Pepino Mosaic Virus (PepMV)

Hazards when used as microbial pesticide

Prepared for:

De Ceuster N.V.

Prepared by:

[redacted]

ENVIRON Netherlands BV

Willem Arntszlaan 115, NL-3734 EE Den Dolder,

the Netherlands

tel. +[redacted]

fax +[redacted]

[redacted]@environcorp.com

http://www.environcorp.com

Date:

28-09-12

Document:

NL11-DI-VIR-20110072

ENVIRON

Project No: NL11-DI-VIR-20110072

Project Status: [redacted]

[redacted]

vrijdag 28 september 2012

This report has been prepared with all reasonable skill, care and diligence, and taking account of the manpower and resources devoted to it by agreement with the client. This report is confidential to the client, and ENVIRON accepts no responsibility whatsoever to third parties to whom this report, or any part thereof, is made known, unless formally agreed by ENVIRON beforehand. Any such party relies upon the report at their own risk.

ENVIRON disclaims any responsibility to the client and others in respect of any matters outside the agreed scope of the work.

CONTENTS

10.1.c Wob juncto 63.2.d Vo 1107/2009

1 Introduction

De Ceuster N.V. (further referred to as the applicant) is applying for registration of a new plant protection product (PPP) based on a plant-pathogenic micro-organism, *viz. Pepino Mosaic Virus* (PepMV), Chilean genotype (CH2), isolate 1906. The PPP contains an isolate of a so-called 'mild' PepMV isolate (isolate 1906 of the CH2 genotype), which is able to protect tomato plants against other isolates of the CH2 genotype of PepMV. This virus isolate has to be considered a new active substance (AS) under the Plant Protection Products Regulation 1107/2009.

The active substance under consideration is an [redacted] *Pepino Mosaic Virus*, strain CH2, isolate 1906. The actual plant protection product will be identical to the active substance.

The applicant has asked ENVIRON to prepare a safety/toxicity evaluation of the intended PepMV, CH2 strain, isolate 1906, based on open literature. At this moment only efficacy data are available and no further dossier data are available (yet). The current document provides a hazard background for viruses used as active substances in plant protection products in general, and PepMV in particular.

10.1.c Wob juncto 63.2.d Vo 1107/2009

2 Intended uses

The intended use of this new PPP is exclusively on tomatoes in greenhouses, not in tunnels or in the open field. The product has been tested in trials by two application techniques. The proposed product is a Liquid (LI) [redacted] *Pepino Mosaic Virus*, strain CH2, isolate 1906, intended for spray applications; [redacted]

2.1 Purpose of the product

PepMV infections, and particularly strain CH2 isolates, have been observed globally, including in its native south America (Chile, Ecuador, Guatemala, Peru), Canada and the USA, Africa (Morocco), Asia (China) and in Europe (19 out of 27 EU member states, as well as Norway, Switzerland and Ukraine) (Werkman & Sansford, 2010). The high infectivity of the virus, and its significant economic damage mean that prevention of infections through hygiene measures is a clear challenge, especially in dense tomato-growing areas. Inoculating tomato crops with PepMV, CH2 strain, isolate 1906, effectively protects these crops from severe damage by other variants of CH2, based on cross-protection. Currently, no other effective – and legally authorized – protective methods are available to tomato growers (Hanssen & Thomma, 2010), and no resistant tomato varieties are commercially available.

3 Requirements for microbial active substances under Directive 2001/36/EC

Regulation 1107/2009 concerns the placing of plant protection products on the market, and thus stipulates that an Annex I inclusion dossier needs to be submitted for PepMV; *data requirements* for micro-organisms under 1107/2009 are laid down in Directive 2001/36/EC. In short, the data-requirements for the active ingredient entail the following subjects:

1. Identity of the micro-organism: covering details on the application and the characterization of the micro-organism
2. Biological properties on the micro-organism: covering information on natural occurrence, history, target organisms, life cycle, infectiveness, relationship to known pathogens, genetic stability
3. Further information on the micro-organism: covering information on fields of use, method of production and quality control, procedures for handling, storage, and decontamination
4. Analytical methods to determine the micro-organism as manufactured and methods to determine and quantify residues (viable and non-viable)
5. Effects on human health: covering medical surveillance information, sensitization information, information on toxicity, pathogenicity and infectiveness
6. Residues on treated products, food and feed: covering persistence and likelihood of multiplications in or on crops
7. Fate and behaviour in the environment: covering persistence and likelihood of multiplication in soil, water and air.
8. Effects on non-target organisms: covering possible effects on birds, bees, aquatic organisms, earthworms, non-target arthropods.

In this report we will focus on the subjects 1, 2, 5, 6, 7 and 8 of the data requirements; subjects 3 and 4 have no direct bearing on the human health and environmental hazards of plant viruses in general and PepMV in particular. Note that in 2005 the detailed data requirements as set in the EU have been transferred into OECD data requirements. As such some wordings have changed and some requirements were added or deleted. When preparing the dossier, the OECD data requirements should be followed.

4 Short introduction to virology

4.1 Viruses in general[1]

Viruses are small infectious agents that lack the ability for autonomous replication; they can replicate only by 'hijacking' the cellular machinery of higher organisms. Viruses are (much) smaller than other microorganisms, and most are too small to be seen with a light microscope. In fact viruses were originally identified as infectious material that was too small to be retained by filters that would retain bacteria (Beijerinck, 1898 - see e.g. Bos, 1999).

Viruses have evolved into a plethora of different forms, to infect all types of cellular organisms, including plants, animals, or bacteria, and are found throughout the biosphere (and some even claim, beyond - see Hoyle and Wickramasinghe as quoted in Wickramasinghe, 2003). The origins of viruses in the evolutionary history of life are unclear (Rybicki, 2008).

Viruses have played key roles in shaping the history of life on our planet by shuffling and redistributing genes in and among organisms and by causing diseases in animals and plants. They have been the culprits in many human diseases, including smallpox, flu, AIDS, certain types of cancer, and the ever-present common cold (Soni, 2007; Wagner *et al.* 2008). Viruses can infect virtually any type of cells: bacteria, fungi, protozoa, plants, animals and humans. True parasites, viruses are basically little more than molecular syringes moving genetic information from one cell to another. Some viruses enter a host and leave virtually unnoticed. Others cause disease and destroy the host (Mahy, 1991; Mahy & Regenmortel, 2010; Soni, 2007; Wagner *et al.* 2008).

Viruses spread in many ways, through vectors, inhalation of airborne particles, ingestion of contaminated water or food (generally indicative of a fecal-oral route) or through direct host-host contact, including, but not limited to sexual transmission. Plant viruses are almost always transmitted through vectors (insects, nematodes) that feed on plants, or opportunistically by 'agents' that damage plants, since plant viruses rely on existing or externally effected damage to cell walls for initial infection. The range of host cells that a virus can infect is called its 'host range'. This can be narrow if a virus is highly host-specific or broad, when a virus is capable of infecting many species. Plant viruses usually employ non-specific methods of infection, and therefore generally have a broad host range s.l.; since in many cases only a few plant species, genera or families show symptomatic infections, the practical host range of plant viruses is defined more by the range of symptomatic infections than by the actually infected host range (Weinstein, 1995).

4.1.1 Origin

As already stated, the origin of viruses is unclear. There are three main hypotheses that try to explain the origins of viruses:

- Regressive hypothesis
  - Viruses may have once been small cells that parasitised larger cells. Over time, genes

1. Some of this material courtesy Wikipedia (http://en.wikipedia.org/wiki/Introduction_to_viruses; http://en.wikipedia.org/wiki/Virus; http://en.wikipedia.org/wiki/Plant_virus). Also Strauss & Strauss, 2008.

not required by their parasitism were lost. The bacteria rickettsia and chlamydia are living cells that, like viruses, can reproduce only inside host cells. They lend support to this hypothesis, as their dependence on parasitism is likely to have caused the loss of genes that enabled them to survive outside a cell.

- Cellular origin hypothesis
  - Some viruses may have evolved from bits of DNA or RNA that 'escaped' from the genes of a larger organism. The escaped DNA could have come from plasmids (pieces of naked DNA that can move between cells) or transposons (molecules of DNA that replicate and move around to different positions within the genes of the cell).
- Coevolution hypothesis
  - This is also called the virus-first hypothesis and proposes that viruses may have evolved from complex molecules of protein and nucleic acid at the same time as cells first appeared on earth and would have been dependent on cellular life for billions of years.

4.1.2 Morphology

A complete virus particle, known as a virion, can consist of either two or three parts: the nucleic acid genetic material (either DNA or RNA), surrounded by a protective coat. This coat, which is an enclosure made up of one or more repeating protein molecules is also known as a capsid; the identical protein subunits that make up the capsid are called capsomers. Capsomers are encoded by the viral genome; the shape of the capsid formed from these capsomers, by self-assembly or assisted by additional, viral genome-coded proteins, serves as the basis for morphological distinction. In some cases, a virus may additionally have an envelope of lipids that encompasses the coat of virus particles after it emerges from an infected cell; this envelope consists of lipids from the infected cell's membrane.

4.1.3 Classification

A major basis of classifying viruses is by genome type and structure, which can vary widely; as a group they contain more structural genomic diversity than any other group of organisms. A virus can have a DNA or RNA genome, with the vast majority of genomes being based on RNA. Plant viruses tend to have single-stranded RNA genomes and bacteriophages tend to have double-stranded DNA genomes. Genomes can be circular or linear, independent on the type of nucleic acid. DNA genomes are usually traditional, in that they need to be transcribed into RNA. Single-strand DNA needs to be transcribed into double-strand before it can be transcribed into RNA. Interestingly, some viral ssDNA is ambisense, meaning that both strands of the double-strand intermediate are transcribed into viable RNA.

RNA viruses can have positive sense RNA, which can be translated directly, or negative sense, which needs to be transcribed into positive sense RNA first. Some RNA viruses, known as retroviruses, require a transcribed DNA intermediate for replication.

Genome size can vary enormously. The smallest viral genomes – found in ssDNA circoviruses – code for only two proteins and have a genome size of only 2 kilobases, while the largest – mimiviruses – have genome sizes of over 1.2 megabases and code for over one thou-

sand proteins.

Due to a number of intentional and non-intentional mechanisms, virus genomes are highly variable; this, among other things, accounts for their rapid evolution.

Plant viruses are classified, among other things, by their mode of transmission, and by the specific vector they use; such as a specific insect, nematode, or fungal species, or by mechanical transmission or air transport. Potyviruses e.g. are transmitted by aphids, begomoviruses by whiteflies, tospoviruses by thrips, and potexviruses rely on mechanical transmission.

4.1.4 Infection

Animal viruses

Viruses that infect animal cells operate according to the following sequence:

- Attachment: specific binding between viral capsid proteins and specific receptors on the host cellular surface. This specificity determines the host range of a virus.
- Penetration: virions enter the host cell through receptor-mediated endocytosis or membrane fusion.
- Uncoating: a process in which the viral capsid is removed, resulting in the release of the viral genomic nucleic acid.
- Replication: multiplication of the genome.
- Assembly (often self-assembly) of the virus particles.
- Release of viral particles from the host cell by lysis, a process that kills the cell by bursting its membrane and cell wall if present.

