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Risk assessment of the plant protection product PROMAN – with the active ingredient metobromuron

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

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Norwegian Scientific Committee for Food Safety (VKM)
Po 4404 Nydalen
N – 0403 Oslo
Norway

Phone: +47 21 62 28 00
Email: vkm@vkm.no

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www.vkm.no
www.english.vkm.no

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Authors preparing the draft opinion

Marit Låg (chair), Hubert Dirven, Jan Ludvig Lyche, Asbjørn M Nilsen

(Authors in alphabetical order after chair of the working group)

Assessed and approved

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

Torsten Källqvist (chair), Katrine Borgå, Hubert Dirven, Ole Martin Eklo, Merete Grung, Jan Ludvig Lyche, Marit Låg, Asbjørn M Nilsen, Line Emilie Sverdrup

(Panel members in alphabetical order after chair of the panel)

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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

Proman is a new product containing the active substance metobromuron. The intended use is as a broad spectrum selective herbicide for potatoes grown outdoors.

VKM's Panel on Plant Protection Products has discussed the questions raised by The Norwegian Food Safety Authority in the Terms of reference and has stated the following opinion:

On the relevance of the carcinogenic effects observed in the rat carcinogenicity study; fibrosarcomas in females and pheochromocytomas and Leydig cell tumours in males:

It is the opinion of VKM's Panel for Plant Protection Products that the relevance of the observed incidence in mammary gland tumours and Leydig cell tumours in the rat carcinogenicity study is strengthened by the fact that the tumours are observed in hormone responsive tissues. The panel concludes that the carcinogenic effects observed in the rat carcinogenicity study are likely to be relevant for tumours that are influenced by the endocrine system, also in humans.

On the higher incidences of still dumbbell-shaped centres of thoracic vertebrae and non-ossification of the 13th rib observed in the rat developmental toxicity study and whether these are considered to be malformations:

VKM's Panel on Plant Protection Products has discussed the classification of the different types of incomplete ossifications and concluded that incomplete ossification of sternebrae and non-ossification of the 13th rib in rats should by itself be considered to be variations, and not adverse developmental effects. On the other hand, the Panel agrees with ECHA that the "thoracic vertebral centres still dumbbell-shaped" should be considered as malformations, due to limited data and understanding of the mechanism underlying the observed slow reversal of these anomalies. Furthermore, it is the view of the Panel that the different types of retarded ossification induced by the exposure of metobromuron should be considered as a whole when assessing for developmental effects.

On the establishment of the NOAEL for the developmental toxicity study in rats and the reference value (ARfD):

VKM's Panel on Plant Protection Products supports the proposal of an ADI value of 0.008 mg/kg bw/day based on a NOAEL of 0.8 mg/kg bw/day from the 2-year study in mouse, and

AOEL of 0.016 mg/kg bw/day based on the NOAEL of 1.6 mg/kg bw/day from the 1-year feeding study in dog. An UF of 100 is applied. The panel suggests on the other hand an ARfD of 0.03 mg/kg bw based on a LOAEL of 10 mg/kg bw /day with the observations of incomplete ossification in the rat developmental study. An UF of 300 is applied.

On the possible anti-androgenic potential of metobromuron.

It is the view of the Panel that the rat carcinogenicity study suggests that metobromuron may possess endocrine disrupting potency. The data from the Hershberger *in vivo* rat study and the *in vitro* studies is also suggestive of a weak anti-androgenic effect. Thus, it is the opinion of the VKM Panel on Plant Protection Products that an anti-androgenic effect of metobromuron cannot be excluded.

Key words: VKM, risk assessment, Norwegian Scientific Committee for Food Safety, pesticide, metobromuron, Proman

Sammendrag på norsk

Proman er et nytt plantevernmiddel med virkestoffet metobromuron som det er søkt bruksgodkjenning for i Norge. Den tiltenkte bruken er som ugressmiddel for poteter dyrket utendørs. VKMs faggruppe for plantevernmidler har følgende oppfatning på de spørsmål som Mattilsynet stiller:

