



Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

Risk assessment of the insecticide/acaricide Milbeknock with the active substance milbemectin

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1 Contributors

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

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2 Summary

Milbeknock is a new insecticide/acaricide containing the new active substance milbemectin, which is a microbial fermentation product of *Streptomyces*. It is applied for control of mites and leafminers. The intended use is as a foliar spray in fruits (apples/pears), strawberries (only after harvest) and in ornamental plants growing in greenhouse and outdoors. The risk assessment was finalized at a meeting November 24, 2011, by VKM's Scientific Panel on plant protection products (Panel 2). Panel 2 is in particular asked by the Norwegian Food Safety Authority to look at the following: 1) The human health risk for operators related to the properties of the active substance and the product. 2) The degree of oral absorption. 3) Acute toxicity. 4) The reproduction and developmental toxicity. 5) Establishment of reference values (ADI, AOEL and ARfD). 6) The fate and behaviour in the environment and environmental risk with regard to the properties of Milbeknock and milbemectin. 7) Bioavailability of milbemectin. 8) The microcosm study.

VKM Panel 2's conclusion is as follows:

Health

Panel 2 proposes to set the absorption to 50%, which is in agreement with the EU DAR report (propose 47%). For acute toxicity, Panel 2 concludes that milbemectin probably has a LD50 for dogs between 100 and 200 mg/kg body weight (bw)/day. Dogs seem to be the most sensitive experimental species and should be considered for hazard classification purposes. With respect to reproduction and developmental toxicity, Panel 2 concludes that the documentation of the role of the P-glycoprotein transporter is not convincing, and that the CF-1 mice study cannot be used to support the argumentation of no developmental toxicity put forward by the applicant. The following reference values have been estimated: ADI of 0.03 mg/kg bw/day; AOEL of 0.015 mg/kg bw/day and ARfD of 0.067 mg/kg bw/day for milbemectin. Provided that personal protection equipment is used, the AOEL for operators is not exceeded.

Environment

With respect to environmental fate, the opinion of the Panel is that the relatively rapid degradation in soil indicates a significant bioavailability in both soil and water-sediment systems. Further it concludes that 0.058 µg/L should be regarded as NOEC_{population} for the microcosm study. Panel 2 concludes that there is a medium risk of toxic effects on aquatic organisms due to exposure of milbemectin sprayed in fruits with the proposed application regime, provided that a buffer zone of 30 meters to surface water is applied. There are minimal risks of toxic effects on aquatic organisms with sufficient buffer zones in other applied crops. Panel 2 further considers the risk for foliage dwelling non-target predators and parasitoids to be high, and the risk to earthworms to be medium.

The strong sorption to soil suggests that the bioavailability in soil may be limited. However, the opinion of the Panel is that the relatively rapid degradation in soil indicates a significant bioavailability. Also, for a substance with such a high log K_{ow} the reported water solubility is relatively high, which will contribute to the availability for biological uptake. The toxicity observed in the microcosm study indicates high bioavailability also in a water/sediment system.

3 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 October 18, 2011 for VKM to perform a risk assessment on use of the pesticide Milbeknock containing the active substance milbemectin. Both the environmental and the health risk assessments of the product were finalized by VKM's Panel 2 at a meeting on November 24, 2011.

4 Terms of reference

Milbeknock is a new insecticide/acaricide in Norway containing the active substance milbemectin, and is applied for control of mites and leafminers. The intended use is as a foliar spray in fruits (apples/pears), strawberries (only after harvest) and in ornamental plants growing in glasshouses and outdoors. In connection with this, The Norwegian Food Safety Authority would like an assessment of the human health risk for operators related to the properties of the active substance and the product and the fate and behaviour in the environment and environmental risk. Panel 2 is in particular asked to look at the following:

- The human health risk for operators related to the properties of the active substance and the product. Panel 2 is in particular asked to look at the following:
 - The degree of oral absorption.
 - Acute toxicity.
 - The reproduction and developmental toxicity.
 - Establishment of reference values (ADI, AOEL and ARfD).
- The fate and behaviour in the environment and environmental risk with regard to the properties of Milbeknock and milbemectin. Panel 2 is particularly asked to look at the following:
 - Bioavailability of milbemectin
 - The microcosm study.

5 Risk Assessment

5.1 BACKGROUND DOCUMENTATION

Panel 2's risk assessment is based on the Norwegian Food Safety Authority's evaluation (2011) of the documentation submitted by the applicant. The Norwegian Food Safety Authority publishes both their evaluation of Milbeknock and their final regulatory action on the registration of the pesticide product at their homepage www.Mattilsynet.no

5.2 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 VKMs Panel 2. 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 Panel 2's conclusions or risk assessment.

5.2.1 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 for animal to human. Then the limits are compared to the operator exposure and human exposure to possible residues in food.

The UKPoem and the German model estimate of exposure are used to estimate the 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). Panel 2 uses the 75 percentile of exposure assessment for both UK poem and German model. Panel 2 has to base their assessment on the models whenever exposure data for the product is not presented.

Panel 2 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, Panel 2 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 case the estimated exposure significantly exceeds AOEL, 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	

Panel 2 may take into consideration critical co-formulants of the product when the degree of 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.

5.2.2 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 photodegradation, 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 evt. HQ_C is the ratio 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.

Panel 2 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, Panel 2 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.

5.3 SUMMARY BY THE NORWEGIAN FOOD SAFETY AUTHORITY (HAZARD IDENTIFICATION, HAZARD CHARACTERIZATION AND ASSESSMENT OF EXPOSURE)

Milbeknock is a new product containing the new active substance milbemectin, which is consisting of the microbial fermentation products of *Streptomyces*. Milbeknock is an emulsifiable concentrate (EC) formulation containing 9.3 g/L of the active ingredient.

The product is an acaricide/insecticide, and is applied for control of mites and leafminers. The intended use is as a foliar spray in fruits (apples/pears), strawberries (only after harvest) and in ornamental plants growing in glasshouses and outdoors.

The Standardised Area Dose is 250 ml product (2.33 g milbemectin) per decare, and is based on the applied use in strawberries. The recommended maximum dose rate in fruits (apple/pear) is 190 ml product (1.77 g milbemectin) per decare depending of tree height. In ornamentals the recommended maximum dose rate is 200 ml product (1.86 g milbemectin) per decare.

The product is applied for spraying at a maximum frequency of up to two times in fruits and berries and up to four times in ornamentals.

Spider mites and Liriomyza species (leafminers) have in general high risk of developing resistance to chemical agents. To ensure maximum and prolonged effectiveness and to minimize the likelihood of resistant strains of pests developing, it is recommended that products with a different mode of action are incorporated into annual spray programs.

