Netherlands Food and Consumer
Product Safety Authority
Ministry of Economic affairs

Pest Risk Analysis

EU internal movement of true potato seed (TPS) of registered TPS varieties: probability of association of regulated pests and analysis of risk reduction options

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Utrecht, the Netherlands

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Summary

Reason for performing the PRA; PRA area
Currently, seed potatoes are used as the main source of propagation material for potato production in the EU. However, several breeding companies are in the process of registering varieties propagated by botanical or true seeds (TPS varieties). Phytosanitary requirements are in place for EU internal movement of seed potatoes but there are currently no such requirements for TPS of official potato varieties. Therefore, the present PRA evaluated the probability of transmission of regulated pests via EU internal movement of TPS. The PRA identified and evaluated options to prevent such transmission. The PRA area was the EU.

Identification of regulated pests present in the EU
The study included those potato pests that are present in the EU and are regulated with regard to Council Directive 2000/29/EC including those organisms for which emergency measures apply or organisms for which recently a Pest Risk Assessment has been completed by EFSA[1] or EPPO[2] but which have not (yet) been included in the Annexes of Council Directive 2000/29/EC. Twenty pests were identified including three insect species (*Epitrix similaris*, *E. cucumeris* and *Leptinotarsa decemlineata*), three bacterial species (*Clavibacter michiganensis* ssp. *sepedonicus*, *Ralstonia solanacearum* and *Candidatus* Liberibacter solanacearum), one fungal species (*Synchytrium endobioticum*), five nematode species (*Globodera pallida*, *G. rostochiensis*, *Meloidogyne chitwoodi*, *M. fallax* and *Ditylenchus destructor*), seven pospiviroid species (*Chrysanthemum stunt viroid, Citrus exocortis viroid, Columnea latent viroid* (CLVd), *Potato spindle tuber viroid, Tomato apical stunt viroid, Tomato chlorotic dwarf viroid*) and one phytoplasma species (Potato stolbur phytoplasm). Although not or no longer known to be present in the EU, *Pepper chat fruit viroid* and *Tomato planta macho viroid* (including isolates of the former *Mexican papita viroid*) were included because they cause similar symptoms in potato as the other pospiviroids under experimental conditions and measures should preferably be effective against all possible pospiviroid species that may affect potato.

Probability of association with true potato seeds
It was assessed that the probability of seed transmission was very low or negligible for all pests identified except:

- *Clavibacter michiganensis* ssp. *sepedonicus* (Cms)
- *Ralstonia solanacearum* (Rs)
- *Potato spindle tuber viroid* (PSTVd)
- Pospiviroids other than PSTVd:
  Chrysanthemum stunt viroid (CSVd)
  Citrus exocortis viroid (CEVd)
  Columnea latent viroid (CLVd)
  Pepper chat fruit viroid (PCFVd)
  Tomato apical stunt viroid (TASVd)
  Tomato chlorotic dwarf viroid (TCDVd)
  Tomato planta macho viroid (TPMVd)

TPS may become infested[3] by Cms and Rs through contamination of the seed crop (mechanical transfer from contaminated tools, equipment, surface water etc.). As far as known, seed infection or transmission of the species via contaminated seeds has not been shown but neither can be excluded. For PSTVd, seed transmission has been shown.

[1] EFSA: European Food Safety Authority
[2] EPPO: European and Mediterranean Plant Protection Organisation
[3] For the purpose of this study "infest" can either mean "infect" or "contaminate"; i.e. an infested seed may be contaminated or infected with the pathogen.

The other eight pospiviroid species have many similarities with PSTVd and might also be transmitted via TPS. Natural infection of potato with these species has, however, not been recorded so far.

Synchytrium endobioticum is a soil-borne pathogen but spores can be spread by wind. Fruits might become contaminated by such spores (and subsequently seeds after extraction). Implementation of existing measures against this pathogen, which prohibit the cultivation of potato plants and only allow the cultivation of resistant cultivars in a safety zone around infested plots (Council Directive 69/464/EEC), is however considered sufficiently effective to reduce the risk of seed contamination to a negligible level.

Evaluation of risk reduction options including current phytosanitary requirements

Clavibacter michiganensis ssp. sepedonicus (Cms) and Ralstonia solanacearum (Rs)
Use of contaminated tools, equipment, surface water etc. is considered the most relevant pathway for infection of plants of *Solanum tuberosum* with Cms or Rs. Articles 18.1 and 18.5 of Annex IVAII of Council Directive 2000/29/EC include specific requirements to prevent infection of tubers of *Solanum tuberosum* with Cms and Rs but not for seeds. Article 18.3 includes specific requirements for TPS but not in relation to Cms or Rs. Article 24 requires a pest free production place for Cms for plants grown in the open air but TPS is expected to be produced under protected conditions. Council Directives 93/85/EEC and 98/57/EC include the requirements of an officially approved programme for the production of seed potatoes which has been found free of Cms and Rs but not in relation to the production of TPS of potato varieties. Therefore, options to reduce the probability of infection of a seed crop were evaluated.

PSTVd and other pospiviroids
The use of genetic material from gene banks or other sources is currently considered the highest risk for introduction of PSTVd in potato breeding programmes. The current requirements in Annex IVAII of Council Directive 2000/29/EC (article 18.3) target this pathway and also applies to TPS. However, PSTVd and six other pospiviroids are known to be present in various plant species in the EU and a seed crop may become contaminated by mechanical transmission. Although the probability of such an event is considered low, it is assessed to be higher for a crop grown under protected conditions (seed crop) than for a crop grown under field conditions (seed potatoes) because of the higher ambient temperature. Therefore, options to reduce the probability of infection of a seed crop were evaluated.

Risk reduction options
The following options are considered to reduce the risk of infestation with Cms, Rs and pospiviroids to a very low level:

Production of TPS in a
- Pest free area
  or
- Pest free production site.

TPS is produced under protected conditions which makes it easier to prevent contamination of the production site by taking hygiene measures than under field conditions. It is assessed that by implementation of strict hygiene measures the probability of crop infection with each of these pests will be very low and lower than for seed potatoes even when current phytosanitary regulations for potato tubers are taken into account.

In addition, the breeding stock of TPS varieties could be tested for presence of pospiviroids. Though such a requirement would be stricter than the current requirements for the production of seed potatoes this would provide additional assurance of freedom of viroids that could be transmitted in true seed.

Suggestion to amend Annex IVAII of Council Directive 2000/29/EC

Suggestion for inclusion of one new article (article 18.1b) in Annex IV part II of Council Directive 2000/29/EC for TPS similar to that of the current article 18.1 for seed potatoes and small amendments of the current articles 18.2 and 18.3:

Plant products and other objects	**Special requirements**
18.1b True seeds of *Solanum tuberosum* L.	Official statement that the Union provisions to combat *Synchytrium endobioticum* (Schilbersky) Percival have been complied with; and (a) either the true seeds originate in areas where *Clavibacter michiganensis* ssp. sepedonicus (Spieckermann and Kotthoff) Davis et al. *Ralstonia solanacearum* (Smith) Yabuuchi *et al., Potato spindle tuber viroid*[1] [the other relevant pospiviroid species[2] could be added if they will be listed in Annex IAII or IIAII of 2000/29/EC] are not known to occur; or (b) true seeds have been produced at a production site, where appropriate measures have been taken to prevent infestation with *Clavibacter michiganensis* ssp. sepedonicus (Spieckermann and Kotthoff) Davis et al. *Ralstonia solanacearum* (Smith) Yabuuchi *et al., Potato spindle tuber viroid*[1] [the other relevant pospiviroid species[2] could be added if they will be listed in Annex IAII or IIAII of 2000/29/EC] and no symptoms of disease caused by those harmful organisms have been observed on the plants at the site of production since the beginning of the last cycle of vegetation.

[1] *Potato spindle tuber viroid* is currently listed in Annex IAI but is known to be present in the EU and should be moved to either Annex IAII (regulated for all plants and products) or IIAII (regulated on certain plants and plant products).

[2] *Chrysanthemum stunt viroid, Citrus exocortis viroid, Columnea latent viroid, Tomato apical stunt viroid* and *Tomato chlorotic dwarf viroid.* (The pospiviroid species *Pepper chat fruit viroid* and *Tomato planta macho viroid* are not or no longer known to be present in the EU).

Amendment of the current articles 18. 2 and 18.3 (amendments in bold):

Plant products and other objects	**Special requirements**
18.2 Tubers **and true seeds** of *Solanum tuberosum* L.. intended for planting, other than tubers **or seeds** of those varieties officially accepted in one or more Member States pursuant to Council Directive 70/457/EEC of 29 September 1970 on the common catalogue of varieties of agricultural plant species (1)	*No changes*
18.3 Plants of stolon or tuber-forming species of *Solanum* L., or their hybrids, intended for planting, other than those tubers of *Solanum tuberosum* L. specified in Annex IV(A)(II)(18.1), **(18.1b)**, or (18.2), and other than culture maintenance material being stored in gene banks or genetic stock collections	*No changes*

(1) OJ L 225, 12.10.1970, p. 1. Directive as last amended by Directive 98/96/EC (OJ L 25, 1.2.1999, p. 27).

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1. Introduction

The objective of the pest risk analysis (PRA) was to evaluate the probability of transmission of EU-regulated pests though botanical or true potato seeds (TPS) and to evaluate options to reduce this risk of transmission. The PRA is intended to consider the need of phytosanitary requirements for the production and movement of TPS varieties within the EU and hence is limited to those regulated pests that are present in the EU.