Det er faggruppens mening at betydning for mennesker av de observerte brystkjertel- og Leydigcelle-svulster i kreftforsøk på rotter styrkes ved at svulster knyttet til eksponering for metobromuron finnes i hormon-følsomme vev. Faggruppen konkluderer med at det er sannsynlig at de kreftfremkallende effekter som er observert i rotter også kan være relevante for mennesker. Faggruppen har videre diskutert klassifisering av de ulike typer ufullstendig forbeining i rottestudiene, og er av den oppfatning at ufullstendig forbeining av brystbein og bortfall av forbeining av det 13. ribben hos rotter isolert sett bør anses å være variasjoner, og ikke misdannelser. Derimot er faggruppen enig med ECHA (European Chemicals Agency) i at redusert forbeining i ryggvirvel som gir opphav til såkalt «dumbbell-form" bør betraktes som misdannelser, på grunn av sein reversering og mangelfull mekanistisk forståelse av disse endringene. Som følge av dette er det også faggruppens oppfatning at de ulike typer av forsinket forbening som følge av eksponering for metobromuron bør vurderes samlet ved vurdering av stoffets evne til å indusere misdannelser. Dette har betydning for faggruppens oppfatning av fastsettelse av NOAEL og toksikologiske referanseverdier. VKMs faggruppe for plantevernmidler støtter forslag om en ADI-verdi på 0,008 mg/kg kroppsvekt /dag basert på en NOAEL på 0,8 mg/kg kroppsvekt /dag fra to-års forsøk i mus, og AOEL av 0,016 mg/kg kroppsvekt /dag basert på en NOAEL på 1,6 mg/kg kroppsvekt /dag fra 1-års forsøk på hund. Faggruppen foreslår imidlertid en ARfD på 0,03 mg/kg kroppsvekt basert på en LOAEL på 10 mg/kg kroppsvekt basert på observasjoner av ufullstendig forbening i rotteforsøk der en usikkerhetsfaktor på 300 er brukt. Faggruppen mener at de typer svulster som er funnet i kreftforsøk på rotter kan tyde på at metobromuron kan ha hormonforstyrrende egenskaper. Dataene fra et såkalt Hershberger *in vivo* forsøk på rotter og supplerende *in vitro* studier er etter faggruppens syn forenlig med at metobromuron kan ha en svak anti-androgen effekt.

Abbreviations

ADI	Acceptable Daily Intake
AOEL	Acceptable Operator Exposure Levels
ARfD	Acute Reference Dose
Bioforsk	Norwegian Institute for Agricultural and Environmental Research
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
g a.s./ha	Gram active substance per hectare
In vivo	Experiment on living organisms
In vitro	“In glass” – experiment outside an organism – in test tube
LOAEL	Lowest Observed Adverse Effect Level
NOAEL	No Observed Adverse Effect Level
PSA	Prostate specific antigen
RMS	Reporting Member State
TP	Testosterone propionate
UF	Uncertainty Factor
VKM	Norwegian Scientific Committee for Food Safety

Background as provided by the Norwegian Food Safety Authority

Proman is a new product containing the new active substance, metobromuron. Proman is a broad-spectrum selective herbicide for potatoes grown outdoors. The herbicide is applied early in the spring. Metobromuron belongs to the urea class and is classified in HRAC Group C2 and inhibits photosynthesis. Its mode of action involves inhibiting photosynthetic electron transfer at photosystem two (PS II). There is a relatively low risk for development of resistance to metobromuron in Norway. Proman will be a valuable resistance breaker to ALS-herbicides.

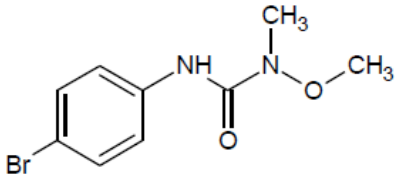
The standardized area dose is proposed set to 4000 ml per hectare (2000 g a.s/ha). Proman should be applied in a volume of 200 L/ha of water with a tractor driven field sprayer. The soil surface should preferably be moist at application.

The Norwegian Institute for Agricultural and Environmental Research (Bioforsk) recommend approval in potato. Due to lack of residue analyses for potato growing under plastic covering with early harvest in mid- summer, Bioforsk suggests specifying on the label that this is for potatoes grown outdoor without cover of plastic or spun bounded polypropylene.

Identity and physical/chemical data

Product name	Proman
Active substance	Metobromuron
Formulation	SC – suspension concentrate
Concentration of active substance	500 g/L
IUPAC-name	3-(4-bromophenyl)-1-methoxy-1-methylurea
CAS number	3060-89-7

Structural formula



Molecular weight		259.1 g/mol
Solubility in water	Moderate:	0.328 mg/l (20 °C)
Vapour pressure	Medium:	2.19×10-4 Pa (25°C) 1.44×10-4 Pa (20°C)
Henrys law constant	Low:	1.14 ×10-4 Pa m3/mol (20°C)
log Pow	Medium:	2.48 (20°C, pH 7.3)
pKa		12.0 (20°C)

Mammalian toxicology

Metobromuron

Toxicokinetics

Absorption: Metobromuron was rapidly and almost completely (> 80%) absorbed from the intestinal tract.

