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HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Scientific Opinions
C2 - Management of scientific committees; scientific co-operation and networks

SCIENTIFIC COMMITTEE ON PLANTS **SCP/MESOTRI/002-Final**

OPINION ON THE EVALUATION OF MESOTRIONE IN THE CONTEXT OF COUNCIL DIRECTIVE 91/414/EEC CONCERNING THE PLACING OF PLANT PROTECTION PRODUCTS ON THE MARKET

(Opinion adopted by the Scientific Committee on Plants, 18 July 2002)

A. Title

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

B. Terms of Reference

The Scientific Committee on Plants (SCP) is requested to respond to the following question in the context of the Commission's work on the implementation of Council Directive 91/414/EEC[1] concerning the placing of plant protection products on the market:

1. Can the Committee comment on the suitability of the rat as an animal model for the extrapolation of the toxicological properties of mesotrione in humans?

2. Can the onset of adverse effects in target organs (in animal models as well as humans) be linked to a certain threshold concentration of tyrosine in plasma? Can the Committee give an estimate of such a threshold concentration in humans?

C. Opinion of the Committee

Opinion on Question 1:

The primary effect of mesotrione in mammals is the inhibition of 4-hydroxyphenylpyruvate dioxygenase (HPD), a key enzyme of the tyrosine catabolic pathway. Inhibition of HPD by mesotrione results in raised plasma tyrosine levels which appear to be responsible for the critical effect seen in rats (ocular toxicity). The plateau levels of plasma tyrosine after mesotrione administration are higher in rats (males> females) than in mice, where they do not reach the threshold for toxic effects, even at the highest dose. The difference in sensitivity between male and female rats as well as between rats and mice can be attributed to differences in tyrosine catabolism in these species. In humans, genetically or pharmacologically abolished or highly reduced HPD is associated with levels of tyrosinaemia comparable to those observed in mice without the occurrence of signs of ocular toxicity. It is concluded that due to similarities in tyrosine kinetics between mice and humans, the mouse can be considered a better animal model than the rat for human risk assessment purposes.

Opinion on Question 2:

The critical effect (ocular toxicity) associated with the administration of mesotrione is mediated by increased systemic levels of tyrosine. The occurrence of such an effect was seen only when plasma tyrosine levels exceeded about 1000 nmol/ml. The ocular sensitivity of the various species to tyrosine plasma levels seems to be rather similar, the difference in overall toxicity of mesotrione among the species being

[1] OJ N° L 230, 19. 8.1991, p. 1.

attributable to the different levels of plasma tyrosine achieved after HPD inhibition by mesotrione.

Available evidence in humans from cases of hereditary diseases affecting the enzymes involved in the tyrosine catabolism indicates that no signs or symptoms of adverse effect are seen in humans when plasma tyrosine levels do not exceed 800-1000 nmol/ml. It is concluded that there is a threshold of plasma tyrosine levels for the expression of ocular effects in humans and such a threshold is not exceeded even with the complete inhibition of hepatic HPD.

A. Title

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

B. Table of contents

C. Background

Mesotrione is a new active substance (a.s.) in the context of Council Directive 91/414/EEC. A draft assessment report (monograph) has been prepared by the Rapporteur Member States (RMS, the United Kingdom) on the basis of a dossier submitted by the notifier (Syngenta). The evaluation was peer reviewed by the ECCO[2] programme. For its assessment, the Committee had been supplied with the source documents listed below.

Source documents made available to the Committee:

1. Draft Assessment report prepared in the context of the possible inclusion of the following active substance in Annex I of Council Directive 91/414/EEC - Volume 1 to 4, December 1999

2. Mesotrione: Addendum to the draft assessment report, Revision 1, May 2001

3. Mesotrione: Addendum to the draft assessment report, Revision 2, September 2001

4. Mesotrione: French position, May 2001

[2] European Commission Co-ordination.

