Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

VKM Report 2017:1

Risk assessment of the biological plant protection product Nemasys C with the active organism *Steinernema carpocapsae*

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

Report from the Norwegian Scientific Committee for Food Safety (VKM) 2007:1
Risk assessment of the biological plant protection product Nemasys C with the active organism *Steinernema carpocapsae*

Opinion of the Panel on Plant Protection Products of the Norwegian Scientific Committee for Food Safety
13.02.2017

ISBN: 978-82-8259-258-1
Norwegian Scientific Committee for Food Safety (VKM)
Po 4404 Nydalen
N – 0403 Oslo
Norway

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

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

Suggested citation: VKM (2017) Risk assessment of the biological plant protection product Nemasys C with the active organism *Steinernema carpocapsae.* Opinion of the Panel on Plant Protection Products of the Norwegian Scientific Committee for Food Safety. VKM Report 2017:1, ISBN: 978-82-8259-258-1, Oslo, Norway. Available online: www.vkm.no

Risk assessment of the biological plant protection product Nemasys C with the active organism *Steinernema carpocapsae*

Authors preparing the draft opinion

Torsten Källqvist (chair), Hubert Dirven, Edgar Rivedal, May-Guri Sæthre, Micael Wendell

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

Assessed and approved

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

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

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

Acknowledgment

May-Guri Sæthre from the Panel of Plant health of the Norwegian Scientific Committee for Food Safety is acknowledged for her valuable work on this opinion. Project manager from the VKM secretariat has been Edgar Rivedal

Competence of VKM experts

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

Table of Contents

Summary

Nemasys C with the nematode *Steinernema carpocapsae* as the active organism is sought be be used as a plant production product in Norway. Nemasys C is intended for use against the codling moth (*Cydia pomonella*) and oriental fruit moth (*Cydia molesta*), the larva stage in shore flies (Ephydriae), pupal stage in large pine wevils (*Hylobius abietes*) and larva including; *Spodoptera* spp., *Chrysodeixis chalcides*, *Mamestra brassicae* and *Duponchelia fovealis* in greenhouses, domestic gardens, outdoor crops and lawns, orchards and forest plantations.

In this regard, the Norwegian Food Safety Authority has asked for the opinion of VKM on specific issues related to the assessment of potential health and environmental risk; in particular if the nematode is naturally occurring in Norway, the potential for establishing and spreading under Norwegian conditions, possible taxonomic challenges, and potential for health risk related to its use.

The assessment was finalized in January 2017 and adopted by VKM's Panel on Plant Protection Products.

VKM's conclusions are as follows:

Distribution; Evaluate if the nematode is naturally occurring in Norway

Steinernema carpocapsae has been identified in a soil sample from an orchard in Western Norway (Sogn) in 2001. It is not likely that the occurrence on that location is caused by application of *S. carpocapsae* as a biological control agent, since this species has been applied only in a research project at a distant location in Norway in 2006, i.e. later than the species was found in Sogn. Thus, VKM considers that *S. carpocapsae* occurs naturally in Norway. The distribution of the species within Norway is not known, since no systematic mapping has been performed.

Assess the potential for establishment and spread of the nematode under Norwegian conditions specified for use in greenhouses and outdoor cultivation.

Steinernema carpocapsae may be able overwinter at least in coastal areas of Southern Norway, and therefore local establishment of the species is possible. The potential for spreading from treated areas is considered to be low because of low active mobility and low potential for reproduction in soil. A possible route for long-range transport is by anthropogenic dislocation of growing medium e.g. with potted plants. The risk of speading is

lower in greenhouse cultivation than outdoor, provided that the growing medium (eg. soil) from the culture is handed properly.

Consider possible taxonomic challenges related to the risk assessment

The taxonomy of *S. carpocapsae* is well established. The nematode can be identified based on morphological characteristics and the DNA sequence of the internal transcribed spacer region of the ribosomal gene array (ITS rDNA). (Adams and Nguyen 2002). There are no taxonomic challenges related to assessment of this nematode.

The human health risk for operators related to the properties of NEMASYS C and with the nematode *Steinernema carpocapse* as the active organism.

The use of plant protection products containing entomopathogenic nematodes against insects has not been associated with health effects on humans. The symbiotic bacteria *Xenohabdus* spp*.* has not been linked to pathogenic effects in humans. It is therefore the view of VKM that the use of the nematode *S. carpocapsae* with the symbiotic bacteria *Xenohabdus nematophilus* will pose a minimal health hazard for operators.