Distribution: Seven days after the administration, the tissue residues were low, except for blood. Metobromuron seems to associate with blood cellular components. These findings were independent of sex, route and/or pre-treatment.

Metabolism: Metobromuron was completely metabolized and was not found in urine or faeces. There were no relevant differences between the sexes.

Elimination: Metobromuron was rapidly eliminated mainly via the urine and to a lesser extent via the biliary route in the faeces.

Acute toxicity

Metobromuron is of low acute toxicity after dermal and inhalation exposure, however is harmful if swallowed and therefore to be classified as Acute tox. 4, H302 and Xn; R22.

Irritation and sensitization

Metobromuron is not considered to be a skin- or eye irritant, however is a skin sensitizer and therefore to be classified as Skin Sens. 1, H317 and Xi; R43.

Genotoxicity

All *in vitro* and *in vivo* genotoxicity studies were shown to be negative. Based on these studies, metobromuron is not considered to be genotoxic.

Sub-chronic toxicity

In the repeated dose studies by dietary and dermal route metobromuron in rodents and dogs showed consistently signs of toxic haemolytic anaemia, with sulf- and met haemoglobin formation over a relatively wide range of doses. Spleen, liver and kidney are the target organs (secondary to haemolytic anemia). Generally these effects showed reversibility even under continued treatment.

In view of the haemolytic effects already appearing at relatively low levels, a classification Xn; R 48/22 “Harmful: danger of serious damage to health by prolonged exposure if swallowed”, equivalent to STOT RE2. H373 is proposed.

Chronic toxicity and carcinogenicity

Treatment of rats with metobromuron over 12/24 months at dietary levels of 150 and 250 ppm induced toxic haemolytic anaemia, manifested primarily by the occurrence of Heinz bodies and met haemoglobin. In pathology increased spleen weights were found in females. Histopathology revealed increased hemosiderin deposition in the liver and spleen. Females were more sensitive than males. There were increases in mammary gland tumours in females and pheochromocytomas in males, which were above the historical controls. There was also an increase in Leydig cell tumours, though this was within the historical controls.

In mice treatment with metobromuron up to 50 ppm did not reveal any oncogenic potential. The tumours seen in this study were spontaneous and rather age-related that occurred most likely independent of the test substance. The incidences were also within the range of variation of the historical controls and literature data according to the study report.

On the basis of increased incidences of mammary gland fibrosarcoma and pheochromocytomoas in the rat study, a classification Xn; R40 “Limited evidence of a carcinogenic effect”, equivalent to Carc. 2. H351 is proposed.

Reproductive and developmental toxicity

Metobromuron caused haemolytic anemia (met haemoglobin, Heinz bodies, histology of spleen and liver) in the two generation reproduction study in parental animals. However, it did not affect reproduction parameters or development of offspring in a two-generation reproduction study in rats.

The developmental toxicity of metobromuron was investigated in two studies in the rat and in the rabbit study. In rats incomplete ossification of vertebrae (dumbbell-shaped) and non-ossification of the 13th rib were observed at the lowest dose without maternal toxicity. In the rabbit study, high post-implantation loss was observed at maternal toxic dose.

On the basis of increased incidence of dumbbell-shaped incomplete ossification of vertebrate in the rat study, a classification Xn; R63 “Possible risk of harm to the unborn child”, equivalent to Repr. 2. H361d is proposed.

Special studies

A special study was performed to address two relevant points: 1.The reversibility of haemolytic effects induced by metobromuron. 2. The potential effect on testes and sperms.

The results showed that the administration of metobromuron for a period of 28 days induced changes in haematology parameters which were reversible within 8 weeks after the end of the treatment period.

No test item-related effects were found in the testes following macroscopic or microscopic examination. There were no test item-related effects upon sperm staging.

