OPINION OF THE SCIENTIFIC COMMITTEE ON PLANTS REGARDING THE INCLUSION OF ISOXAFLUTOLE IN ANNEX 1 OF DIRECTIVE 91/414/EEC CONCERNING THE PLACING OF PLANT PROTECTION PRODUCTS ON THE MARKET
(SCP/ISOXA/012-Final)

(Opinion adopted by the Scientific Committee on Plants on 18 May 1999)

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TERMS OF REFERENCE

The draft proposed inclusion of isoxaflutole in Annex I to Directive 91/414/EEC had been referred to the Scientific Committee on Plants for consultation with the following specific questions to the Committee:

1. Relevance of metabolite RPA 203328 (point 2.1 of doc. 5713/VI/97); most Member States accepted the evaluation of the Rapporteur Member State presented in doc. 5713/VI/98 rev.2 addendum.
2. Further statistical analysis of tumour incidence in the 2 year rat study (point 4.2 of doc. 5713/VI/97); the Rapporteur Member State still has to make the details of its evaluation available.
3. Adverse foetal effects in relation to classification (point 4.4 of doc. 5713/VI/97); the Rapporteur Member State still has to re-evaluate.

CONTEXT OF THE QUESTION

The draft Commission Directive on the inclusion of isoxaflutole in Annex I of Directive 91/414/EEC concerning the placing of plant protection products on the market was submitted to the Committee for consultation. The Committee had been supplied with a dossier provided by Rhône Poulenc AGRO, the monograph prepared by the Dutch authorities including also the document Addendum to Isoxaflutole Monograph of March 1998, the result of the "Peer Review" report involving several Member States and the draft Commission Directive.

Isoxaflutole is a new herbicide for pre-planting and pre-emergence control of grasses and broadleaf weeds in corn (maize). Isoxaflutole is a pigment photosynthesis inhibitor.
Information available to the Committee at the time of writing of this report indicated that the maximum rate and number of application was as follows:

Maize: One application per season with a maximum of 100 g ai/ha.

OPINION OF THE COMMITTEE

Following a review of the data supplied, the Committee decided to deal with the following aspects:

1. Relevance of metabolite RPA 203328 (point 2.1 of doc.5713/VI/97) and of other main metabolites.

2. Assessment of the statistical analysis of the results of the 2-year carcinogenicity study in the rat (point 4.2 of document 5713/VI/97) and the relevance of these results for the human risk assessment.

3. Assessment of the adverse foetal effects in relation to classification (point 4.4 of document 5713/VI/97).

1. RELEVANCE OF MAIN METABOLITES

1.1. General considerations on the relevance of metabolites of active substances

Toxicological relevance of metabolites

The metabolites formed in the environment by biotic or abiotic degradation of chemical substances may be the same or different to those formed in animal species when they were administered the substance. When the nature and proportion of metabolites formed in the environment are the same (or broadly similar) to those appearing in whole animal testing, the toxicological testing for the doses used can be considered as relevant in assessing the risk arising for man through exposure to the metabolites in the environment. When on the contrary, the metabolism in the environment gives rise to different chemical species which are not present in the animal metabolism, a separate specific assessment would be needed in order to evaluate and predict the risk for man.

When a specific assessment is needed, the expected toxicity of the compound may be preliminarily assessed by structure-activity relationship analysis and, based on the results, a specific testing regime may need to be designed. Such a regime may require a limited set of tests to include, as a minimum, acute toxicity, subacute toxicity, screening for mutagenicity, metabolism in rodents. The results from these tests should determine the need for more extensive chronic testing (multi-tier approach) and may involve similar testing as would be required for an active substance in the framework of Directive 91/414/EEC[1].

An important question concerns the concentration at which a given metabolite should be considered of such relevance so as to require separate testing. A fully objective approach to this question is impossible. Taking, for instance, 10% as the trigger value for the presence of an individual metabolite as the threshold value for toxicological significance, means that it is believed that the metabolite is unlikely to be more than tenfold more toxic than the parent compound. While this assumption would be valid in most cases, there is no reason why a given metabolite cannot prove the exception to this rule and it would be difficult to predict with certainty the cases in which such exceptions could occur. Thus, a prudent evaluation policy should involve a case-by-case evaluation approach, based on sound toxicological science rather than the rigid adherence to strict trigger values.

Relevance to Groundwater

The entry of active substances, their metabolites, breakdown or reaction products [in the following called 'metabolites' for short] into groundwater has to be evaluated for inclusion of

[1] OJ No L230, 19,8.1991, P.1.

an active substance in Annex I of Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market.

The legal framework for the subsequent authorisation of the intended uses provides two different criteria:

- Annex VI of directive 91/414/EEC and further EC-Directives, e. g. Council Directive 80/778/EEC of 15 July 1980 relating to the quality of water intended for human consumption, establishing a limit value of 0.1 µg/l for a single active substance
- Annex VI of directive 91/414/EEC where "**relevant** metabolites, breakdown or reaction products" are subject to an effects assessment.