Key words: VKM, (benefit and) risk assessment, Norwegian Scientific Committee for Food Safety, Norwegian Food Safety Authority/Norwegian Environment Agency (stryk det som ikke passer)

Sammendrag på norsk

Det er søkt om tillatelse for bruk av preparatet Nemasys C, med nematoden *Steinernema carpocapsae* som aktiv organisme, som plantevernmiddel i Norge. Nemasys C er tenkt brukt som insektmiddel mot eplevikler (*Cydia pomonella*) og ferskenvikler (*Cydia molesta*), vannfluelarver (Ephydriae), puppestadiet av grasnutebiller (*Hylobius abietis*) samt larver av *Spodoptera* spp., *Chrysodeixis chalcides*, *Mamestra brassicae* og *Duponchelia fovealis*, i veksthus, hager, plener, ulike avlinger, frukthager og skogplantasjer.

I denne forbindelse har Mattilsynet bedt om en uttalelse fra VKM om konkrete problemstillinger knyttet til vurdering av potensiell helse- og miljørisiko; om nematoden forekommer naturlig i Norge, nematodens evne til å etablere og spre seg under norske forhold, mulige taksonomiske utfordringer og eventuell helserisiko knyttet til bruk av preparatet.

Prosjektgruppens arbeid ble avsluttet i januar 2017 og rapporten godkjent av VKMs Faggruppe for plantevernmidler.

VKMs konklusjoner er som følger:

Distribusjon; Om nematoden er naturlig forekommende i Norge

Steinernema carpocapsae ble funnet i en jordprøve fra en frukthage i Vest-Norge (Sogn) i 2001. Det er ikke sannsynlig at dette funnet er forårsaket av bruk av *S. carpocapsae* som plantevernmiddel, siden denne arten bare har vært brukt i et forskningsprosjekt på et annet sted i Norge i 2006, det vil si etter at arten ble funnet i Sogn. Det er derfor VKM sin oppfatning at *S. carpocapsae* forekommer naturlig i Norge. Utbredelsen av arten i Norge er ikke kjent, men anses å være begrenset.

Nematodens evne til etablering og spredning under norske forhold

Steinernema carpocapsae vil trolig kunne overvintre i kystområdene i Sør-Norge, og lokal etablering av arten er derfor sannsynlig. Potensialet for spredning fra behandlede områder anses imidlertid å være lite, på grunn av lav aktiv mobilitet og lavt potensiale for reproduksjon. En mulig spredningsvei er gjennom jordforflytning, for eksempel med potteplanter. Risiko for spredning er lavere ved bruk i drivhus enn ved dyrking utendørs, forutsatt at vekstmediet blir håndtert på riktig måte.

Mulige taksonomiske utfordringer knyttet til risikovurderingen

Taksonomien for *S. carpocapsae* er godt etablert. Nematoden kan identifiseres både basert på morfologiske kriterier og DNA-sekvensering av deler av et ribosomalt gen-array (ITS rDNA). Det er derfor ingen taksonomiske utfordringer knyttet til karakterisering av denne nematoden.

Potensiell helserisiko for brukere av preparatet

Bruk av plantevernmidler med entomopatogene nematoder har ikke vært assosiert med helseeffekter. Den symbiotiske bakterien *Xenohabdus* spp. har heller ikke vært knyttet til sykdomsfremkallende effekt hos mennesker. Det er derfor VKMs oppfatning at bruk av nematoden *S. carpocapsae* med den symbiotiske bakterien *Xenohabdus nematophilus* vil ha minimal helsefare for operatørene.

Terms of reference as provided by the Norwegian Food Safety Authority/

NEMASYS C is a biological plant protection product with the nematode *Steinernema carpocapse* as the active organism. Nemasys C is intended for use against the codling moth (*Cydia pomonella*) and oriental fruit moth (*Cydia molesta*), the larva stage in shore flies (Ephydriae), pupal stage in large pine wevils (*Hylobius abietes*) and larva including; *Spodoptera* spp., *Chrysodeixis chalcides*, *Mamestra brassicae* and *Duponchelia fovealis* in greenhouses, domestic gardens, outdoor crops and lawns, orchards, ornamentals and forest plantations.

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

- Distribution; Evaluate if the nematode is naturally occurring in Norway
- Assess the potential for establishing and spreading of the nematode under Norwegian conditions specified for use in greenhouses and outdoor cultivation.
- Consider possible taxonomic challenges related to the risk assessment
- The human health risk for operators related to the properties of NEMASYS C and with the nematode *Steinernema carpocapse* as the active organism.

Assessment

1 Introduction

1.1 Applicant and producer

Norwegian applicant:

BASF A/S, Ved Stadsgraven 15, 1300 Copenhagen S, Denmark

Producer/ supplier: BASF

1.2 Product and trade name

Application of the biological control product Nemasys C containing the nematode species, *Steinernema carpocapsae*.