Alternatively, some viruses may, after infection has taken place, also undergo a lysogenic cycle where the viral genome is incorporated by genetic recombination into a specific place in the host's chromosome. The viral genome is then known as a ‘provirus’. Whenever the host divides, the viral genome is also replicated. The viral genome is mostly silent within the host; however, at some point, the provirus or prophage may give rise to active virus, which may lyse the host cells, releasing non-enveloped virions.

Enveloped viruses typically are released from the host cell by budding, rather than by lysis. During this budding process the virus acquires its envelope, which is a modified piece of the host’s cell membrane, or sometimes other, internal membrane.

Plant viruses

Viruses that infect plants are much simpler; they have *no* specific mechanism for attaching to cells, or to bind to specific receptors on a cell membrane - in fact, since plant cells have cell walls, there are no specific structures on plant cells to facilitate such processes. Therefore, for infection to take place, plant viruses need a ‘pre-existing’ breach in a plant cell’s cell wall. This cell wall damage can be made for them by their vector or vehicle, or can be made by an unassociated ‘third party’, like a farm worker (superficially) damaging plant

leaves while doing field or greenhouse re-entry work.

4.2 Plant viruses[1,2]

Plant viruses are viruses affecting (higher) plants. Like all other viruses, plant viruses are obligate intracellular parasites that do not have the molecular machinery to replicate without a host. Plant viruses are an important group of plant pathogens in agriculture worldwide. They infect and may cause diseases in plants and are responsible, in Europe and other continents, for considerable economic damage in different crops including vegetables, grains and ornamentals (Agrios, 2005).

As stated above, plant cells have cell walls that do not offer points of recognition, contact, or attachment and entry for viruses, in contrast to animal cell membranes. Plant viruses therefore lack the 'tools' to autonomously enter a cell – they totally rely on 'pre-existing' cell damage to enter and infect a cell. This damage may be effected by the vector for viruses that rely on vectors for transmission, or by 'vehicles' or 'third parties' (such as farmers damaging plants inadvertently or by deliberate agricultural practices, such as pruning or grafting) for viruses that are transmitted by other means.

4.2.1 Vectors

Plant viruses are often spread from plant to plant by organisms, known as vectors. A vector is an organism that acts as intermediate host to a plant virus and that damages the virus's host plant 'on purpose', usually through feeding on plant material. In a few cases, plant viruses may in fact infect their intermediate vector host, but they usually travel along as 'commensals'. Different plant viruses use different routes to infect a plant, such as through vectors (insects, nematodes) that feed on plants, or opportunistically by 'vehicles'. Mechanically transmitted viruses travel from plant to plant with 'vehicles' that just happen to visit and damage the host plants, like bumblebees or crop workers. Such viruses will generally rely on 'third parties' to provide the plant damage necessary to infect their new host.

Insects form the most important type of plant virus vectors. In fact, one class of viruses, the Rhabdoviridae, has been proposed to actually be insect viruses that have evolved to replicate in plants. The chosen insect vector of a plant virus will often be the determining factor in that virus's host range: it can only infect plants that the insect vector feeds upon. Soil-borne nematodes also have been shown to transmit viruses. They acquire viruses by feeding on infected roots, and can transmit them both non-persistently and persistently, but there is no evidence of viruses being able to replicate in nematodes. Examples of viruses that can be transmitted by nematodes include tobacco ringspot virus and tobacco rattle virus. Viruses can be spread by direct transfer of sap by contact of a wounded plant with a healthy one. Such contact may e.g. occur during agricultural practices, as by damage caused by tools or hands. For PepMV, mechanical transmission through sap is the primary mode of transmission.

Other, less common vectors are parasitic protozoa, fungi and molds, plasmodiophorids, and mites. Virus 'transmission' can also take place through pollen and seed.

---

2. http://www.microbiologybytes.com/virology/Plant.html

4.2.2 Transmission of plant viruses

Transmission of plant viruses is dependent on a triple interaction between the plant, the virus and a transmission vector (or vehicle or third party). Moreover, plant viruses normally face bigger infection 'challenges' than human/animal viruses, since plant cells have robust cells walls, therefore fusing with the cell membrane is not possible without aid. Regardless, virus transmission pathways play a crucial role for their survival, since to insure continued existence viruses **must** transmit from one host to another during every season and from field to field or region to region.

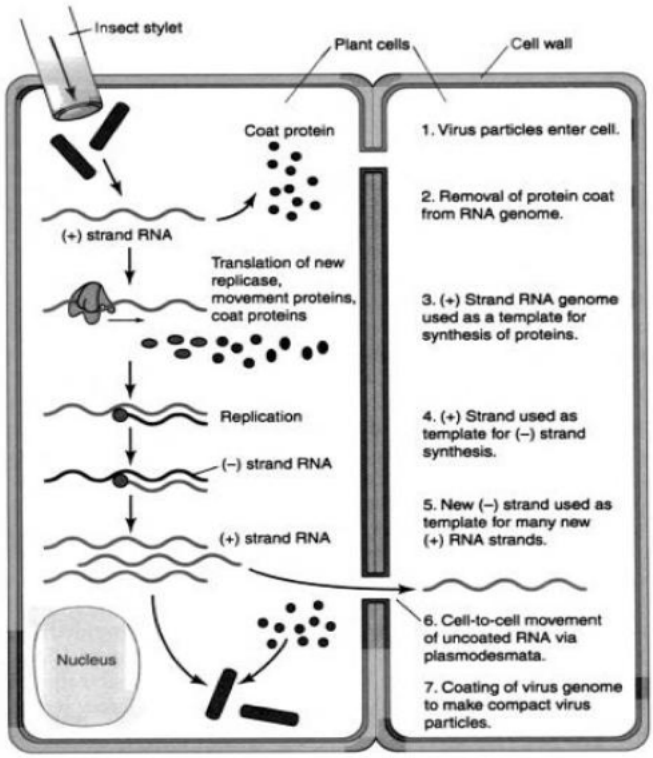


Figure 1: Simplified life cycle of an RNA virus

Depending on the way they are transmitted, plant viruses are classified as non-persistent, semi-persistent and persistent. In non-persistent transmission, viruses become attached to the distal tip of the stylet of the insect and on the next plant it feeds on, it inoculates it with the virus. Semi-persistent viral transmission involves the virus entering the foregut of the insect. Those viruses that manage to pass through the gut into the haemolymph and then to the salivary glands are known as persistent. There are two sub-classes of persistent viruses: propagative and circulative. Propagative viruses are able to replicate in both the plant and the insect (and may have originally been insect viruses), whereas circulative can not.

Once inside a plant, neighbouring cells can be infected through mechanisms that make use of plasmodesmata, microscopic channels linking cells within a plant, or via the vascular system. Plasmodesmata are a.o. capable of transporting diffusable material from one cell to the next, and viruses use specialized movement proteins, such as the TGB proteins found in PepMV, to shuttle viral genomes from one (infected) cell to the (uninfected) next through these organelles (see e.g. Boevink & Oparka, 2005, and Lucas, 2006).

4.3 Plant vs. animal viruses

In contrast to animal viruses, that as we have seen possess highly specialized mechanisms to recognize, bind to, and infect specific cells, plant viruses thus have none of these infection mechanisms. Instead, they have, much more simple, mechanisms to aid in propagation within the plant once they have established an initial infection, through entry into a plant cell via mechanical damage to the cell caused by a vector, vehicle, or third party. Since they lack any mechanism to autonomously infect a new host individual, they lack the possibility to infect animal cells. And even if they would succeed in infecting damaged animal cells (similarly to how they initially infect a plant host), and subsequently in successfully reproducing within this cell (which is not very likely since they also lack the mechanism to hijack the cell's reproductive machinery efficiently), they would still lack the means of propagating this infection beyond the initial, damaged cells, to intact cells, since they still lack any mechanism to bind to and enter intact cells. And since animal cells, contrary to plant cells, lack structures that physically link the inside of one cell to the inside of the next cell, plant viruses cannot use their movement proteins to move from one cell to the next and propagate an infection in this way.

4.4 Effects of plant viruses on humans

Relatively little explicit documented information can be found on the absence of infectivity of plant viruses to humans and other (vertebrate) animals, beyond the widely stated claim that plant viruses do not infect animals and are generally considered harmless to humans – in popular science writing as in scholarly articles and virology and pathology textbooks.

Van der Riet (1997) published a primarily hypothetical paper, philosophizing on the possibility of existence of what he called phytonoses, illnesses of humans caused by plant pathogens, in analogy with zoonoses (illnesses of humans caused by animal pathogens). In this highly controversial paper, he suggested that mycoplasma-like plant pathogens may possibly be infectious to humans. He had to concede however that infection of humans by other types of plant pathogens, including plant viruses was unknown.

Some information, primarily circumstancial, on the absence of pathological effects of plant viruses to humans is available. Fuchs (2008) e.g., writing on the hazards of virus-resistant transgenic plants, explicitly claims that "*Numerous observations suggest that a viral protein in a transgenic plant does not pose any threat to allergenic safety. Most notable is that virus-infected conventional crops have been consumed by humans with no apparent ill effects since plants and plant products have been food.*"

Zhang *et al.* (2006) investigated the viral load in fecal samples of healthy people, and concluded that evidently healthy people harbour high levels of plant viral DNA from many different plant pathogens in their intestines. Presumably, these viruses originate from their diet. The health status of the investigated subjects strongly suggests that these (and other) plant viruses are *not* pathogenic in humans. Interestingly, the most common plant viral genome encountered in fecal matter was *Pepper mild mottle virus* (PMMoV). It was established that fecal PMMoV was infectious to host plants, confirming that the stools contained viable virus particles rather than virus breakdown products.

These findings are particularly interesting in the light of the widely discredited findings by

Colson *et al.* (2010), who fed a group of 304 adults and 208 children with 21 commercialized pepper containing products. These products were intentionally inoculated with *Pepper mild mottle virus* (PMMoV) with the purpose of assessing the presence of this virus in the stool of volunteers. Besides testing for the presence and viability of the virus in the stool, researchers also investigated the presence of Anti-PMMoV IgM antibodies. In addition, a case-study was carried out to associate the presence of biological and clinical symptoms with the presence of PPMoV in the stool.

PMMoV RNA was detected in stool from 22 of 304 (7.2%) adult patients and 1 of 208 children. Regarding the case-study, researchers detected a positive correlation between the detection of clinical signs (fever, abdominal pain and pruritus) and the detection of Anti-PMMoV IgM antibodies. PMMoV-positive patients (39% for both clinical features) had a statistically significant prevalence of fever, abdominal pains and pruritus over patients with PMMoV-negative stool (13 and 7% respectively). The only child with positive PMMoV RNA stool also presented the same symptoms.

The study did not receive many positive reviews, and many experts did not agree with the conclusions of the authors. The number of patients in which PMMoV RNA was very low, only 7.2% of adults and 1 child out of 208. Experts referred to other possible causes for the clinical and biological symptoms in the 'positive' patients, such as sensitivity of the patients to spicy food, or the presence of an infectious agent, since food products were not tested for general innocuousness. Another possibility could be an allergic reaction or a toxic cofactor. In order to overrule these hypotheses, the authors mention the positive correlation of a specific immune response to this symptoms. However, the fact that Anti-PMMoV antibodies were detected in the patients is not an indication of infectivity, only of systemic exposure (i.c. uptake of virus particles across the GI tract). There is indeed evidence of systemic exposure to the virus and a response of the patient immune system. An individual's immune response can be triggered not only by viruses or bacteria, but also by chemical substances with specific structures or by tissues or cells of other individuals (transplant patients).