The product is harmful for several biological control agents used for mite control, and this should be instructed on the label.

5.3.1 IDENTITY AND PHYSICAL/CHEMICAL DATA

Product name Milbeknock

Active substance milbemectin

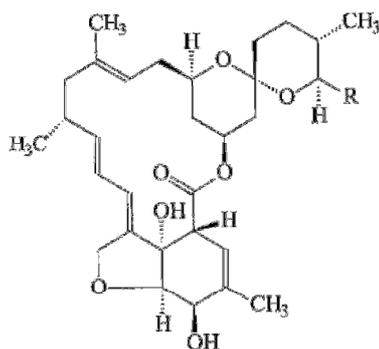
Formulation EC formulation

Concentration of active substance 9.3 g/Litre

IUPAC-name Milbemectin consists of two milbemycin isomers: <30% milbemycin A₃ (MA₃): (10E,14E,16E,22Z)-(1R,4S,5'S,6R,6'R,8R,13R,20R,21R,24S)-21,24-dihydroxy-5',6',11,13,22-pentamethyl-3,7,19-trioxatetracyclo[15.6.1.1^{4,8}.0^{20,24}]pentacos-10,14,16,22-tetraene-6-spiro-2'-tetrahydropyran-2-one ; and >70% milbemycin A₄ (MA₄): (10E,14E,16E,22Z (10E,14E,16E,22Z)-(1R,4S,5'S,6R,6'R,8R,13R,20R,21R,24S)-6'-ethyl-21,24-dihydroxy-5',11,13,22-tetramethyl-3,7,19-trioxatetracyclo[15.6.1.1^{4,8}.0^{20,24}]pentacos-10,14,16,22-tetraene-6-spiro-2'-tetrahydropyran-2-one

CAS number MA₃: 51596-10-2; MA₄: 51596-11-3

Structural formula



Milbemycin A₃: R=CH₃
Milbemycin A₄: R=CH₂CH₃

Molecular weight

Milbemycin A₃ 528.7

Milbemycin A₄ 542.7

Solubility in water

Milbemycin A ₃ : Moderate,	2.68 mg/l (20 °C)
Milbemycin A ₄ : Moderate,	4.55 mg/l (20 °C)

Vapour pressure

Milbemycin A ₃ : Low,	9.7x10 ⁻¹² Pa (20 °C)
Milbemycin A ₄ : Low,	4.3x10 ⁻¹⁰ Pa (20°C)

Henry's law constant

Milbemycin A ₃ : Low,	2.56x10 ⁻³ Pa m ³ /mol
Milbemycin A ₄ : Low,	1.55x10 ⁻³ Pa m ³ /mol

log Pow

Milbemycin A ₃ : Very high,	6.54 (25°C)
Milbemycin A ₄ : Very high,	7.0 (25°C)

pKa

-

5.3.2 MAMMALIAN TOXICOLOGY***Milbemectin****Toxicokinetics**Absorption*

Based on the milbemycin A₄ excretion in urine and bile, the absorption seems to be 47 % of a single low dose in both sexes and 30/40% in males/females at a single high dose. Thus absorption seems to be saturated at higher doses. Peak concentration in blood/plasma was reached after 2-3 hours.

Distribution

The concentration of the substance was higher in tissues (except brain) than in blood/plasma at all time points. Most tissues had a residue peak at 2 hours, but reproductive fat had a peak at 6 hours after dosing. Tissue residues increased disproportionately more than the increase in dose. Repeated dosing gave the same tissue distribution as single dosing. There was no accumulation.

Metabolism

Hydroxylation was the main metabolic pathway and different single-, di-, and trihydroxymetabolites were formed. The main metabolites were 13-hydroxy-MA₃ and -MA₄. MA₃ seems to be more rapidly metabolised than MA₄. There was a minor glucuronidation pathway.

Elimination

The main route of elimination was via bile, and a smaller amount was excreted via urine. There was a higher percentage of excretion of MA₃ than MA₄ in urine. Males had higher urine excretion than females, especially at low doses. There was rapid excretion the first 24 hours followed by a prolonged low excretion, and the elimination was more rapid at low dose than at high dose (reflected in the concentrations in blood). Repeated dosing gave the same elimination pattern as single doses.

Acute toxicity

Milbemectin is of moderate acute toxicity in the rat after oral and inhalation exposure and of low dermal toxicity. Milbemectin appears more toxic to dogs than to rats.

Irritation/sensitisation

Milbemectin was not found to be a skin- or eye irritant nor a skin sensitiser.

Genotoxicity

All *in vitro* and *in vivo* genotoxicity studies were negative. Milbemectin is not considered to be a genotoxic substance.

Sub-chronic toxicity

The dog was the most sensitive species with a LOAEL of 10 mg/kg bw/day and the lowest NOAEL of 3 mg/kg bw/day. In the short term studies effects on liver, kidney, central nervous system and body weight were seen in the rat, mouse and dog. Effects on the adrenals were seen in the rat, dog and rabbit (dermal study). Rats had in addition effects on the uterus, testes and immune system, but the most sensitive parameter was elevated cholesterol. Elevated cholesterol was also seen in dogs. The central nervous system seems relatively more vulnerable in the dog than in rodents, in which effects on other organs were seen at lower dose levels than effects on the CNS.

Chronic toxicity/oncogenicity

The long-term toxicity and carcinogenicity study in the rat gave systemic effects as increased kidney weight in males and effects on adrenals and uterus in females as the most sensitive parameters. At the highest dose level there was also effect on body weight and blood parameters. There was an increase in endometrial polyps and adenocarcinomas in the uterus.

In the mouse elongated incisors, reduced body weight gain and reduced food consumption (females only), were seen at 2000 ppm in a 1.5-year oncogenicity study. The central nervous system, liver, kidney and adrenals were the target organs. There were no neoplastic changes.

Reproductive toxicity and teratology

The two-generation study in rats showed effects on parental body weights and food consumption in parental animals in the high dose group. The high dose level gave reduced litter size and live birth index in the F2 generation. Body weight and body weight gain in the lactation period was affected in both F1 and F2 pups. The F2 generation was more affected than the F1 generation. There were not seen structural abnormalities in the offspring. In the rat, the offspring is more sensitive for milbemectin than the mother. This effects can, however, be regarded as not relevant for humans.

In the rat teratogenicity study, the maternal toxicity was manifested by a decrease in mean maternal body weight and food consumption. There were no effects on the foetuses.

