.

indication of DNA adduct formation by iprovalicarb was found in uterus, whole urinary bladder or urinary bladder epithelium.

It is concluded that iprovalicarb is unlikely to be genotoxic to somatic cells of mammals and any carcinogenicity is highly unlikely to occur through a DNA reactive mechanism.

4. Risk characterisation

In its initial evaluation of iprovalicarb, the Committee concluded that the data set presented could not rule out the relevance to humans of the tumours observed in rats. Iprovalicarb acts by an unknown, but non-genotoxic mechanism that has no counterpart in mice, therefore a Margin of Safety can be determined for these effects.

In the process of risk assessment, an ADI[5] was derived from the liver toxicity study above mentioned. Expected human exposure has been studied or modelled and exposure levels for consumers and operators are available.

In order to characterise the risk and determine a Margin of Safety, the following figures should be taken into consideration:

Data for calculation of consumer Margin of Safety:

$NOAEL_{carc}$		31.7 mg/kg bw/day (female rat)
$LOAEL_{liver}$ –		2.62 mg/kg bw/day (dog)
ADI_{carc}	(Uncertainty factor 1000)	0.0317 mg/kg bw
ADI_{liver}	(Uncertainty factor 200)	0.0131 mg/kg bw
TMDI		0.004736 mg/kg bw

Assessment:

$NOAEL_{carc}$ vs. TMDI	6693
$LOAEL_{liver}$ vs TMDI	553
ADI_{carc} vs TMDI	6.69
ADI_{liver} vs TMDI	2.77

Data for calculation of operator Margin of Safety:

$NOAEL_{carc}$	31.7 mg/kg bw/day (female rat)
$LOAEL_{liver}$ –	2.62 mg/kg bw/day (dog)
$AOEL_{carc}$	0.0317 mg/kg bw
$AOEL_{liver}$	0.0131 mg/kg bw/day
POEM model Operator exposure (gloved)	0.0103 mg/kg bw
POEM model Operator exposure (ungloved)	0.3754 mg/kg bw

Assessment with exposure gloved:

$NOAEL_{carc}$ vs $POEM_{gloved}$	3 077
$LOAEL_{liver}$ vs $POEM_{gloved}$	254
$AOEL^{6}_{carc}$ vs $POEM_{gloved}$	3.08
$AOEL_{liver}$ vs $POEM_{gloved}$	1.27

[5] Acceptable Daily Intake.
[6] Acceptable Operator Exposure Level.

Assessment with exposure ungloved:

$NOAEL_{carc}$ vs $POEM_{ungloved}$	84.44
$LOAEL_{liver}$ vs $POEM_{ungloved}$	6.98
$AOEL_{carc}$ vs $POEM_{ungloved}$	0.08
$AOEL_{liver}$ vs$POEM_{ungloved}$	0.03

From the above figures it can be concluded that a sufficient Margin of Safety exists for the consumers having regard to the human exposure likely to arise from the intended use. In fact, the ADI derived from general toxicity is lower than the ADI that would be derived based on carcinogenicity. The overall ratio between NOAEL for carcinogenicity and TMDI has a value of 6693. The ratio ADI/TMDI is greater than 1 irrespectively of the type of ADI considered.

In the case of operators, a sufficient Margin of Safety only exists when operators wear gloves. In fact, the ratio between the NOAEL based on carcinogenicity and the estimated exposure when using gloves has a value of 3077. The ratio AOEL/POEM exposure is greater than 1 irrespectively of the type of AOEL considered.

5. Conclusions

In its initial evaluation of iprovalicarb, the Committee concluded that the data set presented could not rule out the relevance to humans of the tumours observed in rats. Iprovalicarb acts by an unknown, but non-genotoxic mechanism that has no counterpart in mice, therefore toxicity has a threshold dose and there is a level of exposure below which the probability of adverse effects is negligible.. The Committee considers that a sufficient Margin of Safety exists having regard to the consumers' exposure likely to arise from the intended use of iprovalicarb. In the case of operators, a sufficient Margin of Safety only exists when operators wear gloves.

Because the term "Margin of Safety" is used in the literature with various meanings (see annex), the Committee is of the opinion that the European Commission should establish a formal definition of Margin of Safety**.**

E. ANNEX: GENERAL CONDIDERATIONS ON MARGING OF SAFETY

Several different schemes have been developed by national agencies and international organisations for the derivation of levels of exposure to substances, including pesticides, considered to present minimal risk to exposed populations. These schemes include reference dose or concentrations (U.S. Environmental Protection Agency), tolerable daily intakes or concentrations (Health Canada), tolerable or acceptable daily intakes (International Programme on Chemical Safety (IPCS), a joint programme of WHO[7], ILO[8] and UNEP[9]) and Margins of Safety (European Union). The last is stated to be a comparison of levels of effect with estimated exposures. It appears, however, that there has been no European Commission promulgation that this is indeed what was intended by the term.

The EC First Report of the Scientific Steering Committee working group on the Harmonisation of Risk Assessment Procedures, Part 2 (SSC, 2000) states the following (p

[7] World Health Organisation.
[8] International Labour Organisation.
[9] United Nation Environment Programme.

75): "The 'Margin of Safety' approach has a long history of use (for example a factor of 100 between the NOAEL and an acceptable intake for chronic exposure of the general population), and is defensible based on recent analyses. However, it does not allow interpretation of the risks associated with higher exposures, or the exposure of potentially sensitive sub-groups." This definition in the Harmonisation document would appear to equate Margin of Safety with a comparison of the NOAEL and the ADI or AOEL. It takes no account of estimated exposure, as the Harmonisation document understands the European Union intends.