For the identification of metabolites liable to leach in the context of Annex I inclusion, the SCP considers it appropriate to draw on information which is provided for in the evaluation of active substances, e.g. from soil metabolism and from leaching studies. Specific evaluation is generally required for those metabolites which occur in aerobic soil metabolism studies at any time at levels exceeding 10 %, or which on annual average basis exceed 0.1 µg/l in the leachate from lysimeter studies, field studies or in groundwater monitoring, or which are otherwise known to have critical properties (e.g. toxic effects at even lower concentrations). With respect to leaching potential, the refined assessment should apply the same principles and methods as for the active substance (e.g., scenarios, model calculations) except where there is sufficient information to indicate that the concentration to be expected in groundwater will cause no toxicological or ecotoxicological effects.

Ecotoxicological relevance

With regard to ecotoxicological relevance, it is the opinion of the Committee that metabolites, like parent compounds, are chemical substances introduced into the environment where they may not normally occur, or not in such quantities. They occur there as a direct consequence of the use of an active substance in a plant protection product. Both the parent compound and the metabolites might cause undesirable effects in the environment or, in other words, may be of 'ecotoxicological relevance'. There is, therefore, no scientific reason to deviate from those accepted principles which are used in most countries for the evaluation and assessment of active substances. Hence the ecotoxicological relevance of any metabolite should be based on their toxicology and exposure in the compartment(s) in which they occur.

1.2. Leaching behaviour of isoxaflutole and its main metabolites

Isoxaflutole is mobile and degrades fairly rapidly. Due to the rapid degradation, entry of the active substance into groundwater at concentrations exceeding 0.1 µg/l is not to be expected.

The degradation of isoxaflutole leads to 3 principle transformation products:

RPA 202248	2-cyano-3-cyclopropyl-1-(2-methylsulphonyl-4-trifluoromethylphenyl)propane-1,3-dione
RPA 205834	2-aminomethylene-1-cyclopropyl-3-(2-methylsulfonyl-4-trifluoromethylphenyl)-propane-1,3-dione
RPA 203328	2-methanesulphonyl-4-trifluoromethylbenzoic acid

RPA 202248
Model calculations reflecting worst case conditions of use show that the intended uses are unlikely to cause contamination of groundwater by RPA 202248 at concentrations exceeding 0.1 µg/l.

RPA 205834
In soil, RPA 205834 is only a minor metabolite. In soil transformation studies it is found at maximum amounts of 2 %. In field soil dissipation studies, this metabolite was not found at any site tested (Bologna/Italy, Goch/Germany, Manningtree/UK and Mereville/France). In one aged leaching study, RPA 205834 occurred at levels < 1 % in the loam sediment. Although leaching studies and model calculations with this metabolite have not been carried out, it can be concluded that RPA 205834 will not reach groundwater at concentrations exceeding 0.1 µg/l.

In surface waters, RPA 205834 is more important. In aerobic water/sediment studies, it occurred at concentrations > 10 % in the water phase and in the sediment. DT_{50}[2] values for the whole system ranged from 52 - 97 days. Under anaerobic conditions, DT_{50} for the sediment was 235 days and for the whole system 131 days.

Due to this behaviour (10% threshold value exceeded and persistence in water/sediment studies), further ecotoxicological evaluation for RPA 205834 with respect to surface waters is necessary (see section 1.3 below).

RPA 203328
The leaching behaviour of RPA 203328 is a point of concern. Model calculations using realistic worst case conditions for the intended uses and DT_{50} field data, show that the entry of RPA 203328 into groundwater cannot be excluded. The expected concentration in groundwater exceeds 0.1 µg/l.
Model calculations performed by Rhône Poulenc AGRO (RPA) and the Rapporteur Member State (RMS) seem to give different results. However, a detailed evaluation of those calculations clearly shows that the 0.1 µg/l value was exceeded in both cases.

Hence, an evaluation of the toxicological and ecotoxicological relevance of RPA 203328 is necessary (see below).

<u>Conclusion</u>

According to the information supplied to the Committee on the intended use pattern of isoxaflutole, contamination of groundwater exceeding the 0.1 µg/l limit is unlikely for the parent substance as well as for the metabolites RPA 202248 and RPA 205834. However, it is the opinion of the Committee that metabolite RPA 203328 can leach into groundwater at concentrations > 0.1 µg/l.

[2] Disappearance time for first 50% of compound

1.3. Toxicological properties of isoxaflutole metabolites

Two major metabolites of isoxaflutole have been taken into consideration:
RPA 202248 (2- cyano-3-cyclopropyl-1-(2-methylsulphonyl-4- trifluoromethylphenyl) propane-1,3-dione)
RPA 203328 (2-methanesulphonyl-4-trifluoromethylbenzoic acid).