1.2.1 Associated organism and formulation

The product is formulated with 13% cross-linked polyacrylate which acts as an inert carrier. The active organism, *S. carpocapsae* is symbiotically associated with the bacteria *Xenorhabdus nematophila*, (Akhurst 1980). *Xenorhabdus* cells are asporogenous, rod-shaped cells (0.3–2×2–10µm), and Gram negative. They are facultatively anaerobic, with both respiratory and fermentative types of metabolism (Boemare 2002)

1.3 Properties for use as a plant protection product

Entomopathogenic nematodes are obligate parasites of insects. The only free-living stage occurring naturally in soil outside the insect host is the third larval stage (also called infective juvenile or dauer larvae). Infective juveniles carry symbiotic entomopathogenic bacteria in their intestine and are capable of invading and killing their host within 1-2 days.

The life cycle (Figure 1) begins when infective juveniles encounter a host in the soil, for example a root feeding insect larva. The nematode enters the host through natural openings or through the host cuticle. Once inside the body cavity the associated symbiotic bacteria are released initiating pathogenicity and reproduction of nematodes and bacteria. Death of the host usually occurs quickly within 2 days at around 20°C. Nematodes feed and reproduce within the host cadaver until nutrients are depleted whereby a new generation of infective juveniles are formed, carrying fresh bacterial cells of the symbiotic bacteria. The life cycle is usually completed within 2 weeks at 20°C and a new generation of infective juveniles can be found in the soil surrounding the host cadaver.

The infective juveniles are both the dispersive and resistant life stage of *S. carpocapsae* and have high short term mortality. This high mortality of the infective juveniles is offset by the high fecundity within the hosts. Tens of thousands to hundreds of thousands of infective juveniles can emerge from a single, large host cadaver.

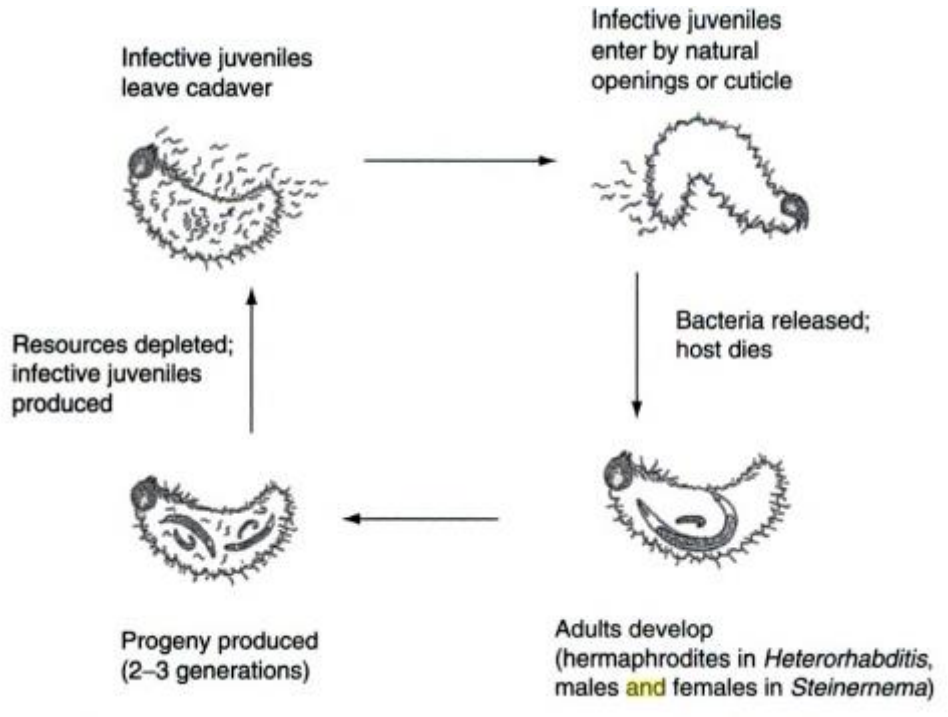

Figure 1. Life cycle of entomopathogenic nematodes (From Griffin et al. 2005)

1.3.1 Target pests

Nemasys C is intended for use against the codling moth (*Cydia pomonella*), oriental fruit moth (*Cydia molesta*), the larva stage in shore flies (Ephydriae), pupal stage in large pine wevils (*Hylobius abietes*) and larva including; *Spodoptera* spp., *Chrysodeixis chalcides*, *Mamestra brassicae* and *Duponchelia fovealis.*

1.4 Status in Norway

Steinernema carpocapse has not previously been registered as a biological control agent under the present Norwegian regulation, which came into force in 2001. However, dispensation was given for use in forest plantation under a research project in 2006 (Haukeland 2007). *Steinernema carpocapsae* is on the "List of biological control agents widely used in the EPPO region" (http://archives.eppo.int/EPPOStandards/biocontrol_web/bio_list.htm#biolist)

1.5 Area for use

Greenhouses, domestic gardens, outdoor crops and lawns, orchards and forest plantations, domestic gardens, outdoor crops and lawns.

