Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

Risk assessment of the metabolite M44 of bixafen, an active substance in the fungicide Aviator Xpro EC 225

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Summary

Aviator Xpro EC 225 is a new fungicide for use in cereals, containing the new active substance bixafen and the already approved active substance prothioconazole. Prothioconazole was assessed by the Norwegian Scientific Committee for Food Safety (VKM) in 2006, and bixafen in spring 2013.

In the assessment of the environmental fate and behaviour of bixafen, it was concluded that a metabolite coded M44 has potential for groundwater contamination. The applying company has later provided documentation on this metabolite, and the VKM panel was asked to assess the relevance of this metabolite in accordance with the "Guidance Document on the Assessment of the Relevance of Metabolites in Groundwater of Substances Regulated under Council Directive 91/414/EEC" (Sanco/221/2000 – rev.10 – final, 25 February 2003). The Norwegian Food Safety Authority would like an assessment of the health risk for consumers related to groundwater contamination with M44. In particular, the panel was asked to focus on the assessment of health risk for consumers related to malformations reported in the rabbit studies, and the establishing of reference values.

The risk assessment was finalized in a meeting on December 13, 2013 by VKM's Scientific Panel on Plant Protection Products.

VKM's conclusions are as follows:

VKM has previously expressed a general concern related to pesticides where ingredients or metabolites of the active ingredient are capable of reaching ground-water. This concern is certainly relevant for bixafen, one of the active ingredients in Aviator.

The presented data from *in vitro* experiments do not provide the necessary assurance that the bixafen metabolite M44 is negative for genotoxic activity. No data for *in vivo* testing of the metabolite have been presented. It is therefore the opinion of VKM that the submitted studies are not adequate to evaluate possible health risk of the metabolite M44 in accordance with the relevant Guidance Document.

NOAEL is suggested to be set to 100 mg/kg bw/day based on the induction of malformations in the rabbit reproduction toxicity study.

An ADI of 0.1 mg/kg bw/day is proposed for the metabolite M44 based on the use of a 1000 fold uncertainty factor to the NOAEL of 100 mg /kg bw/day in the rabbit reproduction study.

VKM concludes that the "misshapen intraparietal bone" and "severely malformed vertebral columns and/or ribs" alterations should be considered treatment related.

Background

VKM performs risk 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 notifier. The Norwegian Food Safety Authority takes the final regulatory action regarding registration or deregistration of pesticides based on VKMs risk assessment, along with a comparative assessment of risk and benefits, and the availability of alternatives (the principle of substitution).

The Norwegian Food Safety Authority submitted a request on April 18, 2013 for VKM to perform a risk assessment on use of the pesticide Aviator Xpro EC 225 containing the new active substance bixafen and the already approved active substance prothioconazole. The application is for use as a fungicide in cereals. Prothioconazole was assessed by the Norwegian Scientific Committee for Food Safety in 2006, and is therefore not included in this report. The risk assessment was finalized in June, 2013.

Terms of reference

The new plant protection product Aviator Xpro EC 225 containing the new active substance bixafen was assessed by Panel 2 of the Norwegian Scientific Committee for Food Safety in spring 2013. In the assessment of environmental fate and behaviour, it was concluded that a metabolite coded M44 has potential for groundwater contamination. The company has later provided documentation on the metabolite.

The Panel is therefore asked to assess the relevance of this metabolite in accordance to the "Guidance Document on the Assessment of the Relevance of Metabolites in Groundwater of Substances Regulated under Council Directive 91/414/EEC" (Sanco/221/2000 – rev.10 – final, 25 February 2003). The Norwegian Food Safety Authority would like an assessment of the health risk for consumers in regards to groundwater contamination with M44. In particular, the panel is asked to focus on the following:

- The adequacy of the studies submitted in order to evaluate health risk for consumers.
- Establishment of the NOAELs and the reference value (ADI).
- Should the higher incidences of the malformations "misshapen interparietal bone" and "severely malformed vertebral column and/or ribs" be considered treatment related or not?

1 Background 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 of Aviator Xpro EC 225 and their final regulatory action on the registration of the pesticide product at their homepage www.Mattilsynet.no.

2 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 notifier. The resulting report

on hazard identification, hazard characterization and assessment of exposure, from which the summary is included in the present document, is then reviewed by VKM. 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 (2011). The fourth step (risk characterization) is based on the three first steps and is VKM's conclusions or risk assessment.

2.1 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 uses the 75 percentile of exposure assessment for both UK poem and German model. VKM has to base the assessment on the models whenever exposure data for the product is missing.

VKM makes use of 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).

