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Opinion of the Panel on Plant Protection Products of the Norwegian Scientific Committee for Food Safety

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Assessed and approved

The opinion has been assessed and approved by Panel on Plant Protection Products of VKM. Members of the panel are:

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Competence of VKM experts

Persons working for VKM, either as appointed members of the Committee or as external experts, do this by virtue of their scientific expertise, not as representatives for their employers or third party interests. The Civil Services Act instructions on legal competence apply for all work prepared by VKM

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Summary

The VKM Panel for plant protection products considered Frupica SC in a meeting on 25.11.2010, and found the active ingredient problematic with regard to carcinogenic effects and possible genotoxicity. M11 is a metabolite of mepanipyrim which is the active ingredient the plant protection product Frupica SC. The Norwegian Food Safety Authority has asked the applicant for further assessment of the genotoxic potential of the metabolite M11. The applicant has submitted a rat liver *in vivo* Comet assay of the metabolite, and the panel has been requested to consider if the genotoxic properties of mepanipyrim and the metabolite M11 is adequately documented.

The metabolite M11 caused positive findings in *in vitro* studies for bacterial mutation and chromosomal aberrations. Three *in vivo* studies (Micronucleus, unscheduled DNA synthesis and Comet assay) did not show evidence of genotoxicity. Based on the documentation available, VKMs Panel on Plant Protection Products concludes that mepanipyrim and the metabolite M11 should not be considered genotoxic *in vivo*. The lack of demonstrated *in vivo* genotoxicity makes it likely that mepanipyrim induces liver tumors in rats and mice by a mechanism that involves a threshold below which tumors are not expected to develop. This conclusion is strengthened by the finding of a promoter-like behavior of mepanipyrim for induction of gamma-glutamyl-transpeptidase positive foci in rat liver.

Key words: VKM, risk assessment, Norwegian Scientific Committee for Food Safety, Frupica SC, mepanipyrim, metabolite M11, genotoxicity

Sammendrag på norsk

VKM's Faggruppe for plantevernmidler vurderte plantevernmiddelet Frupica SC i et møte 25. november 2010, og fant den aktive ingrediensen mepanipyrim problematisk med hensyn til kreftfremkallende effekt og mulig gentoksisitet. M11 er en metabolitt av mepanipyrim, og Mattilsynet har bedt søker om utfyllende informasjon for vurdering av gentoksisk potensiale av metabolitten M11. Søker har innlevert et nytt Comet-forsøk på metabolitten, og faggruppen er bedt om å vurdere om mulig gentoksisitet for mepanipyrim og metabolitten M11 er tilstrekkelig undersøkt.

Metabolitten M11 er vist å forårsake mutasjoner i bakterier og kromosomavvik i pattedyrceller *in vitro*. Tre *in vivo* studier (mikrokjerne, tilfeldig DNA syntese og Comet test) viser imidlertid ikke tegn på gentoksisitet. Basert på tilgjengelig dokumentasjon konkluderer faggruppen med at det aktive stoffet mepanipyrim og metabolitten M11 kan vurderes å være uten gentoksiske effekter. Mangel på *in vivo* gentoksisitet gjør det sannsynlig at mepanipyrim induserer leversvulster hos rotter og mus ved en mekanisme som innebærer at det finnes en terskel for doser der mepanipyrim forventes å framkalle svulster. Denne konklusjonen styrkes av funn av en promotor-lignende effekt av mepanipyrim når det gjelder induksjon av gamma-glutamyl-transpeptidase-positive områder i rottelever.

Abbreviations

CHL	Chinese Hamster Lung
CHO	Chinese Hamster Ovary
EFSA	European Food Safety Authority
EU	European Union
GLP	Good Laboratory Practice
In vivo	Experiment on living organisms
In vitro	"In glass" – experiment outside an organism – in test tube
OECD	Organisation for Economic Co-operation and Development
UDS	Unscheduled DNA Synthesis
VKM	Norwegian Scientific Committee for Food Safety

Background as provided by the Norwegian Food Safety Authority

The VKM Panel for plant protection products considered the plant protection product Frupica SC with the active substance mepanipyrim at its meeting 25.11.2010. The Norwegian Food Safety Authority has asked the applicant for further testing of the metabolite M11 of mepanipyrim for genotoxicity. The applicant has now submitted a Comet assay of the metabolite and concluded that M11 should not be regarded to be genotoxic *in vivo*.