Currently, seed potatoes[4] are used as the main source of propagation material for commercial cultivation of potatoes in the EU. However, several breeding companies in the Netherlands are in the process of registering varieties propagated by true seeds (TPS varieties). TPS can be used for commercial production and trade of potato propagation material as an alternative to seed potatoes. Selection processes and trials are currently ongoing to develop varieties which have suitable marketable traits. It is expected that by the beginning of 2016 this will lead to the first registered potato variety which can be traded as TPS in Europe. Phytosanitary requirements are in place for EU internal movement of seed potatoes but there are currently no such requirements for internal movement of TPS. Therefore, this document evaluates the probability of transmission of regulated pests via TPS within the EU and identified and evaluated options to prevent such transmission. More specifically, the objectives of the present PRA were:

(i) to assess the probability of association with TPS of pests that are present in the EU and that are currently regulated as part of Council Directive 2000/29/EC[5] as amended (including those for which emergency measures apply with regard to article 16.3 of the Directive) or which have recently been evaluated by EFSA or EPPO,

(ii) to evaluate the efficacy of the current requirements in Council Directive 2000/29/EC or emergency measures to reduce the risk of these pests in association with true potato seeds,

(iii) to identify and evaluate additional risk reduction options for those pests of which the risk associated with true potato seeds is considered insufficiently reduced by current regulations.

The PRA does not include an inventory of all possible pests that may be associated with true potato seeds and neither assesses the potential impact of the pests identified. It assesses the probability of association with TPS of those pests that have been qualified as a quarantine pest for potato with regard to Council Directive 2000/29/EC and are present in the EU and potato pests which are not (yet) regulated but have recently evaluated by EFSA or EPPO. Not included are for example non-regulated viruses occurring in the EU, which have been found to be transmitted via TPS, like *Alfalfa mosaic virus* (Valkonen, 1992), *Cherry leaf roll virus* (Crosslin et al, 2010 and *Tomato black ring virus* (or *Beet ringspot virus*, Jeffries, 1998), or might become transmitted via seeds as a result of mechanical transfer from infested[6] seed coats, like potexviruses and tobamoviruses.

In the next paragraphs some global background information on potato breeding for TPS varieties is provided, an overview of the current EU regulation concerning potato and a detailed outline of the content of the present document.

[4] Definition of seed potatoes (FAO, 2010): "tubers (including minitubers) and potato micropropagative material of cultivated tuber-forming *Solanum* spp. for planting".

[5] Council Directive 2000/29/EC of 8 May 2000 on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the community

[6] For the purpose of this study "infest" can either mean "infect" or "contaminate"; i.e. an infested seed may be contaminated or infected with the pathogen.

Potato breeding for true potato seed varieties

In the EU, potato varieties are propagated vegetatively through seed potatoes. Propagation of these potato varieties by true seed is impossible, due to heterozygosity of the varieties. Most of these varieties can produce seed but the seedlings would not be true to the variety. Instead, they would segregate into a wide array of different genotypes.

Over recent decades some organisations/companies have developed TPS varieties, mainly for developing countries. They have done this by breeding potato genotypes that are highly homozygous and produce fairly uniform progeny. TPS offers the advantage of better storability and cheaper transportation when compared to seed potatoes. In markets where these advantages are more important than strict uniformity of the crop, TPS varieties have gained acceptance. In India for example, an estimated 40% of potato crops is grown from TPS. In Europe however, the lack of strict uniformity of the current TPS varieties has prevented wide market acceptance. It has also prevented official European registration of these TPS varieties because varieties must be uniform for 100% to be accepted for registration. At present there is no registered trade of TPS in the EU.

Recently, breeding companies in the Netherlands have developed TPS varieties that are sufficiently genetically uniform and stable and can be propagated and traded as TPS varieties (see also Cioloca et al., 2013). One of these companies has selected tetraploid potato material over several generations which now have a high level of homozygosity and can serve as parents to produce potato hybrid varieties. The company has already started an application for EU registration of the first hybrid varieties which may be completed in 2016.

Breeding in polyploid species, such as potato (*Solanum tuberosum*), is a relatively slow process compared to diploid species. As a consequence, breeding new varieties of potato requires up to eight or twelve years (Tiemens-Hulscher et al., 2013). Still, market demands for disease resistance, flavour, size and other qualities are diverse and continuously changing (Tiemens-Hulscher et al., 2013). This has triggered the development of more rapid potato breeding systems. A Dutch company has developed such a new approach, using a non-GM (non-Genetically Modified) technology to breed fully self compatible diploid potato breeding lines. This company has first crossed a gene for self-compatibility from a wild *Solanum* species into potato. It has then combined this new trait with selection against recessive deleterious alleles which are highly frequent in potato. By doing this, the company has produced vigorous and self fertile homozygous diploid parents. These pure lines can be maintained by selfing and can be crossed to produce F1 hybrid TPS varieties. The F1 hybrid varieties will be very uniform, as are those produced for example for tomato and aubergine. The technology allows breeders to make use of the advantages of breeding at the diploid level such as more rapid introgression of new traits, in particular polygenic traits (Tiemens-Hulscher et al., 2013). Diploid potatoes are normally less productive than tetraploids (Tiemens-Hulscher et al., 2013), but if necessary the F1 hybrid varieties can be turned into tetraploids.

TPS is currently not produced on a large scale in the EU because no commercial TPS-varieties are registered at the moment. The production conditions under which TPS may be produced for commercial varieties will probably resemble the production system for tomato seeds which takes place under protected conditions. The parent plants for TPS production will be propagated by tissue culture when the parental genotypes have been selected by traditional breeding techniques. When pure inbred lines are used following the new technique described above parent plants will be raised from true seeds. A potato mother plant produces on average 3000-4000 true seeds and TPS can be stored for at least 5 years.

TPS may either directly be used as propagation material for the production of ware or processing potatoes or first be used for the production of seed potatoes. The latter because potato plants grown directly from true seed are smaller and produce lower yields than tuber-grown plants (Renia, 1997; Cioloca et al., 2012)

Current EU requirements for plants for planting of *Solanum tuberosum* (potato)

Council directive 2002/56/EC "on the marketing of seed potatoes" provides general requirements for the marketing of seed potatoes but does not regulate the internal movement of TPS varieties. Annexes I and II of Council Directive 2000/29/EC list several pests that are relevant for potato and these are either regulated for all plants and products (Annex I) or for certain plants and products (Annex II). Import of all plants for planting of stolon- or tuber forming species of *Solanum* L. or their hybrids into the EU (including true potato seeds) is prohibited with the exception of potato tubers from Switzerland (Annex III, articles 10-11). Requirements for internal movement of plants for planting of *Solanum tuberosum* are listed in Annex IVAII (articles 18.1 – 18.6). From these articles, only article 18.3 is relevant for the commercial production of TPS. Articles 18.1, 18.2 and 18.5 only concern "Tubers of *Solanum tuberosum* L.". Article 18.1 provides phytosanitary safeguards for the production of seed potatoes of varieties officially accepted in one or more Member States while article 18.2 concerns tubers of advanced potato selections. Article 18.4 concerns plants for planting that are stored in gene banks or genetic stock collections and hence not the commercial propagation of TPS varieties. Article 18.5 applies to tubers not intended for planting. In article 18.6, including specific requirements in relation to Potato stolbur mycoplasm, seeds are exempted.

Article 18.3 includes specific requirements for quarantine conditions and testing of specific plants for planting including TPS (but not for tubers of *Solanum tuberosum* L.). In the case of TPS the plants must be tested for PSTVd and a number of viruses, including viruses that are not known to be present in the EU. Although not indicated in the text, these requirements must probably be seen within the context of related requirements specified in items 18.4 (genetic stock collections) and 18.2 (advanced selections of tubers of *Solanum tuberosum*). Thus, article 18.3 is considered to regulate that TPS which is retrieved from gene banks should be tested for several pathogens before use in breeding programmes ("plants shall have been held under quarantine conditions … found free of any harmful organism in quarantine testing"). This is different from a TPS variety, which is the end-product of a multi-annual breeding programme within the EU of which the original breeding input material has already been tested for listed organisms according to requirements specified in articles 18.2 – 18.4.

In conclusion, no specific EU phytosanitary requirements are in place for TPS varieties. Any such requirements should at least provide similar phytosanitary safeguards for EU regulated harmful organisms for potato as specified for seed potatoes in annex IVAII, article 18.1.

In addition to the more general requirements for potato plants in Directives 2002/56/EC and 2000/29/EC, there are four Directives and one Commission Decision for specific potato pests:

- Council Directive 69/464/EEC on the control of *Synchytrium endobioticum*, the causal agent of potato wart disease,
- Council Directive 93/85/EEC on the control of *Clavibacter michiganensis* ssp. *sepedonicus*, the causal agent for potato ring rot,
- Council Directive 98/57/EC on the control of *Ralstonia solanace*arum, the causal agent of brown rot,
- Council Directive 2007/33/EC on the control of potato cyst nematodes.

- Commission Implementing Decision 2012/270/EC (as amended by 2014/679/EC) as regards emergency measures to prevent the introduction and the spread within the Union of *Epitrix cucumeris* (Harris), *Epitrix similaris* (Gentner), *Epitrix subcrinita* (Lec.) and *Epitrix tuberis* (Gentner)

These Directives and Commission Decision that include specific requirements for potato plants in relation to these pests will be discussed in the present document where relevant.

