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Norwegian Scientific Committee for Food Safety

VKM Report 2014: 06

Risk assessment of the biological plant protection products Nemasys G and Nemasys H with the active organism *Heterorhabditis Bacteriophora*

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) 2014: 06
Risk assessment of the biological plant protection products Nemasys G and Nemasys H with the active organism *Heterorhabditis Bacteriophora*.

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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.

Acknowledgements

Solveig Haukeland at the Norwegian Institute for Agricultural and Environmental Research (Bioforsk) is acknowledged for contribution to the environmental risk assessment of the nematode *Heterorhabditis bacteriophora*. Lucy Robertson at the Norwegian School of Veterinary Science and Ingeborg Aaberge at the Norwegian Institute of Public Health are acknowledged for contribution on health risk aspects of the nematode *Heterorhabditis bacteriophora*.

Assessed by

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Summary

Nemasys G and Nemasys H with the nematode *Heterorhabditis bacteriophora* as the active organism is applied for as a plant protection product in Norway. Nemasys G is intended for use against the garden chafer (*Phyllopertha horticola*) in lawns and Nemasys H against black vine weevil (*Otiorhynchus sulcatus*) in strawberries and ornamentals.

VKM was requested by the Norwegian Food Safety Authority to consider the possible health and environmental risk related to the properties of Nemasys G and Nemasys H; in particular to evaluate if the nematode is naturally occurring in Norway, the potential for establishing and spreading under Norwegian conditions, possible taxonomic challenges and assessment of the health risk related to its use.

The assessment was finalized in October 2014 by VKM's Panel on Plant Protection Products.

VKM's conclusions are as follows:

Natural occurrence of the nematode:
The nematode *Heterorhabditis bacteriophora* has not been observed in Norway, but is widespread world-wide in regions with continental and Mediterranean climates. In Europe it has been isolated in France, Germany, Hungary, Italy, Moldavia, Spain, Switzerland and the UK.

Potential for establishing and spreading of the nematode under Norwegian conditions:
The thermal preference of *H. bacteriophora* restricts its establishing. Short term establishment in the growing season is possible in parts of Norway. This nematode has however poor long term survival in soil, especially in the absence of suitable hosts, and the potential for long term establishment in Norway is considered to be low.

Taxonomic challenges:
There are no taxonomic challenges related to assessment of this nematode.

Human health risk for operators:
Exposure of user is considered to be low. The use of plant protection products containing Heterorhabditid nematodes against insects has not been associated with health effects on humans. The symbiotic bacteria *Photorhabdus luminescens* has not been linked to pathogenic effects in humans. It is therefore the view of VKM that the use of the nematode *Heterorhabditis bacteriophora* with the symbiotic bacteria *Photorhabdus luminescens* will have minimal health risk for operators.

Background

VKM performs risk assessments in the context of pesticide registration of invertebrate biological control agents (IBCA) cf. Regulation § on Pesticides 4. The Norwegian Food Safety Authority, National Registration Section, is responsible for reviewing and evaluating the documentation submitted by the applicant. The Norwegian Food Safety Authority takes the final regulatory action regarding registration or deregistration of IBCAs 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 24, 2013 for VKM to perform a risk assessment on use of Nemasys G and Nemasys H with the nematode *Heterorhabditis bacteriophora* as the active organism. The risk assessment was finalized in xxx (month) 2014.

Terms of reference

Nemasys G and Nemasys S H are new biological plant protection products with the nematode *Heterorhabditis bacteriophora* as the active organism. Nemasys G is intended for use against the garden chafer (*Phyllopertha horticola*) in lawns and Nemasys H against black vine weevil (*Otiorhynchus sulcatus*) in outdoor and indoor strawberries and ornamentals.

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

- The fate and behaviour in the environment and the ecotoxicological effects and risks with regard to the properties of Nemasys G and Nemasys H with the nematode *Heterorhabditis bacteriophora* as the active organism. The Panel is in particular asked to consider the following:
    - Evaluate if the nematode is naturally occurring in Norway
    - Assess the potential for establishing and spreading of the nematode under Norwegian conditions
    - Consider possible taxonomic challenges related to the risk assessment

- The human health risk for operators related to the properties of Nemasys G and Nemasys H with the nematode *Heterorhabditis bacteriophora* as the active organism.