Terms of reference as provided by the Norwegian Food Safety Authority

The Norwegian Food Safety Authority has asked the VKM Panel for plant protection products to assess the result from the submitted Comet assay of the metabolite M11 of mepanipyrim. The Panel is also requested to consider if the question of genotoxic properties of metabolite M11 is adequately documented (Mattilsynet, 2014a).

Assessment

1 Introduction

The plant protection product Frupica with the active substance mepanipyrim was assessed by VKM Panel on Plant Protection Products in 2011 (VKM, 2011). The active ingredient mepanipyrim and its metabolite M11 (for chemical structures see Figure 1-1) was considered problematic with regard to carcinogenic effects and genotoxicity. It was the opinion of the Panel that it cannot be excluded that the finding of liver tumours in both rats and mice may be relevant for humans. Mepanipyrim was however not shown to be genotoxic *in vivo*, and it is therefore possible that it may act as a promoter, and not an initiator of tumour formation, and that there may exist a threshold dose for tumour formation from exposure to mepanipyrim. This possibility was strengthened by the finding that mepanipyrim was found to increase the frequency of gamma-glutamyl-transpeptidase positive foci in rat liver when the animals were exposed to mepanipyrim after exposure to diethyl-nitrosamine and hepatectomy. No increase in the number of gamma-GTP positive foci were seen when mepanipyrim was given as a single dose prior to continued exposure to phenobarbital. It was however also the opinion of the Panel that further investigation of the genotoxic potential of the metabolite M11 formed in rats would strengthen the assumption of an existing threshold dose for tumour formation by the parent substance mepanipyrim.

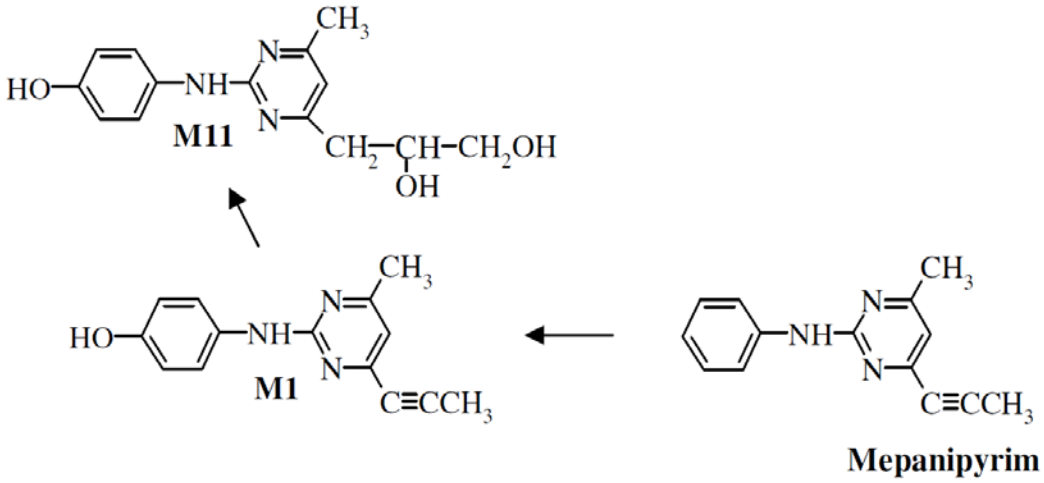

Figure 1-1 Proposed metabolism of Mepanipyrim to metabolite M11 in the rat

Mepanipyrim has tested negative for genotoxicity as measured by point mutations in Salmonella (Ames test), bacterial DNA damage in E. Coli, unscheduled DNA synthesis (UDS) in HeLa cells, point mutations in Chinese hamster V79 cells, and in an *in vivo* micronucleus assay in bone marrow in mice.

On the other hand, mepanipyrim has resulted in non-reproducible effects for chromosomal aberrations in CHO (Chinese hamster ovary) cells *in vitro*, and increased the number of

chromosomal aberrations in two of three time points(6 & 48h, but not at 24h) in an *in vivo* study in bone marrow of rats (5000 mg/kg, single dose).

Several mammalian metabolites were tested in a bacterial mutation assay, but only metabolite M11 which has been shown to be present in urine of rats, was found to produce point mutations in Ames test (in the TA98 strain) in the presence of metabolic activation and induce chromosomal aberrations in CHL (Chinese hamster lung) cells (with and without metabolic activation). Testing of the metabolite in the bacterial mutation assay using TA1535, TA1537, TA1538 and TA100 and in E Coli with and without metabolic activation were negative. Two *in vivo* experiments, micronucleus (mice) and UDS (rat liver) were negative.