1.6 Taxonomy and origin of biological control agent

Class/Order/Family

Phylum: Nematoda, Class: Secernentea, Subclass: Rhabditia, Order: Rhabditida Family: Steinernematidae (Cabi)

Genus and species name:

Steinernema carpocapse (Weiser 1955). (Originally named *Neoaplectana carpocapsae*)

Common name:

Entomopathogenic nematode is the common name for biocontrol nematodes in the families Heterorhabditidae and Steinernematidae. There is no common name for the different species. In Norway the common name for entomopathogenic nematodes is 'nyttenematoder'.

1.7 Countries where *Steinernema carpocapsae* has been introduced as a biological control agent

Nemacys C is marketed in the following European countries: Belgium, Finland, France, Germany, Italy, the Netherlands, Poland, Portugal, Spain, Slovenia, Sweden and UK.

2 Hazard identification and characterisation

2.1 Original area and distribution of the species

Steinernema carpocapsae was first described by Weiser (1955) who isolated the nematode from larvae of the codling moth, *Carpocapsa pomonella* in the Czech Republic (Nguyen and Hunt 2007). The distribution of the species is wide in the temperate regions, and it has been found in Europe, the American continents, Asia and Australia. According to a review by Hominick (2002) *S. carpocapsae* has been reported from the following European countries: Austria, Azores, Czech Republic, France, Georgia, Germany, Italy, Poland, Russia, Slovak Republic, Spain, Sweden, Switzerland and UK. Countries where surveys of entomopathogenic nematodes have been performed without detection of *S. carpocapsae* include Belgium, Denmark, Greece, Hungary and Ireland. However, as pointed out by Hominick (2002), it is difficult to state with confidence that a species does not exist in a locality, as it may be so rare that finding it is a function of sampling effort.

After the review by Hominick (2002), natural occurrence of *S. carpocapsae* in Belgium was reported by Ansari et al. (2007). In Norway, *S. carpocapsae* was identified in a soil sample from an orchard in the coastal area of Western Norway in 2001 (Haukeland et al. 2006). According to the authors, this is the first report of the species from the Nordic countries, since the previous report of this species from Northern Sweden had been shown to be an erroneous identification.

The free living stage of *S. carpocapsae* is found mainly in the soil. According to the Swedish evaluation of the product, the existence of nematods is highest in sandy/loamy soils, where there is more space between the soil particles than in more compact soils (Kemi 2013).

2.2 Dispersion in soil

After release from an insect larva, the infective juvenile *S. carpocapsae* remains in the soil in ambush of a prey. Compared to other entomopathogenic nematodes, *S. carpocapsae* shows lower active mobility in soil. This may be explained by the ambushing type of host-finding behaviour (Poinar and Grewal 2012). The mobility is further dependent on the soil structure and is higher in sandy soils than in silty clay loam soil (Georgis and Poinar 1983). Laboratory studies of the vertical dispersal in sand showed that when applied on the sand surface approx. 90 % of the infective juveniles remained within 1 cm of the surface after 48 hours. In lateral dispersal experiments between 77 and 95% remained within 2 cm of the point of application after 48 hours while 0.2-1.3% were found between 8-10 cm from the application point. Moyle and Kaya (1981). In a field study *S. carpocapsae* were recovered 30.5 cm

from the point of application after seven days, indicating that some juveniles moved as much as 4.35 cm a day (Poinar and Hom 1986).

2.3 Temperature dependence

Saunder and Webster (1999) who studied the effect of temperature on the infection of larvae of the greater wax moth, *Galleria mellonella*, by entomopatogenic nematodes showed that infection, reproduction, and development of *S. carpocapsae* were fastest at 20 to 24 °C. Infection was reduced at lower temperatures, but occurred still between 8 and 16 °C. After 33 days at 8 °C, the mortality of larvae infected by *S carpocapsae* was 94%. However, maturation of *S. carpocapsae* juvenils was very limited at 8 °C and no reproduction was observed.