1 Background documentation

Norwegian Food Safety Authority has compiled the documentation submitted by the applicant and performed an initial assessment. The Norwegian Institute for Agricultural and Environmental Research (Bioforsk) was contracted to prepare an eco-toxicological assessment of the nematode *Heterorhabditis bacteriophora*, the active species in the product, for VKMs Panel on Plant Protection Products. Lucy Robertson at the Norwegian School of Veterinary Science and Ingeborg Aaberge at the Norwegian Institute of Public Health have contributed to the assessment of human health risks of the nematode. The present opinion of VKMs Panel on Plant Production Products is based on these contributions.

2 Identity etc.

2.1 Applicant and producer

Norwegian applicant:
NORGRO AS, Sælidveien 44, 2322 Ridabu

Producer/ supplier:
BASF (previously Becker Underwood), Harwood Rd. Littlehampton, West Sussex BN 17 7AU, England.

2.2 Application

Application of biological control products: Nemasys H and Nemasys G, both containing one nematode species, *Heterorhabditis bacteriophora*. Nematode G is intended for use against the garden chafer (*Phyllopertha horticola*) and Nemasys H for black vine weevil (*Otiorhynchus sulcatus*).

The previous application for registration of this species was in 2000, evaluated in 2001. *H. bacteriophora* was rejected due to lack of natural occurrence in Norway at the time. The closely related *Heterorhabdits megidis* was approved (indigenous species to Norway). *H. megidis* is however no longer on the market.

H. bacteriophora 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)

2.3 Area for use

Field (turf, strawberries, ornamentals), glasshouse and poly-tunnels (ornamentals, strawberries).

3 Health hazards

3.1 EXPOSURE

The nematodes will be applied in water and will rapidly transfer to and stay in the soil. The degree of user exposure is considered to be low.

3.2 HEALTH RISK

Photorhabdus are Gram-negative bioluminescent members of the Enterobacteriaceae family that live in association with soil-dwelling entomopathogenic Heterorhabditid nematodes that invade and kill insects.

At least three different species of Photorhabdus are described; *P. luminescens*, *P. temperata* and *P. asymbiotica* (Fischer-Le Saux et al., 1999).

Due to their production of toxins with insecticidal activity, and to the specificity of the host infected, which is determined by the nematode, Photorhabdus has been the subject of intensive studies for the development of bio-pesticides (Ehlers, 2001). Usefulness as bio-pesticide has focused on the use of *P. luminescens*, since these bacteria have only been recovered from nematodes isolated from infected insect hosts, and are not considered to be human pathogens.

Recently, other Photorhabdus strains have been obtained from human clinical specimens. Taxonomic studies have indicated that all human clinic isolates of Photorhabdus consist of different subspecies of *P. asymbiotica*. *P. asymbiotica* has been associated with locally invasive soft tissue and disseminated bacterial infections, characterized by multifocal skin and soft tissue abscesses (Gerrard et al., 2004). Published data suggest that *P. asymbiotica* have acquired virulence against man via the acquisition of a plasmid and specific virulence factors with similarity to those shown to play roles in pathogenicity against humans in other bacteria (Wilkinson 2009).

4 Taxonomy and origin of biological control agent

4.1 CLASS/ORDER/FAMILY

NEMATODA: Class: *Chromadorea*/ Order: *Rhabditida*/ Family: *Heterorhabditidae* (Stock & Hunt 2005).

4.2 GENUS AND SPECIES NAME

Heterorhabditis bacteriophora, (Poinar, 1976).

Common name: Entomopathogenic nematode is the common name for biocontrol nematodes in the family *Heterorhabditidae* (includes *H. bacteriophora*) and *Steinernematidae*. There is no common name for the different species. In Norway the common name for entomopathogenic nematodes is ‘nyttenematoder’.

4.3 TAXONOMIC CHALLENGES

None

4.4 INDIGENOUS OR NON-INDIGENOUS (IN NORWAY)

Current status: non-indigenous to Norway (according to Bioforsk survey 2013-2014).