In order to describe the exceeding of maximum tolerated dose, VKM makes use of a scale. The scale is based on the ratio between the estimated exposure based on models or measured exposure in field studies and the Acceptable Operator Exposure Level (AOEL). In cases where the estimated exposure significantly exceeds AOEL, the use of the products may lead to increased risk for health effects.

The following scale is used:

Very high excess of AOEL	more than 500% of the limit
High excess of AOEL	300 – 500% of the limit
Medium excess of AOEL	150-300% of the limit
Moderate excess of AOEL	100-150% of the limit
The limit is not exceeded	

VKM may also consider co-formulants in the product when risk is to be determined. 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.

2.2 ENVIRONMENTAL RISK ASSESSMENT

The environmental risk assessment of pesticides involves predictions of exposure concentrations in various environmental compartments (e.g. soil and surface waters) that may occur after application of the pesticide. These predicted effect concentrations (PECs) are compared to exposure levels that are known to cause toxic effects to important groups of organisms representing the environmental compartments.

The environmental fate and possible ecotoxicological effects of pesticides are investigated in several laboratory- and field experiments. In environmental risk assessments of pesticides, Predicted Environmental Concentrations (PECs) are estimated by use of different scenarios for different parts of the environment (terrestrial, aquatic). The first parameter estimated is usually the initial concentration (PIEC, Predicted Initial Environmental Concentration), e.g. the concentration just after application (usually spraying). PIEC in soil is calculated assuming a homogenous distribution of areal dose in the upper 5 cm soil layer. For surface water, the PIEC is based on deposition of pesticides from spray drift in a standard size water body. The calculations are performed with application of buffer zones between the sprayed area and the water body.

The further exposure regime in different compartments is affected on the fate of the pesticide. The fate is dependent on processes such as photo degradation, hydrolysis, biodegradation and sorption to soil particles. These processes are studied in several standardised laboratory tests. In addition, field tests are used to study the dissipation of the pesticide in various agricultural soils. Based on the experimental fate studies, factors describing different fate processes may be derived and used in models that describe the fate of the pesticide in the soil as well as the transport to surface water and ground water. The concentrations of the pesticide in water are estimated by use of models with relevant scenarios based on EU's FOCUS-scenarios. The models produce maximum PEC and average PEC calculated for specified periods after pesticide application. In the surface water scenarios PEC is also calculated for the sediment phase.

Then the Toxicity Exposure Ratio (TER) is estimated for different groups of organisms. The TER is calculated as the ratio between the toxicity for the organism in question (expressed as LC50, EC50, NOEC etc., depending on organism and study type) and PEC or PIEC. Trigger values for TER, which express the acceptability of the risk for different organisms, have been defined by the EU. The risk is considered minimal when the TER does not exceed the trigger value.

In the terrestrial environment, the risk for toxic effects on bees and non-target arthropods is assessed according to other criteria. Hazard quotients for oral- (HQ_O) and contact toxicity (HQ_C) are estimated for bees. HQ_O and HQ_C are ratios between the standardized area dose of the product (g v.s. /ha) and acute toxicity for the bee (LD50, µg active ingredient/bee). Field experiments and expert evaluation is triggered whenever the hazard quotient is above 50.

For the non-target arthropods, the estimated hazard quotient (HQ) is the ratio between the area dose of the product (g active ingredient/ha), which is multiplied with a factor for multiple applications (MAF, multiple application factor) when appropriate, and the acute toxicity for the organism (LR50, g active ingredient/ha). According to EU, whenever the ratio value exceeds 2, further investigations are triggered.

VKM makes use of a scale in order to describe the risk of exposure for different organisms which live within and outside the spraying field. The scale is based on the ratio between the estimated exposure and the limit or the ratio between the TER and the TER trigger value designated each group of organism.

The following risk scale is used:

Very high risk	more than 500% of the limit
High risk	300 – 500% of the limit
Medium risk	150-300% of the limit
Moderate risk	110-150% of the limit
Minimal risk	the limit is not exceeded

The estimates of exposure concentrations are based on maximal concentrations, which exist during or shortly after spraying. The group of organism assessed (for example birds or leaf dwelling non-target organisms) is not always present during the period of maximal concentration. In the final risk assessment, VKM therefore takes into consideration whether, or to which extent, the organism in question actually will be exposed. This may cause that the risk is assessed lower than indicated by the scale above.

Additionally, uncertainties in the data base both with regard to establishments of limits and models of exposure concentrations are taken into consideration if relevant. This may also cause that the risk is assessed lower or higher than the risk scale. Any deviation from the risk scale is justified in this document.