Entomopatogenic nematodes have been isolated at many sites in northern Europe which indicates that they are capable of withstanding sub-zero conditions (Glazer 2002). In laboratory experiments where *S. carpocapsae* was incubated at –4 °C, the half-life (LT50) was 1.8 days, and the surviving nematodes were still pathogenic after 6 days of freezing (Brown and Gaugler 1998). Field studies show that infective juveniles of *S. carpocapsae* in the soil can survive the winter in Germany where temperature reaches below 0°C for several weeks (Susuluk and Ehlers 2008).

2.4 Persistence in soil

The survival of infective juvenile nematodes in the soil is restricted by the energy reserves, stored internally as lipids and glycogen, as long as no new hosts are available (Glazer 2002). Laboratory studies of the survival of *S. carpocapsae* in soil at pH 6 and 25 °C have showen approximately 25% survival after 8 weeks, which indicates a half-life of approximately one month (Kung et al. 1990). In similar experiments with eight different strains of *S. carpocapsae*, seven strains showed less than 20 % survival after 8 weeks, while the survival of the remaining strain was approximately 50% (Shapiro-Ilan et al. 2006). No experimental data on survival at lower temperatures are available, but because of lower metabolic rates, the stored energy is likely to last longer at low temperature, suggesting longer survival of infective juveniles. According to Kemi (2013), *S. carpocapsae* may survive as long as 24-36 months before it runs out of energy.

Unfavourable abiotic conditions such as extreme temperature or pH values, desiccation and anoxic conditions have been found to reduce the survival of entomopathologic nematodes. Biotic factors such as antagonists and predators may also reduce nematode numbers. The most well-known natural enemies of infective juveniles are predatory mites, collembola and nematode trapping fungi. (Griffin et al. 2005).

Studies performed in the field generally indicate a faster decline of viable infective juveniles then found in laboratory experiments. According to Smits (1996) the numbers gradually decrease at levels of 5-10% per day, which correspond to half-lives of 6.6 - 13 days.

Predation, infection by antagonists, depletion of energy and desiccation are probably the main mortality factors during this period. In most cases, after 2-6 weeks less than 1% of the applied population is still alive. If host insects are present, recycling may occur, and the nematodes may then persist for years at these levels (Smits 1996).

In spite of the fast initial decline in numbers of infective juveniles, nematodes have been shown to persist for at least a year under favourable conditions. Field studies in Germany on the persistence of *Heterohabditis bacteriphora* after application on various crops, showed that survival of the infective juveniles varied between the crops, and that much of the differences could be attributed to the tillage regime (Susuluk and Ehlers 2008). In each crop a reduction of the frequency of positive soil samples was recorded after ploughing. On the plot showing the highest persistence, surviving juveniles were found up to the 25th month after application. This shows that this species were able to survive during the winter in Germany even though the temperature was below 0 °C for several weeks. Susuluk and Ehlers (2008) concluded that host insects are the major factor influencing long term persistence in the German field study.

2.5 Host range

Steinernema carpocapsae have the ability to infect a broad range of insects. Under laboratory conditions it has been shown to kill more than 250 species (Poinar 1986, Kemi 2013). However, the number of species which can be killed under field conditions is greatly reduced due to insect behaviour and environmental factors as well as spatial and temporal difference between the applied nematodes and non-target insects, and there is now a general opinion that entomopathogenic nematodes are more restricted in their host range than laboratory infections suggest (Peters 1996, Hominick 2002).

2.6 Effect on non-target organisms

The effects of *S. carpocapsae* and its symbiotic bacteria *X. nematophilus* have been investigated in laboratory studies with mammals, amphibia, fish, reptiles, acarids, earthworms, molluscs and various beneficial and environmentally important insects. The results as reviewed by the applicant (BASF) showed no evidence of infectivity pathogenicity or toxicity in mammals which was partly explained by the fact that *S. carpocapsae* cannot survive at temperatures above 37 °C, and also that the immune system of warm-blooded animals seem to be able to eliminate the nematodes by encapsulation in macrophages.

Tadpoles of toad and frogs were killed in tests at a concentration of 100 infective juveniles/ml in water Kemarrec and Mauleon 1985, Poinar and Thomas 1988). No NOEC was reported from these studies. However, as pointed out by Poinar and Grewal (2012), the likelihood of these nematods making contact with amphibian larvae would be minimal.

In reptiles (*Anolis marmoratus*), oral application (dose 80 000 infective juveniles) resulted in death of adults and live nematodes were recovered from the body cavity (Kermarrec et al.

1991). However, when individuals were challenged with 5000 infective juveniles every 3 days for 30 days no mortality was observed (Kermarrec and Mauleon 1985).

Tests with beneficial and environmentally important insects verified that *S. carpocapsae* affects a broad range of insects as well as spiders (BASF). However, the reported doses or exposure concentrations in most studies, do not allow comparison to realistic in-field conditions.