A third minor metabolite (RPA205834) was not considered as it is a minor, transformation product in soil which does not leach to groundwater.

RPA 202248 is a major metabolite found in animal (about 80% of the parent compound dose) as well in plants. RPA 203328 is a major metabolite in plants and a minor metabolite in animal.
RPA 202248 has been tested for acute oral toxicity in rats, with resulting LD50 >5000 mg/kg bw, and in an Ames-test that did not show gene mutation in bacteria.

RPA 203328 has been tested for acute oral toxicity in rats, with resulting LD50 > 5000 mg/kg bw. An oral 28-day study in rats indicated a NOAEL of 1117.8 mg/kg bw. No gene mutation was induced at an Ames-test. Noteworthy, a 6-week oral study in rats with the parent compound showed a NOAEL < 25 mg/kg bw.

Conclusion

In the light of the above reported findings and also in consideration that both the metabolites were present in the animal metabolism, although in different proportion, it can be concluded that the toxicological information provided by the testing of isoxaflutole in animal, as reported in the toxicological dossier, can be confidently used to assess the toxicological risk for man.
According to the available data, the presence of these metabolites in the environment is not considered to represent an unacceptable health risk for man.

1.4 Ecotoxicological properties of isoxaflutole metabolites

RPA 202248
In ecotoxicological studies with algae, Daphnia and fish, RPA 202248 was shown to be 10 - 100 fold less toxic to these species than isoxaflutole. Phytotoxicity to crop plants may, however, be very high (there are conflicting reports from the US which have not been submitted to the SCP for full evaluation).

RPA 205834
This metabolite was also shown to be 10 - 100 fold less toxic to algae, Daphnia and fish than the parent substance. However, being structurally very similar to the metabolite RPA 202248 , its phytotoxicity to crop plants and possibly other higher plants may be very high. In the absence of data on its phytotoxicity to higher aquatic plants, risk mitigation measures to protect surface water bodies should take into account the persistence and possible phytotoxicity of this metabolite.

RPA 203328

According to ecotoxicological studies on, algae, duckweed, Daphnia and fish, RPA 203328 is more than 100 fold less toxic to all those species than the parent compound. In the case of duckweed which was the most sensitive species to isoxaflutole, the difference was even more pronounced (1000 fold), which suggests that the metabolite does not retain the specific mode of action of isoxaflutole. Assuming a worst case exposure of 3µg/l, the resulting TER[3] values for the most sensitive species (algae and duckweed) range from 2000 to 3000. Hence, it can be concluded that RPA 203328 has no specific ecotoxicological concern (through drainage to surface water) when considering worst-case groundwater contamination.

Conclusion

RPA 203328, which has been identified as having a potential for leaching into groundwater, does not pose a specific ecotoxicological risk, and can accordingly be considered as non-relevant with respect to the intended uses. As for the two metabolites RPA 202248 and RPA 205834, there may be cause for concern with regard to possible phytotoxicity (remaining herbicidal activity). In the absence of clarifying data on this issue, considerations on risk mitigation with regard to non-target plants should include those metabolites together with the parent substance isoxaflutole.

2. ASSESSMENT ON THE STATISTICAL ANALYSIS OF THE RESULTS OF THE CARCINOGENICITY STUDY AND THEIR RELEVANCE TO THE EVALUATION OF THE RISK FOR MAN.

The data of interest in the 104 week rat dietary study of isoxaflutole concern the liver tumours and the microscopic and macroscopic findings in the thyroid. The study included groups of 75 male and female rats treated at the following dose: 0, 0.5, 2, 20, 500 mg/kg/day (1).

2.1. Liver Tumour Findings

An increased incidence of hepatocellular adenomas and carcinomas was observed at 500 mg/kg/day for males and females with a threshold noted at 20 mg/kg/day.

LIVER TUMOR INCIDENCE IN RATS

	Dose Level (mg/kg body weight/day)				
	Control	**0.5**	**2.0**	**20**	**500**
Histopathology - Males[1]					
Liver Adenomas	2/75 (2.7)	3/75 (4.0)	5/75 (6.7)	6/75 (8.0)	14/75* (18.7)
Liver Carcinomas	5/75 (6.7)	1/75 (1.3)	4/75 (5.3)	2/75 (2.7)	17/75* (22.7)
Histopathology - Females[1]					
Liver Adenomas	4/75 (5.3)	2/75 (2.7)	1/75 (1.3)	0/75 (0)	29/74* (39.2)

[3] Toxicity: exposure ratio

Liver Carcinomas	0/75 (0)	0/75 (0)	1/75 (1.3)	0/75 (0)	24/74* (32.4)

Tumour incidence is expressed as the number of animals with tumours/number of animals examined, with percent tumour incidence in parentheses.

*Statistically different from control.

[1]Includes animals dying during the study.