5. Mesotrione: Mechanism of Toxicity and Relevance to human risk assessment - Syngenta Agrochemicals, July 2001

6. Mesotrione: Swedish comment on Mesotrione, draft monograph - fate and behaviour, 5 July 2000

7. Mesotrione: Belgian comments on toxicology, 5 June 2000

8. Mesotrione: Preliminary Comment of Germany on the monograph, 15 June 2000

9. Mesotrione: EU evaluation for inclusion of new active substances in Annex I of Council Directive 91/414/EEC

10. Mesotrione: German comments on the addendum to the draft assessment report, May 2001, Revision 1, 01 June 2000

11. Mesotrione: Greece - Comments on the draft assessment report of Mesotrione, 26 June 2000

12. Mesotrione: Fate and behaviour in the environment - French comments relative to the evaluation Table, 19 December 2000

13. Mesotrione: French comments on sections "Identity, Physical and Chemical properties and Methods of analysis" of the Monograph Mesotrione, January 2001

14. Mesotrione: French comments on the draft monograph of Mesotrione and evaluation table, 17 November 2000

15. Mesotrione: Response to comments in the Mesotrione reporting and evaluation table, 17 November 2000

16. Mesotrione: Comments of the Netherlands on the Physical and Chemical properties and analytical methods, 21 June 2000

17. Mesotrione: Swedish comments on Mesotrione, draft monograph - ecotoxicology, 05 July 2000

18. Mesotrione: Swedish comments on Mesotrione regarding "fasts track", mammalian toxicology aspects, 7 July 2000

19. Mesotrione: Evaluation Table, 11 October 2001 (SANCO/3000/2000 - rev 3-1)

20. Mesotrione: UK - Additional information for Toxicology Meeting, July 2001

21. Mesotrione: UK revised evaluation table (SANCO/3000 rev 2/2000) and letter (and comments on evaluation table) received from applicant, 22 December 2000

22. Mesotrione: UK revised reporting table (SANCO/2273 rev 2/2000) - evaluation table (SANCO/3000 rev 0/2000), 13 October 2000

23. Mesotrione: reporting table - SANCO/2273/2000 rev 2 - 13 October 2000

24. Mesotrione: Background information on questions concerning mesotrione, August 2001

25. Mesotrione: Evaluation of Mesotrione in the context of Council Directive 91/414/EEC concerning the placing of plant protection product on the market

D. Scientific Background on which the opinion is based

I. QUESTION 1:

Can the Committee comment on the suitability of the rat as an animal model for the extrapolation of the toxicological properties of mesotrione in humans?

Opinion:

The primary effect of mesotrione in mammals is the inhibition of 4-hydroxyphenylpyruvate dioxygenase (HPD), a key enzyme of the tyrosine catabolic pathway. Inhibition of HPD by mesotrione results in raised plasma tyrosine levels which appear to be responsible for the critical effect seen in rats (ocular toxicity). The plateau levels of plasma tyrosine after mesotrione administration are higher in rats (males> females) than in mice, where they do not reach the threshold for toxic effects, even at the highest dose. The difference in sensitivity between male and female rats as well as between rats and mice can be attributed to differences in tyrosine catabolism in these species. In humans, genetically or pharmacologically abolished or highly reduced HPD is associated with levels of tyrosinaemia comparable to those observed in mice without the occurrence of signs of ocular toxicity. It is concluded that due to similarities in tyrosine kinetics between mice and humans, the mouse can be considered a better animal model than the rat for human risk assessment purposes.

II. QUESTION 2

Can the onset of adverse effects in target organs (in animal models as well as humans) be linked to a certain threshold concentration of tyrosine in plasma? Can the committee give an estimate of such a threshold concentration in humans?

Opinion of the Committee:

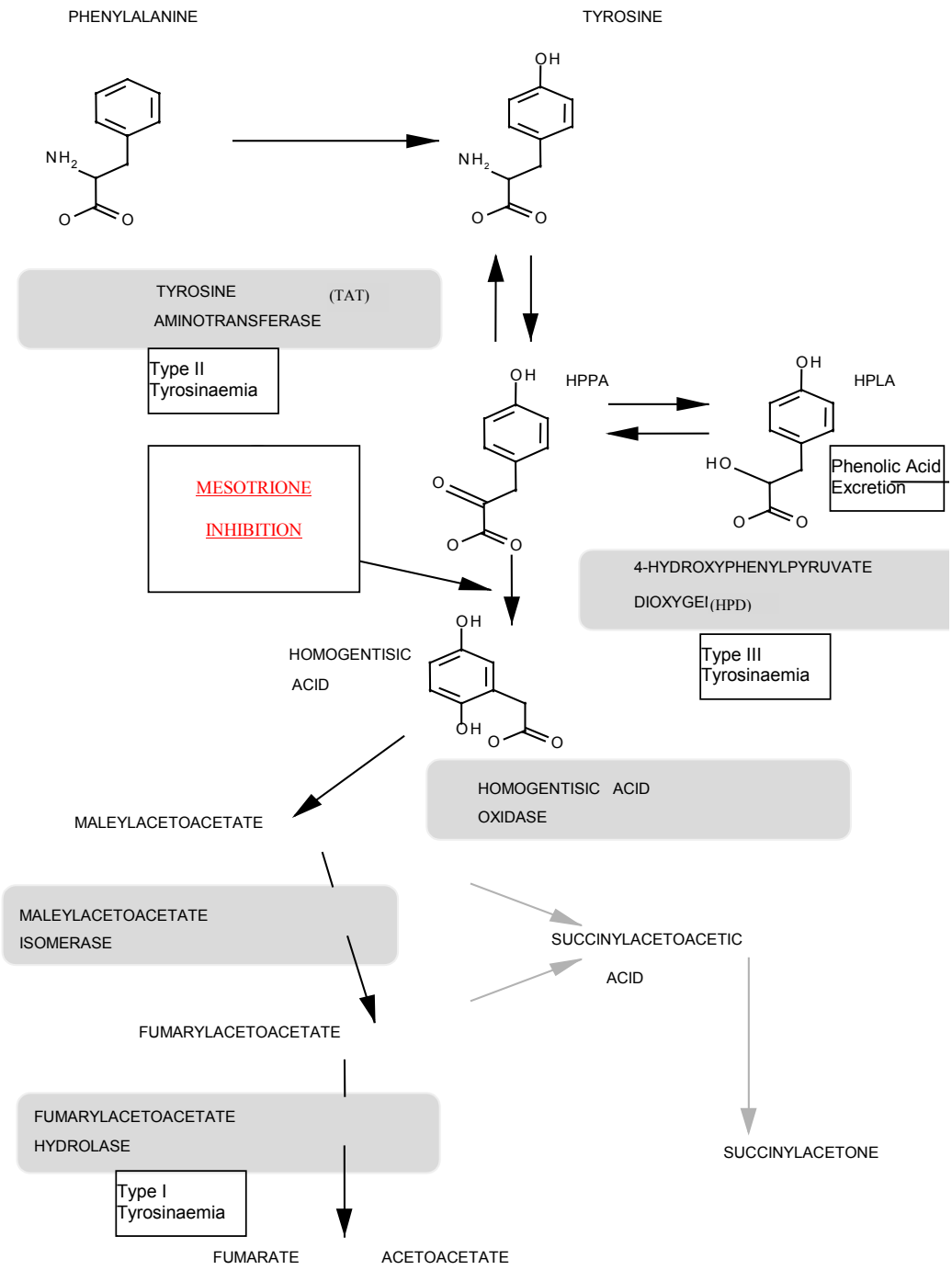
The critical effect (ocular toxicity) associated with the administration of mesotrione is mediated by increased systemic levels of tyrosine. The occurrence of such an effect was seen only when plasma tyrosine levels exceeded about 1000 nmol/ml. The ocular sensitivity of the various species to tyrosine plasma levels seems to be rather similar, the difference in overall toxicity of mesotrione among the species being attributable to the different levels of plasma tyrosine achieved after HPD inhibition by mesotrione.
Available evidence in humans from cases of hereditary diseases affecting the enzymes involved in the tyrosine catabolism indicates that no signs or symptoms of adverse effect are seen in humans when plasma tyrosine levels do not exceed 800-1000 nmol/ml. It is concluded that there is a threshold of plasma tyrosine levels for the expression of ocular effects in humans and such a threshold is not exceeded even with the complete inhibition of hepatic HPD.

I 1. Scientific background on which the opinions are based:

The toxicological effects of mesotrione are attributed to the increase of plasma levels of tyrosine i.e., tyrosinemia, which is a consequence of an inhibition of 4-hydroxyphenylpyruvate dioxygenase (HPD). When HPD is inhibited, tyrosine aminotransferase (TAT) becomes the principal enzyme through which tyrosine can be metabolised although this pathway of metabolism has a low basal activity in rats, particularly in male rats, and does not prevent tyrosine concentrations reaching toxic levels. TAT is the first and rate-determining enzyme for the catabolic pathway, catalyzing the conversion of tyrosine to 4-hydroxyphenylpyruvate (HPPA). HPPA is then converted to homogentisic acid by 4-hydroxyphenylpyruvate dioxygenase (HPD). When this catabolic pathway is restricted by inhibition of HPD, its substrate HPPA is excreted directly into urine or converted to other phenolic acids (i.e. p-hydroxyphenylacetic acid, HPLA and p-hydroxyphenylacetic acid, HPAA) before the excretion into urine. As the reaction mediated by TAT is reversible HPPA can also be converted back to tyrosine. The consequences of the severe and prolonged tyrosinaemia in rats, are the eventual development of the critical effect (ocular toxicity). In contrast, in mouse, the extent of HPD inhibition produced by mesotrione is much less than in rats and TAT basal activity is greater, leading to relatively minor increases in plasma tyrosine levels, with no significant adverse effect. TAT activity in the mouse and humans is similar and is greater than that seen in the rat, likely indicating that humans would not develop the severe tyrosinaemia observed in rats.