In the rabbit teratogenicity studies, there were seen clinical signs (bradypragia and piloerection), reductions in food intake and body weight, deaths, abortions, dead foetuses and reduced foetal weight. There were no teratogenic effects.

Neurotoxicity

Milbemectin may cause neurotoxic effects of concern.

In an acute oral neurotoxicity study in dogs, some evidence for neurotoxicity was found. A decrease in motor activity was observed at all dose levels; at the lowest tested dose, this decreased motoric activity was even observed in the absence of overt systemic toxicity. It appears that the observations were not performed during peak time of the neurotoxic effects. Therefore a full evaluation of the neurotoxic potential of milbemectin cannot be performed. A NOAEL could not be derived in the acute neurotoxicity study. The LOAEL was 20 mg/kg bw. Establishment of an ARfD can be based on this study.

Repeated dose administration via the diet did not result in neurotoxic effects in rats at 4, 8, and 13 weeks of dosing. Based on the available data a NOAEL of 59 mg/kg bw/d was established for repeated dose neurotoxicity of milbemectin in rats.

Special studies

In a pharmacological study in male rats, mice and rabbits, the results were consistent with an action of milbemectin on the central nervous system and at the neuromuscular level.

In another study the abnormal growth of the incisors in rats was investigated. It was found that the treated rats moved very slowly and there was barely any attrition. The lack of gnawing was the cause of apparent elongation of the incisors. There was no clear explanation to why the animals did not gnaw, but many of the possibilities involve effects on the nervous system.

In a third study the involvement of P-glycoprotein in the absorption of milbemectin through Caco-2 monolayers was determined. However, it was not possible to monitor the concentration of milbemectin due to high non-specific binding of MA₃ and MA₄ to the polystyrene and polypropylene plates in the experimental apparatus.

An acute oral toxicity study in female CF-1 mice (3 animals /dose) was performed to investigate whether milbemectin is a substrate for the P-glycoprotein transporter. Two strains were compared: a wild type strain +/+ and a mutant type strain -/- for the expression of a functional *mdr 1* P-glycoprotein. The study shows that milbemectin is much more toxic in the mice strain that lacks the *mdr 1* P-glycoprotein transporter.

Medical data

There are no reports of clinical symptoms or poisoning from the manufacturing or use of milbemectin or Milbeknock.

Impurities and metabolites

Several impurities and metabolites were tested for acute toxicity in the mouse, and some clinical symptoms were observed. All *in vitro* genotoxicity studies were negative.

Milbeknock 1% EC (SI-9009EC)

Co-formulants

The product contains aromatic hydrocarbons, thus the product may cause lung damage if swallowed.

Acute toxicity

Milbeknock 1 % EC was of low acute toxicity by the inhalation, oral and dermal exposure.

Irritation and allergy

Milbeknock 1 % EC is not irritating to the skin or eye, and it is not a dermal sensitiser.

Dermal absorption

No data were submitted. However, based on the data provided on molecular weights (528.7 and 542.7) and the log Kow (6.43 and 7.00, for MA₃ and MA₄ respectively), and according to the guidance document on dermal absorption, dermal absorption of 10% is considered.

Operator, worker and bystander's exposure

The exposure, estimated by the UK POEM, exceeds AOEL with 14 % when spraying pomes without PPE. The use of PPE reduces the exposure under the AOEL. For spraying in greenhouses and in strawberries the AOEL is not exceeded even without use of PPE. For bystanders and re-entry workers the estimated exposure was far below AOEL.

Residues

Residues are not discussed in this report.

5.3.3 ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL EFFECTS

Environmental fate and behaviour

Degradation in soil

The degradation rate of milbemycin A₄ is medium to moderate, DT50: 21-82 days, geometric mean 36.5 days (arithmetic mean: 43 days). DT90: 69-271 days. Bound residue amounted to 40 % of AR at maximum and the mineralization to CO₂ reached a maximum level of 35 % of AR. Two metabolites were identified > 10 % of applied radioactivity (AR); 27-hydroxy-milbemycin A₃/A₄ (max 14 % AR of A₄) and 27-keto-milbemycin A₃/A₄ (max 12 % AR of A₄). The degradation rate (DT50) of the metabolite 27-hydroxy-milbemycin A₄ was calculated to be 18 days with a DT90 estimated to be 59 days.

At 10 °C the degradation rate of milbemycin A₄ is moderate. DT50: 63 days, DT90: 208 days. The degradation rate is low under anaerobic conditions, DT50: 556 days in the soil phase. DT90 of 1835 days is extrapolated well beyond the study duration. Mineralisation and bound

residues amounted to 1.9 and 22 % of AR after 363 days respectively. No metabolites > 5 % of AR.

Photolysis can be an important route of degradation for milbemycin A₄. DT50 was 7.5 days in samples exposed to light and 27 days in dark control samples. Bound residues increased up to 29 % of AR at the end of the study. Mineralisation amounted to 12 % of AR. No metabolites detected > 10 % of AR.

Two acceptable field studies performed in the US have been submitted. The degradation of milbemycin A₃/A₄ is medium to high with DT50: 8-13 days (geometric mean of 8.5 for milbemycin A₃ and 11.4 for milbemycin A₄). Weather conditions are not well described in the two studies and assessing the relevance to Norway is difficult. Swedish authorities have concluded that the studies were not performed under conditions relevant for Sweden.

Sorption/mobility

The sorption of milbemycin to soil can be classified as high to very high with K_d: 12-138 (average 61) and K_{oc}: 1370-4059 (average 2817). 1/n varied from 0.92 to 1.04 with an average of 0.98.

The sorption of the two metabolites, 27-hydroxy-milbemycin A₄ and 27-keto-milbemycin A₄, to soil can be classified as high to very high with K_f: 20-94 (average 55) and 59-246 (average 171) respectively. K_{oc}: 1828-2462 (average 2111) and 5350-7444 (average 6718) respectively. 1/n varied from 0.80 to 0.85 for 27-hydroxy-milbemycin A₄ and 0.95-1.05 for 27-keto-milbemycin A₄.

Based on the amount of radioactivity in the leachate in an aged column study (1.1-3.3 % of AR after 2 days), the mobility can be classified as medium to high in the four tested soils (sand, sandy loam, clay loam, silt loam), but neither milbemycin A₄ nor any of the major degradation products were detected in the leachate.

Degradation in water

Hydrolysis of ¹⁴C- milbemycin A₄ was determined at 50 °C at pH 5, 7 and 9. DT50 at the different pH values were estimated to be 13, 318 and 241 days respectively. The regression coefficients indicate that the DT50 values at pH 7 and 9 are not reliable. 27-hydroxy-milbemycin A₄ and 27-keto-milbemycin A₄ were found at levels of 8.2 and 23 % of AR respectively at pH 5.