Human data

Medical surveillance on manufacturing plant personnel

In its response to the data requirement the notifier provided a report on medical surveillance of manufacturing plant personnel from its manufacturing source where commercial scale production had been limited to date to the batches presented in the 5-batch analysis in vol. 4. No abnormal behaviour and no health complaints of the workers have been noticed and no changes of the worker health status are recorded which could be linked to the production of metobromuron. Especially no indications of sensitization or other allergenic effects have been noticed during the production of metobromuron. In addition the notifier had requested corresponding data from the original owner and manufacturer of metobromuron (now Syngenta) and received the following further information related to medicinal surveillance of manufacturing plant personnel from Syngenta:

Metobromuron was introduced in our database (GEDEX) in 2001, and the last update was in 2007. There was no toxicological data available for metobromuron (from Syngenta, SUVA and CEE). So it was classified C*, that mean workers had to manipulate this product with protective measures of the class C (Class C = substance with VME or OEL between 11 to 100 µg/m. I looked if there has been medical problems related to this substance and I found nothing

Clinical cases and poisoning incidents

There are 3 cases of intentional oral uptake of large quantities of metobromuron (together with metolachlor in 2 cases) reported. The major signs were central or peripheral cyanosis, haemolysis and metabolic acidosis in one case. All survived (Watt B.E, 2005). A 4th similar case has recently been reported by Hsu and Huang (2009). Methaemoglobinaemia developed after 10 hours and hypoxic respiratory failure occurred. Treatment with oxygen, methylene blue and blood transfusions was successful.

Classification and labelling

The proposed classification by the RMS and EFSA is Acute tox. 4, H302 / Xn; R22 (Harmful if swallowed), Skin Sens. 1, H317 / Xi; R43 (May cause an allergic skin reaction), STOT RE2, H373 / Xn; R 48/22 (Harmful: danger of serious damage to health by prolonged exposure if swallowed), and Carc. 2, H351 / Xn; R40 (Limited evidence of a carcinogenic effect).

However, we also propose a classification in Repr. 2. H361d / Xn; R63 (Possible risk of harm to the unborn child) based on the increased incidence of dumbbell-shaped incomplete ossification of vertebrate in the rat study.

Reference values

ADI

The ADI is 0.008 mg/kg bw/day based on the NOAEL of 0.8 mg/kg bw/day from the 2-year study in mouse. An UF of 100 is applied. (EFSA, 2014)

AOEL

The AOEL is 0.016 mg/kg bw/day based on the NOAEL of 1.6 mg/kg bw/day from the 1-year feeding study in dog. An UF of 100 is applied. (EFSA, 2014)

ARfD

EFSA has proposed that the ARfD is 0.3 mg/kg bw based on the NOAEL of 30 mg/kg bw from the developmental studies in rat and rabbit (UF of 100). However, we propose the lower ARfD of 0.1 mg/kg bw based on the NOAEL of 10 mg/kg bw/day from the developmental study in rat (UF of 100).

Metabolites

For the review of metobromuron, additional toxicological information on plant metabolites included in residue definition for risk assessment was requested by EFSA.

The predictions of the QSAR models are confirmed for the acute toxicity and genotoxicity of the compounds under investigation. The acute oral toxicity of 4-bromophenylurea is between 300 and 2000 mg/kg and classifies the compound in the same acute toxicity category 4 (H302) as the parent compound. The oral LD50 of desmethyl-metobromuron and desmethoxy-metobromuron in rat is > 2000 mg/kg and no classification is triggered for these compounds. All three metabolites were negative in the Ames test.

Proman

Co-formulants

Proman does not contain co-formulants occurring above the limit that trigger labelling according to Annex VI of CLP.

Acute toxicity

Proman has low toxicity by oral, dermal or inhalation exposure.

Irritation and sensitization

Proman is found to be neither a skin- or eye irritant nor a skin sensitizer.

Dermal absorption

Rat epidermis is about 3 times more permeable than human epidermis. The potential maximum amount absorbed *in vitro* through human skin was found to be 0.5% of the applied dose for the concentrate and 6.3% for the 1/50 dilution.

Operator, worker and bystander exposure

The AOEL is exceeded in both the UK POEM and the German model without PPE (personal protective equipment) and still exceeded when using PPE (gloves) in the UK POEM. However, the estimated operator exposure to metobromuron is below the AOEL in the German model when PPE and RPE (respiratory protective equipment) were applied during mixing/loading and application.

For bystander and resident the estimated exposure is below the AAOEL/AOEL. Therefore, there is no undue risk for these groups.

For worker the AOEL is exceeded without PPE. However, the estimated exposure is below the AOEL when PPE is applied.

Residues in food or feed

Residues are not discussed in this report.

Terms of reference as provided by the Norwegian Food Safety Authority

Proman is a new product containing the new active substance, metobromuron. The intended use is as a broad spectrum selective herbicide for potatoes grown outdoors.