A number of European plant disease authorities have recently published extensive advisories regarding PepMV, in light of the current wave of PepMV infections in tomato crops. In the UK e.g., Central Science Laboratories (2005) published 'Pest Risk Analysis for *Pepino mosaic virus*', in which they provide a comprehensive overview of the history, epidemiology, and economic impact of PepMV in the UK and Europe, and present a concluding Pest Risk Analysis for the UK and the Netherlands. Not once does CSL mention potential pathological or toxicological effects of PepMV on humans or animals. In fact, no other PepMV-specific publication ever mentions risks to organisms other than (host) plants. Likewise Werkman and Sansford (2010) do not mention any risks to non-plant organisms.

Plant viruses are harmless to humans and other animals because they can reproduce only in living plant cells (Shors, 2009), and are unable to enter cells on their own, relying for cell entry on pre-existing (cell) damage or damage inflicted 'externally', such as by a vector, a vehicle, or a third party. Moreover, it should be well noted that plant viruses are found everywhere in plants and fruits, including food crops, and are therefore commonly consumed by people; however, cases of plant viruses causing diseases in humans have so far never been documented.

5 Details on the micro-organism

Pepino mosaic virus (PepMV), a potexvirus, was first found in Peru in 1974 on pepino (*Solanum muricatum*), an edible fruit known as pear melon (Jones *et al.*, 1980). In 1999, the disease was found for the first time outside of South America, in greenhouse tomato crops (*Solanum lycopersicum*) in the Netherlands and the UK, and has presently become a major disease of greenhouse tomato crops worldwide (van der Vlugt *et al.*, 2000; French *et al.*, 2001; Mumford and Metcalfe 2001; Cotillon *et al.*, 2002; Maroon-Lango *et al.*, 2005; Pagán *et al.*, 2006; Hasiów *et al.*, 2008; Hanssen *et al.*, 2008; Ling, 2006; Ling *et al.*, 2008, Werkman and Sansford, 2010). Four PepMV genotypes have been isolated: the original Peruvian type (LP), the European type, the American type (US1) and the Chilean type (CH2). Within these genotypes various different isolates are identified. A PepMV isolate (CH2 strain, isolate 1906) is intended to be used as the active ingredient.

Infection of tomato plants with PepMV, CH2 strain, isolate 1906 conveys protection to infection with other CH2 genotypes of PepMV. Note that aggressiveness of isolates is not related to simple infectivity/reproductivity levels: the virus titer in plants infected with mild and aggressive isolates is comparable (Hanssen, 2010). Cross-protection not only works within genotypes of PepMV; PepMV, CH2 strain, isolate 1906 conveys full protection against other CH2 variants, and partial protection against LP or EU isolates (Hanssen, 2010; De Nayer *et al.*, 2011).

5.1 Virology of PepMV

5.1.1 Physical properties

PepMV virions are non-enveloped flexuous rods that contain a monopartite, positive sense, single stranded RNA genome of 6.4 kb with a 3' poly-A tail. The genome contains five major open reading frames (ORFs) encoding a 164 kDa RNA-dependent RNA polymerase (RdRp), three triple gene block proteins of 26, 14 and 9 kDa, and a 25kDa coat protein (Hanssen and Thomma, 2010). The TGB proteins in potexviruses and other, closely related viruses, are implicated in within-plant cell-to-cell transmission and infection through plasmodesmata, as well as with suppression of host-defense mechanisms (see e.g. Howard *et al.*, 2004). They interact with host plant-specific structures and mechanisms.

5.1.2 Genome diversity

Four PepMV genotypes, with an inter-genotype RNA sequence identity ranging from 78 to 95%, can be distinguished: the original Peruvian genotype (LP); the European (tomato) genotype (EU), the American genotype US1 and the Chilean genotype CH2 (Hanssen and Thomma, 2010).

PepMV is a moderately adaptable virus, with a relatively host-range and is found primarily under protected cropping conditions. All four currently known PepMV genotypes are present in European tomato production, and recombinantsare observed. As with all RNA viruses PepMV is expected to have a high mutation rate; observations however suggest that the number of mutations over time is rather limited and that most mutations have no biological relevance (Werkman and Sansford, 2010).

5.1.3 Transmission

PepMV is very efficiently transmitted mechanically, by entering the tomato plant cells through natural openings or through wounds in the cell walls; additionally, a low seed transmission rate has been demonstrated. It has furthermore been observed that bumblebees can transmit the virus from infected to non-infected plants; however, transmission by bumblebees is only relevant for spreading the virus within an infected area – there is no risk of spreading the infection beyond such an area, let alone to other regions or countries. (Hanssen and Thomma, 2010).

5.1.4 Host range

Similar to other potexviruses, PepMV has a rather narrow host range. As indicated by its name, PepMV was originally isolated from pepino (*Solanum muricatum*) that showed yellow leaf mosaic symptoms in Peru (Jones *et al.*, 1980). Its host range is thought to be mainly restricted to *Solanaceae* species (Salomone and Roggero, 2002; Soler *et al.*, 2002; Verhoeven *et al.*, 2003). In a survey in central and southern Peru the virus has been identified in natural infections of the wild tomato species *S. chilense*, *S. chmielewskii*, *S. parviflorum* and *S. peruvianum* (Soler *et al.*, 2002). Furthermore, by performing mechanical inoculations, the host range of PepMV was shown to contain eggplant (*S. melongena*), potato (*S. tuberosum*) and species from the genera *Nicotiana* (e.g. *N. benthamiana*), *Datura* (e.g. *D. stramonium*), *Capsicum* (*C. annuum*) and *Physalis* (*P. floridana*) (Salomone and Roggero, 2002; Verhoeven *et al.*, 2003; Jones *et al.*, 1980; Martin and Mousserion, 2002). So far, Basil (*Ocimum basilicum*; *Lamiaceae*) is the only reported natural host that does not belong to the *Solanaceae*, with plants displaying interveinal chlorosis (Davino *et al.*, 2009). The status of basil as host of PepMV is however not clear since attempts to obtain infectious isolates or confirmation of infection failed (Werkman and Sansford, 2010). Thus, so far only species of the *Solanaceae* family are confirmed hosts to PepMV.

Furthermore, in a survey of 42 native weed species growing in or around tomato production sites in Spain, PepMV infection was found in 18 weed species, including those belonging to the *Amaranthaceae* (e.g. *Chenopodium murale*), *Convolvulaceae* (e.g. *Calystegia sepium*), *Brassicaceae* (e.g. *Diplotaxis erucoides*), *Boraginaceae* (e.g. *Heliotropium europaeum*), *Asteraceae* (e.g. *Sonchus tenerrimus*), *Plantaginaceae* (*Plantago afra*), and *Polygonaceae* (*Rumex sp.*) (Córdoba *et al.*, 2004). Interestingly, a recent study revealed that co-inoculation with a EU and a CH2 isolate extended the host range beyond the host range of the single isolates (Gómez *et al.* 2009). More specifically, neither the EU isolate Sp13, nor the CH2 isolate PS5, could establish infection in *N. glutinosa* or *N. tabacum*, while both host plants appeared to be susceptible upon inoculation with the mix of the two isolates (Gómez *et al.*, 2009). No data is found that in natural conditions, the above weeds, if not occuring in close proximity to tomato crops, may already be infected with PepMV.

Taxonomic name and isolate:	*Pepino mosaic virus* (PepMV) Chilean genotype (CH2), isolate 1906, GenBank Accession number JN835466
Species:	*Pepino mosaic virus*
First description:	The original mild type was first isolated in 2006 from a commercial tomato crop

Genotype:	Chilean genotype (CH2)
Genus:	*Potexvirus*
Family:	*Flexiviridae*
Division:	*Virus*

10.1.c Wob juncto 63.2.d Vo 1107/2009

6 Human hazard narrative

The human hazard, exposure and risk assessment of plant protection products is covered in Chapter 5 of Annex II to 91/414 (active substance data) and Chapter 7 of Annex III to 91/414 (product data). Details on the data requirements specifically for (plant protection products based on) micro-organisms can be found in Directive 2001/36/EC. The specific active substance data requirements are summarized here:

6.1 Summary: potential of PepMV to be hazardous to humans with consideration of its pathogenic potential, its ability to infect and pattern of clearance, and its toxicological effects

Viruses and especially plant viruses, by their very nature, cannot be acutely toxic to humans by themselves (i.e. without first infecting a host) – they lack not only potentially toxic components, but also the ability to produce toxic metabolites without the cellular machinery they rely on, i.e. without first infecting a host cell.

For PepMV the complete viral genome sequence is known and the five encoded typical Potexvirus proteins are well understood. None of these proteins shows any homology to known human or animal toxins. It can therefore be stated with certainty that PepMV does not produce toxins, not even after infecting the plant host cell. In addition, the fact that the majority of the tomatoes available on the European markets are infected with a broad variety of PepMV isolates and have been so since the early 2000s indicates that PepMV does not pose any threat to human health. The application of the proposed plant protection product [redacted] PepMV, CH2 strain, isolate 1906) will not lead to a higher incidence of PepMV in either tomato culture or fruits. In fact, application of the proposed plant protection product will reduce the incidence of other, more damaging variants of this plant virus.

Plant viruses in general, and PepMV in particular, are generally regarded as being incapable of infecting humans, and other vertebrates (see Chapter 4). It is furthermore deemed unlikely that PepMV is a sensitizer, since viral proteins are generally not known to be sensitizers; however, since adequate methods to investigate the sensitization potential of viruses are not readily available, PepMV could not be tested on its potential sensitizing properties.