4.5 ASSOCIATED ORGANISMS AND FORMULATION (OR CONTAMINANTS)

Photorhabdus luminescens is associated with *H. bacteriophora*. The association is symbiotic. The bacterial cells are present in the nematode gut and essential to the life cycle and efficacy (pathogenesis) of the product. Both products consist of live infective juvenile stages (see life cycle) of *H. bacteriophora* formulated in a water soluble gel (10% water).

4.6 ORIGINAL AREA AND DISTRIBUTION OF THE SPECIES

H. bacteriophora was first described by Poinar (1976) from an infected insect (*Heliothis punctigera*, Noctuidae) at Brecon, South Australia. Its known distribution is mainly from country/region surveys of soil samples followed by baiting with susceptible insect larvae (see also 5.6). Geographically it is widespread occurring world-wide mainly (more frequently) in regions with continental and Mediterranean climates. It is currently present on several continents: Africa, Asia, Australia, Europe, North America (not Canada) and South America (Hominick 2002; Adams et al 2006). In Europe it has so far been isolated in the Azores, France, Germany, Hungary, Italy, Moldavia, Spain, Switzerland and the UK (Ehlers & Hokkanen 1996; Hominick 2002; Ansari et al 2008).

4.7 GEOGRAPHICAL ORIGIN OF ORGANISM USED IN THE PRODUCT

Heterorhabditis bacteriophora ‘GPS 11’ strain (North East Ohio, USA).

4.8 AREAS INTRODUCED TODAY AS IBCA

Europe (Austria, Belgium, Denmark, France, Germany, Italy, Netherlands, (in Norway, briefly in 1990s for use against black vine weevil) Poland, Slovenia, Spain, Switzerland), Australia, Asia, USA and Canada.

5 Biology and ecology

5.1 LIFE CYCLE

Heterorhabditidae are obligate parasites of insects. The only free-living stage occurring naturally in soil outside the insect host is the non-feeding infective juvenile. Infective juveniles carry symbiotic entomopathogenic bacteria in their intestine and are capable of invading and killing their host within 1-2 days. This association can also be described as an obligate (for nematode and symbiont) vector-borne disease of insects.

The infective stage is capable of surviving in the soil environment until it encounters a suitable host. All the other (adult, juvenile and egg) stages in the nematode life cycle live within or on the insect host and have limited survival.

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. In Heterorhabditids (*H. bacteriophora*), the first generation adults are hermaphroditic (hence a single infective juvenile can infect and reproduce in a host). The second generation is amphimictic (males and females). Death of the host usually occurs quickly within 2 days at around 20^{o}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^{o}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 *H. bacteriophora* (entomopathogenic nematodes) 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. It is also this fecundity, and resistant infective stages that has enabled successful mass production of entomopathogenic nematodes.

Pathogenesis: Briefly the pathogenic process depends on characteristics of the insect host, the nematode and the symbiotic bacteria. It is influenced by insect resistance, virulence factors of the bacteria and the nematode’s ability to overcome the defence system of the host. The quality of the product therefore determines the efficacy against the target host; any contaminants will be detrimental to the pathogenic process.

Ecology: Field studies show that the number of infective juveniles recovered from soil following application, declines rapidly. The reasons may be abiotic factors such as desiccation and temperature extremes, while water logged soils may cause detrimental anoxic conditions. 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.

The infective juvenile does not feed but relies on stored energy reserves, especially lipids (40% of body weight). The nematode's life span is largely determined by the quantity and quality of the energy reserves. A good quality nematode product thus ensures infective juveniles with good quality energy reserves.

The rate of activity of the infective juvenile is influenced by temperature and moisture, hence also the rate at which the energy reserves are utilized. In soil with ideal conditions for movement and activity, between approximately 15 – 30^{O}C for *H. bacteriophora* and moist sandy loam soil, infective juvenile longevity will be short, more so if no hosts are encountered. At low temperatures, with adequate soil moisture, infective juveniles will live longer, with optimal temperature for survival between 5 and 15^{O}C. Infective juveniles are moderately freezing tolerant and can probably overwinter in Northern Europe. The estimated half-life of Heterorhabditid nematodes is approximately one month indicating a short survival period in the absence of hosts. Based on data for Heterorhabditid nematodes, it is likely that persistence of an applied *H. bacteriophora* population will depend on frequent (monthly) recycling through new hosts in the growing season and/or survival of infective juveniles over winter, and presence of new hosts the following season. The main limiting factor for persistence in soil is therefore the presence of available hosts.