Photolysis is an important degradation pathway for milbemycin A₄ when comparing irradiated samples to the dark controls. The amount of initially applied radioactivity recovered was much higher in the dark controls (96-101 % AR) than in the irradiated samples (15-33 % AR). Three metabolites > 5 % AR were also identified.

¹⁴C- milbemycin A₄ is not readily biodegradable.

Based on a water/sediment degradation the degradation for the whole system can be classified as moderate with DT50_{system}: 82-89 days, geometric mean 85 days (arithmetic mean 86 days). Bound residues amounted to about 30 % of AR after 100 days in both compartments and mineralization was low with only about 6 % after 100 days. The active substance quickly dissipated from the water phase to sediment. Metabolites were detected at levels < 5 % AR.

Fate in air

Hydroxyl reaction and ozone reaction half life were estimated to be 16.4 and 13.7 minutes respectively for milbemycin A₃ and A₄. Milbemycin A₃ and A₄ both have a vapour pressure of $<1.3 \times 10^{-5}$ Pa and a Henry's law constant of 2.63×10^{-3} and 1.59×10^{-3} Pa m³ mol⁻¹ respectively indicating that significant volatilization is unlikely to occur.

Exposure

PIEC (predicted initial environmental concentration) in soil has been estimated in different crops after either one or two applications. Time Weighted Averages and PEC_{plateau} have also been estimated. Worst case PIEC and PEC_{twa} was calculated to be 0.04 mg a.s./kg soil after two applications in strawberries. PEC_{plateau} was calculated with the Finish PEC calculator to be 0.07 mg a.s./kg soil. Only one application and applications in other crops gave lower PEC-values.

Strawberries were used as a worst case culture in the assessment of groundwater exposure. The results of the modelling show that the tested Norwegian and Swedish scenarios gave a PEC_{gw} \ll 0.001 µg/l. The modelling was run by The Norwegian Food Safety Authority using MACRO (4.4.2)

Groundwater modelling performed in connection with the EU registration was done with PEARL v. 1.1.1 and all the relevant FOCUS scenarios (Hamburg-apples in Germany, Chateaudun for apples in France, Sevilla for apples in Spain and Piacenza for apples in Italy). PEC_{gw} was calculated to be \ll 0.001 µg/l in all scenarios for both milbemycin A₄ and the two metabolites 27-hydroxy-milbemycin A₄ and 27-keto-milbemycin A₄.

Models developed by EU's working group FOCUS estimates predicted environmental concentrations in surface water and sediment in different scenarios. The highest PEC_{sw} values were observed right after the second application, indicating that spray drift is the main route of exposure. The highest PIEC for the water and sediment phases are 0.18 µg a.s./l and 7.9 µg a.s./kg dw respectively in strawberries (leafy vegetables) and 1.3 µg a.s./l and 0.98 µg a.s./kg in the water and sediment phases respectively in pome fruit. Based on the need for setting buffer zones and the fact that drift seems to be the major route of exposure to surface water, PEC values estimated using the drift tables in Rautmann et al. 2001 were used in the risk assessment. In strawberries, with one application, PEC values ranged from 0.21 µg/L with a buffer zone of 1 meter to 0.01 µg/l with a buffer zone of 20 meter. In Pome fruit PEC ranged from 0.5 to 0.03 µg/l at buffer zones of 5 and 30 meter respectively. In ornamentals PECs varied between 0.5 µg/l with a buffer zone of 3 meter and 0.01 µg/l with a zone of 30 meters.

Terrestrial organisms

The active substance milbemectin is a mixture of two microbially produced compounds: milbemycin A₃ and milbemycin A₄, naturally occurring at a ratio of approximately 3:7. All Annex II ecotoxicological studies have been conducted with technical milbemectin, containing the two components milbemycin A₃ and milbemycin A₄ in the appropriate relative amounts. Where there are indications that the plant protection product is more toxic than what can be explained by the content of active substance (or studies are only conducted with the product), or identified metabolites are more toxic than the active substance, these calculations are included in the summary below. If this is not the case, these values and calculations are omitted.

Mammals

Acute toxic to mammals (LD50: 456 mg/kg bw/d). TERacute for the indicator species in orchards is estimated as 156 and TERacute is estimated as 574 for the indicator species in strawberries. These values do not exceed the trigger (<10). Moderate reproductive toxicity, NOEC: 200 mg/kg. TERchronic is estimated to be 209 in orchards and 904 in strawberries. These values do not exceed the trigger (<5).

Birds

Milbemectin is acutely toxic to birds (LD50: 347 mg/kg bw). TERacute for the indicator species in orchards is estimated as 363. For the indicator species in strawberries, TERacute values are estimated as 161 and 275 for herbivorous and insectivorous birds, respectively. These values do not exceed the trigger (<10). Milbemectin has moderate dietary toxicity (LC50: 1922 mg/kg feed), TERshort-term for all indicator species in all crops are estimated as >1000, which do not exceed the trigger (<10). Milbemectin also has a moderate reproductive toxicity (NOEC: 150 mg/kg). TERchronic is estimated to be 281 for the indicator species in orchards, and 250 for herbivorous birds and 213 for insectivorous birds in strawberry fields. These values do not exceed the trigger (<5).

Bees

Very high contact toxicity to bees (LD50: 0.026 µg/bee). High oral toxicity to bees (LD50: 0.40 µg/bee). Hazard quotients for contact and oral exposure are estimated to be 680 and 44.2 for applications in orchards, 896 and 58 for applications in strawberries, and 731 and 47.5 for applications in ornamentals. The hazard quotients for contact exposure exceed the trigger value (>50) in all crops.

In order to assess the risk of Milbeknock 1% EC, a semi-field (cage) test has been carried out. The results indicated no significant increase in mortality after application of 27.9 g a.s./ha (higher than the highest dose applied for in Norway), and no effects on flight intensity, behaviour or brood.

Non-target arthropods

In Tier 1 laboratory acute contact toxicity studies, Milbeknock showed negligible effects on parasitoids and ground dwelling predators at relevant application rates. For foliage dwelling predators and predatory mites, the trigger of >30% effect is exceeded.

Earthworms

Milbemectin is acutely toxic to earthworms (LC50_{corr}: 28.5 mg/kg d.w. soil). TERacute for orchard and ornamentals is estimated to be 1425 and 950, respectively. TERacute for the strawberry scenario is estimated to be 950. These values do not exceed the trigger (<10).