Outline of the document:

This PRA is specifically targeting the phytosanitary risk and possible management options to allow the movement of TPS of official varieties within the EU. The PRA, therefore, focuses on those harmful organisms which are present in the EU and specifically regulated for potato as part of Council Directive 2000/29/EC, as amended. In detail, the current document includes:

- The identification of relevant pests (Chapter 2)
- For each pest identified: assessment of the probability of transmission through TPS and how TPS may become infested (Chapter 3).
- For pests that may be transmitted through TPS:
  - Evaluation of current regulations to prevent infestation of TPS (Chapter 4)
  - Identification and evaluation of options to reduce the probability of seed infestation (Chapter 4)
- Suggestion for amendments of Annex IV part II in relation to the production of TPS (Chapter 5)

To avoid duplication of work, the present PRA refers to recent PRAs, risk assessments or pest categorisations made by EPPO (European and Mediterranean Plant Protection Organisation) and EFSA (European Food Safety Authority) for the organisms identified without always referring to the original research papers cited by EPPO or EFSA.

In this PRA, the probability of transmission through TPS of pests that were considered relevant were compared with the probability of transmission of the same pests via seed potatoes (Chapters 3 and 4). This is different from regular PRAs in which the rating levels for the likelihood or probability of an event (in this case the introduction of a regulated pest into the potato production chain) in a PRA is usually indicated by one absolute but qualitative variable (very low, low, medium etc.). These variables are often not clearly defined which makes it difficult to compare rating levels between risk assessments.

As already indicated above, seed potatoes may first need to be produced from TPS for use as propagation material in the production of table and processing potatoes. Risks from tubers produced from TPS for seed potato sale are not considered in the PRA because these would be required to comply with existing regulations for seed potato production.

2. Identification of pests

The Pest Risk Analysis was limited to potato pests that are present in the EU and:

- are listed in Annex I or II of Council directive 2000/29/EC, or
- for which emergency measures apply with regard to article 16.3 of the directive, or
- have been evaluated by EFSA or EPPO since 2010 and have not (yet) been included in the Annexes.

A total of 22 organisms were selected (Table 2.1). The probability that each of these pests is associated with TPS is discussed in Chapter 3.

Table 2.1. Identification of potato pests present in the EU and (expected to be) included in phytosanitary regulations in the EU

Organism	**EU categorisation or organism evaluated by EFSA or EPPO**
INSECTS & MITES	
Epitrix similaris (Gentner)	Decision 2012/270/EC
Epitrix cucumeris (Harris)	Decision 2012/270/EC
Leptinotarsa decemlineata Say	IB
BACTERIA	
Clavibacter michiganensis ssp. *sepedonicus* (Spieckermann et Kotthoff) Davis et al.	IAII
Ralstonia solanacearum (Smith) Yabuuchi et al.	IAII
Candidatus Liberibacter solanacearum	PRA prepared by EPPO (2012)
(PSEUDO)FUNGI	
Synchytrium endobioticum (Schilbersky) Percival	IAII
NEMATODES	
Globodera pallida (Stone) Behrens	IAII
Globodera rostochiensis (Wollenweber) Behrens	IAII
Meloidogyne chitwoodi Golden et al. (all populations)	IAII
Meloidogyne fallax Karssen	IAII
Ditylenchus destructor Thorne	IIAII
VIRUSES AND VIROIDS	
Potato spindle tuber viroid (PSTVd)	IAI
Pospiviroids other than PSTVd: *Chrysanthemum stunt viroid* (CSVd) *Citrus exocortis viroid* (CEVd) *Columnea latent viroid* (CLVd) *Pepper chat fruit viroid* (PCFVd)* *Tomato apical stunt viroid* (TASVd) *Tomato chlorotic dwarf viroid* (TCDVd) *Tomato planta macho viroid* (TPMVd)* *Although not or no longer known to be present in the EU, PCFVd and TPMVd (including isolates of the former species *Mexican papita viroid*; ICTV Virus Taxonomy, 2014; Verhoeven et al., 2011) were selected. These pospiviroid species causes similar symptoms in potato as the other pospiviroids under experimental conditions and measures should preferably be effective against all possible pospiviroid species that may affect potato.	PSTVd: IAI CSVd: IIAII, regulated for plants of *Dendranthema* (DC.) Des Moul., intended for planting, other than seeds. Other pospiviroids: not specifically mentioned in Annex I or II. Potato viruses and virus-like organisms are mentioned as a group in Annex IAI but natural infections of potato with pospiviroids other than PSTVd are not known to date. All species identified by EFSA-PLHP (2011) able to infect species within the Solanaceae and which may be relevant for potato.
PHYTOPLASMS	
Potato stolbur phytoplasm	IIAII

3. Probability of pest association with true potato seeds

3.1 Insects and mites

Epitrix similaris and *E. cucumeris*

Distribution within the EU. Epitrix similaris is present in (parts of) Portugal and Spain. *E. cucumeris* has only been reported from Portugal (EPPO, 2014). Since May 2012, EU emergency measures have been in place for *E. cucumeris*, *E. similaris*, *E. subcrinita* and *E. tuberis* (European Commission, 2014).

Probability of association with TPS. Eggs, larvae and pupae of *E. similaris* and *E. cucumeris* can be found in soil or subterranean plant parts. Adults (the beetles) are known to feed on the leaves of *Solanum tuberosum* (e.g. EPPO, 2005; Boavida et al., 2013). *E. cucumeris* has been found to feed on the skin of tomato fruit (http://www.forestryimages.org/browse/subimages.cfm?sub=7477, last access 9 February 2015) and both *Epitrix* species might also feed on the fruit of *S. tuberosum*. However, if they do, they will likely fly away when the fruits are harvested and otherwise will most likely be eliminated during the seed extraction process. The probability of association with TPS is assessed to be negligible for both species.

Leptinotarsa decemlineata

Distribution within the EU. Leptinotarsa decemlineata is present in most EU member states (EPPO, 2014).

Probability of association with TPS. Adults of *L. decemlineata* lay their eggs on the lower surface of leaves. Larvae feed on leaves, stems and petioles. The mature larvae pupate in the soil (Smith et al., 1992). If adults would be present on harvested fruits, they will most likely be eliminated during the seed extraction process. The probability of association with TPS is assessed to be negligible.

3.2 Bacteria

Clavibacter michiganensis ssp. *sepedonicus*

Distribution within the EU. *Clavibacter michiganensis* ssp. *sepedonicus* (Cms) has a restricted distribution in several EU member states (EPPO, 2014). In potatoes, Cms is known to cause a vascular disease referred to as "ring rot". EU legislation requires eradication of any finding of ring rot according to specific measures (European Commission 2006a).

Probability of association with TPS. The databases Agris, Agricola, CABabstracts and the internet (Google, first 10 hits) were searched for relevant papers using the search terms ((Corynebacterium and sepedonicus) or (Clavibacter and michiganensis and sepedonicus)) and (seed or seeds) and (potato or (solanum and tuberosum)) and transmission). No publications or reports were found about seed transmission of Cms. However, *C. michiganensis* ssp. *michiganensis* (Cmm), a pathogen of *Solanum lycopersicum* (tomato) is known to be seed transmitted. Like Cmm, Cms might be able to invade fruits and seeds of *Solanum tuberosum.* Cms was selected for further analysis

Pathways[7]:

1. Contamination. Plants on which seeds are produced may become infested by contamination, e.g. introduction of the pathogen by infested equipment. Contamination of containers, equipment and premises is also considered an important mean of contamination of a seed potato crop (EPPO/CABI, 1992a).

Pathways considered very unlikely:

- Parent plant. Plants might have become infected during the breeding process without being detected and eliminated. Seeds raised from such plants might be infected. This pathway seems very unlikely. In fact, during traditional breeding, seed infections might also occur but this has never been reported. This pathway seems very unlikely and is not further considered in the present PRA. Also note that article 18.3 (Annex IVAII of Council directive 2000/29/EC) requires quarantine testing of plant material for Cms but TPS is excluded from this requirement.
- Insects. There is experimental evidence that Cms could be transmitted by insects but this pathway seems irrelevant under practical conditions because spread in the field from plant to plant is very slow (EPPO/CABI, 1992).

<u>*Ralstonia solanacearum*</u>

Distribution within the EU. There is much variation within the bacterial species *Ralstonia solanacearum* (Rs), leading to categorisation of the species into subtaxa. There are several classification systems for these subpopulations. In relation to geographical/climatic categorisation usually the categorisation by Buddenhagen *et al*. (1962) is used. This categorisation is based on biological aspects and separates the species into three "races": race 1, 2 and 3. Rs race 3 has a restricted distribution in several EU member states. Races 1 and 2 of Rs are not present within the EU (EPPO, 2014). Therefore this study focuses on Rs race 3. Where in this paragraph Rs is mentioned, it concerns the Rs race 3 unless stated otherwise. In potatoes, Rs is known to cause a vascular disease referred to as "brown rot". EU legislation requires eradication of any finding of brown rot according to specific measures (European Commission, 2006b).

Probability of association with TPS. The databases Agris, Agricola, CABabstracts and the internet (Google, first 10 hits) were searched for relevant papers using the search terms (Pseudomonas and solanacearum) or (Ralstonia and solanacearum)) and (seed or seeds) and (potato or (solanum and tuberosum)) and transmission). No publications or reports were found about true seed transmission of *Ralstonia solanacearum* in potato. The main means by which Rs can be introduced into a potato crop are (i) the use of (latently) infected seed potatoes and (ii) contamination especially by irrigation with contaminated surface water (EPPO/CABI, 1992). In a broader search (excluding the search term potato) on seed transmission and Rs, evidence for seed infection and disease transmission was found for groundnut (*Arachis hypogaea* L.) (Machmud & Middleton, 1990; Zhang et al., 1993). Machmud & Middleton (1990) reported that bacterial colonies (Rs race not indicated) were isolated from different parts of infected groundnut seeds. Zhang *et al* (1993) reported isolation of Rs (race not indicated) from the embryo of groundnut seeds in one of 20 seed samples from naturally infected groundnut pods.