In this regard, The Norwegian Food Safety Authority would like an assessment of the following:

- The relevance of the carcinogenic effects observed in the rat carcinogenicity study; fibrosarcomas in females and pheochromocytomas and Leydig cell tumors in males.
- Higher incidences of still dumbbell-shaped centres of thoracic vertebrae and non-ossification of the 13th rib observed in the rat developmental toxicity study, and whether these are considered to be malformations.
- Establishment of the NOAEL for the developmental toxicity study in rats and the reference value (ARfD).
- The anti-androgenic potential of metobromuron.

Assessment

1 Introduction

1.1 Background

VKM performs assessments in the context of pesticide registration cf. Regulation on Pesticides § 4. The Norwegian Food Safety Authority, National Registration Section, is responsible for reviewing and evaluating the documentation submitted by the pesticide manufacturer. The Norwegian Food Safety Authority takes the final regulatory action regarding registration or deregistration of pesticides along with a comparative consideration of risk and benefits, and the availability of alternatives (the principle of substitution).

Proman is a new broad-spectrum selective herbicide for potatoes grown outdoors containing the new active substance metobromuron.

1.2 Documentation

VKM's risk assessment is based on the Norwegian Food Safety Authority's evaluation of the documentation submitted by the applicant. The Norwegian Food Safety Authority publishes both their evaluation and final regulatory action on the registration of the pesticide product at their homepage www.Mattilsynet.no.

1.3 Procedure

The first three steps of the risk assessment (hazard identification, hazard characterization and assessment of exposure) are performed by the Norwegian Food Safety Authority and involve an assessment of the documentation submitted by the pesticide manufacturer. The resulting report on hazard identification and hazard characterization, from which the summary is included in the present document, is then reviewed, and specific questions discussed and concluded on by the VKM panel. This review may result in some amendments in the original documents of both the summary and the full report issued by the Norwegian Food Safety Authority.

1.4 HEALTH RISK ASSESSMENT

The assessment of health risk of pesticides is based on the adverse effects produced by the active substance and product in several experimental test systems including long term animal studies. On the basis of this, limits of exposure which represent no health risk are determined. The limits take account of the uncertainties of extrapolating data from animals

to humans and are compared to the operator exposure and human exposure to possible residues in food.

The UKPoem and the German model are used to estimate operator exposure. The models are based on a limited number of studies and are not validated. Thus, the models may not always be sufficiently representative for Norwegian conditions. The limitations of model estimates of exposure are taken into consideration when the calculated level of exposure is close to the threshold limit for acceptable operator exposure (Acceptable Operator Exposure Level; AOEL).

VKM may use a higher safety factor when calculating AOEL and ADI in cases where the product contains critical active substances with serious adverse inherent properties (toxic to reproduction or carcinogenic).

VKM may also consider co-formulants in the product. Consequently, if a product contains critical co-formulants it may be assessed to represent higher risk than what the inherent properties of active substances imply.

2 Hazard identification and characterisation

VKM's Panel on Plant Protection Products has discussed the specific questions raised by The Norwegian Food Safety Authority (Mattilsynet, 2014a) in meetings 24. October and 21. November 2014.

The assessment made by VKM is mainly based on documentation supplied by the manufacturer (European Commission, 2012; European Commission, 2013; European Food Safety Authority, 2014), processed by the Norwegian Food Safety Authorities (Mattilsynet, 2014b) and shown in the reference list. In addition, literature search in PubMed using the search phrase "metobromuron" resulted in 41 hits, but none of them contained data that added to the data used in the assessment.

This is the opinion of the panel for each of the points raised in Terms of reference:

2.1 The relevance of the carcinogenic effects observed in the rat carcinogenicity study; fibrosarcomas in females and pheochromocytomas and Leydig cell tumours in males.

The rat carcinogenicity study showed increases in mammary gland tumours in female rats (150 ppm; 11.1 mg/kg bw) and pheochromocytomas in male rats (150 ppm; 9.3 mg/kg bw) that were above the historical controls. Dose dependent increase in Leydig cell tumours (50 ppm; 3.1 mg/kg bw) were also observed, although this was within the reported range of historical controls. The data for these three tumour types are shown in Table 2.2-1.

In the carcinogenicity study in mice, treatment with metobromuron up to 50 ppm did not reveal any oncogenic potential. The tumours seen in the mouse study were spontaneous and age-related, and most likely independent of exposure to the test substance. The incidences were also within the range of variation of the historical controls and literature data according to the study report.