According to IPCS (IPCS, 1999) the Margin of Safety is the comparison of an effect level with the estimated exposure. However, a level of effect is determined experimentally and, hence, its precision is dependent upon a number of factors, not least of which is the dose intervals employed. Consequently, it is prudent (although subject to the same weakness of precision as the effect level) to use as a starting point the NOAEL, from which is derived the ADI and AOEL.

The NOAEL is the highest dose at which the toxic effect is not observed. It is based on toxic effects of functional importance or pathological significance, rather than adaptive responses. The NOAEL has been defined as the highest observed dose or concentration of a substance at which there is no detectable adverse alteration of morphology, functional capacity, growth, development or life-span of the target (IPCS, 1994). The NOAEL depends on the sensitivity of the methods used, the size of the exposed groups and the dose intervals (IPCS, 1999). It does not take into account characteristics of the dose-response curve.

Once the NOAEL has been determined, uncertainty factors are applied that are designed to take account of variability between individuals of the test species (a 10 x factor), differences between species (a factor of 3 x for inhalation route and 10 x for the oral route), weaknesses that may be perceived in the NOAEL determination (e.g., LOAEL to NOAEL is allocated a 3 – 10 x factor), the gravity of the effect upon which the NOAEL is based (a 5 – 10 x factor for teratogenicity, a 3 – 10 x factor for non-genotoxic carcinogenicity). Their application at least boarders upon risk management. A scheme for their application is given in the aforementioned document, p 57. *Since these factors are applied as compensation for uncertainties in the NOAEL*, it can be considered that the ADI or AOEL should be the value against which the estimate of exposure is compared when arriving at the Margin of Safety. The decision as to what Margin of Safety is acceptable is, however, very clearly a risk management issue outside the terms of reference of this Committee. Nevertheless, it may comment that the magnitude of an acceptable Margin of Safety must depend in part upon the toxicological endpoint being considered.

In a review of risk assessment to human, conducted by IPCS (1999), a distinction is drawn between non-neoplastic (threshold) effects and neoplastic (non-threshold) effects. The implication of this distinction is that no neoplastic effect is a threshold effect. The rationale for this distinction is not given and it is not clear that it was ever intended. Furthermore, it is stated in the review that even a simple distinction between "genotoxic" and "non-genotoxic" neoplastic effects is becoming increasingly problematical and there are possible thresholds for aneugenic, genotoxic effects. This is an issue that has been discussed in this Committee and an agreement reached that consideration of a lack of a threshold should be confined to DNA-reactive genotoxic agents, or at least the lack of a threshold should not apply to carbendazim-related substances (e.g., carbaryl) or other substances that act through a similar mechanism (SCP/BENOMYL/010). For substances that are not neoplastic and for neoplastic substances that are not so because of DNA-reactivity, it is generally assumed that there is a level of exposure below which the probability of an adverse effect is negligible.

There are at least three ways in which Margin of Safety can be defined. These are:

1. NO(A)EL vs. ADI or AOEL
2. NO(A)EL vs. TMDI
3. ADI or AOEL vs. TMDI

To establish a Margin of Safety in any one of these ways, two characteristics must be applicable to a substance. These are:

1. a NOAEL, from which the ADI and AOEL can be derived (possibilities 1 and 2);
2. a confidently established level of exposure, from which a TMDI can be derived (possibilities 2 and 3) and
3. in the case of a carcinogen, there should be a very low probability that it is DNA-reactive (possibilities 1, 2 and 3).

According to the first definition, Margin of Safety > 300 – 1000 is generally considered acceptable for non genotoxic-carcinogens. The same criterion could be applied to the second definition. In contrast, a Margin of Safety of unity would be sufficient for the third definition. Any ratio < 1 would be unacceptable. Note these apparently differing margins are based on the same toxicological database. Because the expectations from the three definitions are different and because there appears to be so much confusion in the literature regarding them, a definition of Margin of Safety should be established by the European Commission.

F. REFERENCES

IPCS, 1994: Assessing Human Health Risks of Chemicals: Derivation of Guidance Values for Health based Exposure Limits. Environmental Health Criteria 170. WHO, Geneva.

IPCS, 1999: Principles of the Assessment of Risks to Human Health from Exposure to Chemicals. Environmental Health Criteria 210. Who, Geneva.
http://www.who.int/pcs/risk-assessment-ehc/docs/ehc210_exposure.htm

Rittinghausen J, Deerberg F. Toxicol Pathol., 1990;18(3):417-422.

SSC, 2000: First Report of the Scientific Steering Committee working group on the Harmonisation of Risk Assessment Procedures, Part 2, October 2000.
http://europa.eu.int/comm/food/fs/sc/ssc/out84_en.pdf

SCTEE, 2001: Position paper on Margins of Safety in human health risk assessment expressed at the 22nd Scientific Committee on Toxicity, Ecotoxicity and the Environment CSTEE plenary meeting Brussels, 06/07 March 2001.
http://europa.eu.int/comm/food/fs/sc/sct/out110_en.html

SCP, 2001: Opinion of the Scientific Committee on Plants on the evaluation of iprovalicarb in the context of Council Directive 91/414/EEC for placing plant protection products on the market (opinion adopted on 7 March 2001)
http://europa.eu.int/comm/food/fs/sc/scp/out94_ppp_en.pdf

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