2.2. Mechanistic studies on liver enzyme induction

In a short-term study to assess the P450 induction potential of isoxaflutole, Sprague-Dawley CD male rats were administered test material with diet for two weeks at levels equivalent to doses of 10, 100, and 400 mg/kg/day. Isoxaflutole produced maximal induction of the *CYP*2B1 and *CYP*2B2 isoforms of P450 at 100 and 400 mg/kg/day indicating a metabolic overload in the liver at these doses. Minimal induction was noted at 10 mg/kg/day.

2.3. Opinion of the SCP on the analysis of the results on liver

*CYP*2B1 and *CYP*2B2 are the same isoforms induced by phenobarbital, an agent which also produces liver tumours in rodents, and isoxaflutole appeared to be at least as potent as phenobarbital in the induction of these isozymes. This type of induction is of a reversible nature, quickly subsiding on the cessation of treatment, and can be regarded as an adaptive response to the presence of high levels of xenobiotic chemicals requiring metabolism (McClain, 1990). Although induction of microsomal enzymes is unlikely to cause tumours *per se*, the promotion of spontaneous preneoplastic liver lesions is believed to be the underlying mechanism leading to an increase in liver neoplasms (Williams, 1990). Such non-genotoxic carcinogens appear to act as promoters through indirect mechanisms involving increased cell proliferation or prolonged cellular proliferation with inhibition of programmed cell death (apoptosis). Based on the enzyme induction data, the highest dose tested in the rat carcinogenicity study, i.e. 500 mg/kg/day, resulted in maximal enzyme induction and was the only dose at which liver tumours were significantly increased. The next lower dose of 20 mg/kg/day would produce considerably less than maximal enzyme induction. Since tumours were not observed at this dose level, this indicates that a threshold exists for such an effect.

2.4. Thyroid Tumour Findings

The observed effects consisted of carcinoma, adenoma, follicular cell hyperplasia, cystic follicular hyperplasia and other macroscopic abnormalities. The results of the study were analysed comparing incidences in each specific group with that in the control group, testing for overall between-group variation, and testing for dose-related trend, taking account of between-groups differences in survival.

Statistical Analysis provided by the Notifier (Lee, P.N., 1992)
When the endpoint "carcinoma or adenoma " was analysed, the following data were noted:

Dose (mg/kg)	0	0.5	2	20	500
Males					
Number	3	2	7	8	17
Females					
Number	1	1	2	4	5
Total					
Number	4	3	9	12	22

The statistical analysis of the data concerning "carcinoma or adenoma " indicated the following outcomes:

1. a strongly significant positive trend in males and both sexes combined ($p<0.001$) and a moderately significant trend in females (one tailed $p<0.05$).
2. in males and total rats, statistically significant differences versus the control group for the 500 mg group ($p<0.01$)

When the endpoint "tumour or any hyperplasia" was analysed, the following data were noted:

Dose (mg/kg)	0	0.5	2	20	500
Males					
Number	8	9	14	27	37
Females					
Number	3	4	6	8	17
Total					
Number	11	13	20	35	54

The statistical analysis of the data concerning "tumour or any hyperplasia" indicated the following outcomes:

1. a strongly significant positive trend in both sexes ($p<0.001$).
2. by combining the two sexes, statistically significant differences versus the control group for the 500 mg and 20 mg dose groups ($p<0.001$) and for the 2 mg dose group ($p<0.05$ one-tailed test).
3. in the male rats, a significant difference versus the control in the 500 mg and 20 mg dose groups ($p<0.001$) while the 2 mg group increase in frequency (14 versus 8) was not significant.

Interpretations of these findings provided by the Notifier are as follows:

P.N.Lee Statistics and Computing Ltd, 15 Sept. 1997: "Overall the results demonstrate a clear effect of treatment at 20 and 500 mg/kg/day. While they suggest the possibility that 2 mg/kg/day may have a weak effect on the less severe grades of lesion, this is not convincingly demonstrated by the data"

Addendum to Isoxaflutole Monograph, March 1998: "When looking at the lower part of the dose-response, it appears there are no clear dose-response relationships for both carcinoma and adenoma induction up to 20 mg/kg bw/d. The tumour incidence at 2 mg/kg bw/d (i.e. adenomas and carcinomas combined) may be 'borderline' significant from a biological point of view. However, a ten-fold increase in dose to 20 mg/kg bw/d clearly is not reflected in a parallel increase in tumour induction. This suggests that the incidence observed at the lower dose of 2 mg/kg bw/d was not treatment-related. When looking at the historical tumour incidence figures of this testing lab, these do not exclude the number of tumours observed in

the present study at 2 mg/kg bw/d. Also, at this dose no increase in any hyperplastic response was found, a condition often met with non-genotoxic carcinogens acting via a 'promoting'-type of mechanism.

Conclusion of the Notifier and Rapporteur Member State.