3 Summary by the Norwegian Food Safety Authority (hazard identification, hazard characterization and assessment of exposure) for bixafen

Aviator Xpro EC 225 is a new product containing two active substances bixafen and prothioconazole. Bixafen is a new active substance. The intended use is as fungicide in spring wheat, winter wheat, triticale, barley, rye and oats.

Aviator Xpro EC 225 has effect against several plant diseases caused by fungi or oomycetes. Aviator Xpro EC 225 is a mixture of two active substances with different mode of action. Both active ingredients have curative and preventive effects. They are systemic and can be quickly translocated within the plant. The standardized area dose is set to 1.25 L per hectare. Aviator Xpro 225 should be applied in a volume of 100 to 300 L/ha of water with a broadcast sprayer using ground directed spraying. Maximal recommended application number is 2 per season with 14-21 days in between.

The Norwegian Institute for Agricultural and Environmental Research recommend approval in spring wheat, winter wheat, triticale, barley, rye and oats.

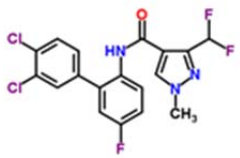
3.1 IDENTITY AND PHYSICAL/CHEMICAL DATA

Product name: Aviator Xpro EC 225

Active substances: Bixafen and prothioconazole

Formulation:	Emulsion concentrate EC
Concentration of active substance:	Bixafen 75g/l, prothioconazole 150 g/l
IUPAC-name (bixafen):	N-(3′,4′-dichloro-5-fluorobiphenyl-2-yl)-3-(difluoromethyl)-1-methylpyrazole-4-carboxamide
CAS number (bixafen):	581809-46-3

Structural formula:



Molecular weight:	414.21 g/mol	
Water solubility:	Low	0.00049 g/l (20 °C)
Vapour pressure:	Low	4.6 x 10^{-8} Pa (20°C)
Henrys law const.:	Low	3.89 x 10^{-5} Pa m^3 mol^{-1}
log Pow:	High	3.3 (25 °C)
pKa:	-	

3.2 MAMMALIAN TOXICOLOGY

3.2.1 BIXAFEN

3.2.1.1 *Toxicokinetics*

Absorption: Bixafen was rapidly absorbed after oral administration. Based on the recoveries in bile, urine and carcasses, approximately 86% and 83% of the administered dose were absorbed by male and female rats, respectively. At low doses, the female rats AUC were

approximately twice as high as those for males. Saturation of the absorption processes was noted at high doses.

Distribution: Maximum concentrations in the organs and tissues were observed between 1 and 8 hours after dosing. A rapid decline of the radioactivity concentrations in all organs and tissues was observed between 1 and 48 hours after administration. Residues levels in the organs and tissues were generally low with highest level in in the liver. There was no evidence of bioaccumulation in males or females.

Metabolism: Bixafen-desmethyl, formed by desmethylation of the pyrazole ring was the most predominant metabolite identified. Hydroxylation of bixafen and bixafen-desmethyl occurred at different positions, especially in the fluoro-phenyl ring. Hydroxy compounds were further conjugated with glucuronic acid. N-conjugation of bixafen-desmethyl with glucuronic acid was also found. Bixafen was also found to be conjugated with glutathione, and this was a major metabolic reaction in bile. Bixafen-desmethyl was also conjugated with glutathione. Cleavage occurs up to 4% of the administered dose.

Excretion: Approximately 93-99% of the administered dose had been eliminated in urine and faeces 72 hour after dosing. For all treatments, the urinary excretion was low (<3%) but female rats excreted approximately twice the amount of the administered radioactivity in urine than males. Biliary excretion was approximately 83% in males and 56% in females at 48 hours after dosing.

3.2.1.2 *Acute toxicity*

Acute toxicity of bixafen is low. No mortalities or clinical signs of toxicity were observed in the acute oral or dermal studies. Clinical signs of toxicity were observed at the maximum achievable concentration in the inhalation study and included bradypnoea, laboured breathing, piloerection, flaccid paralysis of hindlimbs and ungroomed coat. Gross necropsy revealed mild discolouration of the lungs.

3.2.1.3 *Genotoxicity*

A complete battery of *in vitro* and *in vivo* genotoxicity studies was conducted with bixafen. Based on these studies, bixafen is not genotoxic.

3.2.1.4 *Subchronic toxicity*

In the short-term studies on rats, a dose related increase in protrombine time, reduction of bilirubin levels, slight enzyme induction and increased liver weight were observed. Centrilobular hepatic hypertrophy and thyroid follicular cell hypertrophy was also dose related. Increases in platelets, cholesterol and γ-glutamyl transferase activity were observed at the high dose level.