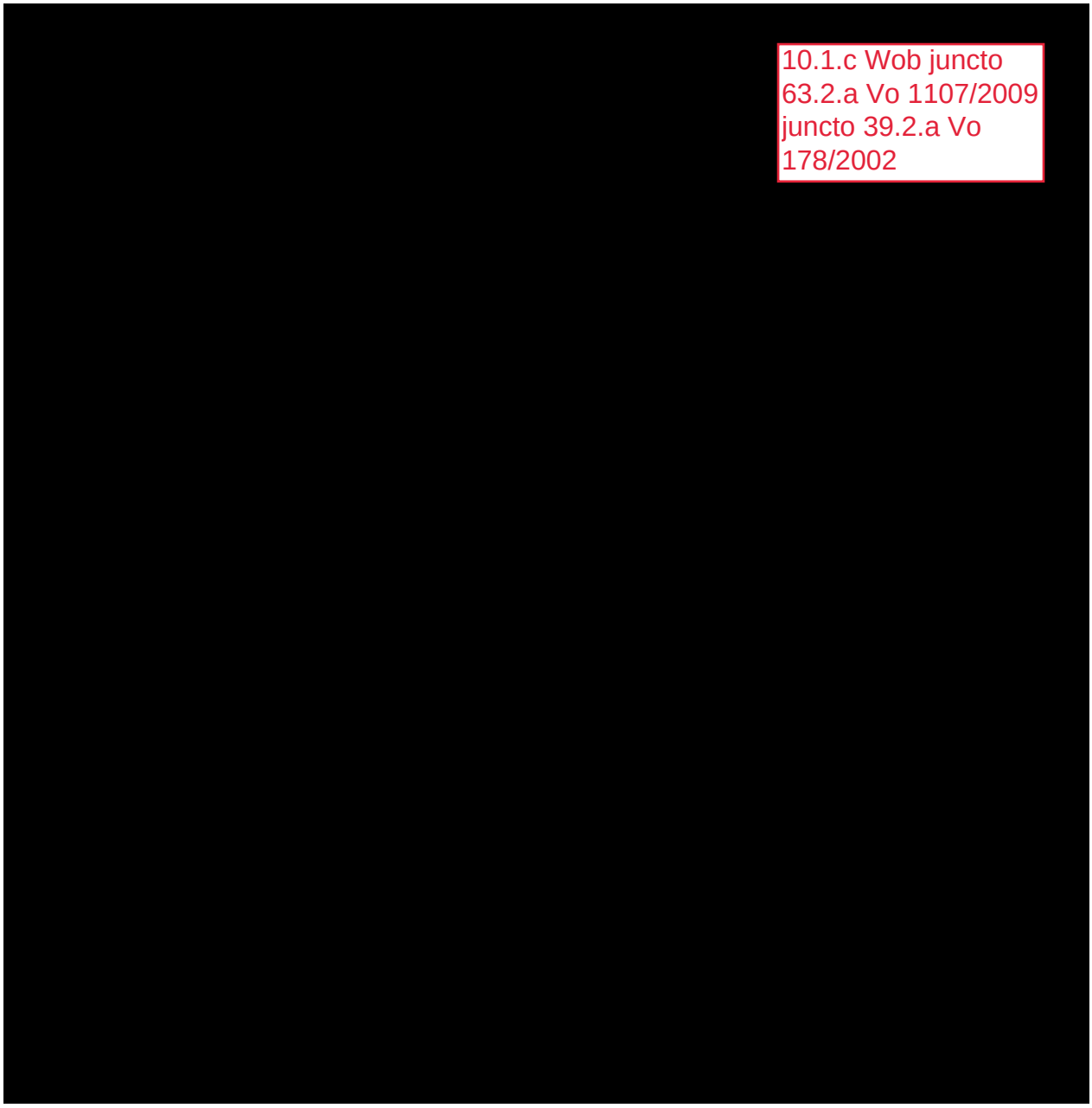
The available occupational exposure surveillance information (see Section 6.2) corroborates the absence of toxicity and other potential hazards to humans. As such, it is considered that the human safety of PepMV can be supported with the available information, and that no additional hazard studies are required. It should be noted here that PepMV, more specifically CH2 strains of PepMV, is ubiquitous in all major tomato-growing regions and countries in the EU, and as such it is clear that a large part of the EU population is routinely exposed to PepMV in one form or another.

6.2 Occupational health surveillance report on workers during production and testing of PepMV (including sensitisation/allergenicity observations)

Pepino mosaic virus is a widely distributed plant virus that has been initially isolated from Pepino (*Solanum muricatum*) in Peru in 1974 (Jones *et al.*, 1980). Its host range is thought to be mainly restricted to *Solanaceae* species.

PepMV is efficiently transmitted mechanically and it is highly contagious in tomato, because it easily can be spread by the standard crop handling procedures in greenhouses, through contaminated tools, hands and clothing and by direct plant-to-plant contact (Hanssen, 2010). Since it is such an abundant plant virus in tomato cultivation, it is reasonable to assume that people are routinely exposed to the virus either professionally (farm personnel, researchers etc.) or via consumption of tomatoes. However, to date, there are no known incidences of adverse health effects of exposure to PepMV, including hypersensitivity or chronic sensitisation.

The following explicit information is available regarding exposure of working personnel (farmers and researchers) to PepMV, CH2 strain, isolate 1906 type isolate, during working experiments in Scientia Terrae and Proefcentrum Hoogstaten. Tomato crops produced were occasionally sold in auction markets to the general public.



10.1.c Wob juncto 63.2.a Vo 1107/2009 juncto 39.2.a Vo 178/2002

A medical officer annually examined the involved personnel by performance of standard medical tests. A confirmation of the aforementioned results by the medical officers is provided in Appendix 1, supporting the claims that exposures of workers to the PepMV, CH2 strain, isolate 1906 during the years 2006–2011 did not result in any known incidence of adverse health effects, including hypersensitivity or chronic sensitisation.

6.3 Basic toxicity studies

The requirements for the basic toxicological data set (acute toxicity, intraperitoneal/subcutaneous single dose, genotoxicity testing, cell culture study, information on short-term toxicity) are waived based on the following rationale. No literature is available in the public domain providing evidence for suggesting that plant viruses might pose a toxicological hazard to humans and mammals in general. Routine exposure of farm personnel, laboratory researchers, as well as consumers, to several abundant plant viruses has not resulted in any known adverse effects of toxicological significance. Even more so, occupational exposure of personnel to the PepMV, CH2 strain, isolate 1906 or/and exposure of the general public after consumption of the infected tomatoes did not result in any known adverse health effects.

Viruses do not have a cellular structure, and hence, they cannot reproduce through cell division; instead, they exclusively depend on the metabolic system of their host cell for replication, by delivering their own genetic information into this system (Wagner *et al.*, 2008, textbook in basic virology; Soni SK, 2007, textbook). A cell-free virus preparation therefore consists only of viral coat protein and viral RNA. Due to the lack of a metabolic system, viruses are incapable of producing metabolites/toxic compounds *per se*, compounds which might ultimately exert any direct adverse effects on their hosts **prior** to infection, for instance via injury of tissues. Once a virus enters its host cell and starts to replicate, viral components, as well as by-products may accumulate within the cell; the extent of this will strongly depend on the type of virus. Such by-products may be (cyto-) toxic. However, infection of the host cell is a pre-condition for the production of such toxins, since the virus does not have its own metabolic system. One characteristic example is the vaccinia virus (poxvirus family) cytotoxin. Wolstenholme *et al.* (1977) demonstrated that whereas extracts of vaccinia-infected cells rendered from the virus were toxic to uninfected cells, extracts from uninfected cells were not cytotoxic under the same test conditions, suggesting that the toxin is produced only within the cell.

For PepMV the complete viral genome sequence is known[3] and the proteins that are encoded by this genome are just five well known, typical plant Potexvirus proteins, *viz.* the coat protein, three Triple Gene Block proteins, a Replicase protein containing an RNA dependent RNA polymerase region, as detailed in the previous chapter. None of these proteins shows any homology to known human or animal toxins. It can therefore be stated with certainty that PepMV does not produce toxins, not even after infecting the plant host cell.

Moreover, the fact that the majority of the tomatoes available on the European markets is infected with a broad variety of PepMV isolates and has been so since the early 2000s indicates that PepMV does not pose any threat to human health. Given the nature of the PepMV virus, and its absolutely minimal genome, it is highly unlikely that replication of PepMV in tomato cells would result in relevant by-products.

Viruses have been widely studied; hence, it is highly unlikely that if secretion of particular toxins **prior** to infection with a known virus would be a relevant process, this would not have been observed and reported upon. Based on the above argumentation, it can be concluded it is highly unlikely that exposure to viruses, and even more so to plant viruses, which are incapable of infecting mammalian cells, may result to any **direct** adverse effects of **toxicological** significance in humans. Moreover, as explained in Section 5.1.1, the whole PepMV virus genome is known and understood, and all genes code for products that are active only in plants, and do not produce secondary metabolites. Therefore, it is not deemed necessary to test *Pepino mosaic virus* for its **toxic** potential to mammals.

6.4 Pathogenicity

Viruses and microorganisms are ubiquitous and worldwide. Humans are exposed daily to millions of these agents, yet major part of these organisms are not able to infect and cause diseases. While infection is the capacity of an organism to invade and colonize a host (White, 2005), pathogenicity relates to the ability of an organisms, bacteria or virus, to cause a disease (Bannister *et al.* 2006). Another important terminology to be used to characterize a virus, is virulence. Virulence is used to compare the frequency and degree of severity of an organism to provoke diseases (White, 2005; Smith, 1972).

As the definitions state, a pathogen is an agent that is able to invade a host and cause disease. In order for a **virus** to be a pathogen (human, plant or animal), it needs first to infect the host, because there is no other way a virus can exert a pathogenic influence on a host. As explained extensively in the previous chapter, plant viruses do not infect mammals (including humans), since they do not possess receptors that bind to human cells. Thus the pathogenicity potential of the PepMV, Chilean genotype (CH2) can be considered irrelevant, as this virus has no ability to infect humans.

6.5 Sensitisation

Sufficient information needs to be provided as regards to the allergenic potential of the micro-organism via the dermal and inhalative route. However, no suitable testing methods are

3. Many complete sequences of the PepMV genome are publically available in GenBank

available. Therefore, according to the Directive 2001/36/EC, *Pepino mosaic virus*, like all PPP microorganisms, should be regarded as a potential sensitizer by default. Nonetheless, it is doubtful whether such a classification is applicable for viruses, and especially plant viruses.

No information is available in the open literature reporting on sensitization or allergic reactions to plant viruses. Since many viruses are pathogenic, and will invoke a normal immune response, and are quite extensively studied, it is extremely unlikely that sensitization to viruses, if extant, would have gone totally unnoticed. It can furthermore be argued that there would be a definitive evolutionary selection pressure on viruses to not be sensitizing, since this would greatly interfere with their capacity for infectivity.

Some **animal** viruses have been occasionally associated with the induction of allergic sensitization in animal models (Riedel *et al.*, 1996; Sakamoto *et al.*, 1984; Schwarze & Gelfand, 2002). However, none of the studies have shown any direct sensitisation of the virus per se; instead, viral infections appeared to trigger and enhance allergic reactions to a real sensitizer.

In humans, viral infections have been often associated with the exacerbation of asthma (Clare *et al.*, 2004). For example, Frick *et al.* (1979) found a clear correlation between viral respiratory tract infection and the start of allergic sensitisation in children. Likewise, Nicholson *et al.* (1993) observed that acute viral respiratory infections may trigger wheezing and enhancement of asthma in adults. It should be noted here that all of these findings are for (animal) viruses that explicitly infect the respiratory tract.

Potential correlation of sensitization with viral infections has been widely studied both in animal models and in humans. Despite the fact that some viral infections may enhance the allergic symptoms (mainly asthma) to an allergen, there is no reported incidence of direct sensitisation to a virus, i.e. production of IgE antibodies against the virus itself. Even so, a prerequisite for such observations is primarily the infection of the host by the virus. It is rather unlikely that plant viruses will infect human or mammalian cells, due to the different infectivity processes and thus, it can be explained why such enhancement effects have not been described after exposure to plant viruses. Particularly, as regards to PepMV, regular exposure of farm and research personnel did not result in any known incidence of hypersensitivity or chronic sensitisation (see Section 6.2 for details).

Taken all together, it is very unlikely that viruses, i.c. PepMV, may provoke sensitisation and consequently, allergic reactions to humans.