A firm conclusion about the exact NOEL[4] in this study cannot be made with certainty. However, based on both statistical and biological considerations, there are insufficient grounds to regard the tumourigenic and hyperplastic effects observed at 2 mg/kg bw/d as treatment-related."

2.5. Opinion of the SCP on the analysis of the results on thyroid

There is no doubt that the effects observed in this study (hyperplastic changes, adenomas and carcinomas) have to be considered as treatment-related, as there was a clear dose-response relationship in both sexes, highly significant also from the statistical point of view. In view also of the likely mechanism of action of isoxaflutole on thyroid (see below), it is justified to group hyperplastic changes together with adenomas and carcinomas and consider this endpoint in the analysis of results.
The statement that the 2 mg/kg group increase should not be treatment-related is not supported by the SCP for the following reasons:

a) The highly significant response to the trend test is the crucial finding of the study. This finding means that there is a positive relation linking the dose and the response and every difference of responses between a group and the subsequent one along the dose-response relationship, contributes to the overall significance of the test. In other words, the experimentally determined dose-response curve is dose dependent.
b) The next question is at which dose does the curve start? From biological considerations, there are no obvious reasons why the effects should start at 20 mg or 2 mg in this study. Actually, the shape of a dose-response curve starting at 2 mg/kg (as the experimental results seem to suggest), would make sense from the biological standpoint as a dose-response curve generally increases gradually and has a shape like that found in this study. However this question must also be addressed from a statistical point of view.
c) In the attempt to test the effectiveness of a given dose, one can test that single dose group against the control group. By combining the two sexes and considering the endpoint "hyperplastic changes, adenomas and carcinomas", the 2 mg group increase is statistically significant in the one-tailed test. The one-tailed test is the correct one to apply as the hypothesis under test is that the 2 mg group response (20) is greater than the control response (11) and not that it is greater or lower. It has to be remarked, however, that the single testing of a dose group versus the control is not a very informative procedure, as this testing *per se* ignores the information provided by the behaviour of the other dose-groups, which in this case is of extreme value in judging about the probability that the observed difference is due to chance. As an example, one would consider in a totally different way a dose-response sequence of 11,13,20,35,54 and another one (speculative) such as 11,13,20,12,16, although both have a 20 versus 11 comparison. In addition, the repeated testing of single groups versus the same control group has implications also on the significance level to be used in each single test.
d) The argument that the increase in response from 2 to 20 mg (9 to 12 for tumours and 20 to 35 for all lesions) is not 'parallel' to the dose increase (a factor of ten) has no meaning as

[4] No observed effect level

the relation between dose and response must not necessarily be linear. In fact, the increase in response from 20 mg to 500 mg (12 to 22 and 35 to 54, respectively) is not quantitatively proportional to the dose increase either, but everyone agrees on its reality.

e) The fact that the response observed in the present study at 2 mg lies in the range of the historical tumour incidence figures of the testing lab, is not an argument that can be used to disregard the validity of this figure under the specific conditions of testing in which it was obtained. Each toxicological study has a control group because that group has to serve as reference for that study. If there are reasons to believe that the control group of a study is not adequately serving the purpose of being the reference (for instance it is unusually "healthy"), the study must be repeated. A comparison with control groups outside the study is not acceptable and is against the theory of experimental testing. When looking at the data of this study, moreover, one notes that the incidence value observed for the control group was very similar to the incidence value of the 0.5 mg group, which is another group belonging to the no-effect zone of the dose-response curve and can be regarded as if it were another control group. Thus it is very unlikely that the incidence observed in the control group has to be considered abnormally low.

Conclusions

The SCP is of the opinion that the NOEL of the 2-year study of isoxaflutole carcinogenicity in rats should be considered to be 0.5 mg/kg bw/day.

2.6. Mechanism of action of isoxaflutole on thyroid and its relevance to man

2.6.1. Hormonal imbalance and thyroid tumours

In response to the secretion of thyroid stimulating hormone (TSH) the thyroid gland secretes the thyroid hormones thyroxine (T4) and tri-iodothyranine (T3). Once released the thyroid hormones act upon many different target cells. In mammalian cells, under normal physiological conditions T4 is secreted by the thyroid into the systemic circulation in greater quantities than T3 to produce approximately 10 times higher levels (Thomas and Williams 1993). T3 is the more potent hormone on target tissues and is formed predominantly by 5'-monodeiodination of T4 in the peripheral tissues (approximately 80%) and the thyroid (approximately 20%) (Engler and Burger 1984, Atterwill and Aylard 1995).

In man, other primates and dogs **but not rats** there is a high affinity binding protein i.e. thyroxine binding globulin (TBG) which binds T4, and to a lesser extent, T3. In man, free and bound T3 and T4 are in dynamic equilibrium in the circulation and because of the differences in affinities for the binding proteins, there is more unbound T3 (approximately 0.4% of the total T3) than T4 (approximately 0.04% of total T4) (Atterwill and Aylard 1995).