Table 2.1-1 Tumour types with increased incidence after exposure to metobromuron in the rat carcinogenicity study (Data taken from Table 5.1.6.10 and 5.1.6.11 in the report from The Norwegian Food Safety Authority)

Tumour type	Neoplastic findings in rats (50 animals/group)						Historical control
		Dose level in ppm					
	Sex	0	5	15	50	150	
Mammary gland fibrosarcoma	Female rats	0	1 (2%)	0	1 (2%)	3 (6%)	0-4%
Pheochromocytoma	Male rats	3 (6%)	2 (4%)	4 (8%)	4 (8%)	6 (12%)	0-4%
Leydig cell tumours	Male rats	8	11	12	16	18 (36%)	16-52%

The use of historical controls when assessing the relevance of tumour incidences is a subject for discussion. Considering differences in environmental factors and feed composition between studies it may not be scientifically valid to compare data from a specific study with previous results. It is the view of the Panel that such comparisons should be done with care. When considering the dose-dependent increase in Leydig cell tumours in the rat experiment shown in Table 1, it is the opinion of the Panel that the reported historic controls, in this case going up to 52%, should not be used to disqualify the observed dose-dependent increase in Leydig cell tumours. In addition, published data shows that the herbicide linuron, which have a similar structure to that of metobromuron also induce endocrine disrupting effects (Gray et al., 2001) as well as Leydig cell tumours. The Leydig cell tumours induced by linuron have been suggested to take place through an anti-androgenic mechanism (Cook et al., 1993).

Pheochromocytomas have been shown to be inducible by hormones and other non-genotoxic agents in rats, but the relevance of this to humans is questionable (Greim et al., 2009; Tischler et al., 2004).

2.1.1 Conclusion

It is the opinion of VKM's Panel for Plant Protection Products that the relevance of the observed incidence in mammary gland tumours and Leydig cell tumours in the rat carcinogenicity study is strengthened by the fact that the increases in tumours associated with exposure to metobromuron are observed in hormone responsive tissues. The panel concludes that the carcinogenic effects observed in the rat carcinogenicity study are likely to be relevant for tumours that are influenced by the endocrine system, also in humans.

2.2 Higher incidences of still dumbbell-shaped centres of thoracic vertebrae and non-ossification of the 13th rib observed in the rat developmental toxicity study and whether these are considered to be malformations.

Metobromuron caused haemolytic anemia (met haemoglobin, Heinz bodies, histology of spleen and liver) in the parental animals, but did not affect the reproduction parameters in a rat two-generation reproduction study. The developmental toxicity of metobromuron was investigated in two studies in rats and one in rabbits. Data from the rat development study is shown in Table 2.2-1.

Table 2.2-1 Observations on still incomplete ossifications in the rat developmental studies (Data taken from Table 5.1.8.2 in the report by The Norwegian Food Safety Authority)

		Parameters; number (%)				
Dose (mg/kg)	# skeletons examined	Calcaneus	Sternebrae	Vertebrae dumbbell	Vertebrae bipartite	13th rib
0	183	47 (25.7)	57 (31.1)	8 (4.4)	1 (0.5)	13 (7.1)
10	193	55 (28.5)	65 (33.7)	11 (5.7)	2 (1.0)	28 (14.5)*
30	180	44 (24.4)	80 (44.4)*	13 (7.2)	1 (0.6)	20 (11.1)
90	173	47 (27.2)	71 (41.0)	23 (13.3)*	2 (1.2)	28 (16.2)*
Historic controls	5545	6.8-65.6	4.8-88.3	0.0-5.5	0.15	na

*Fischer exact test, $p<0.01$

Incomplete ossification of sternebrae, vertebrae (dumbbell-shaped) and non-ossification of the 13th rib in rats were observed. In the rabbit study, post-implantation loss was observed at maternally toxic doses.

Incomplete ossifications have often been regarded as variations. It is the opinion of the Panel that incomplete ossification of sternebrae and non-ossification of the 13th rib in rats should by itself be considered as variations, and not as adverse developmental effects.

It is however debated whether still dumbbell-shaped centres of thoracic vertebrae are malformations or aberrations (retardations, variations or deviations). These anomalies have been observed in term foetuses, and still being present 21 days after birth (PND 21), thereby suggesting that either they are not transient, or that they are very slowly reversible changes. There are few studies on the postnatal persistence of foetal anomalies in laboratory animals. Uncertainties about the postnatal fate (persistence/health effects) of some of the foetal abnormalities detected at term remain as a major obstacle for classification. ECHA (European Chemicals Agency) has now described “thoracic vertebral centres still dumbbell-shaped” as malformations.