6.6 Toxicity studies on metabolites

Plant viruses are viruses affecting plants. Like all other viruses, they are intracellular parasites, lacking the molecular machinery to replicate without a host. Since viruses do not have a metabolic system, they are incapable of producing their own metabolites; hence, this requirement is deemed as non-relevant for viruses. Additionally, plant viruses do not infect humans (see Chapter 4) so the production of secondary transcription products is not expected. Metabolic breakdown of virus particles (i.e. digestion of the coat proteins and the genome DNA or RNA) is not expected to generate any relevant metabolites.

7 Consumer hazard narrative

Again the requirements for possible residues and their effects on the treated products, food and feed are addressed at the active substance level (Annex II Chapter 6).

7.1 Metabolism of the micro-organism

Contrary to cellular micro-organisms, that can autonomously produce secondary metabolites, viruses, lacking any transcription, protein synthesis, and biochemical cellular machinery, cannot produce metabolites. Therefore, metabolism of the micro-organism in and of itself is not a relevant issue when evaluating (plant) viruses. However, infection of the host plant with a plant virus might result in altered metabolic profiles of the host cells.

Since viruses are intracellular parasites that require the host's cell machinery to multiply, infection of a plant with a virus commonly induces several alterations in the physiology, as well as the gene expression, of the host. Nevertheless, plant defence response to pathogens is not very specific in comparison to the complex specific response of mammalian organisms. Therefore, and considering the fact that plant viruses are widespread among the plant kingdom, secondary plant metabolites produced in response to viral infections in general, and to PepMV infection in particular, are not anticipated to result in significant exposure issues for consumers.

PepMV is a highly infectious potexvirus and a major disease of greenhouse tomato (*Solanum lycopersicum*) (Hanssen *et al.*, 2011). Transcriptional changes in response to inoculation of tomato seedlings with a mild and an aggressive PepMV isolate have been examined by Hanssen *et al.* (2011). PepMV inoculation leads to a repression of photosynthesis in the plants. Defence responses were higher when the aggressive type was used. Analysis of plant metabolism in PepMV-infected tomatoes showed a reduced amount of carotenoids and elevations in the concentrations of alkaloids and phenylpropanoids, which are associated with the pathogen defence of the plant. All of these effects were more pronounced in the interaction of the tomato host plant with a aggressive PepMV isolate than in the interaction with the mild isolate 1906.

Since PepMV is a naturally occurring virus of tomatoes, which can be present in a very high percentage of the total production of the crop, intentional introduction of the PepMV, CH2 strain, isolate 1906 is not expected to result in a substantially different metabolic profile of the crop from that of a non-treated crop; in fact, intentional infection of tomatoes with PepMV, CH2 strain, isolate 1906 may lead to reduced levels of alkaloids and phenylpropanoids when compared to tomatoes that are infected with wild isolates of CH2 (which may be a significant fraction of all tomatoes currently on the market).

7.2 Adverse effects of the microorganism or any residual traces to humans

As argued in this document (see Chapter 4) plant viruses are viruses affecting plants and they are not known to infect mammalian cells (see e.g. Shors, 2009). As all viruses, they are metabolically inert and they entirely depend on their host's metabolic system for their replication. Therefore, they are incapable of secreting any potentially toxic substances (metabolites) by themselves, which could be deleterious to their host, before, or without, infection.

Moreover, as explained in Section 5.1.1, the whole PepMV virus genome is known and understood, and all genes code for products that are active only in plants, and do not produce secondary metabolites (Hanssen and Thomma, 2010).

Virus-infected food plants have always been part of the food chain, and it is common knowledge that most crop plants may be infected by at least one virus (Scholthof et al., 1993; Gergerich and Dolja, 2006; see also http://www.dpvweb.net/intro/index.php). Humans have been continuously exposed to plant viruses through ingestion of infected food commodities (Zhang *et al.*, 2006). Moreover, routine exposure of working personnel, e.g. farmers and laboratory researchers, to several abundant plant viruses has not resulted in any known adverse effects. Hitherto, there have been no reports of adverse human health effects associated with consumption or exposure in general to plant viruses (see Chapters 4 & 6).

Particularly, exposure of working personnel (farmers and researchers) to PepMV, CH2 strain, isolate 1906 during experiments and field trials performed in Scientia Terrae and Proefcentrum Hoogstaten (2006-2011) did not result in any known adverse health effects (see Section 6.2). Tomato crops produced through these experiments were occasionally sold to the general public through auction markets. No health problems have been recorded amongst people after consuming the tomato crop infected with PepMV, CH2 strain, isolate 1906.

PepMV, CH2 strain, isolate 1906 is a naturally occurring isolate of the virus, which has not exerted any hazardous effects to humans. Exposure of humans to concentrations of the virus considerably higher than under uninfected conditions, due to consumption of tomato fruits inoculated with PepMV, CH2 strain, isolate 1906 as an active ingredient of a PPP, does not pose any additional risk to human health, since the virus is by nature not hazardous to humans. Note that unintentional infection with wild, aggressive isolates of PepMV is common in tomatoes, and that virus titers in tomatoes unintentionally infected with these isolates are in the same order as virus titers in tomatoes intentionally infected with PepMV, CH2 strain, isolate 1906; consumers of tomatoes are therefore commonly exposed to significant levels of PepMV. The requirement for residue trials is deemed not relevant for PepMV, CH2 strain, isolate 1906, based on the above justification.

8 Fate and behaviour in the environment

The PepMV, CH2 strain, isolate 1906 is applied once per season to young tomato plants grown in greenhouses. The virus only survives within plants (*Solanaceae* being the primary host range), and does not survive outside of its host.

PepMV infection in tomatoes is primarily an issue in protected cultivation. In the southern part Europe tomato is commercially grown outdoors as well as under protection. Most reports on PepMV in tomato in northern Europe, Southern Europe and North America are on crops grown under protected cultivation. The only report of an infection with PepMV in outdoor tomato cultivation originates from Cyprus, although there is not yet any information available on the possible survival of PepMV in the field. Infections with PepMV in outdoor tomato cultivation probably occur more often although no further data are available. All Additionally there have been reports of findings of PepMV in weeds in infected tomato fields in Spain. However, due to the unsymptomatic nature of these infections, and the fact that they are all the result of local transmission through 'vehicles' the environment is not considered as being at risk as a result of PepMV. Survival of PepMV outside of a host plant, in the field, or for a prolonged time on a 'vehicle' is deemed unlikely (Werkman and Sansford, 2010).

8.1 Soil

Current day greenhouse tomato culture is a substrate culture, where tomato plants are usually grown on rock wool or sometimes on coconut hull fibre; no commercially important greenhouse tomato culture on exposed soil exists today in Belgium and the Netherlands (Centraal Bureau voor de Statistiek, 2009). Additionally, the growth medium is covered with plastic, preventing the virus to reach the growth medium during application. Exposure to soil, either inside the greenhouse, or outside, is therefore considered irrelevant.

8.2 Water

It is assumed that the amount of virus that will reach surface water through a possible discharge of drainage water from storage tanks will be insignificant. The drainage water in tomato culture greenhouses is disinfected before recycling or discharge by UV light (Runia, 2000). Also the influence of UV light during application will decrease the survival of PepMV, CH2 strain, isolate 1906.

8.3 Air

In the tomato cultures in greenhouses the release to the environment through air is very limited. All entrances are controlled and the only air-exchange with the outside is located at the ventilation windows several meters above the plants. The virus does not survive long outside plant material and is broken down by UV light. Exposure of the environment through air is considered insignificant.

9 Ecotoxicology

PepMV is a plant virus with no identified animal vector (note that the observed opportunistic transmission by bumblebees does not classify as a true vector and as such has no relation to any ecotoxicological issues), and no known animal hosts; no indications exist that PepMV will be infectious to higher organisms, including insects. In addition, there are no known toxins or metabolites produced by any known isolate of PepMV that could be harmful to animals or the environment.

9.1 Effects on birds and mammals

PepMV is a plant virus with no identified animal vector, and no known animal hosts; no indications exist that PepMV will be infectious to birds or mammals. Additionally, risks to birds or mammals are not expected, because the specific application technique (spraying in closed greenhouses) will exclude the direct exposure of birds and mammals, since no birds or mammals will be present in the greenhouse. PepMV occurs widely in cultured tomato plants, and presumably also in other cultured and wild *Solanacea*; no effects on birds or mammals have been observed so far. A study to the effect on birds is therefore considered not relevant. Studies to the effects on mammals are usually covered by the studies performed in the context of human toxicology. As previously argued, explicit human toxicology studies are deemed irrelevant, and consequently, specific studies with mammals in the context of ecotoxicology are also deemed irrelevant.

9.2 Effects on aquatic organisms

PepMV is a plant virus with no identified animal vector, and no known animal hosts; no indications exist that PepMV will be infectious to aquatic organisms. Additionally, risks to aquatic organisms are not expected, because application in closed greenhouses will exclude significant emission to surface water. PepMV does not survive outside its plant host, will not infect aquatic organisms, and will not propagate in surface water. Studies to the effect on aquatic organisms are therefore considered not relevant.

9.3 Effects on honeybees

PepMV is a plant virus with no identified (invertebrate) vector, and no known invertebrate hosts (with the exception of the already mentioned opportunistic transmission by bumblebees); as such, no indications exist that PepMV will be infectious to honeybees or other pollinators. Note that tomatoes are commercially pollinated by bumblebees. However, risks to pollinators are not expected, because application of PepMV will not coincide with the presence of pollinators in the greenhouse; tomato plants are to be treated with PepMV either as seedlings or upon or shortly after planting in a greenhouse. Many tomato culture greenhouses are already infected with (other isolates of) PepMV; the available information does not suggest any effects of infected tomato plants on pollinators (Lacasa *et al.*, 2003; Shipp *et al.*, 2008). Studies to the effect on honeybees are therefore considered not relevant.

9.4 Effects on nontarget plants (NTP)

PepMV is a virus with a narrow recognized host range, essentially only *Solanaceae*. PepMV infections have been observed in several weeds adjacent to tomato growing areas in Spain, however, these are non-symptomatic infections, and should therefore not be considered an adverse effect by itself. Since no effects of PepMV infections on any infected plant species outside its host range have been observed, it is deemed unlikely that a risk to non-target plants exists; studies to the effects on non-target plants are therefore considered not relevant.

9.5 Effects on nontarget arthropods (NTA)

PepMV is a plant virus with no identified animal vector, and no known animal hosts; no indications exist that PepMV will be infectious to nontarget arthropods. Additionally, risks to nontarget arthropods are not expected, because application in closed greenhouses will not coincide with the presence of nontarget arthropods of commercial relevance (natural enemies, pollinators) in the greenhouse; application of PepMV occurs at the seedling stage or at or shortly after planting. Nontarget arthropods are employed, if at all, much later in the growing season. Many tomato culture greenhouses are already infected with (other isolates of) PepMV; the available information does not suggest any effects of infected tomato plants on nontarget arthropods. Studies to the effect on nontarget arthropods are therefore considered not relevant.

9.6 Effects on earthworms

PepMV is a plant virus with no identified animal vector, and no known animal hosts; no indications exist that PepMV will be infectious to earthworms. Additionally, risks to earthworms are not expected, because application in closed greenhouses in substrate culture will exclude residues in soil, either inside or outside the greenhouse. PepMV does not survive outside its plant host, will not infect soil organisms, and will not propagate in soil. Studies to the effect on earthworms are therefore considered not relevant.