In the short-term studies on mice one female was found dead at the high dose level. This was related to haemorrhage. Dose related reduction in albumin and elevated ALAT and ASAT activities and liver effects were also observed. Focal/multifocal squamous cell hyperplasia in the stomach was observed at high dose level.

In the short-term studies in the dog, increased liver weight and enlarged centrilobular hepatocytes with vacuolated cytoplasm were observed. Haematological changes indicating anaemia were noted at the high dose level.

3.2.1.5 *Chronic toxicity and carcinogenicity*

In the initial 2-year chronic toxicity and carcinogenicity study, significant mortality and signs of haemorrhage were observed early in the study. The analysis of the diet showed levels of vitamin K < 0.3 mg/kg. The haemorrhagic syndrome was attributed to the lack of vitamin K in the diet. All males were therefore terminated without necropsy. The female rats remained in the study, but the diet was supplemented with 15.7 ppm vitamin K. At the high dose, two females died and this was attributed to haemorrhagic syndrome possibly caused by the low vitamin K3 content in the diet at the beginning of the study. Total bilirubin levels were reduced at all dose levels. A dose-related increase in cholesterol levels was also observed. Histopathological findings in the liver included centrilobular to panlobular hepatocellular hypertrophy and an increased incidence and/or severity of hepatocellular brown pigments and multinucleated hepatocytes. In the thyroid, there were increased incidences and/or severity of follicular cell hypertrophy, colloid alteration, brown pigments in follicular cells, follicular cell hyperplasia and an increase in the incidence of follicular cell tumours (adenoma & carcinoma incidences combined).

In the complementary male rat chronic toxicity and carcinogenicity study (supplemented with 8.2-10.6 ppm vitamin K), prothrombin times were significantly decreased. Dose-related reductions in total bilirubin were evident at all dose levels throughout the study. The cholesterol levels were also increased. The microscopic findings in the liver included increased incidences of centrilobular to panlobular hepatocellular hypertrophy, hepatocellular brown pigments, eosinophilic hepatocellular alterations and hepatic cystic degeneration. In the thyroid, there were increased incidences of follicular cell hypertrophy, colloid alteration, brown pigments in follicular cells and follicular cell hyperplasia. There was no evidence of any increase in the incidence of thyroid tumours.

In the carcinogenicity mice study, increased mortality in males occurred at the high dose level during the first twenty weeks of the study. The analysis of the diet showed levels of vitamin K < 0.3 mg/kg. The haemorrhagic syndrome was attributed to the lack of vitamin K in the diet. The diet was then supplemented with 15.7 ppm vitamin K. Several changes to the haematological parameters were observed. Microscopic findings in the liver included increased incidence of centrilobular hepatocellular hypertrophy and vacuolation in both sexes. In addition, male livers had increased incidences of hepatocellular brown pigments, single cell degeneration necrosis, multinucleated hepatocytes and mononuclear infiltrate. In the thyroid, there were increased incidences of follicular cell hyperplasia in both sexes. There was no evidence on any compound induced tumours in either sex.

In the chronic/carcinogenicity studies in rat and mice increased mortality was observed in males. This was attributed a haemorrhagic syndrome occurring early in the study period. The notifier analysed the diet and found levels of vitamin K < 0.3 mg/kg. The notifier conducted studies where diet was supplemented with vitamin K. These studies showed that bixafen did not result in any significant changes to blood coagulation parameters (see 5.1.10 Mechanistic/supporting studies).The notifier then attributed the haemorrhagic syndrome observed in male rats and male mice in short-term and chronic studies to the lack of vitamin K in the diet.

The notifier argued further that the occurrence of haemorrhagic syndrome and the increased mortality in males, but not in the females, were due to a combination of vitamin K deficiency and enzyme induction seen in males. According to the notifier a literature search provided evidence for a synergistic effect of diets deficient in vitamin K and presence of liver enzyme

induction, occurring preferentially in male rats, when exposed to a wide variety of chemical compounds that induce liver metabolising enzymes, when there is low vitamin K in the diet. The chemicals that cause this synergistic response have in common induction of cytochrome P450 2B enzymes. It is hypothesised that induction of CYP450 2B and/or CYP450 3A4 isoenzymes (i.e. those induced by PB) could result in reduction in one or more coagulation factors normally activated by the vitamin K cycle.

The EU rapporteur member state (RMS, UK) concluded that while some information is supportive of the argumentation of the notifier, it is possible that bixafen has some inherent ability to impair blood coagulation in animals on a diet containing normally adequate levels of vitamin K. The RMS supported this conclusion by the following:

- The quantitative data on vitamin K content of the diets are not available for most of the studies
- The supplementation level of 16 ppm vitamin K is in excess of the minimum recommended level for the vitamin K content of rodent diets (about 1 mg/kg in the diet)
- It is not clear what levels of vitamin K are appropriate for the testing and identification of a compound with anticoagulant or suspected anticoagulant properties in rodents
- The magnitude of enzyme induction is not particularly great.