Studies of the metabolite M11's ability to induce chromosomal aberrations *in vivo* as well as point mutations in mammalian cells, are not available. Such studies would have helped to clarify the possible role of the metabolite, considering the results from the *in vitro* test for chromosomal aberrations and bacterial test for point mutation (Ames test).

VKMs Panel on Plant Protection Products has discussed the specific questions raised by The Norwegian Food Safety Authority in meetings 24. October and 21. November 2014.

The assessment made by VKM is mainly based on documentation supplied by the manufacturer and shown in the reference list. In addition, literature search in PubMed using the search phrase "mepanipyrim" resulted in 27 hits, but none of them was related to genotoxicity and did not contain information that added to the data used in the assessment.

2 Hazard characterisation

The Norwegian Food Safety Authority demanded additional testing of the metabolite M11 in an *in vivo* Comet assay and for ability to induce DNA adducts *in vivo*. These demands were based on the conclusion of the VKM panel (VKM, 2011), the EU data requirements, and the EFSA recommendations for genotoxicity tests (EFSA, 2012). The demand is further justified by the ability of the Comet assay to study organ-specific genotoxicity effects, in this case possible effects on the liver.

EFSA recommends that in case of positive *in vitro* results an appropriate *in vivo* study is performed. Suitable *in vivo* tests are the mammalian erythrocyte micronucleus test, transgenic rodent test, and Comet assay (EFSA, 2012).

The applicant has performed a comet assay with the metabolite M11 (Kumiai Chemical Industry, 2012, 2013), but has not tested the metabolite M11 for ability to induce DNA adducts. The applicant argues that no standardized methods are available for measurement of DNA adducts, and that the presence of DNA adducts are not general predictors for carcinogenic effects (Japan Agro Services, 2013).

2.1 Comet study

At present no approved OECD guideline for the performance of Comet studies is available. OECD has however published a draft guidance for the performance and evaluation of Comet studies (OECD, 2012).

The performed study meets the GLP requirements and is in line with the Draft OECD guidance with respect to study design and interpretation of results. Results on DNA damage are reported for the endpoints % tail DNA as well as ‘Tail length’ and ‘Olive tail moment’.

Metabolite M11 did not induce DNA damage under the conditions of the Comet assay in this study as illustrated in Table 2.2-1 and documented in the Comet assay report (Kumiai Chemical Industry, 2012).

Table 2.1-1 Results from the *in vivo* rat liver Comet assay. The table is taken from a note written by the Norwegian Food Safety Authority (Mattilsynet, 2014b).

Substance	Dose (mg/kg, p.o.)	Organ	Number of animals	Number of cells analysed	% tail DNA (Mean ± S.D.)	
0.5 w/v% CMC-Na	0		5	500	1.63 ± 0.32	
Test substance	500		5	500	1.80 ± 0.33	(D)
	1000	Liver	5	500	1.66 ± 0.41	
	2000		5	500	1.61 ± 0.28	
EMS	200		5	500	17.82 ± 2.40	*(AW)

0.5 w/v% CMC-Na: Negative control (0.5 w/v% Carboxymethylcellulose sodium ,10 mL/kg)
EMS: Positive control (Ethyl methanesulfonate, 10 mL/kg)
*: Significant difference from negative control ($p \leq 0.025$)
(AW): Aspin-Welch t-test
(D): Dunnett test

3 Uncertainties

The exact mechanism by which mepanipyrim causes liver tumors in rat and mice is unknown so that the possible human relevance of the mechanism cannot directly be assessed.

It is also not known if the metabolite M11 is formed in humans.

4 Conclusions

Metabolite M11 caused positive findings in *in vitro* studies for bacterial mutation and chromosomal aberrations. Three *in vivo* studies (Micronucleus, Unscheduled DNA synthesis and Comet assay) didn't show evidence of genotoxicity. Based on the documentation available it is concluded that mepanipyrim and the rat metabolite M11 are not genotoxic *in vivo*. The absence of *in vivo* genotoxicity makes it likely that mepanipyrim induce liver tumors in rats and mice by a mechanism that involves a threshold below which tumors are not expected to develop. This conclusion is strengthened by the finding of a promoter-like behavior of mepanipyrim for induction of gamma-glutamyl-transpeptidase positive foci in rat liver.

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