10 Conclusions

Based on considerations of general virology, the difference between plant and animal viruses, specific knowledge about PepMV virus, information obtained as a result of occupational exposure to this specific PepMV, CH2 strain, isolate and anecdotal evidence based on the ubiquitous nature of PepMV virus infections in commercial tomato crops, it is concluded that it is unlikely that PepMV virus, when used as a plant protection product to improve tomato resistance to natural infections with other PepMV isolates, poses a health risk to humans (operators, workers, bystanders, or consumers) or the environment.

11 References

Agrios GN, 2005. Plant pathology. Fifth Edition. Elsevier Academic Press

Bannister B, Gillespie S, Jones J, 2006. Infection: microbiology and management. Third Edition. Blackwell Publishing Ltd.

Boevink P, Oparka KJ, 2005. Virus-Host Interactions during Movement Processes. Plant Physiol. 138: 1815-1821.

Bos L, 1999. Beijerinck's work on tobacco mosaic virus: historical context and legacy, Phil.Trans. R. Soc. Lond. B, 675-685

Centraal Bureau voor de Statistiek, 2009. De Nederlandse economie 2008. CBS, Den Haag/Heerlen.

Central Science Laboratories, 2005. Pest risk analysis for Pepino mosaic virus. CSL, Sand Hutton, York, UK (from CSL web site: http://www.fera.defra.gov.uk/plants/plantHealth/pestsDiseases/documents/pepino.pdf).

Clare SM, Simpson A, Custovic A, 2004. Allergens, Viruses, and Asthma Exacerbations. Proceedings of the American Thoracic Society 1: 99-104.

Colson P, Richet H, Desnues C, Balique F, Moal V, Grob J-J, Berbis P, Lecoq H, Harlé J-R, Berland Y, Raoult D, 2010. *Pepper mild mottle virus*, a plant virus associated with specific immune responses, fever, abdominal pains, and pruritus in humans. PLoS ONE 5(4): e10041. doi:10.1371/journal.pone.0010041.

Córdoba MC, Martínez-Priego L, Jordá C, 2004. New natural hosts of *Pepino mosaic virus* in Spain. Plant Dis. 88: 906.

Cotillon AC, Girard M, Ducouret S, 2002. Complete nucleotide sequence of the genomic RNA of a French isolate of *Pepino mosaic virus* (PepMV). Arch. Virol. 147: 2231-2238.

Davino S, Accotto GP, Masenga V, Torta L, Davino M, 2009. Basil (*Ocimum basicilum*), a new host of *Pepino mosaic virus*. Plant Pathol. 58: 407.

De Nayer F, Moerkens R, Goen, K, Vanbriel M, Wittemans L, Paeleman A & Hanssen I, 2011. Vaccinatie met mild CH2-isolaat biedt goede bescherming tegen PepMV. ProeftuinNieuws 14: 36-37.

French CJ, Bouthillier M, Bernardy M, Ferguson G, Sabourin M, Johnson RC, Masters C, Godkin S, Mumford R, 2001. First report of *Pepino mosaic virus* in Canada and the United States. Plant Dis. 85: 1121.

Frick OL, German DF, Mills J, 1979. Development of allergy in children. I. Association with virus infections. Journal of Allergy and Clinical Immunology 63: 228-241.

Fuchs MF, 2008. Why virus resistance in transgenic plants, and are there risks associated with the release of virus-resistant transgenic plants? Cornell University

Gergerich RC, Dolja VV, 2006. Introduction to Plant Viruses, the Invisible Foe. The Plant Health Instructor. DOI: 10.1094/PHI-I-2006-0414-01

Gómez P, Sempere RN, Elena SF, Aranda MA, 2009. Mixed infections of *Pepino mosaic virus* strains modulate the evolutionary dynamics of this emergent virus. J. Virol. 83: 12378-12387.

Hanssen IM, 2010. *Pepino mosaic virus*: an endemic pathogen of tomato crops, Doctoral thesis, Wageningen University, Wageningen, the Netherlands, ISBN 978-90-8585-557-6, 167 pages.

Hanssen IM, van Esse HP, Ballester A, Hogewoning SW,Ortega Parra N, Paeleman A, Lievens B, Bovy AG, Thomma BPHJ, 2011. Differential tomato transcriptomic responses induced by *pepino mosaic virus* isolates with differential aggressiveness. Plant Physiol. 156: 301-318.

Hanssen IM, Gutiérrez-Aguirre I, Paeleman A, Goen K, Wittemans ., Lievens B, Vanachter AC.C, Ravnikar M, Thomma BPHJ, 2010. Cross-protection or enhanced symptom display in greenhouse tomato co-infected with different *Pepino mosaic virus* isolates. Plant Pathol. 59: 13-21.

Hanssen IM, Paeleman A, Vandewoestijne E, Van Bergen L, Bragard C, Lievens B, Vanachter AC RC, Thomma BPHJ, 2009. *Pepino mosaic virus* isolates and differential symptomatology in tomato. Plant Pathol. 58: 450–460.

Hanssen IM, Paeleman A, Wittemans L, Goen K, Lievens B, Bragard C, Vanachter ACRC, Thomma BPHJ, 2008. Genetic characterization of *Pepino mosaic virus* isolates from Belgian greenhouse tomatoes reveals genetic recombination. Eur. J. Plant Pathol. 121: 131-46.

Hanssen IM, Thomma BPHJ, 2010. *Pepino mosaic virus*: a succesful pathogen that rapidly evolved from emerging to endemic in tomato crops. Mol. Plant Pathol. 11: 179-189.

Hasiów B, Borodynko N, Pospieszny H, 2008. Complete genomic RNA sequence of the Polish *Pepino Mosaic Virus* isolate belonging to the US2 strain. Virus Genes 36: 209–214.

Howard AR, Heppler ML, Ju H-J, Krishnamurty K, Payton ME, Verchot-Lubicz J, 2004. *Potato virus X* TGBp1 induces plasmodesmata gating and moves between cells in several host species whereas CP moves only in *N. benthamiana* leaves. Virology 328:185–197.

Jones RAC, Koenig R, Lesemann DE, 1980. *Pepino mosaic virus*, a new Potexvirus from pepino (*Solanum muricatum*). Ann. Appl. Biol. 94: 61-68.

Lacasa A, Guerrero MM, Hita I, Martinéz MA, Jordá C, Bielza P, Contreras J, Alcázar A, Cano A, 2003. Implicationes de los abejorros (*Bombus* spp.) en la dipersión del virus del mosaico del pepino dulce (*Pepino mosaic virus*) en cultivos de tomato. Bol. Sanid. Veg., Plagas, 29: 393–402.

Ling K-S, 2006. Molecular characterization of two *Pepino mosaic virus* variants from imported tomato seed reveals high levels of sequence identity between Chilean and US isolates. Virus Genes 34: 1-8.

Ling K-S, Wintermantel WM, Bledsoe M, 2008. Genetic composition of *Pepino mosaic virus* population in North American greenhouse tomatoes. Plant Dis. 92: 1683-1688.

Lucas WJ, 2006. Plant viral movement proteins: Agents for cell-to-cell trafficking of viral genomes. Virology 344: 169-184.

Mahy BWJ, 1991. Seminars in virology. Volume 2 Number 1. Saunders Scientific Publications.

Mahy BWJ, Regenmortel MH., 2010. Desk encyclopedia of plant and fungal virology. Academic Press

Maroon-Lango CJ, Guaragna MA, Jordan RL, Hammond J, Bandla M, Marquardt SK, 2005. Two unique US isolates of *Pepino mosaic virus* from a limited source of pooled tomato tissue are distinct from a third (EU like) US isolate. Arch. Virol. 150: 1187-1201.

Martin J, Mousserion C, 2002. Potato varieties which are sensitive to the tomato strains of *Pepino mosaic virus* (PepMV). Phytoma, la défense des végétaux 552: 26-28.

Mumford RA, Metcalfe EJ, 2001. The partial sequencing of the genomic RNA of a UK isolate of *Pepino mosaic virus* and the comparison of the coat protein sequence with other isolates from Europe and Peru. Arch. Virol. 146: 2455-2460.

Nicholson KG, Kent J, Ireland DC, 1993. Respiratory viruses and exacerbations of asthma in adults. Brit. Medical J. 307: 982–986.

Pagán I, Córdoba-Sellés MC, Martinez-Priego L, Fraile A, Malpica JM, Jordá C, García- Arenal F, 2006. Genetic structure of the population of *Pepino mosaic virus* infecting tomato crops in Spain. Phytopathology 96: 274-279.

Riedel F, Krause A, Slenczka W, Rieger CHL, 1996. Parainfluenza-3-virus infection enhances allergic sensitization in the guinea-pig. Clinical and Experimental Allergy 26: 603-609.

Runia W, 2000. UV-ontsmetter doodt pepinomozaïekvirus bij lagere dosis. Groenten en Fruit 28 april 2000, 19.

Rybicki, E, 2008. Where did viruses come from. Scientific American Online, March 27 (http://www.scientificamerican.com/article.cfm?id=experts-where-did-viruses-come-fr).

Sakamoto M, Ida S, Takishima T, 1984. Effect of influenza virus infection on allergic sensitization to aerosolized ovalbumin in mice. Journal of Immunology 132: 2614-2617.

Salomone A, Roggero P, 2002. Host range, seed transmission and detection by ELISA and lateral flow of an Italian isolate of *Pepino mosaic virus*. J. Plant Pathol. 84: 65-68.

Scholthof K, Scholthof HB, Jackson AO, 1993. Control of Plant Virus Diseases by Pathogen-Derived Resistance in Transgenic Plants. Plant Physiol. 102:7-12.

Schwarze J, Gelfand EW, 2002. Respiratory viral infections as promoters of allergic sensitization and asthma in animal models. European Respiratory Journal 19: 341-349.

Shors T, 2009. Understanding viruses. Jones and Bartlett Publishers, Sudbury, MA.

Shipp JL, Buitenhuis R, Stobbs L, Wang K, Kim WS, Ferguson G, 2008. Vectoring of Pepino mosaic virus by bumble-bees in tomato greenhouses. Ann. Appl. Biol. 53: 149–155.

Smith H, 1972. Mechanisms of virus pathogenicity. Bacteriological Reviews. 291-310.

Soler S, Prohens J, Díez MJ, Nuez F, 2002. Natural occurrence of *Pepino mosaic virus* in *Lycopersicon* species in Central and Southern Peru. J. Phytopathol. 150: 49-53.

Soni S.K., 2007. Microbes A source of energy for 21st Century. New India Publishing Agency.

Strauss JH, Strauss EG, 2008. Viruses and human disease. Elsevier Academic Press, London, UK.

van der Riet F de St J, 1997. Diseases of plants transmissible between plants and man (phytonoses) exist. Medical Hypotheses, 49: 359-361

van der Vlugt RAA, Stijger CCMM, Verhoeven JTJ, Lesemann DE, 2000. First report of *Pepino mosaic virus* on tomato. Plant Dis. 84: 103.

van der Vlugt RAA, 2009. *Pepino mosaic virus*. Hellenic Plant Protection Journal 2: 47-56.