EFSA has also concluded that the haemorrhagic effect observed at high doses in rats and mice does show that this is an intrinsic property of bixafen that may be masked by an excess of vitamin K in the diet.

3.2.1.6 *Reproductive toxicology and teratogenesis*

In the range finding one-generation rat study, there were treatment-related reductions on maternal body weight and body weight gain, some evidence of increased APTT times in males, increased liver and thyroid weights and reduced thymus weights. During lactation, pup weights were reduced (birth weight was not affected) and changes in organ weights were observed.

In the rat two-generation study, there were treatment-related reductions on maternal body weight and body weight gain, and parental organ weight changes (e.g. increased liver, kidney and spleen weight and reduced thymus weight). Hepatic hypertrophy (centrilobular and/or diffuse) was observed, this was associated with a decrease in vacuolisation. F1 and F2 pup body weights were reduced during the lactation periods, and the reductions in F1 pups persisted into adulthood. It is unclear whether the organ weight changes observed in F2 pups were treatment related or secondary to reductions in pup weight. Since birth weight was unaffected by treatment and the reductions in pup weight was seen already from lactation day 4 (in the range-finding study), this effect might be caused by either the presence of test material in the dam's milk, a change in the amount of milk produced by the dams or a change in the nutritional status of the milk. It cannot be excluded that the reduced pup weight during the lactation period is cause by the presence of the test material in the dam's milk.

The numbers of litters with stillborn pups were increased in the F1 and F2 generations when compared to concurrent controls, and were also slightly outside the historical control data for the laboratory. During the assessment in the EU this finding was regarded as inconsistent and insignificant, with minimal impact on overall numbers in the next generation, and the

reproductive NOAEL was set at 2500 ppm. However, based on the increased number of stillborn at the highest dose level (2500 ppm), a NOAEL at 400 ppm should be considered.

It should be noted that the Purina diet used in both reproduction studies contained 1.3 ppm vitamin K (as menadione). There was some evidence of an effect on coagulation parameters in the range finding study, but coagulation parameters were not evaluated in the main multigenerational study.

In the rat developmental study, maternal body weight loss, reduced foetal weight and delayed skeletal development were observed. In the rabbit developmental study, the maternal effects included marked mortality (abortions/failure to maintain pregnancy and sacrificed for humane reasons), clinical signs, body weight loss and reductions in body weight gain, increased liver weight, liver (white foci and prominent lobulation) and urinary bladders findings (enlarged/purulent contents). The rabbit foetal findings included an increase in the number of runts, reduced foetal weight and visceral and skeletal findings. Adopting a precautionary approach, the increased percentage of litters with short innominate arteries and a dose related increase in the percentage of foetuses and litters with extra sternebral ossification site(s) at the mid and top dose levels are regarded as treatment related, because they are outside the historical control range.

3.2.1.7 *Neurotoxicity*

Not relevant.

3.2.1.8 *Mechanistic/supporting studies*

Male rats administered bixafen in diet (containing <0.3 ppm vitamin K3) exhibited a haemorrhagic syndrome (increased PT and APTT values) and a high rate of mortality. The addition of 16 ppm vitamin K to the diet significantly lowered these values after two weeks.

The test material induced slight increases in serum TSH. A slight reduction of T3 and T4 was also seen. There was an increase in BROD activity. A slight increase was also observed in mean UDPGT activity.

3.2.1.9 *Humane data*

No data reported.

3.2.1.10 *Reference values*

ADI: Based on the NOAEL of 2.0 mg/kg bw/day determined for male rats and an assessment factor of 100, an ADI of 0.02 mg/kg bw/day can be proposed for bixafen.

AOEL: Based on the NOAEL of 12.9 mg/kg bw/day determined for the 90-day rat study an assessment factor of 100, a short-term systemic AOEL of 0.13 mg/kg bw/day can be proposed for bixafen.

ARfD: Based on the NOAEL of 20.0 mg/kg bw/day determined for the rat development study an assessment factor of 100, an ARfD of 0.20 mg/kg bw/day can be proposed for bixafen.

3.2.1.11 *Metabolites*

Cereals is not a re-entry culture, therefore there is no risk of workers being exposed to plant metabolites. The exposure of crop inspectors to these metabolites is considered to be minimal, since the metabolites were found only in very low amounts in a 3N rotational crop study.