Milbeknock has a high chronic toxicity to earthworms (NOEC_{corr}: 0.11 mg/kg d.w. soil). TERchronic for orchards is estimated to be 11. TERchronic for ornamentals is estimated to be 6. These values do not exceed the trigger (<5). TERchronic for strawberries is estimated to be 3. This value exceeds the trigger (<5). TERchronic for strawberries recalculated based on a single application results in a TER of 6 which does not exceed the trigger.

Microorganisms

Neither mineralization nor nitrogen transformation by soil microflora of soils treated with milbemectin up to 75 g a.s./ha (3 x the maximum expected concentration) differed from untreated soils by greater than 25 % (trigger) after 28 days.

Terrestrial plants

Twenty tests are available for a number of crop species. In all treatments effects on emergence, shoot length and shoot weight were below the trigger of > 50% effect at the maximum application rate.

Aquatic organisms

All PEC-values below are based on single application drift values from Rautmann et al. (2001), since FOCUS modeling has shown that drift gives the highest PEC-values. TER calculations have been performed mostly on single species tests, but also with the microcosm study (for invertebrates).

Fish

Milbemectin showed extreme acute toxicity to fish (96h LC50: 4.4-35 µg a.s./L) and extreme chronic toxicity (ELS NOEC: 0.65 µg a.s./L). Milbeknock showed extreme acute toxicity to rainbow trout (96h LC50: 5.7 µg a.s./L). All TER calculations for milbemectin, both acute and chronic, pass the EU triggers (acute: 100, chronic: 10) with 5-30 meter buffer zones.

Invertebrates

Milbemectin showed extreme acute toxicity to *Daphnia magna* (48h EC50: 11 µg a.s./L) and extreme chronic toxicity to *D. magna* (21d NOEC: 0.12 µg a.s./L). Milbeknock showed extreme acute toxicity to *D. magna* (48h EC50: 3.43 µg a.s./L) and very high toxicity to other invertebrates (LC50: 49.3-187 µg a.s./L). All TER calculations for milbemectin pass the EU trigger with 3-20 meter buffer zones, except TERs for chronic exposure from use in ornamentals (TER:9) and pome fruit (TER:4) which fail the trigger (10) even with 30 meter buffer zones.

Sediment dwelling organisms

Milbemectin showed extreme chronic toxicity to *Chironomus riparius larvae* (28d NOEC: 6.3 µg/L(spiked water)). Milbeknock showed extreme acute toxicity to *Chironomus riparius larvae* (48h EC50: 30.1 µg a.s./L) and medium acute toxicity to the oligochaeta Tubificidae (48h EC50: 1142 µg a.s./L). All TER calculations for milbemectin pass the EU trigger with 1-5 meter buffer zones.

Aquatic plants

Milbemectin showed high toxicity to duckweed (14d EC50: >620 µg a.s./L). All TER calculations for milbemectin pass the EU trigger with 1-3 meter buffer zones.

Algae

Milbemectin showed no effects on algae at the highest tested concentration (72h EC50: >2000 µg a.s./L, NOEC: 2000 µg a.s./L). Milbeknock showed very high toxicity to algae (72h EC50: 220 µg a.s./L). All TER calculations for milbemectin pass the EU trigger with 1-3 meter buffer zones.

Microcosm studies

A microcosm study representing a plankton-dominated community was submitted. The company suggests a NOEAEC of 3.68 µg a.s./L. The Swedish Authority (KemI) suggests a

NOEC of 0.058 µg a.s./L, but argues that since it cannot be concluded that the most sensitive organisms were present in the microcosms, the study cannot be used to override the results from the single species tests. The Norwegian Food Safety Authority agrees that the NOEC should be 0.058 µg a.s./L. All TER calculations for milbemectin pass the Nordic/Baltic microcosm trigger with 20-30 meter buffer zones, except the TER for use in pome fruit (TER:1.8) which fail the trigger (3) even with a 30 meter buffer zone.

Bioconcentration

Milbemectin shows a moderate potential for bioconcentration; in bluegill sunfish average whole fish BCF was 76 and 114 for milbemycin A₃ and milbemycin A₄, respectively. Rapid depuration occurred (CT50: 0.7-1.1 days).

Dossier quality and completeness

The dossier is complete and is adequate as a basis for an evaluation of the active substance, metabolites and product.

5.4 PANEL 2'S ASSESSMENT ON HEALTH

5.4.1 SUMMARY OF HUMAN TOXICITY/INHERENT PROPERTIES

In the terms of reference it was stated that Panel 2 in particular should look at the following:

The human health risk for operators related to the properties of the active substance and the product. Panel 2 is in particular asked to look at the following:

- The degree of oral absorption.
- Acute toxicity.
- The reproduction and developmental toxicity.
- Establishment of reference values (ADI, AOEL and ARfD).

Panel 2 discussed these points in-depth:

The degree of oral absorption

Based on the milbemycin A₄ excretion in urine and bile, the absorption seem to be 47 % of a single low dose in both sexes and 30/40% in females/males at a single high dose. This may point to a saturated absorption of milbemycin A₄ at larger doses. The Norwegian Food Safety Authority writes in the evaluation report: "The metabolites found in bile were the same as in faeces from non-bile cannulated rats. In urine there were found several dihydroxy-metabolites of milbemycin A₄. The parent compound was not found in bile samples from any group or in faeces from the low dose group. In the high dose group, the mother substance in faeces was detected as 31% (males) and 37% (females). Since all mother substance was transformed at the low dose, and not all can be accounted for in bile and urine, there seems to be another excretion process involved, presumably intestinal secretion". Furthermore, the Rapporteur Member State (RMS, NL) states in the DAR page 59-60 "it cannot be excluded that the actual absorbed amount systemically available is much less (than 47 %). It could be that most of the radiolabel had not been present in the post-hepatic systemic circulation. The parent compound may have become hydrolysed upon first passage of the liver (first pass metabolism) followed directly by excretion into the bile-duct. This mechanism is supported by the absence of any parent compound in the urine"

The opinion of Panel 2:

Based on the delivered reports the degree of absorption of milbemectin is questionable. At the low dose (2.5 mg/kg bw) most of the compound was metabolized either in the liver or in the intestine. Thus, there seems to be a high first pass metabolism, and thereby low systemic uptake. At the high dose (25 mg/kg bw) the parent compound was detected in faeces (30-40%). There seems to be a saturated absorption of milbemectin and 30-40% of the dose has not been absorbed. However, with regard to the use of milbemectin the absorption at low dose is most realistic for the exposure. Panel 2 discussed possible mechanisms that could explain the findings of metabolites in the gastrointestinal tract: secretion of metabolites over the mucous gland cells in the intestine by P-glycoprotein, excretion into the bile-duct or biotransformation of milbemectin in the intestine. Intestinal efflux mediated by transporters may be a reason why not all metabolites are accounted for in bile and urine. Panel 2 did not find it possible to conclude on which mechanisms that were involved.

