Food and Environment Research Agency (Fera) Pest Risk Analysis for *Columnea latent viroid*

STAGE 1: PRA INITIATION

1. What is the name of the pest?

Columnea latent viroid. Named as such as it was first isolated from *Columnea erythropae* (Owens *et al*., 1978).

Synonyms:
CLVd (ICTV, 2005).

Common names of the pest:
None.

Taxonomic position:
Viroids; Family *Pospiviroidae*; Genus *Pospiviroid.*

Special notes on nomenclature or taxonomy:
Twenty-nine species of viroid are known to exist and species can be assigned to one of two families, namely the *Avsunviroidae* and the *Pospiviroidae*. CLVd belongs to the *Pospiviroidae. Pospiviroidae* species are generally considered to have a wider host range than the *Avsunviroidae* species.

Two variants of CLVd are known to exist:

CLVd-bru
This variant was initially designated CLVd-B because it was isolated from an ornamental plant: (symptomless) *Brunfelsia undulata* (Spieker, 1996). It is now known as CLVd-bru (ICTV, 2005).

CLVd-nem
This variant was initially designated CLVd-N because it was isolated from another ornamental plant which was also symptomless: *Nematanthus wettsteinii* (Singh *et al*., 1992). It is now known as CLVd-nem (ICTV, 2005).

Tomato isolates
The first records of CLVd in tomato (*Lycopersicon esculentum*) were made by Verhoeven *et al*. (2004) who found five CLVd-like isolates in symptomatic

plants originating from the Netherlands and one in plants from Belgium. Tomato isolates of CLVd have not been assigned as separate strain(s) to date, (ICTV, 2005).

Phylogenetic analysis indicates that there are two separate lineages of CLVd (Verhoeven *et al*., 2004). The authors consider that one lineage contains the ornamental isolates ('CLVd-Brun' = CLVd-bru, 'CLVd-Nem' = CLVd-nem, and the isolate from *C. erythropae* named in this paper as 'CLVd-Col'; no longer mentioned in ICTV, 2005). The other lineage contains the tomato isolates.

Studies have not been carried out to examine whether differences exist in pathogenicity or virulence between the different forms of CLVd. However, they have been individually tested for their pathogenicity to a limited range of plant species (see section 9 below).

2. What is the pest's status in the EC Plant Health Directive (Council Directive 2000/29/EC) (Anon., 2000)?
CLVd is not listed in the EC Plant Health Directive.

3. What is the recommended quarantine status of the pest in the lists of the European and Mediterranean Plant Protection Organisation (EPPO)?
CLVd is not recommended for consideration for regulation as a quarantine pest by EPPO and is not on the EPPO Alert List.

4. What is the reason for the PRA?
In 2007, there were four UK outbreaks of CLVd in glasshouse grown tomato plants, which were symptomatic (cv. Santa) (EPPO, 2008; Nixon *et al*., 2009). The PRA has been undertaken to assess the risk of further entry into the UK, as well as the risk of establishment in and economic impact to tomato, and other known and potential hosts.

5. What is the PRA area?

UK	✓	EU[1]		EPPO[2]	

STAGE 2: PEST RISK ASSESSMENT

6. What is the pest's present geographical distribution?
Due to the asymptomatic nature of the pathogen in 3 of the 4 natural hosts, the full geographic distribution of CLVd is uncertain.

Table 1 summarises the presumed geographical distribution. See text below

[1] If the PRA area is the EU then it excludes locations such as the French DOMS, Spanish Canary Isles and Portuguese Azores and Madeira.
[2] EPPO = The whole EPPO region concentrating on the European and Mediterranean area, i.e. EPPO west of the Ural Mountains.

for further details.

Table 1. Distribution of *Columnea latent viroid*

North America	Canada, USA
Central America	No record
South America	No record
Caribbean	No record
Europe	Belgium UK (2007 outbreaks eradicated); 2009 outbreak subject to eradication) France Germany The Netherlands (eradicated)
Africa	No record
Asia	No record – but see text below
Oceania	No record

The pathogen was first reported in 1978 as an unknown viroid isolated from asymptomatic *C. erythrophae* from a commercial nursery in the USA (Owens *et al*., 1978) and this was further characterised by Hammond *et al*. (1989).