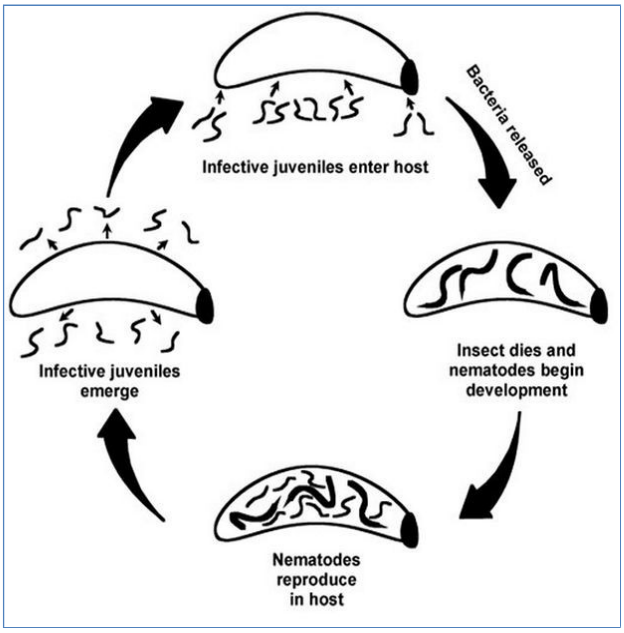

Figure 1. Schematic diagram of *Heterorhabditis bacteriophora* life-cycle (http://microbewiki.kenyon.edu/index.php/File:Shapiro.cornell.process.jpg)

5.2 POSSIBLE OVERWINTERING STAGE

Non-feeding infective juvenile stage overwinters.

5.3 CLIMATIC CONDITIONS

H. bacteriophora is distributed world-wide but appears to be less common in Northern Europe, Northern USA and has so far not been found in the Nordic countries or Canada (see 4.7). This fits well with the studies conducted so far on thermal preferences for development of the species.

Temperature: In laboratory experiments using *Galleria mellonella* as host, thermal preferences for
H. bacteriophora development ranged between 10 and 32^{o}C for infection (mortality), 15 – 32^{o}C for establishment (nematodes entering the host) and 15-30^{o}C for nematode reproduction in the host.
Laboratory studies have shown that the free-living infective juveniles of *H. bacteriophora* are moderately freezing tolerant. In a fast freezing regime the lethal temperature was minus 5^{o}C. Survival was enhanced by slow freezing and showed survival at minus 13^{o}C. This suggests that *H. bacteriophora* infective juveniles can overwinter in Northern Europe. For successful development (establishment), however, several studies show conclusively that *H. bacteriophora* is less cold tolerant, and requires at least 12^{o}C (soil temperature) to successfully infect, kill and reproduce in the host and finally produce viable infective juveniles (Grewal et al 1994, Susurluk and Ehlers 2008).

Soil moisture: Movement and dispersal of infective juveniles of *H. bacteriophora* is restricted in soils with low moisture content. This may result in increased survival of infective juveniles in host cadavers. However, for efficacy of the product dry conditions are not favourable to infection of target hosts.

5.4 MECHANISMS FOR DISPERSAL

The main mechanism for dispersal is nematode (infective juvenile) movement through soil in search of hosts. Research so far has indicated that infective juvenile movement is somewhat limited and usually much less than 1 meter in distance. As mentioned previously infective juveniles rely on their internal fat reserves, as they are non-feeding, and depletion of these fat reserves will limit their ability to spread in soil. Populations of entomopathogenic nematodes, including *H. bacteriophora* are thus prone to becoming locally extinct depending on environmental conditions and available hosts. There is little evidence or knowledge on passive dispersal of *H. bacteriophora*, but considering the biology of the species, the infective juvenile stage is the most resistant and may be dispersed with soil (anthropologically). Outside of soil the infective juveniles quickly desiccate and die, hence dispersal above ground (eg with plants) is unlikely (Ehlers 2003).