Figure 1:

Catabolic pathway of tyrosine relevant to mesotrione mechanism of action



I.1.1. Biochemical parameters

Mesotrione was found to be rapidly absorbed in the rat and in mouse following oral administration. Clearance was rapid and followed first order kinetics. Excretion of radioactivity was primarily in the urine (44.0-67.0% and 40.6-69.8% in rat and mouse respectively), although significant amounts were also detected in faeces (11.2-30.5% and 20.9-37.7% in rat and mouse respectively). Biliary excretion was more extensive in rat males (10.3-14.1%) than females (2.0-3.7%) rats. No further significant differences were noted in the proportions of radioactivity excreted between sexes or dose groups. Tissue residues 72 hours following administration were low with the exception of the liver and kidney. A typical absorption figure of 68% and 70% following oral administration was determined in the rat and mouse respectively.

I.1.2. Male rats

Administration of mesotrione was found to rapidly induce tyrosinaemia in male rats due to inhibition of 4-hydroxyphenylpyruvate dioxygenase (HPD). HPD activity was reduced to approximately 30% of control values at a dietary level of 0.5 ppm mesotrione; complete inhibition of HPD activity was seen at 100 ppm. Plasma tyrosine levels increased steeply between 0.5-10 ppm mesotrione. Further increasing dietary mesotrione concentration had little effect on steady state plasma tyrosine levels. The maximum plasma tyrosine concentration seen in male rats was approximately 3000 nmol/ml (mesotrione ED^{3}_{50} about 7.5 ppm) The toxic effects induced by mesotrione in male rats at around 5 ppm (corneal opacity, increased liver and kidney weights) were found to correlate with increased plasma tyrosine levels. Administration of high doses of tyrosine in the diet was found to cause increased plasma tyrosine levels and similar toxicity, and to exacerbate the toxicity of mesotrione. Following a recovery period, the effects on enzymes and toxicity were found to be reversible.

I.1.3. Female rats

Basal HPD activity in female rats was found to be approximately ten times that of male rats. Maximum inhibition of HPD activity in female rats was found to occur at a dietary level of 1000 ppm mesotrione (compared to 100 ppm in males). The maximum plasma tyrosine concentration seen in female rats was approximately 1500 nmol/ml, ED_{50} about 75 ppm (compared to 3000 nmol/ml, ED_{50} about 7.5 ppm in males). Basal TAT activity in female rats was found to be about double than in male rats. Toxic effects induced by mesotrione in the female were found to be less severe than those seen in males, corresponding to the degree of tyrosinaemia. Findings were exacerbated by the administration of high concentrations of dietary tyrosine.

[3] Effect Dose, Median

I.1.4. Mice

Mesotrione was found to induce tyrosinaemia in mice, maximum plasma tyrosine concentrations were found to be approximately 800-nmol/ml in both sexes at 3500 ppm. Toxic effects (i.e. body weight decrease) induced by mesotrione in mice were found to be much less severe than those seen in rats (no ocular toxicity was observed), again corresponding to the degree of tyrosinaemia. Basal HPD activity in mice was lower than in rats, whereas basal TAT activity was found to be 3-5 times higher than that seen in male rats.