Conclusion:

Panel 2 concludes that the degree of absorption is difficult to determine based on the reported studies. The absorption might be as low as 10% (due to high first pass metabolism) or as high as 100% (since only metabolites were observed in the intestinal track at the low dose). Panel 2 proposes to set the absorption to 50%, which is in agreement with EU DAR report (propose 47%). The difficulty in establishing an exact degree of absorption based on the submitted studies is that the mechanism and degree of the first pass metabolism is unclear.

Acute toxicity

Milbemectin is harmful to rat and mouse in studies by oral gavage single dose. LD50 is between 300 -700 mg/kg/bw. It seems that dogs are more sensitive.

The opinion of Panel 2:

The lethal dose for dogs seems to be between 200 and 400 mg/kg/bw observed in an acute toxicity study. LD50 could however not be determined due to few animals. Panel 2 also considered a sub-chronic toxicity study with Beagle dogs where 4 dogs died at 200 and 300 mg/kg bw/day after the first day of administration. Severe treatment-related effects were also observed at 100 mg/kg bw/day. Thus, the LD50 value for dogs is likely to be between 100 and 200 mg/kg bw/ day.

The reproduction and developmental toxicity

RMS proposed in 2005 classification for developmental toxicity (R63) which was discussed in an ECB-meeting (T3) and concluded as follows: "Based on the results from the above acute oral toxicity study in two strains of mice, it is apparent that milbemectin is more toxic when P-glycoprotein is not expressed. The development of the P-glycoprotein transporter is expressed in rats only during late gestation and early lactation whereas in humans P-glycoprotein expression is fully developed by week 28. Adverse effects of milbemectin noted in rats during late gestation and early lactation are not relevant to humans and Sankyo Agro therefore considers that the classification of either R63 or R64 is not appropriate for milbemectin".

The reason for this conclusion is that the applicant has investigated the role of the P-glycoprotein transporter by comparing the acute oral toxicity of milbemectin in two strains of CF-1 mice: a wild type strain +/+ and a mutant type strain -/- for the expression of the *mdr 1a* P-glycoprotein. These mice were of the same two strains used to show the importance of the

P-glycoprotein transporter in expression of the toxicity of abamectin. Doses were selected according to the criteria in OECD 425 (Up and Down procedure). Milbemectin was more toxic in the mutant strain of mice. The NOAEL for clinical signs of toxicity and mortality in wild type mice were 100 and 900 mg/kg respectively, whereas in the mutant mice the NOAEL for clinical signs of toxicity and mortality were only 11 and 100 mg/kg respectively. The applicant also tried to conduct a mechanistic study with P-glycogen transporter but did not succeed.

The opinion of Panel 2:

Panel 2 is more sceptical to the applicant's conclusions than ECB. Panel 2 is of the opinion that the documentation is not convincing, and that the CF-1 mice study cannot be used to support the argumentation put forward by Sankyo Agro. It is not shown in the documentation that the expression of P-glycoprotein has such central role in the developmental toxicity following *in utero* exposure to milbemectin.

Norwegian Food Safety Authority has proposed a NOAEL-value of 12.4 mg/kg bw/day for males, for parental toxicity, offspring toxicity and for reproduction. Panel 2 supports the proposal.

Establishment of reference values:

ADI

An ADI of 0.03 mg/kg bw/day is proposed for milbemectin based on applying a 100-fold uncertainty factor to NOAEL of 3 mg /kg bw/day determined in the 90-days and 1-year dog studies. The critical effects in these studies were an increase in vomiting and in liver weight. The uncertainty factor accounts for interspecies extrapolation (10X) and intraspecies variability (10X). The chronic study in the rat has a lower NOAEL than the dog studies, but the NOAEL in the rat study was based on marginal effects.

The opinion of Panel 2:

Panel 2 supports the choice of ADI.

AOEL

An AOEL of 0.014 mg/kg bw/day is proposed for milbemectin based on applying a 100-fold uncertainty factor to the NOAEL of 3 mg /kg bw/day determined in the 90-days and 1-year dog studies. A correction for incomplete oral absorption of 47 % is applied.

The opinion of Panel 2:

The degree of oral absorption based on the available studies is uncertain. Panel 2 suggests using 50 % oral absorption. The AOEL-value will then be 0.015 mg/kg bw/day

ARfD

An ARfD of 0.03 mg/kg bw/day is proposed for milbemectin based on reduced motor activity in the acute neurotoxicity study in the rat with a NOAEL of 3 mg/kg bw (extrapolated from a LOAEL of 20 mg/kg bw) and a 100 fold uncertainty factor.

The opinion of Panel 2:

The Panel 2 propose an ARfD of 0.067 mg/kg bw/day for milbemectin based on the LOAEL value (20 mg/kg bw) with reduced motor activity in the acute neurotoxicity study in the rat. The LOAEL value is divided by an uncertainty factor of 300 (10x10x3). The value 3 is an extrapolation from a LOAEL value to a NOAEL.

Metabolites and impurities – possible exposure to humans via the environment

Several impurities and metabolites were tested for acute toxicity in the mouse (Slc:ddY), and some clinical symptoms were observed.

5.4.2 RISK CHARACTERIZATION OF HEALTH***Health risk due to human exposure***

Panel 2 has based their risk characterization for operators on the summary from Norwegian Food Safety Authority presented in section 5.3 and on the exposure- and dose-response assessments presented in section 5.2.1 by applying the scale of exceed of AOEL.

Operator, worker and bystander exposure***Operator exposure***

The operator exposure, when using mechanical spraying in pomes and strawberries, was estimated based on the UK POEM and the German model. Exposure, when spraying in greenhouses, was estimated by the Dutch model.

The exposure exceeds AOEL with 14 % when spraying pome fruit without plant protection equipment (PPE). The use of PPE reduces the exposure under the AOEL. For spraying in greenhouses and in strawberries the AOEL is not exceeded even without use of PPE

Re-entry worker exposure

The exposure for re-entry workers harvesting fruits after spraying pomes was estimated by Norwegian Food Safety Authority. The estimated exposure was far below AOEL

Bystander exposure

For estimating bystander exposure the EUROPOEM II model will be used. These are for upward spraying. The estimated exposure was far below AOEL.