5.5 HABITAT RANGE

Heterorhabditid species are frequently found in coastal regions with sandy soil and *H. bacteriophora* is no exception, but this species has also been isolated outside of coastal regions. In Europe it is so far more frequently found in the central and southern parts, but has also been isolated in the UK (Wales) (see 4.7).

5.6 HOST RANGE

In nature the host insect degrades within a short period after infection with entomopathogenic nematodes, leaving us with little information on the natural host range. Our knowledge on natural occurrence of entomopathogenic nematode species (including *H. bacteriophora*) is based on the collection of soil samples in various habitats that are baited with a susceptible host (most often (Pyralidae: *Galleria mellonella*). For *H. bacteriophora* some information on the natural host range is available (see section 6.7).

The host range of *H. bacteriophora* is broad when tested against various insect larvae in laboratory experiments. In the field however the host range is narrower and studies have shown that this species migrates downwards in the soil targeting root feeding larvae. *H. bacteriophora* has been developed as a biological control agent against various insect pests, and appears to be particularly effective against (beetles) Colleopteran species such as *Otiorhynchus sulcatus* and some Scarabidae species.

6 Assessment of risks

6.1 HISTORY OF PREVIOUS RELEASES

First commercial release of *H. bacteriophora* was in 1984. It has since 1990s been widely used in Europe and USA particularly for control of black vine weevil and the garden chafer. In Norway; see section 4.8.

6.2 REFERENCE TO PREVIOUS RISK ASSESSMENTS

Risk assessments on entomopathogenic nematodes (including *H. bacteriophora*) have been assessed several times, first in 1995 & 1996 (Hokkanen & Lynch, Ehlers & Hokkanen) thereafter in 2003 (Hokkanen & Hajek), 2005 (Grewal) and recently in 2011 (Bale).

6.3 OUTCOME OF PREVIOUS RISK ASSESSMENTS

H. bacteriophora is on the positive list of EPPO. All risk assessments have unchanged conclusions (from 1996 to current 2011) summarized as follows, entomopathogenic nematodes (including *H. bacteriophora*) have very limited potential to cause non-target effects because of their limited potential to disperse or persist at the site of application.

6.4 POTENTIAL FOR ESTABLISHMENT (PHYSICAL CONSTRAINS)

The thermal preference of *H. bacteriophora* is 10 to 32^{O}C for infection (mortality), 15 to 32^{O}C for establishment (nematodes entering the host) and 15 to 30^{O}C for nematode reproduction in the host (Grewal et al 1994). The establishment *H. bacteriophora* is therefore restricted to areas or years when soil temperatures are above 12^{o}C during the growing season, in Norway the agricultural (coastal areas) of the southern part of the country may be suitable for establishment at least in the short term (see also 5.3). This species has poor long term survival in soil especially in the absence of suitable hosts (Ehlers & Hokkanen 1996) (see also section 6.6). The potential for establishment in Norway is considered slight.

6.5 POTENTIAL FOR ESTABLISHMENT (RESOURCE CONSTRAINS)

Low availability of suitable hosts may restrict establishment of *H. bacteriophora* even in areas where temperatures are suitable (see 6.4). There are indications that *H. bacteriophora* rely on high host densities for their reproduction (Peters 1996), hence if an *H. bacteriophora* application successfully infects and reduces the host population in an area, further successful establishment depends on new hosts available within a relatively short period of time (see 6.6). Previous studies on the establishment of *H. bacteriophora* after commercial applications show a typical rapid population decline after application, a low potential to persist and an uneven distribution with higher density only in the vicinity of infected insect cadavers (Ehlers 2003). In Norway the main limiting factor for establishment is likely to be low temperatures, but low densities of available hosts may also contribute to limiting the possibility of establishment.

6.6 EVIDENCE FOR ESTABLISHMENT

Research indicates poor survival of *H. bacteriophora* after few (4) weeks. Bacteria can be re-isolated after no more than 12 months without hosts or up to 23 months with available hosts (Susurluk & Ehlers 2008). In general *Heterorhabditis* species have low survival rates (Strong 2002; Ehlers 2003). In Norway there is no evidence of establishment based on analysis of soil samples collected along the coastal southern part of the country (Agder and Rogaland) (unpublished data).