Because of the lack of a high affinity binding protein in rats more T4 remains bound to lower affinity plasma proteins and thus the rat is more susceptible to removal of T4 from the blood, metabolism and excretion. Thus, the serum half-life of T4 in rats is shorter in rats (approximately 6 hours) than in man (approximately 24 hours). To compensate for the shorter half-lives, the basal level and thyroid stimulation by TSH is much greater in rats than mans. Thus, in the rat the thyroid gland is already chronically stimulated and so slight perturbation in the thyroid hormone levels may lead to TSH levels above basal levels. Such perturbations may readily move the gland to increased growth and potentially to neoplastic changes (McClain 1992). Thus, chemical interactions which modify T4 levels may potentially be more likely to generate neoplastic growth in the rat compared to man.

There is a similar morphological progression towards tumour formation in the thyroid of rats and human. Long-term TSH stimulation produces changes in the thyroid in 3 phases:-

a) an initial phase lasting several days

b) a period of rapid growth

c) a period in which growth rate slows down and a plateau is achieved

Following continued TSH stimulation there is a rapid and prolonged increase in thyroid weight and size, manifested by follicular cell hyperplasia (Hill *et al* 1989). The majority of the resulting tumours will be benign but malignant tumours may also be detected.

2.6.2. Potential mechanisms leading to thyroid tumours and their relevance to the risk assessment for man

Non-genotoxic chemicals can potentially induce thyroid tumours by modifications of hormonal balance. Substances which induce enzymes (particularly UDP-glucuronyl transferase) which are responsible for the metabolism of T4 may lead to increased excretion of thyroid hormone. In rats this would lead to increased production of TSH by the pituitary and potentially to prolonged stimulation of the thyroid. In contrast, in humans the increased enzymatic metabolism and excretion of T4 would be buffered by the reservoir of hormone bound to TBG. Thus, when chemical exposure can be shown to lead to increased metabolism and excretion of T4, the consequent induction of thyroid tumours in the rat **will not be relevant to man, at least at the same dose level**.

The enzyme 5'-monodeiodinase catalyses the conversion of T4 to T3 and inhibitors of this reaction can cause hormonal imbalance. This will lead to increases in TSH production by the pituitary to compensate for decreased serum T3. In the rat continued stimulation of the thyroid may lead to the appearance of thyroid tumours. In the case of inhibitors of 5'monodeiodinase there are no known mechanistic differences between man and the rat and man and the mechanism may **be relevant to man**.

Inhibition of iodine uptake by the thyroid leads to a decreased production of thyroid hormones and a consequent increase in TSH production by the pituitary potentially leading to thyroid stimulation and tumour formation in the rat. Extensive epidemiological studies have failed to demonstrate any link between iodine deficiency and human thyroid tumour formation. Thus, this mechanism of thyroid tumour formation can be considered of **limited relevance to man**.

The inhibition of thyroid peroxidase would prevent the organification of the iodine molecule and thus lead to a reduction in the production of thyroid hormone. Such a mechanism could be predicted **as being relevant to man**.

Agents which act by stimulating TSH receptors on the thyroid can cause over-stimulation of the thyroid and thus lead to tumour formation. This mechanism would be characterised by normal or below normal serum levels of TSH with high levels of T4 and T3. Such a mechanism is **not** the result of hormone imbalance and **would be relevant to man**.

Inhibition of T3 and T4 release from the thyroid would be expected to cause decreased serum levels of the two enzymes and potentially to a positive feedback increase in TSH secretion from the pituitary gland. Subsequent stimulation of the thyroid gland could lead to thyroid tumour induction. Such a mechanism could be predicted **as being relevant to man**.

2.6.3. Genotoxicity Studies on Isoxaflutole

Isoxaflutole showed no evidence of genotoxic activity when evaluated in:

a) Bacterial/Mammalian Microsome assay using *Salmonella typhimurium*.

b) Mouse lymphoma TK +/- Point Mutation assay in the presence and absence of S9 mix.

c) Chinese hamster V79 lung cell HPRT Point Mutation assay in the presence and absence of S9 mix.

d) Chromosome Aberration assay in human lymphocytes in the presence and absence of S9 mix.

e) *In vivo* mouse bone marrow micronucleus assay.

f) *In vivo/in vitro* unscheduled DNA synthesis assay in rat hepatocytes.

All the above studies were negative, indicating that isoxaflutole was not genotoxic.

A limited number of genotoxicity studies were also performed with the metabolite RPA2033328. Negative results were obtained.

2.6.4. Carcinogenicity Studies on Isoxaflutole

a) A 2-year dietary study in rats exposed to Isoxaflutole at 0, 0.5, 2, 20, 500mg/kg/bw/day showed thyroid tumours and liver tumours. The findings have been above discussed.

b) A 78-week dietary study in mice exposed to 0, 3.2, 64.4, 977.3mg/kg/bw/day (in males) and 0, 4, 77.9, 1161.1mg/kg/bw/day (in females) showed adenomas and carcinomas of the liver in the highest-dose groups of animals.