2.2.1 Conclusion

VKM's Panel on Plant Protection Products has discussed the classification of the different types of incomplete ossifications and concluded that incomplete ossification of sternebrae and non-ossification of the 13th rib in rats should by itself be considered to be variations, and not adverse developmental effects. On the other hand, the Panel agrees with ECHA that the "thoracic vertebral centres still dumbbell-shaped" should be considered as malformations, due to limited data and understanding of the mechanism underlying the observed slow reversal of these anomalies. Furthermore, it is the view of the Panel that the different types of retarded ossification induced by the exposure should be considered as a whole when assessing for developmental effects.

2.3 Establishment of the NOAEL for the developmental toxicity study in rats and the reference value (ARfD).

The ADI is proposed by EFSA (European Food Safety Authority, 2014) and the Norwegian Food Safety Authority to be 0.008 mg/kg bw/day based on the NOAEL of 0.8 mg/kg bw/day from the 2-year study in mouse. An uncertainty factor (UF) of 100 is applied.

The AOEL is proposed by EFSA (EFSA, 2014) and the Norwegian Food Safety Authority to be 0.016 mg/kg bw/day based on the NOAEL of 1.6 mg/kg bw/day from the 1-year feeding study in dog. An UF of 100 is applied.

EFSA has proposed that the ARfD is 0.3 mg/kg bw based on the NOAEL of 30 mg/kg bw /day from the developmental studies in rat and rabbit (UF of 100). The Norwegian Food Safety Authority proposes a lower ARfD of 0.1 mg/kg bw based on a NOAEL of 10 mg/kg bw/day from the developmental study in rat (UF of 100).

The applicant considers the observed delay in skeletal maturation to be associated with reduction in body weight gain and thus as non-specific effects. However, no significant difference in weight gain between the 10 mg/kg bw/day group and the controls was observed.

The findings of the different incomplete/delayed ossifications in rat are shown in Table 2.2-1. Statistical increase in incomplete ossification of 13th rib, sternebrae and vertebrae dumbbell is observed at 10, 30 and 90 mg/kg bw/day metobromuron, respectively, although increases is also indicated at lower concentrations. After thorough discussions of the findings, the Panel decided to view the different endpoints as mechanistically and phenotypically linked and therefore to suggest that a LOAEL should be set to 10 mg/kg bw/day, and that these data from the developmental study on incomplete ossifications in rat should be used for establishment of the ARfD. The LOAEL is therefore based on the various observations of incomplete ossifications as a whole, and not only on the vertebral centres still dumbbell-shaped. An ARfD of 0.03 mg/kg bw is therefore suggested, using an UF of 300 from the LOAEL of 10 mg/kg bw/day.

The panel considers that the reported inhibitory effect on the gripping reflex, also seen in litters of the lowest dosed animals (15 ppm; 1.1 mg/kg bw/day), strengthens the use of 10 mg/kg bw/day as LOAEL for the developmental effects in rats.

2.3.1 Conclusion

VKM's Panel on Plant Protection Products supports the proposal of an ADI value of 0.008 mg/kg bw/day based on a NOAEL of 0.8 mg/kg bw/day from the 2-year study in mouse, and

AOEL of 0.016 mg/kg bw/day based on the NOAEL of 1.6 mg/kg bw/day from the 1-year feeding study in dog. The panel suggests on the other hand an ARfD of 0.03 mg/kg bw based on a LOAEL of 10 mg/kg bw /day with the observations of incomplete ossification in the rat developmental study.

2.4 The anti-androgenic potential of metobromuron.

To assess for androgenic and anti-androgenic effects of metobromuron in rats, a Hershberger bioassay was performed. This assay is based on studies of changes in the weight of five androgen dependent tissues. Marked effects on mean body weight, mean food consumption and enlarged spleen at necropsy were recorded in the groups receiving 100 mg/kg bw/day metobromuron. Sensitivity of the test system was demonstrated by statistically significant weight differences for all 5 studied organs between the untreated control group and the TP (Testosterone propionate) treated group (androgenic effect), as well as between the TP treated group and the TP + Flutamid exposed group (anti-androgenic effect).

At the two highest doses of metobromuron (30 and 100 mg/ kg bw/day) some reduction of seminal vesicles and prostate weights is indicated. By itself, the panel does not consider this as sufficient evidence for an anti-androgenic effect. But on the other hand, only six animals were used in each experimental group. Although this number fulfils the guideline, the risk of false negatives is evident because of low statistical power. Thus, a weak anti-androgenic effect cannot be excluded based on this *in vivo* study.