Health risk due to residues in products for consumption

Not included in the terms of reference.

5.5 PANEL 2'S ASSESSMENT OF ENVIRONMENT**5.5.1 SUMMARY OF THE ENVIRONMENTAL FATE**

Panel 2 has reviewed the actual documentation and points out the following inherent properties of the product, the active substance and possible metabolites:

Degradation and mobility in soils.

Milbemectin is relative rapidly degraded in soils and has a low mobility due to high soil sorption. The sorption is reversible. Panel 2 does not expect leaching to the groundwater. In areas liable to erosion and run-off, transport of milbemectin sorbed to soil particles can occur. Two metabolites were identified >10 %. The metabolites have a high soil sorption too. An aged column study with ¹⁴C-labelled milbemycin indicates medium to high mobility based on ¹⁴C activity in the leachate, but neither milbemycin nor any of the major degradation products

were detected in the leachate. Therefore Panel 2 will not accentuate the results from this study. Photolysis can be an important route of degradation in surface soil. The degradation of milbemycin is medium to high in field dissipation studies, but Panel 2 does not consider the field studies performed in the US as relevant for Norwegian conditions.

Bioavailability in soils, sediment and water

The strong sorption to soil suggests that the bioavailability in soil may be limited. However, the opinion of the Panel is that the relatively rapid degradation in soil indicates a significant bioavailability. Also, for a substance with such a high log K_{ow} the reported water solubility is relatively high, which will contribute to the availability for biological uptake. The toxicity observed in the microcosm study indicates high bioavailability also in a water/sediment system.

Degradation in water

The aerobic degradation for the water/sediment system can be classified as moderate. The active substance quickly dissipated from the water phase to sediment. Metabolites were not detected at significant levels. The anaerobic degradation is very slow. Photolysis is an important degradation pathway in water.

5.5.2 ENVIRONMENTAL RISK CHARACTERIZATION

The risk characterization of the product's ecotoxicological effects on terrestrial and aquatic organisms made by Panel 2 is based on the summary from the Norwegian Food Safety Authority presented in section 5.3 and exposure-, dose/response assessments and risk scale described in section 5.2.2.

Effects and risk to terrestrial organisms

Panel 2 concludes that there is minimal risk for toxic effects of milbemectin to mammals, birds, plants and soil microorganisms with the proposed application regime.

Bees

Standard laboratory studies show high oral and contact toxicity to bees. The calculated hazard quotients for contact exposure exceeds the trigger value with a hazard quotients for contact exposure exceeding the trigger value (>50) in all crops. However there was no significant increase in mortality and no effects on flight intensity, behaviour or brood in a semi-field (cage) test and Panel 2 therefore considers the risk to bees to be minimal.

Non-target arthropods

Studies showed negligible acute contact effects of Milbeknock to ground dwelling predators at relevant application rates. High effects were seen in acute studies on parasitoids, but not in higher tier studies. For foliage dwelling predators and predatory mites, the trigger of >30% effect was exceeded by more than 300%. Panel 2 therefore considers the risk for foliage dwelling predators and predatory mites to be high and to be minimal for parasitoids and ground dwelling predators.

Earthworms

Milbeknock has a high chronic toxicity to earthworms. TER_{chronic} for use in ornamentals and orchards is estimated to be 6 and 11. The TER_{chronic} values for use in strawberries based

on two applications of Milbeknock within one growing season failed the trigger value, by 170 %, while TER_{chronic} based on a single application, results in a TER of 6 and thus pass the trigger. (minimal risk) Panel 2 considers the chronic risk to earthworms to be medium with two applications.

Effects to aquatic organisms

Laboratory tests on aquatic organisms have shown that milbemectin is extremely toxic to the crustacean zooplankton species *Daphnia magna* (21 d NOEC = 0.12 µg/l). Tests with other invertebrates indicate that insect larvae, gastropoda and tubificidae are less sensitive. The same is true for fish and algae.

A tier 1 risk assessment based on NOEC for *Daphnia* (0.12 µg/l) and the PEC based on drift calculation with 30 m buffer zone and spraying in Pome fruit (0.03 µg/l) gives a TER = 4, which does not pass the EU trigger of 10. The applicant has submitted a microcosm test for possible refinement of the aquatic risk assessment. The test was performed in indoor laboratory plankton-dominated microcosms with a 30 cm water column above a 3 cm sediment layer. The volume of the water was 14 l. The planktonic communities contained microorganisms, algae, cladocerans, copepods, ostracods and rotifers which were obtained from natural populations in nutrient-rich systems. The treatments consisted of two applications of the formulation Milbeknock EC. The interval between the applications was 10 days. The test was terminated 70 days after the second application. The test design included controls and 6 treatments (0.058 – 58.9 µg/l), each with three replicates.

Chemical analysis of the water phase showed that the half-life (DT50) of milbemectin in the water phase was 4-10 days. The results from the study were reported based on the initial nominal concentrations.

Calanoid copepods was the most sensitive taxon showing short term effects at 0.23 µg/l ($NOEC_{\text{population}} = 0.058 \mu\text{g/l}$) and no recovery at 0.92 µg/l. For other copepods and the most sensitive Cladocerans short term effects were seen at 3.68 µg/l ($NOEC = 0.92 \mu\text{g/l}$).

The applicant has argued that the effects observed on the calanoid copepods should be disregarded as it represents only 2% of the total number of taxa identified in the study and furthermore the initial numbers of this group were low (less than 10 /l in the controls) which would tend to make the population inherently unstable and reduce the potential for recovery. The applicant thus suggests a consistent $NOEC_{\text{population}}$ at 0.92 µg/l, and a NOEAEC (No Observed Ecological Adverse Effect Concentration) at 3.68 µg/l. The NOEAEC was selected at the highest tested concentration where recovery of all affected populations (except the calanoid copepods) had occurred within 32 days after the second application.

Panel 2 recognizes that the microcosm test is well performed and reported. The microcosm study confirms the results of the single-species laboratory tests which have shown that milbemectin is extremely toxic to aquatic crustaceans. The fact that the NOECs for various Cladoceran species in the microcosm test are higher than the chronic NOEC for *Daphnia magna* from the laboratory reproduction test may be explained by the different exposure scenarios in the two test systems. In the microcosms the exposure concentrations in the water phase declined after the applications with a half-life of 4-10 days. This decline was probably mainly due to partitioning to the sediment, but this cannot be verified since the concentration of milbemectin in the sediment was not measured.