No increases were observed at 3.2mg/kg/bw/day in males and 4.0mg/kg/bw/day in females.

2.6.5. Mechanistic Studies on Isoxaflutole

a) Liver enzyme activity in rats

Increase in a range of liver xenobiotic metabolising enzymes was observed following dietary administration of 100 and 400 mg/kg/bw/day for 14 days. The findings have been above discussed.

b) Liver enzyme activity in mice

Increase in a range of liver xenobiotic metabolising enzymes was observed following dietary administration of 100, 400 and 1000 mg/kg/bw/day for 14 days.

c) Effects on thyroid of male rats

Following dietary administration of 500 mg/kg/bw/day for 2 weeks there was a significant decrease in T4 hormone levels. There was a significant increase in the phase II enzyme p-nitrophenol UDP glucuronyl transferase activity. Increased systematic clearance of ^{125}I-thyroxin was observed.

2.7. Opinion of the SCP about the mechanism of tumour induction of isoxaflutole and its relevance to man

Isoxaflutole induced thyroid tumours in male rats and liver tumours in rats and mice. The compound was non-genotoxic and the mechanistic data provided indicates that it was capable of inducing liver enzymes.

The liver tumour has to be considered the result of the inductive action of isoxaflutole on the liver cells. This mechanism is of non-genotoxic nature, has a threshold and would occur in man only at doses exceedingly higher than those proved to be effective in the rodents. At concentrations near to or below the non-observed effect level, the liver tumours observed in the rat have no relevance to man.

The liver enzyme induction observed combined with a reduction in T4 hormone levels suggests that thyroid tumours observed are the result of hormone imbalance. Due to differences in the thyroid hormone physiology, the human species is remarkably less sensitive than the rat (or perhaps is totally insensitive) to the tumourigenic action of isoxaflutole on the thyroid. At concentrations near to or below the no-observed effect level, the tumours observed in the rat have no relevance to man.

3. OPINION OF THE SCIENTIFIC COMMITTEE ON PLANTS REGARDING THE DEVELOPMENTAL TOXICITY OF ISOXAFLUTOLE.

The Scientific Committee on Plants has assessed the reproductive toxicity of isoxaflutole as resulting from two developmental toxicity studies, one in rats and one in rabbits, and a two - generation reproduction study in rats.
The Scientific Committee on Plants would like to emphasise that the term *Developmental toxicity* is preferable to the term *Teratology*. *Developmental toxicity* is a wide term taken to include any effects interfering with normal development, both before and after birth. It includes effects induced or manifested pre-natally as well as those manifested post-natally. This term includes embryotoxic/foetotoxic effects such as reduced body weight, growth and developmental retardation, organ toxicity, death, abortion, structural defects (teratogenic effects), functional defects, peri-postnatal effects, and impaired postnatal mental or physical development, up to and including, pubertal development.

3.1. Rat Study

On the developmental toxicity study in the rats two main elements should be considered:

1) The SCP noted with concern the high frequency of delayed ossifications observed in this strain, as deduced from the historical control data. This may raise questions about the appropriateness of the strain used. The delay in ossification observed in the treated foetuses may or may not be a substance-related effect.
2) The dose selection (10, 100 or 500 mg isoxaflutole/kg b.w.) was considered inappropriate because of two main reasons:
    a) In systemic toxicity studies, isoxaflutole at doses of 10 mg/kg body weight in rats was found to cause hematological changes and eye lesions as well as increased liver weight. An increase in liver weight was also observed in a two generation study in rats at 20 mg/ kg body weight. Thus, the use of doses of 100 or 500 mg isoxaflutole/kg body weight was inappropriate due to the likely occurrence of maternal toxicity.

b) The intervals among the selected doses, especially the 10 fold increase between the low and intermediate dose is not optimal. In toxicity studies, the doses should preferably be more closely spaced (two to four fold intervals is considered optimal).

Since the relationship between maternal hepatic effects and the developmental effects has not been sufficiently clarified, such a relationship may be a possible explanation of the observed effects. However, the possibility cannot be excluded that the effects seen in the study were compound related.
The SCP having taken into consideration the incidence and type of effects observed in the rat study and the possible influence of maternal toxicity on these effects, came to the conclusion that the data from the rat study alone do not warrant a classification of isoxaflutole for developmental toxicity.

3.2. Rabbit Study

The following issues related to the developmental toxicity study of isoxaflutole in rabbits were considered by the SCP:

1. Delay in ossification of the so-called *heads of limb-long bones* (in the study, the term *head of limb-long bones* was used to indicate epiphyses) in the light of historical control data. The incidence of this finding in the highest dose group was 13%. The range of the historical control data is 3.9-19.3%.