Infestation of the seed coat of tomato seeds was reported after artificial inoculation of tomato plants (Devi & Menon, 1980). After artificial inoculation, tomato plants developed disease symptoms and Rs was found in high levels on freshly extracted, non-sterilized seeds and in fruit pulp. On surface-sterilized and on dried seeds, the bacterium was found in a considerably lower numbers than on non-treated seeds and the bacterium was not found in the embryo of the seeds. These observations do not support Rs transmission via infected tomato seed but rather indicate contamination of the seed surface when

[7] Pathway in the context of this PRA means: any means that allows infestation of TPS.

seeds are harvested from fruits of highly disease plants. Although no data were found for potato, it is concluded that Rs may become associated with true potato seeds (by contamination of the seed surface) if the seeds are harvested from an infected plant.

Pathways:

1. Contamination. Plants may become infected by contamination, e.g. the use of contaminated equipment and especially by the use of contaminated surface water (EPPO/CABI, 1992). Seeds produced on infected plants may subsequently become infested (external contamination of the seed coat).

Candidatus Liberibacter solanacearum

Distribution within the EU. Candidatus Liberibacter solanacearum (CaLs) has been found in carrot plants (*Daucus carota* L.) and celery plants (*Apium graveolens* L.) in several EU member states (EPPO, 2012, 2015; Nelson et al., 2013). CaLs is not known to be present in potato in the EU except from a few infected potato plants found on or directly adjacent to infected carrot fields. In Finland, CaLs was found in volunteer potato plants in a carrot field heavily infested with CaLs and its vector *Trioza apicalis* and at the edge of a commercial potato field next to a carrot field (pers. comm. A. Nissinen, December 2013). Most likely, the bacterium had incidentally been transmitted from carrot to potato by psyllids that normally feed on carrot. CaLs was recently found in carrot and celery in Austria but was not detected in Solanaceae plants (species not indicated) present in adjacent fields (EPPO, 2015).

Probability of association with TPS. Recently, experimental data indicated seed transmission of CaLs via carrot seeds (Bertolini et al., 2014) but EPPO (2012) did not consider seeds of Solanaceae a pathway for CaLs: "*Ca.* L. solanacearum has not been shown to be transmitted by seed (unpublished work on tomato, pepper and tamarillo; Liefting, pers. comm., 2010-10)". Munyaneza (2012) stated "It appears that Lso [synonym for CaLs] is not transmitted through true seed from infected solanaceous plants (Munyaneza, unpublished data; Liefting, personal communication)". Also, CaLs is not present in potato in the EU except from a few infected potato plants found in the vicinity of infected carrot plants (see above). The probability that CaLs is associated with TPS is assessed to be very low and lower than the probability that seed potatoes would become infected. Seed potato has been considered a pathway for CaLs (EPPO, 2012). Incidental transmission of the pathogen from an infected carrot or celery crop to a seed potato crop seems more likely to occur than to a potato crop producing TPS because TPS is grown under protected conditions and a much larger acreage is needed to produce seed potatoes than to produce TPS. CaLs was not selected for further analysis. It is, however, recommended to conduct more research on CaLs in Europe to decrease the uncertainty about the risk of infected carrot and celery crops for potato in the EU.

3.3 Fungi

Synchytrium endobioticum

Distribution within the EU. Synchytrium endobioticum has a restricted distribution in several EU member states (EPPO, 2014).

Probability of association with TPS. *Synchytrium endobioticum* especially affects the potato tuber but may also attack the stem base and leaves of potato plants (EPPO/CABI, 1992c). Although infection of TPS seems very unlikely, fruits might become contaminated with wind-blown spores. *Synchytrium endobioticum* is listed in Annex IAII of Council directive 2000/29/EC and regulated for all plants and products. Therefore, potato crops (including crops grown for the production TPS) should be free of the pathogen. Council

directive 69/464/EEC on control of Potato Wart Disease includes the prohibition to grow potato plants on infested plots and the requirement that only resistant cultivars may be grown in a safety zone around infested plots. Also because of these requirements, the risk of contamination of TPS is considered negligible and lower than for the production of seed potatoes.

3.4 Nematodes

Ditylenchus destructor

Distribution within the EU. Ditylenchus destructor has a restricted distribution in several EU member states (EPPO, 2014).

Probability of association with TPS. Based on its biology, *D. destructor* is very unlikely to be associated with potato seeds. EPPO (2008): "The nematode attacks subterranean parts of plants (tubers, stolons, bulbs, rhizomes, roots), but may occasionally also invade above-ground parts, mainly the base of stem). *D. destructor* is unable to withstand excessive desiccation unlike *D. dipsaci*." Thus even if a seed lot would become contaminated the species may not survive during drying and storage of the seeds. In general, nematode species that attack potato are not known to be transmitted by TPS (Hooker, 1981). The species was not selected for further analysis.

Meloidogyne chitwoodi and *M. fallax*

Distribution within the EU. Meloidogyne chitwoodi and/or *M. fallax* are known to be present in Belgium, Germany, France, the Netherlands, Portugal and the United Kingdom (EPPO, 2014).

Probability of association with TPS. *Meloidogyne chitwoodi* and *M. fallax* are only known to colonize subterranean plant parts (EPPO/CABI, 1997; EPPO, 1999) and are very unlikely to be associated with seed. The species were not selected for further analysis.

Globodera pallida and *G. rostochiensis*

Distribution within the EU. Globodera pallida and *G. rostochiensis* are present in most EU member states (EPPO, 2014).

Probability of association with TPS. *Globodera pallida* and *G. rostochiensis* are only known to colonize subterranean plant parts (EPPO/CABI, 1992d) and are very unlikely to be associated with seed. The species were not selected for further analysis.

3.5 Viruses and viroids

Potato spindle tuber viroid (PSTVd)

Distribution within the EU. PSTVd is transient or has a restricted distribution in several EU member states. Most findings concern infected ornamental species that do not show symptoms (EPPO, 2014).

Probability of association with TPS. Transmission of PSTVd through TPS has been reported by Hunter et al (1969) and Fernow et al (1970). These studies showed that PSTVd could be detected in pollen, seeds and seedlings of infected plants of potato. In a

study of Singh (1970), infected true seeds were obtained from crossings of infected mother and healthy father as well as healthy mother and infected father plants in potato and tomato. In that study, indications were obtained that in tomato seeds PSTVd was located internally because treatments aimed to remove the viroid from the seed surface did not prevent transmission (no such studies were performed with TPS). It was also shown that immersion of healthy seeds in sap from infected plants, might lead to infection of seedlings. Thus, PSTVd might also be transmitted by contaminated seeds. Furthermore, it was shown that PSTVd could persist in collections of TPS for more than 20 years (Singh et al, 1991). The results of these studies demonstrate that PSTVd can be transmitted through true (potato) seeds. The underlying mechanisms of transmission and the influence of external factors are, however, still not clear (EFSA-PLHP, 2011). PSTVd was selected for further analysis.

Pathways:

1. Parent plants. If the breeding material is infected with PSTVd and this is not eliminated during the breeding process, parents may carry PSTVd and this could be transferred to the seeds produced on these plants. Currently, requirements are in place to test potato material from gene banks or genetic stock collections for PSTVd (art. 18.3 Annex IVAII Council Directive 2000/29/EC). This requirement should be sufficiently effective to reduce the risk of pathway 1 to a very low level but recent findings in a potato breeding programme in the Netherlands (NPPO-NL, 2014) indicate that infected material might still be present outside gene banks or genetic stock collections in the EU. This poses a risk both for traditional breeding and for breeding TPS-varieties. Therefore, the NPPO of the Netherlands recently implemented additional safeguards against possible introduction of PSTVd into commercial potato breeding programmes (EPPO, 2014). These additional safeguards include official testing of all individual breeding plants actively used for crossing. The plants should be tested each year, at each company. Other safeguards concern strict isolation of any new incoming breeding stock from outside the company or other locations, until testing or verification of testing records have been completed.
2. Contamination. Plants may become infected via mechanical transmission, e.g. by introduction of the pathogen through crop handling or contaminated equipment. PSTVd has been detected in various ornamental species which did not express any symptoms. The probability of transfer between ornamental species infected with PSTVd and potato has been rated as "very unlikely to unlikely" by EFSA-PLHP (2011). However, mechanical transmission of PSTVd with diluted plant sap from two infected ornamental species has been shown to be more efficient at 25 than at 15°C (Verhoeven et al., 2010). Therefore, the probability of transmission of PSTVd to a potato crop grown under protected conditions, although still considered low, is higher than under field conditions in areas with relatively cool growing seasons because of the higher ambient temperatures under protected conditions.

<u>Pospiviroids other than PSTVd able to infect (some) Solanaceous species</u>

- *Chrysanthemum stunt viroid* (CSVd)
- *Citrus exocortis viroid* (CEVd)
- *Columnea latent viroid* (CLVd)
- *Pepper chat fruit viroid* (PCFVd)
- *Tomato apical stunt viroid* (TASVd)
- *Tomato chlorotic dwarf viroid* (TCDVd)
- *Tomato planta macho viroid* (TPMVd)

Distribution within the EU. CSVd, CEVd, CLVd, PCFVd, TASVd, and TCDVd have been reported from one or several (depending on the species) EU member states (EFSA-PLHP, 2011). TPMVd has thus far not been reported from EU member states.