Verhoeven JTJ, van der Vlugt R, Roenhorst JW, 2003. High similarity between tomato isolates of *Pepino mosaic virus* suggests a common origin. Eur. J. Plant Pathol. 109: 419-425.

Wagner EK, Hewlett MJ, Bloom DC, Camerini D, 2008. Basic virology. Third edition. Blackwell Publishing.

Weinstein, P., 1995. Introductory biology. Pascal Press

Werkman AW, Sansford CE, 2010. Pest Risk Analysis for *Pepino mosaic virus* for the EU. Deliverable Report 4.3. EU Sixth Framework Project Project PEPEIRA. http://www.pepeira.com.

White L, 2005 Foundations of Nursing. Second Edition. Thomson Delmar Learning.

Wickramasinghe C, 2003. Panspermia according to Hoyle. Astrophysics and Space Science 285: 535-538.

Wikipedia, 2011. Introduction to viruses. http://en.wikipedia.org/wiki/Introduction_to_viruses.

Wikipedia, 2011. Virus. http://en.wikipedia.org/wiki/Virus.

Wolstenholme J, Woodward CG, Burgoyne RD, Stephen J, 1977. Vaccinia virus toxin. Archives of Virology 53: 25-37.

Zhang T, Breitbart M, Lee WH, Run J-Q, Wei CL, Soh SWL,Hibberd ML, Liu ET, Rohwer F, Ruan Y, 2006. RNA Viral Community in Human Feces: Prevalence of Plant Pathogenic Viruses. PLoS Biol 4(1): e3.

Appendix 1

Medical records

10.1.c Wob juncto 63.2.d Vo 1107/2009

"Scientia Terrae vzw verricht sinds 2006 onderzoek rond het tomatenvirus '*Pepino mosaic virus*' (PepMV). [redacted] met als doel een vaccinatiestrategie te ontwikkelen aan de hand van een milde virusvariant. Sinds 2007 worden er proeven uitgevoerd met de milde virusvariant 'CH2 mild', zowel op laboratoriumschaal (in klimaatkamers) als op praktijkschaal (in tomatenserres). De proeven op laboratoriumschaal worden in het onderzoeksinstituut zelf uitgevoerd. De proeven op praktijkschaal worden uitgevoerd in proefcentra of op commerciële tomatenbedrijven, [redacted] Ik verklaar hierbij dat er tijdens de jaarlijkse medische controles van de werknemers van Scientia Terrae vzw geen gezondheidsproblemen of negatieve bijwerkingen ten gevolge van dit virus werden vastgesteld."

10.1.c Wob juncto 63.2.a Vo 1107/2009 juncto 39.2.a Vo 178/2002

[redacted] 10.2.e

Securex

Non-official translation

10.1.c Wob juncto 63.2.d Vo 1107/2009

Scientia Terrae vzw have performed research with the tomato virus 'Pepino mosaic virus' *(PepMV) since 2006.* [redacted] *with the aim of developing a vaccination strategy with a mild virus variant. Since 2007, tests have been performed with the mild virus variant 'CH2 mild', both in laboratory settings (in climate chambers) and in actual cultures (in tomato greenhouses). The laboratory tests are performed within the research institute. The tests in actual cultures are performed at agricultural experimental centres, and at commercial tomato growers,* [redacted] *I hereby declare that during the annual medical checkups of the Scientia Terrae vzw employees no health issues or negative side effects attributable to this virus were observed.*

10.1.c Wob juncto 63.2.a Vo 1107/2009 juncto 39.2.a Vo 178/2002

"Het Proefcentrum Hoogstraten werkt mee aan een onderzoeksproject om tomatenplanten te immuniseren tegen het Pepino mosaic virus. Dit virus veroorzaakt bij tomaten opbrengst- en kwaliteitsverlies! De vaccinatie bestaat uit het vernevelen van een [redacted] van een verzwakte variant op de jonge planten. Na enkele jaren van proefnemingen met dit natuurlijk isolaat werden er geen negatieve effekten, zoals irritatie, hoofdpijn of allergie vastgesteld bij de werknemers."

10.1.c Wob juncto 63.2.d Vo 1107/2009

[redacted] 10.2.e

Preventieadvizeur- Arbeidsgeneesheer, 9 juni 2011

Non-official translation

The agricultura experimental centre 'Hoogstraten' participates in a research project into the immunization of tomato plants to 'Pepino mosaic virus'*. This virus causes yield and quality loss in tomatoes. Vaccination consists of spraying young plants with an* [redacted] *of a*

10.1.c Wob juncto 63.2.d Vo 1107/2009

weakened variant. After several years of tests with this natural isolate, no negative effects, such as irritation, headaches or allergies, were observed in employees.

Appendix 2

Revised Literature Review Report

Overview of the scientific peer-reviewed open literature, as recommended in EFSA Guidance Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009, as published in the EFSA Journal.[4] Note however that this Guidance Document states that:

> *Article 8(5) of Regulation (EC) No 1107/2009 requires that applicants submitting dossiers for the approval of active substances of plant protection products under Regulation (EC) No 1107/2009 shall provide "Scientific peer-reviewed open literature, [...], on the active substance and its relevant metabolites dealing with side-effects on health, the environment and non-target species and published within the last ten years before the date of submission of the dossier..." as determined by the European Food Safety Authority.*

The current document is *not* intended to provide information (alternative or supplemental to submitting test reports) on a specific active substance or its metabolites, but to provide a general biological background on the (absence of) infectivity of plant viruses to animals in general and humans in particular; as such the Guidance Document is in fact not applicable.

Protocol

The objective of this literature review was to provide a biological background for understanding the potential hazard of PepMV, a narrow host range (*Solanum sp.*) plant virus intended to be used in a newly developed plant protection product, to animal and particularly human, non-target organisms. More specifically, the objective was to provide a general understanding of the biology of plant viruses, and the differences, if any, between plant and animal viruses in toxicology, infectivity and epidemiology. As such there is no list of specific end points/data requirements to be covered. Also, since the search purpose was rather broad and general, criteria for determining the relevance of any individual hit in the search process can only be described in rather general terms, where anything that appears to bear on the biology of plant viruses or the interaction of (unmodified) plant viruses and animals is relevant. Relevance was judged primarily by relying on search hit titles – under the assumption that in general, primary (articles) and secondary (reviews, textbooks, etc.) scientific literature may be counted on to provide adequate and informative article, chapter, or book titles. Information from tertiary literature (encyclopedic works) was confirmed by checking against listed (and unlisted) references, and was primarily used as a portal to primary and secondary literature.

Search methods

Since the primary objective was to provide background material for the evaluation of PepMV, the first reference source on general virology as well as on biological aspects of PepMV was the doctoral thesis on the development of PepMV as a plant protection pro-

4. EFSA Journal 2011;9(2):2092

ducts by I.M, Hanssen.[5] This thesis provides references to (almost) all relevant scientific information on PepMV, as independently (informally) checked by Google Scholar and Medline searches using the search terms “Pepino Mosaic Virus”; “PepMV”.

For general virology information, searches were done through Wikipedia and through Google Scholar, using the search terms: virus, virology, “plant virus”, “plant virus” AND “human”. The Wikipedia search resulted in the following two entries being selected as relevant tertiary information: Wikipedia, 2011. Introduction to viruses. http://en.wikipedia.org/wiki/Introduction_to_viruses; Wikipedia, 2011. Virus. http://en.wikipedia.org/wiki/Virus. The Wikipedia entry on plant viruses (http://en.wikipedia.org/wiki/Plant_virus) was less informative. The primary secondary reference retrieved from these encyclopedic sources was Shors (2009).

For general virology as well as for information on infectivity and epidemiology of plant viruses in humans, a Google Scholar search was performed using the following search terms: virus, virology, “plant virus”, “plant virus” AND “human”. It is noteworthy that the latter search results primarily in hits concerned with genetically modified plant viruses or chimaeric plant viruses, not with human / plant virus epidemiology. This particular search results in 8970 hits (omitting citations and patents). To illustrate the nature of the most relevant hits (according to Google Scholar), the first 50 hits are reproduced in Annex 1.

Especially the information presented in paragraph 4.4, on observed (or tentatively observed) effects of plant viruses in humans are based on a Google internet search, keywords [human, health, infection, plant virus, pepmv, pepino mosaic virus], coupled with a targeted search for any relevant references contained in the search hits; the primary Google search hit was the Colson et al. (2010) reference.

References in chapter 5 are primarily based on the information contained in Hanssen, 2010, as well as on a general Google search on “pepino mosaic virus” (see also Annex 2); the primary source resulting from this search was van der Vlugt, 2009.

Many references were obtained from primary sources, and were retrieved through targeted search actions within individual journal archives, or through the literature search service provider STN.

---

5. I.M, Hanssen: Pepino mosaic virus: an endemic pathogen of tomato crops, 2010. Doctoral thesis, Wageningen University, Wageningen, the Netherlands, ISBN 978-90-8585-557-6, 167 pages

Annex 1

First 50 hits on "plant virus" and "human" from Google Scholar

Chimeric plant virus particles as immunogens for inducing murine and human immune responses against human immunodeficiency virus type 1. C Marusic, P Rizza, L Lattanzi, C Mancini… - Journal of …, 2001 - Am Soc Microbiol

Human immunodeficiency virus type 1-neutralizing antibodies raised to a glycoprotein 41 peptide expressed on the surface of a plant virus; L McLAIN, C PORTA… - … and human …, 1995 - online.liebertpub.com

Stimulation of neutralizing antibodies to human immunodeficiency virus type 1 in three strains of mice immunized with a 22 amino acid peptide of gp41 expressed on the surface of a plant virus; L McLain, Z Durrani, LA Wisniewski, C Porta… - Vaccine, 1996 - Elsevier

Hepatitis C virus shares amino acid sequence similarity with pestiviruses and flaviviruses as well as members of two plant virus supergroups; RH Miller, RH Purcell - … of the National Academy of Sciences, 1990 - National Acad Sciences

Association of hepatitis C virus in human sera with β-lipoprotein; R Thomssen, S Bonk, C Propfe, KH Heermann… - Medical microbiology …, 1992 - Springer

Expression in plants and immunogenicity of plant virus-based experimental rabies vaccine; V Yusibov, DC Hooper, SV Spitsin, N Fleysh, RB Kean… - Vaccine, 2002 - Elsevier

Structure and organization of the hepatitis C virus genome isolated from human carriers; A Takamizawa, C Mori, I Fuke, S Manabe… - Journal of …, 1991 - Am Soc Microbiol

High-yield production of authentic human growth hormone using a plant virus-based expression system; M Gils, R Kandzia, S Marillonnet… - Plant biotechnology …, 2005 - Wiley Online Library

Self-assembly in vitro of purified CA-NC proteins from Rous sarcoma virus and human immunodeficiency virus type 1; S Campbell, VM Vogt - Journal of virology, 1995 - Am Soc Microbiol

Influence of three-dimensional structure on the immunogenicity of a peptide expressed on the surface of a plant virus; KM Taylor, T Lin, C Porta, AG Mosser… - Journal of Molecular …, 2000 - Wiley Online Library

Potent and specific inhibition of human immunodeficiency virus type 1 replication by RNA interference; GA Coburn, BR Cullen - Journal of Virology, 2002 - Am Soc Microbiol