2. Systemic toxicity in the rabbit.
   The data provided by the notifier showed that the No Observed Effect Level (NOEL) of isoxaflutole in rabbits was 20 mg/kg/ day. However, the NOEL is only based upon limited parameters from the dams in the developmental toxicity study e.g. mortality, body weight changes and food consumption. Furthermore, no data were available for rabbits on haematology, clinical biochemistry, organ weights or histopathology.
   A high incidence of embryo-lethality and some skeletal changes were observed in the treated groups in comparison to the concurrent control group. All of these findings lie in the range of the historical data.
   The study does not warrant a classification of isoxaflutole for developmental toxicity.

3.3. Two-generation Reproduction Study in Rats

Isoxaflutole was administered in the diet to rats in doses of 0, 0.5, 2, 20 and 500 mg/ kg bw.
No effects on fertility or reproductive organs were seen in any of the generations. The NOEL for systemic toxicity was 2 mg/ kg bw. There was a substance related effect on viability in F1 and F2 pups at 20 and 500 mg/ kg bw. Enlarged renal pelvis was observed in female F2 weanlings at incidences of 3/20, 2/20, 6/20, 6/20 and 8/20 for the doses of 0, 0.5, 2, 20 and 500 mg/ kg bw respectively. The statistical analysis of the data showed a positive trend (Cochran-Armitage trend test, $\alpha = 0.05$).
The NOEL for this effect was 0.5 mg/kg bw.

3.4. Opinion of the SCP on development toxicity of isoxaflutole and its relevance for man

In conclusion, although a category 3 classification for developmental toxicity has been proposed for isoxaflutole by the Rapporteur Member State in the Monograph, the SCP,

taking into account the incidence and type of observed effects in the rat and rabbit studies, and the possible impact of maternal toxicity, is of the opinion that the data raise no concern about possible developmental toxic effects of isoxaflutole for man.

OVERALL CONCLUSION

In summary, the conclusions reached by the SCP are the following:

1. **Relevance of metabolite RPA 203328 (point 2.1 of doc.5713/VI/97) and of other main metabolites:**

According to the available data, the presence of isoxaflutole metabolites in the environment is very unlikely to pose an unacceptable health risk for man. Contamination of groundwater exceeding the 0.1 µg/l limit is unlikely for the parent substance as well as for the metabolites RPA 202248 and RPA 205834. However it is the opinion of the Committee that metabolite RPA 203328 could leach into groundwater at concentrations > 0.1 µg/l.
RPA 203328 which has been identified as potentially leaching into groundwater does not pose a specific ecotoxicological risk, hence it can be considered not relevant under the intended uses. As to the two metabolites RPA 202248 and RPA 205834, there may be cause for concern with regard to possible phytotoxicity (remaining herbicidal activity). In the absence of clarifying data on this issue, considerations on risk mitigation with regard to non-target plants should include those metabolites together with the parent substance isoxaflutole.

2. **Assessment of the statistical analysis of the results of the 2-year carcinogenicity study in the rat (point 4.2 of doc. 5713/VI/97) and the relevance of these results for the human risk assessment:**

Isoxaflutole induced thyroid tumours in male rats and liver tumours in rats and mice. The compound was non-genotoxic and the mechanistic data provided indicates that it was capable of inducing liver enzymes.

The liver tumour has to be considered the result of the inductive action of isoxaflutole on the liver cells. This mechanism is of non-genotoxic nature, has a threshold and would occur in man only at doses exceedingly higher than those proved to be effective in the rodents. At concentrations near to or below the non-observed effect level, the liver tumours observed in the rat have no relevance to man.

The liver enzyme induction observed combined with a reduction in T4 hormone levels suggests that thyroid tumours observed are the result of hormone imbalance. Due to differences in the thyroid hormone physiology, the human species is significantly less sensitive than the rat to the tumorigenic action of isoxaflutole on the thyroid. At concentrations near to or below the non-observed effect level, the tumours observed in the rat have no relevance to man.

3. **Assessment of the adverse foetal effects in relation to classification (point 4.4 of doc. 5713/VI/97):**

Although a category 3 classification for developmental toxicity has been proposed for isoxaflutole by the Rapporteur Member State, the SCP, taking into account the incidence and type of observed effects in the rat and rabbit studies, and the possible impact of maternal toxicity, is of the opinion that the data raise no concern about possible developmental toxic effects of isoxaflutole for man.

ACKNOWLEDGEMENTS

The Committee wishes to acknowledge the contribution of the members of the following working groups who prepared the initial draft opinions:

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

Toxicology including Development Toxicology: Professor M. Maroni (Chairman), and Committee Members Dr. M.-P. Delcour-Firquet, Prof. A. Silva Fernandes, and invited experts Dr. G. Speijers, Dr. O. Meyer, Prof. P. Peters, Dr. R.O. Shillaker, Professor I. Chahoud.

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