Probability of association with TPS. Although natural infections of potato have not been reported, the aforementioned pospiviroids were able to infect potato plants and induce similar symptoms as PSTVd after artificial inoculation (EFSA-PLHP, 2011). EFSA-PLHP (2011) assessed the potential impact of these pospiviroids for potato similar to that of PSTVd although with medium uncertainty. Like PSTVd, these pospiviroids might be seed-transmissible in potato and, therefore, were selected for further analysis.

Pathways:

1. Contamination. The pospiviroid species have been detected in various plant species of which several do not express any symptoms. The probability of transfer between these species and potato has been rated as "very unlikely to unlikely" by EFSA-PLHP (2011). Although still considered low, the probability will likely be higher under protected conditions because of the higher ambient temperatures as compared to field conditions (see above: PSTVd). Several of the pospiviroids have been found in symptomlessly infected ornamentals in the EU and may occur more widespread than currently known.
2. Parent plants. Natural infections of potato plants with the pospiviroids mentioned above (not PSTVd) have never been reported and the pathway "parent plants" is considered less likely for the seven pospiviroids listed above than for PSTVd. However, experimental evidence indicates that (most of the) pospivirioids behave similar to PSTVd in potato and, therefore, the pathway "parents" will be discussed in Chapter 4 (Risk reduction options). Note that at present no requirement is in place to test potato material from gene banks or other origins for pospiviroids other than PSTVd (art. 18.3 Annex IVAII Council Directive 2000/29/EC).

3.6 Phytoplasms

Potato stolbur phytoplasma (Stolbur phytoplasma or '*Candidatus* Phytoplasma solani')

Distribution within the EU. "*Candidatus* Phytoplasma solani" (CPs) is endemic in the Euro-Mediterranean area. It is present with restricted distribution in 10 of the 28 EU member states (EFSA-PLHP, 2014).

Probability of association with TPS. Phytoplasmas are cell wall-less, unculturable bacteria that reside almost exclusively in the phloem of diseased plants (Marcone, 2010). Phytoplasmas are considered neither to be transmitted mechanically, nor by seed (EFSA-PHLP, 2014b). This is based on the fact that there are no vascular contacts between the mother plant and embryo (Bos, 1999). According to the EPPO datasheet "Stolbur is not thought to be transmitted in the true seed of any of its hosts" (EPPO/CABI 1992e). However, recent papers on phytoplasmas report upon the detection of phytoplasma DNA by molecular tests in embryos, seeds and/or seedlings of infected hosts, a.o. lethal yellowing in embryo's of coconut palms (Cordova et al, 2003; Nipah et al, 2007) and different phytoplasmas in seeds and/or seedlings of winter oilseed rape, tomato and corn (Calari et al, 2011). In none of these cases, however, the presence of phytoplasma cells could be shown, nor the ability to induce symptoms in mature plants. Moreover, studies by Faghihi et al (2011) and Tan (2010) for Witches'-broom disease of lime and phyllody of sesame, respectively, did not provide any evidence for seed transmission of these phytoplasma diseases. Therefore, although association of phytoplasma DNA with seeds might be possible as demonstrated for plants other than potato, there is no evidence of transmission of the disease to mature plants. CPs was not selected for evaluation of risk reduction options.

3.7 Conclusions

The following pests were selected for identification and evaluation of risk reduction options:

- *Clavibacter michiganensis* ssp. *sepedonicus*
- *Ralstonia solanacearum*
- PSTVd
- Pospiviroids other than PSTVd (7 species: CSVd, CEVd, CLVd, PCFVd, TASVd, TCDVd, TPMVd)

4. Risk reduction options

4.1 Methods

The present PRA only concerns production and trade of TPS within the EU, i.e. not import of TPS from third countries. Therefore, risk reduction options were evaluated that can be implemented before the product is being traded and no options were considered that can be implemented after entry of consignments such as post-entry quarantine or import inspections. Post-entry measures are usually included when analysing the risk of commodities imported from outside the EU (questions 7.27-7.29 in the EPPO Decision-support scheme for quarantine pests PM 5/3(5))

For each pest selected in Chapter 3, the current phytosanitary requirements were evaluated for efficacy against infestation of TPS. If these requirements were not considered sufficiently effective risk reduction options were identified and evaluated for efficacy and feasibility (Table 4.1).

Table 4.1. Risk reduction options evaluated for selected pests

Risk reduction option[1]	**Corresponding questions in the EPPO-scheme**[2]
Existing phytosanitary measures to reduce the risk of spread within the EU	**7.10**
Options at the place of production	**7.13-7.21**
a. Detection of the pest at the place of production by inspection or testing	
b. Prevention of infestation of the commodity at the place of production: • use of resistant cultivars, • growing the crop in specified conditions (e.g. physical protection), • crop treatments, and/or • harvest at certain times of the year or growth stages	
c. Establishment and maintenance of a pest-free production site, pest- free production place or pest-free production area	
Options after harvest, at pre-clearance or during transport	**7.22-7.26**
a. Detection of the pest in consignments by inspection or testing	
b. Removal of the pest from the consignment by treatment or other phytosanitary procedures (remove certain parts of the plant or plant product, handling and packing methods)	

[1] Table adapted from the PRA-scheme of the NVWA, the Netherlands (e.g. Van der Gaag et al., 2013)
[2] EPPO Decision-support scheme for quarantine pests PM 5/3(5).

4.2 Results

4.2.1 Clavibacter michiganensis ssp. sepedonicus (Cms)

4.2.1.1 Regulatory status for plants and products within the Community

Council Directive 2000/29/EC
Clavibacter michiganensis ssp. *sepedonicus* is listed in Council Directive 2000/29/EC in the following sections:

- Annex I, Part A - Harmful organisms whose introduction into, and spread within, all Member States shall be banned
  - Section II – Harmful organisms known to occur in the community and relevant for the entire community
    (b) Bacteria
    1. *Clavibacter michiganensis* (Smith) Davis et al. ssp. *sepedonicus* (Spieckermann and Kotthoff) Davis et al.
- Annex IV, Part A, section II (IVAII) **–** Special requirements which must be laid down by all member states for the introduction and movement of plants, plant products and other objects into and within all member states - articles 18.1, 18.3, 18.5, 24 (Annex I of this document).

From the articles in Annex IVAII, only article 18.3 is relevant for the production of TPS because the other articles refer to potato tubers (articles 18.1, 18.5) or plants grown in the open air (article 24). Article 24 states for plants grown in the open air: "There shall be evidence that the place of production is known to be free from *Clavibacter michiganensis* ssp. *sepedonicus* (Spieckermann and Kotthoff) Davis et al..." Because TPS is produced under protected conditions (Chapter 1), article 24 does no apply to TPS production. Article 18.3 requires that plant material is subjected to quarantine testing for *C. michiganensis* ssp. *sepedonicus* (Cms) but TPS is excluded (similar requirements are formulated in Commission directive 2008/61/EC[8]).

Council Directive 93/85/EEC
"Council Directive 93/85/EEC has laid down detailed measures to be taken within the Member States against the organism in order to locate it and determine its distribution; prevent its occurrence and spread; and if found, to prevent its spread and to control it with the aim of eradication" (European Commission, 2006). The specified measures (surveys, reporting, measures in case of a finding) apply to both tubers and plants (including TPS). One article (article 8) refers to seed potatoes only. Article 8 requires that seed potatoes should be produced under an officially approved program which has been found free of Cms (Annex II of this document). The Council Directive does not include such a requirement for the production of TPS.

Conclusion.
Several of the requirements in Annex IV of Council Directive 2000/29/EC to control Cms apply to the production of seed potatoes and not to the production of TPS (articles 18.1, 18.2, 18.5). Council Directive 93/85/EEC includes the requirement of an officially approved programme for the production of seed potatoes which has been found free of Cms but such programme is not required for the production of true potato seeds. Thus,

[8] Commission Directive 2008/61/EC of 17 June 2008 establishing the conditions under which certain harmful organisms, plants, plant products and other objects listed in Annexes I to V to Council Directive 2000/29/EC may be introduced into or moved within the Community or certain protected zones thereof, for trial or scientific purposes and for work on varietal selections

no specific requirements are in place to prevent infestation of TPS comparable to those for seed potatoes as laid down in article 18.1 of Council Directive 2000/29/EC and risk reduction options are discussed below.

4.2.1.2 Risk reduction options

Pathways

1. Contamination during production of TPS (introduction of the pathogen by use of infested equipment, clothes etc.)

Detection by visual inspection

Cms can be latently present in potato plants and symptom development is affected by environmental conditions. Visual inspection alone is not considered sufficiently effective because of the possibility of latent infections. It should, however, be noted that visual inspections will probably be more effective in a crop on which TPS is produced than in a seed potato crop. Logsdon (1967) found an increase in symptom development with soil temperature, that ranged from 16 to 25°C and temperatures under protected conditions are probably higher than in the field in the seed potato growing areas in northern Europe. Therefore, symptoms of Cms may be more apparent in a TPS crop (crop on which true potato seeds are produced) than in a seed potato crop. Also, inspections in a TPS crop will be more feasible than in a seed potato crop. The acreage and number of plants is lower and it is easier to walk along plant rows.

Detection by testing

Tests are available for tubers and foliage of potato plants (EPPO, 1990; Commission directive 2006/56/EC[9]). Plants on which seeds are produced (the parental mother line) and (at a lower sampling intensity) also the plants on which pollen are produced (parental father line) could be sampled and tested for presence of Cms. The risk reduction level by testing will very much depend on the sampling intensity. A high risk reduction level may be obtained by sampling and testing each plant on which seed is produced although heterogeneous distribution of the pest in the plant may hamper detection. Generally, the probability of detection will decrease with infection level and especially at low infection levels the organism may remain undetected.