Characterization of self-cleaving RNA sequences on the genome and antigenome of human hepatitis delta virus; MY Kuo, L Sharmeen, G Dinter-Gottlieb… - Journal of virology, 1988 - Am Soc Microbiol

Plant virus gene vectors for transient expression of foreign proteins in plants; HB Scholthof, KBG Scholthof… - Annual review of …, 1996 - annualreviews.org

Antigenomic RNA of human hepatitis delta virus can undergo self-cleavage; L Sharmeen, MY Kuo, G Dinter-Gottlieb… - Journal of virology, 1988 - Am Soc Microbiol

Mechanisms of plant virus evolution; MJ Roossinck - Annual review of phytopathology, 1997 - annualreviews.org

Variability and genetic structure of plant virus populations; F García-Arenal, A Fraile… - Annual review of …, 2001 - annualreviews.org

Production of antiviral and antitumor proteins MAP30 and GAP31 in cucurbits using the plant virus vector ZYMV-AGII; T Arazi, P Lee Huang, PL Huang, L Zhang… - Biochemical and …, 2002 - Elsevier

Identification of a novel GC-rich 113-nucleotide region to complete the circular, single-stranded DNA genome of TT virus, the first human circovirus; H Miyata, H Tsunoda, A Kazi, A Yamada… - Journal of …, 1999 - Am Soc Microbiol

RNA viral community in human feces: prevalence of plant pathogenic viruses; T Zhang, M Breitbart, WH Lee, JQ Run, CL Wei… - PLoS biology, 2005 - dx.plos.org

Evidence that a plant virus switched hosts to infect a vertebrate and then recombined with a vertebrate-infecting virus; MJ Gibbs, GF Weiller - … of the National Academy of Sciences, 1999 - National Acad Sciences

The 35S CaMV plant virus promoter is active in human enterocyte-like cells; MR Myhre, KA Fenton, J Eggert, KM Nielsen… - … Food Research and …, 2006 - Springer

Expression and assembly of a full-length monoclonal antibody in plants using a plant virus vector; T Verch, V Yusibov, H Koprowski - Journal of immunological methods, 1998 - Elsevier

Cowpea mosaic virus: from the presentation of antigenic peptides to the display of active biomaterials; A Chatterji, LL Burns, SS Taylor, GP Lomonossoff… - …, 2002 - content.karger.com

Chimeric plant virus particles administered nasally or orally induce systemic and mucosal immune responses in mice; FR Brennan, T Bellaby, SM Helliwell, TD Jones… - Journal of …, 1999 - Am Soc Microbiol

Triple gene block: modular design of a multifunctional machine for plant virus movement; SY Morozov, AG Solovyev - Journal of General Virology, 2003 - Soc General Microbiol

Intranasal immunization with a plant virus expressing a peptide from HIV-1 gp41 stimulates better mucosal and systemic HIV-1-specific IgA and IgG than oral …; Z Durrani, TL McInerney, L McLain, T Jones… - Journal of …, 1998 - Elsevier

Antitumor activity of DNA vaccines based on the human papillomavirus-16 E7 protein

genetically fused to a plant virus coat protei; Massa, P Simeone, A Muller… - Human gene …, 2008 - online.liebertpub.com

Pokeweed antiviral protein isoforms PAP-I, PAP-II, and PAP-III depurinate RNA of human immunodeficiency virus (HIV)-1; F Rajamohan, TK Venkatachalam, JD Irvin… - … and biophysical research …, 1999 - Elsevier

Pseudomonas aeruginosa outer-membrane protein F epitopes are highly immunogenic in mice when expressed on a plant virus; FR Brennan, TD Jones, LB Gilleland, T Bellaby… - …, 1999 - Soc General Microbiol

Plant–derived vaccine protects target animals against a viral diseas; Dalsgaard, Å Uttenthal, TD Jones, F Xu… - Nature …, 1997 - nature.com

Human respiratory syncytial virus vaccine antigen produced in plants; H BELANGER, N FLEYSH, S COX, G BARTMAN… - The FASEB Journal, 2000 - FASEB

Human influenza virus NS1 protein enhances viral pathogenicity and acts as an RNA silencing suppressor in plants; MO Delgadillo, P Sáenz, B Salvador… - Journal of general …, 2004 - Soc General Microbiol

Self-ligating RNA sequences on the antigenome of human hepatitis delta virus; L Sharmeen, MY Kuo, J Taylor - Journal of virology, 1989 - Am Soc Microbiol

A model for analysing plant-virus transmission characteristics and epidemic development; MJ Jeger, F Van Den Bosch, LV Madden, J Holt - Mathematical Medicine and …, 1998 - IMA

Analysis of the ability of five adjuvants to enhance immune responses to a chimeric plant virus displaying an HIV-1 peptide; TL McInerney, FR Brennan, TD Jones, NJ Dimmock - Vaccine, 1999 - Elsevier

Immunogenic and Antigenic dominance of a nonneutralizing epitope over a highly conserved neutralizing epitope in the gp41 envelope glycoprotein of human immunodeficiency virus type 1: its …; SM Cleveland, E Buratti, TD Jones, P North, F Baralle… - Virology, 2000 - Elsevier

The complete sequence of a human astrovirus; MM Willcocks, TD Brown, CR Madeley… - The Journal of general …, 1994 - ukpmc.ac.uk

The development of cowpea mosaic virus as a potential source of novel vaccines; C Porta, VE Spall, T Lin, JE Johnson… - …, 1996 - content.karger.com

A chimaeric plant virus vaccine protects mice against a bacterial infection; FR Brennan, LB Gilleland, J Staczek… - …, 1999 - Soc General Microbiol

Antigens produced in plants by infection with chimeric plant viruses immunize against rabies virus and HIV-1; V Yusibov, A Modelska, K Steplewski… - Proceedings of the …, 1997 - National Acad Sciences

Yeast as a model host to explore plant virus-host interactions; PD Nagy - Annu. Rev. Phytopathol., 2008 - annualreviews.org

Induction of HSP70 and polyubiquitin expression associated with plant virus

replication; MA Aranda, M Escaler, D Wang… - Proceedings of the …, 1996 - National Acad Sciences

Hepatitis E virus (HEV): molecular cloning and sequencing of the full-length viral genome; AW Tam, MM Smith, ME Guerra, CC Huang… - Virology, 1991 - Elsevier

HSP70 homolog functions in cell-to-cell movement of a plant virus; VV Peremyslov, Y Hagiwara… - Proceedings of the …, 1999 - National Acad Sciences

Inactivated recombinant plant virus protects dogs from a lethal challenge with canine parvovirus; JPM Langeveld, FR Brennan… - Vaccine, 2001 - Elsevier

TTV, a new human virus with single stranded circular DNA genome; S Hino - Reviews in medical virology, 2002 - Wiley Online Library

Sindbis virus proteins nsP1 and nsP2 contain homology to nonstructural proteins from several RNA plant viruses; P Ahlquist, EG Strauss, CM Rice, JH Strauss… - Journal of …, 1985 - Am Soc Microbiol

Virus taxonomy 1996—a bulletin from the Xth International Congress of Virology in Jerusalem; CR Pringle - Archives of virology, 1996 - Springer

Isolation of infective tomato bushy stunt virus after passage through the human alimentary tract; JA Tomlinson, E Faithfull, TH Flewett, G Beards - 1982 - nature.com

Novel plant virus-based vaccine induces protective cytotoxic T-lymphocyte-mediated antiviral immunity through dendritic cell maturation; P Lacasse, J Denis, R Lapointe, D Leclerc… - Journal of …, 2008 - Am Soc Microbiol

Annex 2

Google search hits for "pepino mosaic virus", first page

Scholarly articles for pepino mosaic virus
Pepino mosaic virus - Van der Vlugt - Cited by 6

Search Results

Pepino mosaic virus - European and Mediterranean Plant Protection ...
www.eppo.int/QUARANTINE/Alert_List/viruses/PEPMV0.htmWhy: Pepino mosaic virus (Potexvirus) came to our attention because it was recently found in Europe on glasshouse tomatoes, first in the Netherlands and UK, ...

Pepino Mosaic Virus on Greenhouse Tomatoes
www.agf.gov.bc.ca › Agriculture › Pest ManagementPepino Mosaic Virus - description, distribution, symptoms on tomatoes, how it spreads, management.

PRA Pepino mosaic virus - The Food and Environment Research ...
www.fera.defra.gov.uk/plants/plantHealth/pestsDiseases/.../pepino.pd...File Format: PDF/Adobe Acrobat - Quick View
8 Jun 2004 – Pepino mosaic virus (PepMV) was considered to have the potential to ... An updated PRA on Pepino mosaic virus was requested from the UK ...

Management of Pepino Mosaic Virus in Greenhouse Tomatoes
www.omafra.gov.on.ca/english/crops/facts/01-017.htmPepino mosaic virus (PepMV) was first found in Peru in 1974 on pepino (Solanum muricatum), an edible fruit known as pear melon. In 1999, the disease was ...

Pepino mosaic virus: an endemic pathogen of tomato crops
edepot.wur.nl/133145File Format: PDF/Adobe Acrobat - Quick View
19 Mar 2010 – Genetic characterization of Pepino mosaic virus isolates from Belgian ... Pepino mosaic virus: a successful pathogen that rapidly evolved from ...

ARS | Publication request: Pepino Mosaic Virus on Tomato Seed ...
www.ars.usda.gov/research/publications/publications.htm?seq_no...by K Ling - 2008 - Cited by 9 - Related articles
1 Dec 2008 – Title: Pepino Mosaic Virus on Tomato Seed: Virus Location and ... Interpretive Summary: Pepino mosaic virus (PepMV) is an emerging disease ...

Invasive Species Compendium | Pepino mosaic virus
www.cabi.org/isc/?compid=5&dsid=43661&loadmodule...31 Jul 2012 – Infection of tomato crops with PepMV does not necessarily result in significant economic damage since fruit symptoms may be absent. However ...

Pepino mosaic virus: a successful pathogen that rapidly evolved from
www.ncbi.nlm.nih.gov/pubmed/20447268by IM Hanssen - 2010 - Cited by 15 - Related articles
TAXONOMY: Pepino mosaic virus (PepMV) belongs to the Potexvirus genus of the Flexiviridae family. PHYSICAL PROPERTIES: PepMV virions are ...

Pepino mosaic virus
www.strateco.it/index.php?...13%3Apepino-mosai... - Translate this page
Fin dalla sua comparsa in Europa, alla fine degli anni '90, il Pepino mosaic virus (PepMV) è normato da decisioni comunitarie transitorie (2004/200/CE) ed ...

Pepino mosaic virus - Show DPV and Refs in Frame
www.dpvweb.net/dpv/showdpv.php?dpvno=411Pepino mosaic virus. R.A. Mumford Central Science Laboratory, Sand Hutton, York YO41 1LZ, UK. R.A.C. Jones Department of Agriculture, Baron-Hay Court, ...

Pepino mosaic virus: an endemic pathogen of tomato crops
edepot.wur.nl/133145File Format: PDF/Adobe Acrobat - Quick View 19 Mar 2010 – Genetic characterization of Pepino mosaic virus isolates from Belgian ... Pepino mosaic virus: a successful pathogen that rapidly evolved from ...