Panel 2 does not support the view of the applicant that the observed effects on calanoid copepods should be disregarded since statistically significant effects on numbers of this group

were observed on several consecutive sampling occasions after application. Thus, 0.058 µg/l should be regarded as NOEC_{population} for the study.

Panel 2 does not recommend the use of the NOEAEC as proposed by the applicant as a basis for risk assessment since the potential of recovery as shown in the microcosm test may not be representative for the Norwegian field situation.

The representativeness of the study for potential Norwegian field scenarios is reduced by the following factors:

- Nutrient-rich communities
- Potentially sensitive groups e.g. larger crustaceans are lacking
- High temperature (19-24 °C) which could affect dissipation of milbemectin in the water phase and the recovery potential of affected populations
- Small size of the microcosm units

The opinion of Panel 2 is that a NOEC from a single microcosm test should not be used directly as a basis for risk assessment and that an assessment factor (AF) in the range 2-5 should be used to account for the uncertainty involved in extrapolation to different real field situations. The use of an appropriate AF is a risk management decision but based on the representativeness issues indicated above, a minimum assessment factor of 3 should be considered.

Based on the NOEC_{population} from the microcosm test (0.058 µg/l) and using an AF=3, the environmental concentration should not exceed 0.019 µg/l. This can be compared with the corresponding concentration that can be calculated from the NOEC from the Daphnia reproduction test in tier 1 (0.12µg/l), which divided by the TER-trigger (10) gives 0.012 µg/l. Thus, the outcome of the risk assessment based on the microcosm NOEC gives a similar result as an assessment based on tier 1 data.

Risk characterisation to aquatic organisms

Based on the NOECs for effects on aquatic crustaceans in Tier 1 tests and from the microcosm test, the following risk estimates for various cultures have been calculated.

Pome fruit

Exposure concentrations have been estimated based on spray drift during application in pome fruit which is considered by Panel 2 to be the worst case scenario in terms of exposure. The tier 1 chronic risk assessment with a TER = 4 (with a 30 meter buffer zone) and use of the scale described in chapter 5.2.2 gives a medium risk with 250 % exceed of the limit. The higher tier calculation with the NOEC from the microcosm study gives a TER = 1.8 and a medium risk with 170 % excess of the limit.

Ornamentals

Exposure concentrations have been estimated based on spray drift during application in ornamentals. The tier 1 chronic risk assessment with a TER = 9 (with a 30 m buffer zone) and use of the scale described in chapter 5.2.2 gives a moderate risk with 110 % excess of the limit. The higher tier calculation with the NOEC from the microcosm study gives a TER = 5.8 and thus pass the trigger (minimal risk).

Strawberry

Higher tier calculated $TER_{\text{chronic}} = 5.8$ with 20 m buffer zone and therefore indicates a minimal risk.

Conclusions

For terrestrial organisms, Panel 2 concludes that there is minimal risk for toxic effects of milbemectin to mammals, birds, plants, bees, and soil microorganisms with the proposed application regime.

Panel 2 considers the risk for foliage dwelling predators and parasitoids to be high and to be medium for earthworms (two applications).

Panel 2 further concludes that there is a medium risk of toxic effects on aquatic organisms due to exposure of milbemectin sprayed in fruits with the proposed application regime, provided that a buffer zone of 30 meter to surface water is applied. There are minimal risks of toxic effects on aquatic organisms with sufficient buffer zones in other applied crops.

5.5.3 QUALITY OF THE SUBMITTED DOCUMENTATION

Panel 2 is of the opinion that the documentation submitted to VKM is adequate as a basis for an evaluation of the active substance, the metabolites, and for the technical material.

6 Conclusion

6.1 HUMAN HEALTH

Absorption:

The degree of absorption is difficult to determine based on the reported studies. The absorption might be as low as 10% (due to high first pass metabolism) or as high as 100% (since only metabolites were observed in the intestinal track at the low dose). Panel 2 propose to set the absorption to 50%, which is in agreement with the EU DAR report (propose 47%).

Acute toxicity:

Milbemectin probably has a LD50 for dogs between 100 and 200 mg/kg bw/day. Dogs seem to be the most sensitive experimental animal.

The reproduction and developmental toxicity:

Panel 2 is of the opinion that the documentation of the role of the P-glycoprotein transporter is not convincing, and that the CF-1 mice study cannot be used to support the argumentation of no developmental toxicity put forward by the applicant.

Establishment of reference values:

ADI

Panel 2 supports the choice of ADI of 0.03 mg/kg bw/day

AOEL

The degree of oral absorption based on the available studies is uncertain. Panel 2 suggests using 50 % oral absorption. The AOEL-value will be 0.015 mg/kg bw/day

ARfD

Panel 2 propose an ARfD of 0.067 mg/kg bw/day for milbemectin based on the LOAEL value (20 mg/kg bw) with reduced motor activity in the acute rat neurotoxicity study.

6.2 ENVIRONMENT

Bioavailability:

Despite the very high fat-solubility of the active substance, the water solubility is high. The opinion of Panel 2 is that the biodegradation in soil is higher than expected. This suggests that milbemectin is bioavailable in soil in spite of the high sorption to soil particles. The toxicity observed in the microcosm study is also an indication of bioavailability in a water/sediment system.

The microcosm study:

Panel 2 recognizes that the microcosm test is well performed and reported. The microcosm study confirms the results of the single-species laboratory tests which have shown that milbemectin is extremely toxic to aquatic crustaceans. Panel 2 does not support the view of the applicant that the observed effects on calanoid copepods should be disregarded since statistically significant effects on numbers of this group were observed on several consecutive sampling occasions after application. Thus, 0.058 µg/l should be regarded as NOEC_{population} for the study, and an assessment factor of 3 should be used.

Aquatic risk assessment:

Panel 2 concludes that there is a medium risk of toxic effects on aquatic organisms due to exposure of milbemectin sprayed in fruits with the proposed application regime, provided that a buffer zone of 30 meter to surface water is applied. There are minimal risks of toxic effects on aquatic organisms with sufficient buffer zones in other applied crops.

Terrestrial risk assessment:

Panel 2 considers the risk for foliage dwelling non-target predators and predatory mites to be high and the risk for earthworms to be medium (two applications).

Attachment

Attached is The Norwegian Food Safety Authority's evaluation of the documentation submitted by the applicant, following application for registration of the acaricide/insecticide Milbeknock (milbemectin) 2011 (www.Mattilsynet.no).

References.

T3: Sankyo Agro CO., Ltd, 2006. "Position statement for ECB Meeting 21 March 2006"