Prevention of infestation

Cms may be introduced at a production site of TPS by use of contaminated equipment, clothes etc. Seeds are produced under protected conditions and the risk of Cms can be reduced significantly by implementation of strict hygiene measures at the production place targeting all possible pathways of contamination (Table 4.2; GSPP, 2015):

- Plants
  - any plant not orginating from a pest free production site or area should first be tested for presence of the organism (kept under quarantine conditions) before it enters the production site,
- People
  - Washing of hands before entering the production site
  - Use of protective clothes or change clothes before entering the production site
- Materials
  - Any new tool or equipment brought from outside the production place or site which may carry the pathogen should be cleansed and disinfected (including crates, packaging material etc)
  - Equipment and tools should stay inside the production place or site,

[9] Commission directive 2006/56/EC of 12 June 2006 amending the Annexes to Council Directive 93/85/EEC on the control of potato ring rot

- Water
    - Use of pathogen free water, if needed the water should be disinfected

This option, strict hygiene measures to prevent infestation of the production site, is considered highly effective to prevent infestation of the crop on which seeds are produced.

Establishment of a pest free production site or place
A pest free production site or place could be maintained by strict hygiene measures (see above: prevention of infestation).

Establishment of a pest free area
Highly effective. Surveys as required by Council Directive 93/85/EEC will be needed to confirm absence of the organism.

Options after harvest, at pre-clearance or during transport
Although no data are available for Cms in relation to seed treatments, extraction of seed from fruit debris using fermentation and acid treatments will likely reduce bacterial populations, but internal infections cannot be eliminated by seed treatments (EFSA-PLHP, 2014a). No reports are known on *C. michiganensis* ssp. *sepedonicus* in relation to seed transmission and currently, no testing methods are available for seeds.

Conclusion
The following options are considered to reduce the risk to a very low level:

- Pest free area
- Pest free production site: protected cultivation in combination with strict hygiene measures including testing of plants not originating from a pest free production site or area before entering the production site and verification by visual inspections.

Each of these options is assessed to reduce the probability of contamination of TPS with Cms to a lower level than the current probability of contamination of seed potatoes despite the current existing official programmes in place for tuber production. The reasons for this are: (i) low levels of infestation will be more difficult to detect in a seed potato crop (much larger fields) than in the production of TPS, (ii) seed potatoes are grown in the open where it is more difficult to clean and disinfect machinery, equipment, crates etc than under protected conditions. Also note that current requirements for import of TPS in Canada (besides a permit to import) are country freedom or testing of parent material for seven viruses and viroids and isolation to prevent infection with seed-transmitted viruses and viroids; no requirements are in place for bacterial diseases (CFIA, 2013).

Table 4.2. Summary of possible risk reduction options for *Clavibacter michiganensis* ssp. *sepedonicus* in relation to true potato seed.

Risk reduction options	
Options at the place of production	**Risk reduction and justification**
a. Detection of the pest at the place of production by inspection or testing	Cms may be latently present. Testing methods are available for tubers and foliage but not for seeds. Plants on which seeds are produced can be tested.
b. Prevention of infestation of the commodity at the place of production: • use of resistant cultivars, • growing the crop in specified conditions (e.g. physical protection), • crop treatments, and/or • harvest at certain times of the year or growth stages	Seeds are already produced under protected conditions. Parents and hybrid seeds are maintained and produced under protected conditions. Strict hygiene measures can significantly reduce the risk. (use of resistant cultivars, crop treatments and harvesting time are not applicable)
c. Establishment and maintenance of a pest-free production site, pest- free production place or pest-free production area	See above: protected conditions and strict hygiene measures
Options after harvest, at pre-clearance or during transport	
a. Detection of the pest in consignments by inspection or testing	No seed-testing method yet available
b. Removal of the pest from the consignment by treatment or other phytosanitary procedures (remove certain parts of the plant or plant product, handling and packing methods)	Seed extraction method or seed treatment will reduce surface contamination on the seed but cannot remove (internal) seed infections.

4.2.2 Ralstonia solanacearum (Rs)

4.2.2.1 Regulatory status for plants and products within the Community

Council Directive 2000/29/EC

Ralstonia solanacearum (Rs) is listed in Council Directive 2000/29/EC in the following sections:

- Annex I, Part A - Harmful organisms whose introduction into, and spread within, all Member States shall be banned
  - Section II – Harmful or organisms known to occur in the community and relevant for the entire community
    (b) Bacteria
    2. *Ralstonia solanacearum* (Smith) Yabuuchi et al.
- Annex IV, Part A, section II (IVAII) **–** Special requirements which must be laid down by all member states for the introduction and movement of plants, plant products and other objects into and within all member states - articles 18.1, 18.3, 18.5, 18.7 (Annex I of this document).

Like for Cms*,* no specific requirements are in place for the production of TPS in relation to Rs.

Council Directive 98/57/EC

Council Directive 98/57/EC on the control of *Ralstonia solanace*arum (Smith) Yabuuchi et al. amended by Commission Directive 2006/63/CE includes detailed requirements to determine the distribution of the organism and to prevent its occurrence and spread with the aim of eradication. The specified measures (surveys, reporting, measures in case of a finding) apply to both tubers and plants (including TPS). One article (article 7) only refers

to seed potatoes; it requires that seed potatoes should be produced under an officially approved program which has been found free of the organism (Annex III of this document). The Council Directive does not include such a requirement for the production of true potato seeds.

Conclusion.
Several of the requirements in Annex IV of Council Directive 2000/29/EC to control Rs apply to the production of seed potatoes and not to the production of TPS (articles 18.1, 18.5, 18.7). Directive 93/85/EEC includes the requirement of an officially approved programme for the production of seed potatoes which has been found free of Cms but such programme is not required for the production of true potato seeds. Thus, no specific requirements are in place to prevent infestation of TPS and risk reduction options are discussed below.

4.2.2.2 Risk reduction options

Pathways
1. Contamination during production of TPS (by use of contaminated surface water, tools etc.).

Detection by visual inspection
Rs infection can be symptomless especially at relatively low temperatures. Swanson *et al*. (2005) observed a relation between temperature increase and symptom appearance in Geranium (Pelargonium hortorum). Increase in ambient temperature of latently infected geranium plants from 24°C /19°C (day/night) to 28°C continuously for fourteen days, was followed by wilting of many previously asymptomatic plants. However, after the temperature increase period there were still asymptomatic plants that harboured large numbers of bacteria in their crown tissue, leading to the authors' remark that temperature increase does not reliably convert latent infections of geranium into active symptomatic disease. Also for potato plants a correlation between temperature and symptom appearance could be expected. Hence, visual inspection of the crop alone is not sufficiently effective to detect infections. It should, however, be noted that visual inspections will probably be more effective in a seed crop than in a seed potato crop for the same reasons as stated for Cms (see 5.2.1.2).

Detection by testing
Test are available for tubers and foliage of potato plants (EPPO, 2004a; Commisson Directive 2006/63/CE[10]). Plants on which seeds are produced (the parental mother line) and (at a lower sampling intensity) also the plants on which pollen are produced (parental father line) could be sampled and tested for presence of Rs. The testing scheme prescribed for the detection and identification of Rs in samples of asymptomatic, potato, tomato or other host plants could be followed (Commisson Directive 2006/63/CE). The risk reduction level by testing will very much depend on the sampling intensity. A high risk reduction level may be obtained by sampling and testing each plant on which seed is produced although heterogeneous distribution of the pest in the plant may hamper detection. Generally, the probability of detection will decrease with infection level and especially at low infection levels the organism may remain undetected.

Prevention of infestation
See Cms (5.2.1.2). In addition, surface water in areas where Rs is present could be an important source of infestation (EPPO, 1992b) and should be disinfected before use or its use prohibited. This option (strict hygiene measure to prevent infestation of the production site) is considered highly effective to prevent infection of the crop on which seeds are produced

[10] Commission Directive 2006/63/CE of 14 July 2006 amending Annexes II to VII to Council Directive 98/57/EC on the control of *Ralstonia solanacearum* (Smith) Yabuuchi et al.

Establishment of a pest free production site or place
See above: prevention of infestation.

Establishment of a pest free area
Highly effective. Surveys as required by Council Directive 98/57/EC will be needed to confirm absence of the organism.

Options after harvest, at pre-clearance or during transport
See Cms. If only the seed surface could become infested with Rs, acid extraction of the seeds or similar method will probably be highly effective. No data on disinfection of TPS were found.

Conclusion
The following options are considered to reduce the risk to a very low level:

- Pest free area
- Pest free production site: protected cultivation in combination with strict hygiene measures including the use of pathogen free water and testing of plants not originating from a pest free production site or area before entering the production site and verified by visual inspections.

Each of these options is assessed to reduce the probability of contamination of TPS with Rs to a lower level than the current probability of contamination of seed potatoes despite existing official programmes. The reasons for this are: (i) low levels of infestation will be more difficult to detect in a seed potato crop (much larger fields) than in the production of TPS, (ii) seed potatoes are grown in the open where it is more difficult to clean and desinfest machinery, equipment, crates etc than under protected conditions.

Application of an acid extraction method to obtain TPS from seeds or a similar method that eliminate bacteria from the outer surface of the seeds will further reduce the risk of contamination with Rs.