I.1.5. Threshold plasma tyrosine concentrations for toxic effects

In both rat sexes the consequence of mesotrione dosing is the inhibition of the enzyme HPD and increase in activity of TAT. In males, however, inhibition of HPD (which has relatively lower activity) occurs at lower dose levels compared with females. The consequence of these differences is that plasma tyrosine elevation occurs in males at much lower mesotrione dose levels than in females (and plasma tyrosine elevation is greater in males (3000 nmol/ml) than in females (1500 nmol/ml).
In mice, basal HPD activity is lower but basal TAT activity is higher than in rat. Following inhibition of HPD by mesotrione (60% in mice versus 97% in rats), mice maintain maximum plasma tyrosine levels at approximately 800 nmol/ml. Even under conditions of maximal inhibition of HPD, when maximal tyrosinaemia is expressed, mice do not develop the ocular effects as seen in rats. Indeed, the organ weight effects are only detected following prolonged dosing at limit dose levels (7000 ppm; equivalent to to >1000 mg/kg bw) of mesotrione and in presence of a maximum achievable steady state plasma tyrosine level.
These data indicate that there is a threshold plasma tyrosine level which must be exceeded before the toxic findings seen in the studies occur. The available data from the rat and mouse indicate this plasma tyrosine threshold is approximately 1000 nmol/ml.

Table 1:

Summary of principal findings following Mesotrione administration to rat and mouse

	Rat M	Rat F	Mouse M	Mouse F
Plasma tyrosine levels (nmol/ml)	↑ 0.5 ppm	↑ 5 ppm	↑ 10 ppm	↑ 10 ppm
Kidney weight	↑ 5 ppm	-	-	-
Liver weight	↑ 5 ppm	↑ 1000 ppm	-	-
Corneal opacity	↑ 5 ppm	↑ 100 ppm	-	-
Body weight	-	↓ 2500 ppm	↓ 7000 ppm	↓ 7000 ppm
TAT activity	↑ 3 ppm	↑ 10 ppm	↑ 100 ppm	↑ 100 ppm
HPD activity	↓ 0.5 ppm	↓ 5 ppm	↓ 1 ppm	↓ 1 ppm

Table 2:

Enzyme activities and maximum plasma tyrosine concentrations

SPECIES		HPD ACTIVITY µg oxygen/min/mg/prot.		TAT ACTIVITY nmol HPPA/min/mg/p.		Maximum Tyrosine plasma concentration (nmol/ml) following HPD inhibition by mesotrione	
		Basal	At the max level of tyrosine	Basal	At the max level of tyrosine	Dose	Max level of tyrosine nmole/ml
Rat	M	0.27	0.008 (2.9%) (↓33.27 times)	1.7	2.3 (35%) (↑1.35 times)	100 ppm	3000(ED_{50} ≅ 7.5 ppm)
	F	2.4	0.057 (2.4%) (↓42.1 times)	3.4	4.3 (26%) (↑1.26 times)	1000 ppm	1500 (ED_{50}≅ 75 ppm)
Mouse	M	0.07	0.02 (28.6%) (↓3.5 times)	7.8	10.35 (32%) (↑1.32 times)	3500 ppm	800
	F	0.15	0.05 (33.3%) (↓3.0 times)	10.5	15.44 (47%) (↑1.47 times)	3500 ppm	800
Dog	M	-		- -		600 ppm	1100
	F	-				1300 ppm	1300
Human	M			4.5–7.3#			800-1200♥
	M					0.1 mg/kg b.w.	130* (40 - 50)§
	M					0.5 mg/kg b.w.	150* (40 - 50)§
	M					4.0 mg/kg b.w.	280* (40 - 50)§

Cytosolic TAT activity in human liver
* 24 plasma peak
§ pre-dose values
♥ 1 mg/kg bw NBTC

I.1.6. Relevance to humans

There are several sources of information where plasma tyrosine concentrations have been measured in humans under conditions where HPD activity is minimal indicating that a maximum steady state tyrosinaemia of about 800 nmol/ml is maintained during complete inhibition of HPD.

In human volunteer studies, a single oral dose of mesotrione (0.1, 0.5 and 4.0 mg/kg bw.) was shown to induce a dose-related tyrosinaemia peak within 12 hours. At the highest dose level of 4 mg/kg bw, plasma tyrosine concentrations increased to a maximum of about 300 nmol/ml (≈6 times background levels) and returned to background levels by 48 hours. The observed plasma half-life for mesotrione was approximately 1 hour. A

significant proportion of the administered dose was excreted rapidly as unchanged mesotrione in urine.