6.7 HOST RANGE: WILD HOSTS KNOWN

<u>Coleoptera:</u>
Scarabaeidae: *Popillia japonica, Amphimallon solstitiale, Cyclocephala hirta* and *Phyllophaga* sp., Chrysomelidae: *Diabrotica balteata*, Curculionidae: *Curculio caraye* and *Diaprepes abbreviates*

<u>Lepidoptera:</u> Noctuidae: *Heliothis punctigera* and *Helicoverpa zea*. Pyralidae: *Diatrea grandiosella*

6.8 HOST RANGE: ORGANISMS TESTED IN CONTROLLED EXPERIMENTS

Many laboratory experiments show wide host range in controlled laboratory trials. Field trials indicate best effect against Coleopteran pest species.

6.9 TARGET AND NON-TARGET HOST PLANTS

H. bacteriophora lives in the soil ecosystem either as a non-feeding free living infective stage searching/waiting for a host or as developing reproducing stages within an insect host. Plants are not affected by *H. bacteriophora*.

6.10 ABILITY TO DISPERSE

Low (see section 5.4).

6.11 DIRECT AND/OR INDIRECT NON-TARGET ENVIRONMENTAL EFFECTS

Heterorhabditis bacteriophora is an entomopathogenic nematode species and well known biological control agent of in particular weevil pests such as the black vine weevil (*Otiorhynchus sulcatus*). It is the active ingredient of Nemasys G and Nemasys H. The table in section 6.11 summarizes the risks regarding this nematode species and shows that the risk to non-target organisms is either very low or transient in nature. The main reason is the limited potential for this nematode to disperse or persist at the site of application.

RISK	RATING[a]
Risk to NTOs (non-target organisms):	
In untreated field	0
In treated fields:	2
In the soil	3
In cryptic environments	2
On foliage	1
Vertebrates:	
Warm blooded	0
Cold blooded	2
Invertebrates:	
Arthropods	
Predators	2
Parasitoids	2
Pollinators	1
Endangered species	1
Others	1
Non-arthropods	
Earthworms	0
Others	1
Plants	0
Competitive displacement of indigenous Entomopathogenic nematode in treated fields:	
Temporary	2
Permanent	0
Changes in ecosystem balance:	
Local temporary suppression of NTOs	2
Permanent suppression of NTOs	0

Contamination of ground water	1
Gene transfer from exotic symbiotic bacteria to other soil bacteria	2
Biological 'pollution' with new Entomopathogenic nematode species	4
General biodiversity	1

From: Hokkanen and Hajek 2003
[a]Scale: 0 = no risk, 1 = remote, 2 = slight, 3 = moderate, 4 = high, 5 = very high risk

7 Conclusion

VKM has evaluated available data on the fate and behaviour in the environment, as well as the ecotoxicological effects of the nematode *Heterorhabditis bacteriophora*, the active organism in Nemasys G and Nemasys H, and concludes as follows:

Natural occurrence of the nematode:
The nematode has not been observed in Norway, but is widespread world-wide in regions with continental and Mediterranean climates. In Europe it has been isolated in France, Germany, Hungary, Italy, Moldavia, Spain, Switzerland and the UK.

Potential for establishing and spreading of the nematode under Norwegian conditions:
The thermal preference of *H. bacteriophora* restricts the establishing to areas or years with soil temperatures above 12 °C, and short term establishment in the growing season is therefore possible in parts of Norway. This species has however poor long term survival in soil, especially in the absence of suitable hosts, and the potential for long term establishment in Norway is considered to be low.

Taxonomic challenges:
There are no taxonomic challenges related to assessment of this nematode.

Human health risk for operators:
Exposure of user is considered to be low. The use of plant protection products containing Heterorhabditid nematodes against insects has not been associated with health effects on humans. The symbiotic bacteria *Photorhabdus luminescens* has not been linked to pathogenic effects in humans. It is therefore the view of VKM that the use of the nematode *H. bacteriophora* with the symbiotic bacteria *P. luminescens* will have minimal health risk for users.

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