4.2.3 PSTVd

4.2.3.1 Regulatory status for plants and products within the Community

Council Directive 2000/29/EC
Potato spindle tuber viroid is listed in Council Directive 2000/29/EC in the following sections:

- Annex I, Part A - Harmful organisms whose introduction into, and spread within, all Member States shall be banned
- Section I – Harmful or organisms not known to occur in the community and relevant for the entire community
  (d) Viruses and virus-like organisms'
  2. Potato viruses and virus-like organisms such as:
  (e) Potato spindle tuber viroid
- Annex IV, Part A, **–** Special requirements which must be laid down by all member states for the introduction and movement of plants, plant products and other objects into and within all member states – article 18.3 (Annex I of this document).

Article 18.3 requires that each plant ("each unit of material") other than culture maintenance material being stored in gene banks or genetic stock collections should be tested for PSTVd (and a number of other viruses). Testing of each single seed is not possible (testing will destroy each unit of material) but mother plants on which the seeds are produced could be sampled and tested or otherwise plants raised from seeds (under quarantine conditions) could be sampled and tested. As already indicated in chapter 1

(Introduction), article 18.3 has probably been written in the context of genetic material coming out of gene banks for use in breeding programmes and not in the context of commercial production of potato varieties. Testing of genetic material for PSTVd is also a requirement for import of TPS for research or breeding purposes (Annex III of Commission directive 2008/61/EC). In principle, these requirements should reduce the risk of pathway 1 (infected parental material through use of infected genetic sources in breeding programmes) to a very low level both for breeding of traditional potato varieties as for TPS varieties.

Commission decision 2007/410/EC
Commission decision of 12 June 2007 on measures to prevent the introduction into and the spread within the Community of PSTVd has laid down specific measure to locate infected plants of *Brugmansia* spp. and *Solanum jasminoides* with the aim of eradication of PSTVd in these species. These measures aim to prevent the spread of PSTVd within the EU. The measures have thus far not been able to eradicate PSTVd in the EU. EFSA-PLHP (2011): "In summary, PSTVd appears to be present in solanaceous ornamentals in a number of EU MS and may still have been under-reported in the EU MS surveys, because of limitations in sampling (number of samples per country, sampled species...)." This Commission decision will be discontinued (ScoPAFF, 26 – 27 March 2015).

Conclusion
Current requirements in Annex IV part II of Council Directive 2000/29/EC (article 18.3) and Commission Directive 2008/61/EC reduces the probability of introduction of PSTVd into breeding programmes for potato varieties (including TPS varieties) through use of infected material (pathway 1) to a very low level. Therefore, no risk reductions will be discussed in relation to pathway 1 (parental material). However, PSTVd is present in ornamentals in the EU (EFSA-PLHP, 2011). Transfer between ornamental species and a potato crop grown under protected conditions is assessed to be higher than under field conditions and risk reduction options will be discussed in relation to the pathway 2 "contamination".

4.2.3.2 Risk reduction options

Pathways for which options were evaluated
1. Parents. No options evaluated for reasons stated above.
2. Contamination (transfer from infected ornamental plants or other species) during production of TPS.

Detection by visual inspection
"The severity of symptom expression of PSTVd in the growing plant and in daughter tubers is affected by potato variety, PSTVd strain and growing conditions" (EFSA-PLHP, 2011). Therefore, visual inspection of the seed crop is considered not sufficiently effective.

Detection by testing
Several tests are available and could be used to test for presence of the PSTVd in leaves and stems of potato plants used for seed production (EPPO, 2004b; IPPC, 2015). As stated already for Cms and Rs the sampling intensity will greatly affect the risk reduction level.

Prevention of infestation
Hygiene measures can prevent infestation by contamination (mechanical transfer). Such measures can be similar to those for bacteria (see above) but disinfectants that are effective against bacteria may not be effective against PSTVd. Sodium hypochlorite is effective against both PSTVd and bacteria (O'Neill & Mumford, 2006; Matsuura et al.,

2010; EFSA-PLHP, 2011). Lack of official registration of sodium hypochlorite at dosages sufficiently effective against PSTVd may, however, hamper application.

Establishment of a pest free production site or place
A pest free production place or site could be maintained by strict hygiene measures (see above: prevention of infestation).

Conclusion
As already concluded above, the current requirement (article 18.3 in Annex IVAII of Council Directive 2000/29/EC) is considered sufficiently effective to reduce the probability of PSTVd introduced into breeding programmes by use of material from gene banks or stock collections (pathway 1) to a very low level because each unit should be tested. Testing of the parents of the TPS variety for PSTVd could, however, be recommended to further reduce the risk of PSTVd infections.

The probability of contamination by mechanical transmission (transfer from another plant species) during maintenance of the parents and production of TPS is assessed to be low. Currently, no specific requirements are in place to reduce the probability of infection of a seed potato crop by mechanical transmission from another plant species but the probability of mechanical transmission is assessed to be higher for a glasshouse crop (higher temperatures). The probability of transfer to a seed crop under protected cultivation can be reduced to a very low level by implementation of strict hygiene measures. In case plants show suspicious symptoms, plants could be sampled and tested for presence of PSTVd.

4.2.4 Pospiviroids other than PSTVd

4.2.4.1 Regulatory status for plants and products within the Community

Council Directive 2000/29/EC
The pospiviroids CSVd, CEVd, CLVd, PCFd, TASVd, TCDVd and TPMVd are not specifically listed in Annex I or II of Council Directive 2000/29/EC in relation to potato (CSVd is regulated for plants of *Dendranthema* (DC) Des Moul. intended for planting, other than seeds). PSTVd is specifically mentioned in Annex IAI under "Potato viruses and virus-like organisms such as" but not the other pospiviroids.

4.2.4.2 Risk reduction options

Pathways for which options are evaluated
1. Contamination (transfer from infected ornamental plant or other species)
2. Parent plants (requirements are in place to test genetic material for presence of PSTVd but not for other pospiviroids).

Detection by visual inspection
Like for PSTVd, visual inspection of the seed crop is considered not sufficiently effective (see above).

Detection by testing
Generic tests for all relevant pospiviroids (including PSTVd) are available and could be used to test for the presence of pospiviroids in leaves and stems of plants on which the seeds are produced (Botermans et al, 2013; Luigi et al, 2014; Monger et al, 2010; Olivier et al, 2014; Torchetti et al, 2012; Verhoeven et al, 2004). At present, no international harmonised protocol for pospiviroids is available, although some generic tests are also included in the IPPC diagnostic protocol for PSTVd (IPPC, 2015). As stated above for the other pathogens, the sampling intensity will greatly affect the risk reduction level.

Prevention of infestation
See above: PSTVd.

Establishment of a pest free production site or place
See above: PSTVd.

Conclusion
See above: PSTVd.

4.3 Conclusions

4.3.1 Risk reduction options

The following pests that are regulated or may be regulated in the near future and are present in the EU have been identified that may be transmitted with TPS:

- *Clavibacter michiganensis* ssp. *sepedonicus* (Cms)
- *Ralstonia solanacearum* (Rs)
- Pospiviroids (8 species: CSVd, CEVd, CLVd, PCFd, PSTVd, TASVd, TCDVd and TPMVd; TPMVd is not known to be present in the EU but was selected because it shows many similarities with the other pospiviroids)

TPS coming out of gene collections should be tested for presence of PSTVd according to the current provisions indicated in article 18.3 of Annex IVAII of Council directive 2000/29/EC. For potato plants and tubers several requirements apply including an official certification programme to reduce the risk of contamination with Cms and Rs. For the production of TPS varieties no such programme exists and infestation of true seeds may occur during seed production of TPS varieties by mechanical transfer (contamination). The following options are considered to reduce the risk of contamination of the seed crop with each of the pests mentioned above to a very low level:

Production of TPS in a

- Pest free area
  or
- Pest free production site:

TPS is produced under protected conditions which makes it easier to apply strict hygiene measures to prevent infestation of the crop with regulated pests than under field conditions. It is assessed that by implementation of strict hygiene measures (e.g. use of protective clothes, pathogen free water and only allow entrance of new or disinfected materials) the probability of crop infestation with each of these pests will be very low.

In addition, parents could be systematically tested for pospiviroids. Such a requirement would be more strict than current regulations for the production of seed potatoes which do not include such a requirement (only for the original material as laid down in article 18.3 Annex IVAII Council Directive 2000/29/EC).

4.3.2 Other conclusions

Article 18.3 of Annex IVAII of Council directive 2000/29/EC includes specific requirements for TPS. In the case of TPS, the plants ("each unit of the material") should have been tested for a number of viruses, including viruses that are not known to be present in the EU, and PSTVd. It does not indicate what kind of material article 18.3 applies to but the list of viruses including ones that are not present in the EU and requirements specified in items 18.4 (genetic stock collections) and 18.2 (advanced selections of tubers of *Solanum tuberosum*) suggest that the article has been written in

the context of plant material coming out of gene banks fore use in breeding programmes. In the case of the production of registered TPS varieties it is considered redundant to test each plant raised from seed for the pathogens listed in article 18.3. It is, therefore, suggested to include one new article, specifically for TPS, before article 18.3 comparable to those already existing for seed potatoes (articles 18.1 and 18.2) and exclude the TPS specified in the new article from article 18.3. Text suggestions for the new article and small amendments of articles 18.2 and 18.3 are provided in Chapter 5.