No effects were found on four species of earthworms in laboratory tests where the worms were exposed in 100 mL soil to which 4000 infective juveniles of *S. carpocapsae* were added (Capinera et al. 1982).

Mortality of two species of molluscs were found in laboratory tests with *S. carpocapsae* (Javorska 1993). When the slug *Deroceras agreste* was exposed to *S. carpocapsae* in soil at a concentration of 200 infective juveniles/cm^2 soil surface, mortality of slugs was observed after 4 days and reached 100% after 6 days.

The potential environmental impacts of entomopathogenic nematodes were reviewed by Barbercheck and Millar (2000). They concluded that the non-target species most likely to be affected are those with soil-dwelling stages active when the nematodes are applied. However, only small and temporal reductions in field populations of non-target species have been associated with applications of entomopathogenic nematodes (Georgis et al. 1991, 1993; Ropek and Jaworska, 1994, Campbell et al. 1995, Chevaliere and Webster 2006).

The environmental safety of using entomopathogenic nematods for biocontrol has also been addressed at a combined OECD and COST workshop on scientific and regulatory policy issues. The consensus view of the participants was that: "entomopathogenic nematodes (EPNs) possess specific biological and ecological features, which make their use in biological control exceptionally safe. All the scientific evidence available supports the conclusion that EPNs are safe to the environment, as well as to production and application personnel, the general public and the consumers of agricultural products treated with them. Only a few potential, but very remote, risks could be identified. Therefore, it was recommended that EPNs should not be subject to any kind of registration. The introduction of non-indigenous nematode species, however, should be regulated. Species should be accurately identified, and details of the origin, known distribution, probable host range and safety to the user must be provided. In addition, an expert opinion, based on available information, of the possible impact on non-target organisms must be available" (Ehlers and Hokkanen 1996).

2.7 Human Health

2.7.1 Allergenicity of Nematodes

The experience gained from biopesticide production suggests that there is a very small but real risk of allergenicity associated with entomopathogenic nematodes. At Biosys, a large nematode production company where over 75 people worked with more than five species of nematodes over a 12-year period, only one case of possible allergic responses was recorded. This person had dermal reactions from handling concentrated nematode solutions during the harvesting, cleaning and storage stages of production. In an Australian research laboratory producing at least ten species of entomopathogenic nematodes for more than 20 years, one person reported a mild allergic response whenever *S. carpocapsae* infective juveniles came into contact with his eye (R.A. Bedding, Australian Capital Territory, Australia, 2000, personal communication). This person had no response from nematode contacts with other tissues and has continued working with these nematodes for the past 12 years (Akhurst and Smith 2002).

2.7.2 Allergenicity of Xenorhabdus

Allergenic responses to *Xenorhabdus* have been noted in one person who reacted with asthmatic symptoms that resulted from an allergy to *X. nematophila* (Akhurst and Smith 2002). This individual, who was developing nematode formulations, was only able to work in the main facility for a few hours each day. While the nematodes and their symbionts may, like any organism, evoke an allergic response in sensitive individuals, there is no indication that they are hyperallergenic or allergenic for people. Anecdotal reporting of adverse events in people working closely with these bacteria for many years suggests that the risk of allergenicity is low (Akhurst and Smith 2002).

2.7.3 Risk of infection in humans of Xenorhabdus

Xenorhabdus nematophila is only found associated with *S. carpocapsae*. The upper threshold for growth of these bacteria in nutrient broth is 35°C (Boemare 2002), which suggests that it should not be able to infect warm-blooded vertebrates. There have been no reports of *Xenorhabdus* being isolated from human clinical sources. However, Akhurst and Smith (2002) argued that it is too early to conclude that no members of the genus *Xenorhabdus* ever infect humans since it is possible that there has simply been a failure to identify clinical *Xenorhabdus*. Nevertheless, if such infections occur, they are rare.

3 Uncertainties

Uncertainties related to the current assessment concerns mainly the the distribution of *S. carpocapsae*, both regionally and within Norway. Entomopathogenic nematodes are aggregated rather than random in distribution. The most common technique used to isolate the nematodes from soil is to use host larvae (*Galleria*) as baits. This bioassay technique allows isolation of entomopathogenic species from the numerous other nematodes present in soil. However, even a susceptible host recovers only a portion of a nematode population, and if several species of entomopathogenic nematodes are present, the least numerous ones may not be detected. Hence, it is difficult to state with confidence that a species does not exist in a locality, as it may be so rare that finding it is a function of sampling effort (Hominick 2002). The results of surveys in Europe indicate that *S. carpocapsae* is rare, especially in the Northern countries and that the distribution is patchy. Thus, extensive sampling would be required to clarify the distribution. As described in section 2.1, *S. carpocapsae* has only been found once in Norway. In this study (Haukeland 2006) 160 soil samples were taken in apple orchards in Western Norway. *Steinernema* species were found in 19 of the samples, while *S. carpocapsae* was found in only one. Although the present distribution of the species remains unknown, the fact that it has been recorded once is taken as a confirmation that the species is present in Norway.