Anti-androgenic effects of metobromuron was also assayed for using the *in vitro* T47D-Luc reporter gene assay (stable transfection), and in the prostate specific antigen (PSA) expression assay. Metobromuron was in these assays observed to inhibit dihydroxy-testosterone induced effects, either as decreased luciferase activity (T47D-Luc reporter gene assay) or decreased formation of PSA. These two *in vitro* assays are developed as tests for quantitative comparison of endocrine activity, and the results from both assays could be taken to suggest a weak anti-androgenic effect of metobromuron, in support of the results from the Hershberger bioassay discussed above.

The applicant argues that there is no indication of any modulating effect on other hormonal systems, i.e. thyroid/parathyroid, adrenal gland, pancreas and others. The panel is however

not aware of that other methods than the Hershberger assay have been used to test for potential endocrine effects.

The studies discussed above suggest that metobromuron may possess endocrine disrupting potency. The reported induction of cancers in hormone-regulated tissues is further in support of this. Published data also show that the herbicide linuron, which has a similar structure to that of metobromuron, induces endocrine disrupting effects (Gray et al., 2001), and also induces Leydig cell tumourigenesis, which is suggested to take place through an anti-androgenic mechanism (Cook et al., 1993).

The panel noted that the percent of male live foetuses showed a dose dependent decrease following exposure to metobromuron, 50.9 % (control), 49.8 % (10 mg/kg), 46.3 (30 mg/kg) and 45.0 (100 mg/kg) with no change in mean foetal weight, also might be compatible with anti-androgenic activity.

2.4.1 Conclusion

The rat carcinogenicity study indicates that metobromuron may possess endocrine disrupting potency. The data from the Hershberger *in vivo* rat study and the *in vitro* studies is suggestive of a weak anti-androgenic effect. Thus, it is the opinion of the VKM Panel on Plant Protection Products that an anti-androgenic effect of metobromuron cannot be excluded.

3 Exposure / Intake

Not part of terms of reference

4 Risk characterisation

Not part of this evaluation

5 Uncertainties

The uncertainties are discussed under chapter 2 Hazard identification and characterisation

6 Conclusions (with answers to the terms of reference)

VKM's Panel on Plant Protection Products has discussed the specific questions

in the Terms of reference and has the following conclusions:

The relevance of the carcinogenic effects observed in the rat carcinogenicity study; fibrosarcomas in females and pheochromocytomas and Leydig cell tumours in males.

It is the opinion of VKM's Panel for Plant Protection Products that the relevance of the observed increase in mammary gland tumours and Leydig cell tumors in the rat carcinogenicity study is strengthened by the fact that the increases in tumours associated with exposure to metobromuron are observed in hormone responsive tissues. The panel concludes that the carcinogenic effects observed in the rat carcinogenicity study are likely to be relevant for tumors that are influenced by the endocrine system, also in humans.

Higher incidences of still dumbbell-shaped centres of thoracic vertebrae and non-ossification of the 13th rib observed in the rat developmental toxicity study and whether these are considered to be malformations.

VKM's Panel on Plant Protection Products has discussed the classification of the different types of incomplete ossifications and concluded that incomplete ossification of sternebrae and non-ossification of the 13th rib in rats should by itself be considered to be variations, and not adverse developmental effects. On the other hand, the Panel agrees with ECHA that the "thoracic vertebral centres still dumbbell-shaped" should be considered as malformations, due to limited data and understanding of the mechanism underlying the observed slow reversal of these anomalies. Furthermore, it is the view of the Panel that the different types of retarded ossification induced by the exposure of metobromuron should be considered as a whole when assessing for developmental effects.

Establishment of the NOAEL for the developmental toxicity study in rats and the reference value (ARfD).

VKM's Panel on Plant Protection Products supports the proposal of an ADI value of 0.008 mg/kg bw/day based on a NOAEL of 0.8 mg/kg bw/day from the 2-year study in mouse, and

AOEL of 0.016 mg/kg bw/day based on the NOAEL of 1.6 mg/kg bw/day from the 1-year feeding study in dog. The panel suggests on the other hand an ARfD of 0.03 mg/kg bw based on a LOAEL of 10 mg/kg bw with the observations of incomplete ossification in the rat developmental study. An UF of 300 is applied.

The anti-androgenic potential of metobromuron.

The rat carcinogenicity study indicates that metobromuron may possess endocrine disrupting potency. The data from the Hershberger *in vivo* rat study and the *in vitro* studies is suggestive of a weak anti-androgenic effect. Thus, it is the opinion of the VKM Panel on Plant Protection Products that an anti-androgenic effect of metobromuron cannot be excluded.

7 References

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