3.2.1.12 *Co-formulants*

One co-formulant is harmful if swallowed and its content in Aviator Xpro EC 225 is above the classification limit.

3.2.2 AVIATOR XPRO EC 225

3.2.2.1 *Acute toxicity*

The studies conducted with Aviator Xpro EC 225 showed low toxicity by the oral and dermal rout of exposure. The product was not found irritating to the skin, nor found to be a dermal sensitizer. However, the product was found to be irritating to the eyes, and should be classified as eye irritating in category 2 (H319: Causes serious eye irritation) according to the CLP criteria.

3.2.2.2 *Classification and labelling*

Aviator Xpro EC 225 should be classified as an eye irritant in category 2 (H319: Causes serious eye irritation), according to the CLP criteria.

3.2.2.3 *Dermal absorption*

No dermal absorption study has been conducted with the product Aviator Xpro EC 225. Therefore, EFSA's default values for dermal absorption (25% for the concentrated product and 75% for the spray dilution) have been used in the exposure calculations.

3.2.2.4 *Operator, worker and bystander exposure*

The AOEL for bixafen was exceeded in the UK Poem and in the German model without PPE (personal protective equipment). However, the estimated exposure was below the AOEL for bixafen when PPE was applied during mixing/loading and application. No re-entry activities are envisaged for the intended use, and the exposure estimates show no risk for bystanders. The combined level of exposure to the respective active substances (bixafen and prothioconazole) is acceptable for operators using field crop sprayers when personal protective equipment is worn.

3.2.3 RESIDUES IN FOOD OR FEED

The active substance prothioconazole and the product Proline EC 250 were evaluated by the Norwegian Scientific Committee for Food Safety in 2006.

Bixafen is a new active substance and Bayer CropScience AG has applied for approval in EU. The RMS (the United Kingdom) checked the completeness of the dossier and provided its initial evaluation of the dossier on bixafen in the Draft Assessment Report (DAR), which was received by the EFSA on 19 July 2011.

The European Commission (see Draft review report for the active substance bixafen finalised in the Standing Committee on the Food Chain and Animal Health at its meeting on 15 March

2013) supported the approval of the active substance bixafen. The EU concluded that it is appropriate to approve bixafen; however, it is necessary to include certain conditions and restrictions. Member States shall pay particular attention to the residues of bixafen and of its metabolites in rotational crops.

4 Risk characterization

4.1 SUMMARY OF HUMAN TOXICITY/INHERENT PROPERTIES

The plant protection product Aviator Xpro EC 225 with the active substance bixafen was assessed by Plant Product Panel of the Norwegian Scientific Committee for Food Safety (VKM) in spring 2013. In the consideration of environmental fate and behavior, it was concluded that the metabolite M44 has potential for groundwater contamination. In this and previous assessments, VKM has expressed a general concern related to pesticides where ingredients or metabolites of the ingredients are capable of reaching the ground-water. This general concern is certainly also relevant for bixafen, the active ingredient in Aviator.

The company has later provided additional documentation on the metabolite, and in the terms of reference VKM has been requested to assess the health risk for consumers with regard to groundwater contamination with metabolite M44 of bixafen in accordance with the "Guidance Document on the Assessment of the Relevance of Metabolites in Groundwater of Substances Regulated Under Council Directive 91/414/EEC" (Sanco/221/2000 – rev.10 – final, 25 February 2003).

In the EU Drinking Water Directive (Council Directive 98/83/EC) it is stated that the concentration of pesticides and their **relevant** metabolites in groundwater or other types of drinking water must not exceed 0.1 µg/L. The intention of the above mentioned Guidance Document is to define the term "*relevant metabolite*", and to provide an approach towards the assessment of drinking water contaminants.

Thus, a main task of the evaluation of drinking water contaminants is to determine if the metabolites should be considered as relevant, or alternatively as non-relevant. Different provisions exist for relevant and non-relevant substances.

Whether a metabolite is relevant (and thus subject to the 0.1 µg/L limit) or not is generally determined by use of criteria for biological and toxicological properties. A metabolite or degradation product is considered relevant if there is reason to assume that it has comparable properties to that of the active substance with regard to target activity, or alternatively, if it has severe toxicological properties such as genotoxicity, reproduction toxicity, carcinogenicity or general toxicity.

A stepwise procedure should be used:

1. Exclude degradation products of no concern based on structural considerations.
2. Identify groundwater contaminants of concern that exceeds the limit of 0.1 µg/L.
3. Hazard assessment
    a. Compare target activity of metabolite and parent substance
    b. Screen for genotoxicity
    c. Screen for toxicity

On the basis of these steps, relevance of the metabolite is determined.