4 Conclusions (with answers to the terms of reference)

Distribution; Evaluate if the nematode is naturally occurring in Norway

Steinernema carpocapsae has been identified in a soil sample from an orchard in Western Norway (Sogn) in 2001. It is not likely that the occurrence on that location is caused by application of *S. carpocapsae* as a biological control agent, since this species has been applied only in a research project at a distant location in Norway in 2006, i.e. later than the species was found in Sogn. Thus, VKM considers that *S. carpocapsae* occurs naturally in Norway. The distribution of the species within Norway is not known, but is considered to be limited.

Assess the potential for establishment and spread of the nematode under Norwegian conditions specified for use in greenhouses and outdoor cultivation.

Steinernema carpocapsae may be able overwinter at least in coastal areas of Southern Norway, and therefore local establishment of the species is possible. The potential for spreading from treated areas is considered to be low because of low active mobility and low potential for reproduction in soil. A possible route for long-range transport is by anthropogenic dislocation of growing medium e.g. with potted plants. The risk of speading is lower in greenhouse cultivation than outdoor, provided that the growing medium (eg. soil) from the culture is handled properly.

Consider possible taxonomic challenges related to the risk assessment

The taxonomy of *Steinernema carpocapsae* is well established. The nematode can be identified based on morphological characteristics and the DNA sequence of the internal transcribed spacer region of the ribosomal gene array (ITS rDNA) (Adams and Nguyen 2002). There are no taxonomic challenges related to assessment of this nematode.

The human health risk for operators related to the properties of NEMASYS C and with the nematode *Steinernema carpocapsae* as the active organism.

The use of plant protection products containing entomopathogenic nematodes against insects has not been associated with health effects on humans. The symbiotic bacteria *Xenohabdus* spp*.* has not been linked to pathogenic effects in humans. It is therefore the view of VKM that the use of the nematode *Steinernema carpocapsae* with the symbiotic bacteria *Xenohabdus nematophilus* will pose a minimal health hazard for operators.

5 References

Adams, B.J. and Nguyen, K.B. (2002). Taxonomy and Systematics in Gaugler, R. (ed.) *Entomopatogenic Nematodes*. CABI Publishing, Wallingford, UK. pp. 1-34.

Akhurst, R. J. (1980). Morphological and functional dimorphism in Xenorhabdus spp., bacteria symbiotically associated with the insect pathogenic nematodes Neoaplectana and Heterorhabditis. *Journal of General Microbiology. 121*: 303-309.

Akhurst R. and Smith K. (2002). Regulation and Safety. In: Gaugler R. (ed) *Entomopathogenic Nematology*. Kluwer Academic Publishers, Boston, pp. 311-332.

Ansari, M. A., Waeyenberge, L. and Moens, M. (2007). Natural occurrence of *Steinernema carpocapsae*, Weiser 1955 (Rhabtita: Steinernematidae) in Belgian turf and its virulence to Spodoptera exigua (Lepidoptera: Noctuidae). Russian *Journal of Nematodology* 15 (1), 21-24.

Barbercheck M.E. and Millar L.C. (2000). Environmental impacts of entomopathogenic nematodes used in biological control in soil. In: Follett P.A. and Duan J.J. (eds) *Nontarget effects of biological control.* Kluwer Academic Publishers, Boston, pp. 287-308.

BASF. Review of product safety and host range: Nemasys C (*Steinernema carpocapsae*). Documentation provided by the applicant.

Boemare, N. (2002). In Gaugler R. (ed.) *Entomatopatogenic Nematology.* CABI Publishing, Wallingford, pp. 35-56.

Brown, I. and Gaugler, R. (1998). Survival of Steinernematid nematodes exposed to freezing.*Journal of Thermal Biology* 23(2), 75-80.

Campbell JF, Lewis E, Yoder F, Gaugler R. (1995). Entomopathogenic nematode (Heterorhabditidae and Steinernematidae) seasonal population dynamics and impact on insect populations in turfgrass. *Biological Control* 5:598–606.

Capinera, J.L., Blue, S.L. and Wheeler, G.S. (1982). Survival of earthworms exposed to Neoaplectana carpocapsae nematodes. *Journal of Invertebrate Pathology 39*, pp. 419-421.