In the medical literature there are reports on hereditary diseases where enzymes involved in tyrosine catabolism are deficient. Although severe tyrosinaemia resulting in ocular effects has been reported in humans, it is restricted to patients whose tyrosine catabolic rate is reduced due to a very low activity of TAT (Tyrosinaemia Type II, or the Richner-Hanhart syndrome, a rare metabolic disorder). This leads to very high levels of plasma tyrosine (up to 3300 nmol/ml plasma) and herpetiform corneal ulcers and painful punctate keratoses of digits, palms and soles. It is diagnosed by these characteristic clinical symptoms and by significant and permanent (above 1000 nmol/ml) elevation in plasma tyrosine. As these patients have a low clearance rate for tyrosine, treatment of this disease is to restrict the input rate of tyrosine until plasma tyrosine concentrations fall to about 800 nmol/ml, at which point the ocular effect resolves. This reduced tyrosine intake rate is achieved by feeding the patients diets low in tyrosine or phenylalanine. Therefore there is also evidence in humans of a threshold level of tyrosine, similar in concentration to that occurring in animals, which must be exceeded before lesions develop.

Recently a very few cases of Tyrosineamia Type III, a hereditary condition where there is a deficiency of 4-hydroxyphenylpyruvate dioxygenase (HPD), have been reported. Affected children present elevated levels of tyrosine and its phenolic metabolites in blood and urine. Since some of these children presented mild neurological defects, concern has been raised about the possibility that elevated plasma tyrosine levels or its metabolites might affect the developing nervous system (Rüetschi et al, 2000). However, no correlation between plasma tyrosine levels and clinical signs was found (Rüetschi et al, 2000), and available experimental data with mesotrione including developmental and reproductive studies do not indicate nervous system effects.

Another hereditary disease of tyrosine catabolism which, unless treated, has a fatal outcome is Tyrosinaemia Type I, which is characterised by a deficiency of the enzyme fumarylacetoacetate hydrolase (FAAH). A restriction at this stage of tyrosine catabolism leads to a build up of maleylacetoacetate (MAA) and fumarylacetoacetate (FAA), which are responsible for severe injury to the liver and kidney. Treatment with a diet restricted in phenylalanine and tyrosine does not prevent the fatal outcome and, until recently, liver transplantation was the only effective therapy available. However the administration of NBTC, a chemical analogue of mesotrione, induces beneficial clinical effects by preventing the formation of these hepatotoxic and nephrotoxic metabolites (MAA and FAA) through irreversible inhibition of human HPD. To date, some 200 children have been treated with NBTC to ensure that HPD activity is minimal in these patients. Plasma tyrosine measurement in these patients showed a steady state tyrosine concentration below 800 nmol/ml, and although in 5 cases transient and minor ocular effects have been reported, there is no clear evidence that they are associated with NBTC treatment. Likewise, for the treatment of hereditary Tyrosinaemia Type I, a human volunteer study was conducted in 10 healthy male adults. NBTC was administered at 1 mg/kg b.w., the dose level at which NBTC causes complete inhibition of HPD. Plasma tyrosine concentrations as high as 1200 nmol/ml were seen to be followed by a reduction to a steady state level of about 800 nmol/ml and remained stable for 2 weeks when a second dose of NBTC was administered. No signs or symptoms of toxicity were noted in either study.

The information from all these observations in humans where HPD activity is minimal or abolished indicates that the resulting steady state tyrosinaemia is in the order of about 800 nmol/ml and is not associated with ocular or dermal signs.

I.2. Conclusions

The primary effect of mesotrione in mammals is the inhibition of 4-hydroxyphenylpyruvate dioxygenase (HPD), a key enzyme of the tyrosine catabolic pathway. Inhibition of HPD by mesotrione results in raised plasma tyrosine levels which appear to be responsible for the critical effect seen in rats (ocular toxicity). The plateau levels of plasma tyrosine after mesotrione administration are higher in rats (males> females) than in mice, where they do not reach the threshold for toxic effects, even at the highest dose. The difference in sensitivity between male and female rats as well as between rats and mice can be attributed to differences in tyrosine catabolism in these species. In humans, genetically or pharmacologically abolished or highly reduced HPD is associated with levels of tyrosinaemia comparable to those observed in mice without the occurrence of signs of ocular toxicity. It is concluded that due to similarities in tyrosine kinetics between mice and humans, the mouse can be considered a better animal model than the rat for human risk assessment purposes.

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References

Rüetschi U, Cerone R, Pérez-Cerda C, Schiaffino MC, Standing S, Ugarte M, Holme E. Mutations in the 4-hydroxyphenylpyruvate dioxygenase gene (HPD) in patients with tyrosinemia type III. Hum Genet, June 1, 2000; 106(6): 654-62

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