If the metabolite is considered non-relevant, the following two steps are performed:

4. The EU Scientific Committee on Plants has proposed a toxicological threshold of concern of 1.5 µg/person/day or 0.02 µg/kg body weight/day, in line with the established value from US-FDA. With a consumption of 2 liters of water per day, this relates to an upper limit for the non-relevant metabolite of 0.75 µg/L.
5. Non-relevant metabolites with a groundwater concentration between 0.75 and 10 µg/L may be assessed by a specific refined procedure as described in the Guidance Document.

The panel is especially asked to consider:

- The adequacy of the studies submitted in order to evaluate health risk for consumers.
- Establishment of the NOAELs and the reference value (ADI).
- Should the higher incidences of the malformations "misshapen interparietal" and "severly malformed vertebral column and/or ribs" be considered treatment related or not?

4.1.1 THE ADEQUACY OF THE STUDIES SUBMITTED IN ORDER TO EVALUATE HEALTH RISK FOR CONSUMERS.

The metabolite in question is 3-(difluoromethyl)-1H-pyrazole-4-carboxylic acid, referred to as M44. M44 has been assessed in the EU as a metabolite (M700F200) of the active substance fluxapyroxad, which is identical to M44. The applicant has provided access to parts of this data package, to be used for the assessment of M44 and bixafen in Norway.

Quantification of groundwater contamination was peer-reviewed by VKM in the assessment of bixafen in spring 2013, and the maximum PECgw value for M44 was set to 1.92 µg/L, which is in accordance with calculations in the DAR. The applicant has suggested use of a new maximum PECgw value in the documentation submitted September 2013, based on a lower application rate of Aviator in Norway compared to the EU. The new PECgw calculations have however not been assessed by Mattilsynet or VKM, since the new study was submitted after the fate assessment of bixafen was completed in spring 2013.

The PECgw value of 1.92 µg/L for M44 exceeds the trigger value of 0.1 µg/L, thus requires further assessment to determine its relevance with regard to ground water contamination, in accordance with the Guidance Document described above.

1. *Exclude degradation products of no concern based on structural considerations.*
   M44 does not meet the criteria of being of no concern based on structural considerations.

2. *Identify groundwater contaminants of concern that exceeds the limit of 0.1 µg/L.*
   M44 exceeds the limit of 0.1 µg/L for groundwater contamination.

3.
   a. *Compare target activity of metabolite and parent substance*
      - M44 showed no activity against the cereal diseases *Pyrenophora teres*, *Puccinia triticina* and *Septoria tritici*, and it is concluded that the metabolite M44 does not have comparable target activity to that of the parent substance, bixafen.

   b. *Screening for genotoxicity*

- M700F002 was tested for mutagenicity in four strains of *Salmonella typhimurium* (TA1535, TA1537, TA98 and TA100) and one strain of *Escherichia coli* (WP2 uvrA) with and without metabolizing S9-mix. No indication of a response was reported.

- M700F002 was evaluated for clastogenic effects *in vitro* in Chinese hamster V79 cells, with and without metabolizing S9-mix. Three out of four experiments could be evaluated.

  Experiment 1: Increased numbers of aberrant metaphase cells with and without S9-mix. The increase with S9-mix is statistically significant.
  Experiment 2: Increased numbers of aberrant metaphase cells without S9-mix. The increase at highest dose (1.6 mg/ml) is statistically significant.
  Experiment 4: Statistically significant increase in the number of aberrant metaphase cells with S9-mix.

  It is the opinion of VKM that the experiments carried out with M700F002 in Chinese hamster V79 cells are not sufficient to conclude that the metabolite does not induce clastogenic effects. Furthermore, VKM is skeptical to the consideration of "historic controls" in *in vitro* assays for genotoxicity.

- M700F002 was tested for induction of gene mutations Chinese hamster CHO cells with and without metabolic S9-mix in two independent experiments.
  No statistically significant increases in mutant frequency were observed.

c. *Screening for toxicity*
No acute or 28 day toxicity study has been provided or is required for the metabolite, since the parent compound bixafen has been observed to have low toxicity.

The metabolite M700F002 has been administered in the diet to rats for 90 days (0, 100, 300 and 1000 mg/kg/day). Exposure to 1000 mg/kg bw/day resulted in reduced thymus weights in male rats, but without association with histopathological findings.
Thus, the metabolite is not likely to possess severe toxic properties.

Conclusion: Based on the data presented from the testing of the metabolite for clastogenic effects in Chinese hamster V79 cells, it is the opinion of VKM that the data from the *in vitro* experiments presented does not provide the necessary assurance that the metabolite M700F002/ M44, are negative for genotoxic activity. No data for *in vivo* testing of the metabolite have been presented.