Chevalier, R. and Webster, J.M. (2006). Temporary Changes in Populations of Soil Organisms after Field Application of Entomopathogenic Nematodes. *Journal of Nematology 38*(4): 466–473.

Ehlers R.U. and Hokkanen H.M.T. (1996). Insect biocontrol with non-endemic entomopathogenic nematodes (Steinernema and Heterorhabditis spp.): Conclusions

and recommendations of a combined OECD and COST workshop on scientific and regulatory policy issues. *Biocontrol Science and Technology 6*: pp. 295-302.

Georgis, R. and Poinar, G.O. (1983). Effect of Soil Texture on the Distribution and Infectivity of Neoaplectono carpocopsoe (Nematoda: Steinernematidae). *Journal of Nematology 15*(2):308-311.

Georgis R, Kaya HK, Gaugler R. (1991). Effect of steinernematid and heterorhabditid nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) on nontarget arthropods. *Environmental Entomology 20*:815–822.

Griffin, C.T., Boemare, N.E. & Lewis, E.E., (2005). Biology and behaviour. In: Grewal, P.S., Ehlers, R.-U. & Shapiro-Ilan, D.I. (eds). *Nematodes as biocontrol agents*. CABI Publishing, Wallingford, UK. pp. 47 – 75.

Haukeland, S. Klingen, I. & Brurberg, M.B. (2006). An overview of entomopathogenic nematodes in the Nordic countries including a first report of *Steinernema carpocapsae* (Steinerneatidae: Rhabditida). *Russian Journal of Nematology 14*, 139–146.

Haukeland, S. (2007). Bruk av nyttenematoder til bekjempelse av gransnutebillen (Hylobius abietis). Bioforsk Plantehelse, Ås. (http://www.skogoglandskap.no/filearchive/haukeland_forskning-3-07.pdf)

Hominick W.M. (2002). Biogeography. In Gaugler R. (ed.) *Entomatopatogenic Nematology.* CABI Publishing, Wallingford, UK. pp. 115-144.

Jaworska, M. (1993). Laboratory infection of slugs (Gastropoda: Pulmonata) with entomopathogenic nematodes (Rhabditida: Nematoda). *Journal of Invertebrate Pathology 61*, pp. 223-224.

Kemi (2013). Evaluation of *Steinernema carpocapsae*; Evaluation for authorisation 2012. *Kemikalieinspektionen Diarienummer F-3865-B12-00004*.

Kermarrec, A. and Mauléon, H. (1985). Potential noxiousness of the entomogenous nematode Neoplectana carpocapsae Weiser to the Antillan toad Bufo marinus L. *Mededelingen van de Faculteit Landbouwwetenschappen, Rijksuniversiteit (Gent); 50*, pp. 831-838.

Kung, S-P., Gaugler, R. and Kaya, H.K. (1990). Influence of Soil pH and Oxygen on Persistence of Steinernema spp. *Journal of Nematology 22*(4):440-445.

Moyle, P.L. and Kaya, H.K. (1981). Dispersal and infectivity of the entomogenous nematode, Neoaplectana carpocapsae Weiser (Rhabditida: Steinernematidae), in sand. *Journal of Nematology 13*(3): 295-300.

Poinar, G.O. and Home, A. (1986). Survival and Horizontal Movement of Infective Stage Neoaplectana carpocapsae in the Field. *Journal of Nematology 18*(1):34-36.

Poinar, G.O. and Grewal P.S. (2012). History of Entomopathogenic Nematology. *Journal of Nematology* 44(2): 153–161.

Ropek, D. and Javorska, M. (1994). Effect of an entomopathogenic nematode, Steinernema carpocapsae Weiser (Nematoda, Steinernematidae), on carabide beetles in field trials with annual legumes. *Anzeiger für Schädlingskunde Pflanzenschutz Umwelteschutz* 67: 97-100

Saunders J.E. and Webster, J.M. (1999). Temperature Effects on Heterorhabditis megidis and *Steinernema carpocapsae* Infectivity to Galleria mellonella. *Journal of Nematology 31*(3):299–304.

Shapiro-Ihlan, D.I., Stuart, R.J. and McCoy, C.W. (2006). A Comparison of Entomopathogenic Nematode Longevity in Soil under Laboratory Conditions. *Journal of Nematology 38*(1):119–129e

Smits, P.H. (1996). Post-application persistence of Entomopathogenic nematodes. Biocontrol Science and Technology 6(3): 378-388.

Weiser (1955). Neoaplectana carpocapsae n. sp. (Anguillulata, Steinernematidae) novy cizopasnic housenik obalece jablecneho, Carpocapsa pomonella L. Vestnik Cesk. Zool. Spolecnosti 19: 44-52.