5. Suggestion for amendment of Annex IV part II of Council directive 2000/29/EC

Suggestion to amend Annex IVAII of Council Directive 2000/29/EC

Suggestion for inclusion of one new article (article 18.1b) in Annex IV part II of Council Directive 2000/29/EC for TPS similar to that of the current article 18.1 for seed potatoes and small amendments of the current articles 18.2 and 18.3:

Plant products and other objects	**Special requirements**
18.1b True seeds of *Solanum tuberosum* L.	Official statement that the Union provisions to combat *Synchytrium endobioticum* (Schilbersky) Percival have been complied with; and (a) either the true seeds originate in areas where *Clavibacter michiganensis* ssp. sepedonicus (Spieckermann and Kotthoff) Davis et al. *Ralstonia solanacearum* (Smith) Yabuuchi *et al., Potato spindle tuber viroid*[1] [the other relevant pospiviroid species[2] could be added if they will be listed in Annex IAII or IIAII of 2000/29/EC] are not known to occur; or (b) true seeds have been produced at a production site, where appropriate measures have been taken to prevent infestation with *Clavibacter michiganensis* ssp. sepedonicus (Spieckermann and Kotthoff) Davis et al. *Ralstonia solanacearum* (Smith) Yabuuchi *et al., Potato spindle tuber viroid*[1] [the other relevant pospiviroid species[2] could be added if they will be listed in Annex IAII or IIAII of 2000/29/EC] and no symptoms of disease caused by those harmful organisms have been observed on the plants at the site of production since the beginning of the last cycle of vegetation.

[1] *Potato spindle tuber viroid* is currently listed in Annex IAI but is known to be present in the EU and should be moved to either Annex IAII (regulated for all plants and products) or IIAII (regulated on certain plants and plant products).

[2] *Chrysanthemum stunt viroid, Citrus exocortis viroid, Columnea latent viroid, Tomato apical stunt viroid* and *Tomato chlorotic dwarf viroid,* (*Pepper chat fruit viroid* and *Tomato planta macho viroid* are not or no longer known to be present in the EU).

Amendment of the current articles 18. 2 and 18.3 (amendments in bold):

Plant products and other objects	**Special requirements**
18.2 Tubers **and true seeds** of *Solanum tuberosum* L.. intended for planting, other than tubers **or seeds** of those varieties officially accepted in one or more Member States pursuant to Council Directive 70/457/EEC of 29 September 1970 on the common catalogue of varieties of agricultural plant species (1)	*No changes*
18.3 Plants of stolon or tuber-forming species of *Solanum* L., or their hybrids, intended for planting, other than those tubers of *Solanum tuberosum* L. specified in Annex IV(A)(II)(18.1), **(18.1b)**, or (18.2), and other than culture maintenance material being stored in gene banks or genetic stock collections	*No changes*

(1) OJ L 225, 12.10.1970, p. 1. Directive as last amended by Directive 98/96/EC (OJ L 25, 1.2.1999, p. 27).

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Annex I: Council directive 2000/29/EC, special requirements for Clavibacter michiganensis ssp. sepedonicus, Ralstonia solanacearum and Potato spindle tuber viroid

Special requirements for *Clavibacter michiganensis* ssp. *sepedonicus, Ralstonia solanacearum* and *Potato spindle tuber viroid* in Annex IV, part A, section II – Plants, plant products and other objects originating in the community in Council Directive 2000/29/EC.

Plant products and other objects	**Special requirements**
18.1 Tubers of Solanum tuberosum L., intended for planting	Official statement that: ……. (b) either the tubers originate in an area known to be free from ***Clavibacter michiganensis* ssp. *sepedonicus*** (Spieckermann and Kotthoff) Davis et al. or the Union provisions to combat ***Clavibacter michiganensis* ssp. *sepedonicus*** (Spieckermann and Kotthoff) Davis et al. have been complied with; (d) (aa) either, the tubers originate in areas in which ***Ralstonia solanacearum*** (Smith) Yabuuchi *et al.*is known not to occur; or (bb) in areas where ***Ralstonia solanacearum*** (Smith) Yabuuchi *et al.* is known to occur, the tubers originate from a place of production found free from ***Ralstonia solanacearum*** (Smith) Yabuuchi *et al.*, or considered to be free thereof, as a consequence of the implementation of an appropriate procedure aiming at eradicating ***Ralstonia solanacearum*** (Smith) Yabuuchi *et al.*;
18.3 Plants of stolon or tuber-forming species of Solanum L., or their hybrids, intended for planting, other than those tubers of Solanum tuberosum L. specified in Annex IV(A)(II)(18.1) or (18.2), and other than culture maintenance material being stored in gene banks or genetic stock collections	(a) The plants shall have been held under quarantine conditions and shall have been found free of any harmful organisms in quarantine testing; (b) the quarantine testing referred to in (a) shall: (aa) be supervised by the official plant protection organisation of the Member State concerned and executed by scientifically trained staff of that organisation or of any officially approved body; (bb) be executed at a site provided with appropriate facilities sufficient to contain harmful organisms and maintain the material including indicator plants in such a way as to eliminate any risk of spreading harmful organisms; (cc) be executed on each unit of the material, —

	by visual examination at regular intervals during the full length of at least one vegetative cycle, having regard to the type of material and its stage of development during the testing programme, for symptoms caused by any harmful organisms, — by testing, in accordance with appropriate methods to be submitted to the Committee referred to in Article 18: — in the case of all potato material at least for — Andean potato latent virus, — Arracacha virus B. oca strain, — Potato black ringspot virus, — **Potato spindle tuber viroid**, — Potato virus T, — Andean potato mottle virus, — common potato viruses A, M, S, V, X and Y (including Y $_{o}$, Y $_{n}$ und Y $_{c}$) and Potato leaf roll virus, — ***Clavibacter michiganensis* ssp. *sepedonicus*** (Spieckermann and Kotthoff) Davis *et al*., — ***Ralstonia solanacearum*** (Smith) Yabuuchi *et al.*, — **in the case of true seed potato at least for the viruses and viroid listed above;** (dd) by appropriate testing on any other symptom observed in the visual examination in order to identify the harmful organisms having caused such symptoms; (c) any material, which has not been found free, under the testing specified under (b) from harmful organisms as specified under (b) shall be immediately destroyed or subjected to procedures which eliminate the harmful organism(s); (d) each organisation or research body holding this material shall inform their official Member State plant protection service of the material held.
18.5 Tubers of *Solanum tuberosum* L., other than those mentioned in Annex IV(A)(II)(18.1), (18.1.1), (18.2), (18.3) or (18.4)	There shall be evidence by a registration number put on the packaging, or in the case of loose-loaded potatoes transported in bulk, on the vehicle transporting the potatoes, that the potatoes have been grown by an officially registered producer, or originate from officially registered collective storage or dispatching centres located in the area of production, indicating that the tubers are free from ***Ralstonia solanacearum*** (Smith) Yabuuchi *et al.* and that (a) the Union provisions to combat *Synchytrium endobioticum* (Schilbersky) Percival, and (b) where appropriate, the Union provisions to combat ***Clavibacter michiganensis* ssp. *sepedonicus*** (Spieckermann and Kotthoff) Davis

	et al., and (c) the Union provisions to combat *Globodera pallida* (Stone) Behrens and *Globodera rostochiensis* (Wollenweber) Behrens are complied with.
18.7. Plants of *Capsicum annuum* L., *Solanum lycopersicum* L., *Musa* L., *Nicotiana* L., and *Solanum melongena* L., intended for planting, other than seeds	Without prejudice to the requirements applicable to the plants listed in Annex IV(A)(II)(18.6) where appropriate, official statement that: (a) the plants originate in areas which have been found free from ***Ralstonia solanacearum*** (Smith) Yabuuchi *et al.*, or (b) no symptoms of ***Ralstonia solanacearum*** (Smith) Yabuuchi *et al.* have been observed on the plants at the place of production since the beginning of the last complete cycle of vegetation.
24. Plants with roots, planted or intended for planting, grown in the open air	There shall be evidence that the place of production is known to be free from ***Clavibacter michiganensis* ssp. *sepedonicus*** (Spieckermann and Kotthoff) Davis *et al*. and *Synchytrium endobioticum* (Schilbersky) Percival.

Annex II: article 8 Council Directive 93/85/EEC

Article 8
1 . Member States shall prescribe that seed potatoes shall meet the requirements of Directive 77 / 93 / EEC and shall derive in direct line from material obtained under an officially approved programme which has been found free of the organism in official or officially supervised testing using the method set out in Annex I.

The aforesaid testing shall be carried out:
— in cases where the contamination affects seed potato production, on the plants of the initial clonal selection,
— in other cases, either on the plants of the initial clonal selection or on representative samples of the basic seed potatoes or earlier propagations.

Annex III: article 7 Council Directive 98/57/EC

1. Member States shall prescribe that seed potatoes shall meet the requirements of Directive 77/93/EEC and shall derive in direct line from potato material obtained under an officially approved programme which
has been found free of the organism in official or officially supervised testing using the relevant method set out in Annex II.

The aforesaid testing shall be carried out by a Member State:

(a) in cases where there have been confirmed finding(s) of the organism in their own seed potato production,
    (i) by testing of the earlier propagations, including the initial clonal selection and systematic testing of basic seed potato clones; or
    (ii) in cases where it has been established that there is no clonal relationship, by testing of all basic seed potato clones or earlier propagations including the initial clonal selection, and
b) in other cases, either on each plant of the initial clonal selection or
on representative samples of the basic seed potatoes or earlier propagations.

2. The following provisions may be adopted in accordance with the
procedure laid down in Article 16a of Directive 77/93/EEC:

— the detailed rules of application of paragraph 1, second subparagraph, point (a),
— the rules concerning the representative samples provided for in paragraph 1, second subparagraph, point (b).