Thus, it is the view of VKM that the submitted studies are not adequate to evaluate the possible health risk of the metabolite M44 in accordance with the above mentioned Guidance Document.

4.1.2 ESTABLISHMENT OF THE NOAELS AND THE REFERENCE VALUE (ADI).

Repeated dose 90-day oral toxicity study in Wistar rats

M700F002 were administered in the diet to rats for 90 days (0, 100, 300 and 1000 mg/kg/day). Alterations in some clinical parameters were observed. The changes were however sporadic with relative large variation, and is therefore not considered treatment related. Exposure to 1000 mg/kg bw/day resulted in reduced thymus weights in male rats, but without association with histopathological findings.

VKM concludes that on the basis of the observations reported, it is reasonable to set NOAEL to 300 mg/kg/day, with reference to the reduced thymus weight observed in the 90 days experiment in male rats.

Reproduction toxicity

Oral administration of M700F002 to Crl:KBL (NZW) rabbits during pregnancy resulted in maternal toxicity at 1000 mg/kg/day, observed as reduced bodyweight gain and increased maternal mortality.

VKM concludes that maternal NOAEL should be set to 300 mg/kg bw/day, based on maternal mortality.

Severely malformed vertebral columns and/or ribs were observed at 300 and 1000 mg/kg bw/day. These observations were above the historical control and were not observed in the control or at 100 mg/kg bw/day. A clear dose-response relationship was not demonstrated, but it is the view of VKM that it cannot be excluded that these malformations were treatment related. Sporadic soft tissue malformations were observed in all dose groups including the control. None of these malformations displayed a dose-response. The incidences were within the historical control range in the study, and were not considered treatment related.

VKM concludes that the developmental NOAEL should be set to 100 mg/kg bw/day based on the induction of malformed vertebral columns and/or ribs.

An ADI of 0.1 mg/kg bw/day is proposed for the metabolite M44 based on applying a 1000 fold uncertainty factor to NOAEL of 100 mg /kg bw/day based on a reproduction study in rabbits. The uncertainty factor accounts for interspecies extrapolation, intraspecies variability, serious and irreversible effects, and data gaps.

4.1.3 SHOULD THE HIGHER INCIDENCES OF THE MALFORMATIONS "MISSHAPEN INTERPARIETAL" AND "SEVERLY MALFORMED VERTEBRAL COLUMN AND/OR RIBS" BE CONSIDERED TREATMENT RELATED OR NOT?

The number of "misshapen intraparietal bone" alterations was enhanced and above the historic control in the highest dosed animals (1000 mg/kg bw/day). The increased frequencies of "severely malformed vertebral columns and/or ribs" observed at the mid- and high-dose (300 and 1000 mg/kg bw/day) were also above the historical control. This type of

malformation was not observed in the control or the low dose group (100 mg/kg bw/day). Although a clear dose-response relationship was not demonstrated, it is the view of VKM that these malformations should be considered treatment related.

A number of soft tissue malformations were observed in all dose groups including control. However, none of these malformations displayed a dose-response and the incidences were within the historical control ranges for the age and sexes of animals used in the study, and were not considered treatment related.

VKM concludes that the "misshapen intraparietal bone" and "severely malformed vertebral columns and/or ribs" alterations should be considered treatment related.

5 Conclusion

5.1 HEALTH

VKM has previously expressed a general concern related to pesticides where ingredients or metabolites of the active ingredient are capable of reaching ground-water. This concern is certainly relevant for bixafen, the active ingredient in Aviator.

VKM concludes that the presented data from *in vitro* experiments do not provide the necessary assurance that the bixafen metabolite M44 is negative for genotoxic activity. No data for *in vivo* testing of the metabolite have been presented. It is therefore the opinion of VKM that the submitted studies are not adequate to evaluate possible health risk of the metabolite M44 in accordance with the relevant Guidance Document.

NOAEL is suggested to be set to 100 mg/kg bw/day based on the induction of malformations in a rabbit prenatal developmental toxicity study.

An ADI of 0.1 mg/kg bw/day is proposed for the metabolite M44 based on the use of a 1000 fold uncertainty factor to the NOAEL of 100 mg /kg bw/day in the rabbit reproduction study.

VKM concludes that the "misshapen intraparietal bone" and "severely malformed vertebral columns and/or ribs" should be considered treatment related.

6 Documentation

The documentation submitted by the applicant in the process of evaluation of the bixafen metabolite M44 has been compiled and evaluated by The Norwegian Food Safety Authority. (www.Mattilsynet.no)