Later, a strain of the pathogen, designated CLVd-N, was found on symptomless *N. wettsteinii* plants in Canada (Singh *et al*., 1992) (now CLVd-nem; ICTV, 2005).

Another strain of the pathogen, designated CLVd-B was isolated from symptomless *B. undulata* in Germany (Spieker, 1996) (now CLVd-bru; ICTV, 2005).

Hadidi *et al*. (2003) list the pathogen as present in the USA, Germany and Canada; no information is available to confirm the most recent status of the viroid in these countries.

Sampling of selected *Columnea* cultivars obtained from European nurseries (country/countries of origin not described) indicated the presence of a CLVd-related viroid (Hammond *et al*., 1989). However, no further information is available for these findings.

Six CLVd isolates proposed as a new strain of the species CLVd, as yet undesignated, were identified retrospectively in symptomatic diagnostic samples from tomato crops originating from the Netherlands (five isolates), and a Belgium tomato crop (one isolate). All findings from the Netherlands were reported as subsequently eradicated. (Verhoeven *et al*., 2004).

Because the original isolate came from *C. erythropae*, an epiphytic plant originating in Central America, 120 samples of *Columnea* spp. collected in

Costa Rica were tested, but no viroids could be detected (Hammond *et al*., 1989).

There are indications from other sources that CLVd may be present in other countries but there are no published records for any of these, details are given below:

The UK outbreaks in tomato plants in 2007 were all in crops that had been grown from seed originating in Taiwan, arriving in the UK via France. However, seed transmission for CLVd has not been proven (Hadidi *et al*., 2003). There are no published records of CLVd in Taiwan. However, subsequent to the first version of this PRA a record has been published of CLVd in tomato crops in western France occurring in the summer of 2007. The origin of infection of these crops is unknown (Steyer *et al*., 2009). In the summer of 2009, a UK fruit crop of tomato cultivars (cv.'s) Angelle and Santazian was found to be infected with CLVd; this crop is subject to eradication. There are no further details of this outbreak available at present. (P. Reed, Fera, UK, *personal communication*, 2009).

Nucleotide sequence accessions are present on the National Centre for Biotechnology Information (NCBI) database (NCBI, undated) for CLVd (strains not assigned) from tomato plants in Portugal, accession number EF015581. However, there are no published records of CLVd in Portugal.

NCBI accessions are also recorded for two tomato isolates numbered AM698093 and AM698094. These derive from tomato seeds imported into Thailand which were tested in post-entry quarantine and destroyed once infection with CLVd was detected (P. Tangkanchanapas, Plant Quarantine Research Group, Department of Agriculture, Bangkok, Thailand, personal communication, 2009). The origin of the seed is unknown.

7. Is the pest established or transient[3] in the PRA area? (Include information on interceptions and outbreaks here).

The pest is considered to be transient in the PRA area in tomato crops. In 2007 there were four confirmed outbreaks of the viroid in the UK in glasshouse tomato crops (cv. Santa). These were the first findings of CLVd in the UK. (EPPO, 2008; Nixon *et al*., 2009). These outbreaks were eradicated. There were no findings in the UK in 2008. However, in 2009 a single outbreak occurred in a tomato crop (cv.'s Angelle and Santazian). This is subject to eradication. The status in ornamental hosts in the UK is unknown as the natural hosts (that are known about) are asymptomatic and no surveys have been undertaken to date to determine the status of CLVd on plants grown in UK ornamental nurseries.

Further information on potential pathways of entry including the possible entry via seed is given in sections 12 and 13.

[3] Transience: presence of a pest that is not expected to lead to establishment (ISPM No., FAO, Rome)

8. Is there any reason to suspect that the pest is already established in the PRA area?

Not in tomato crops. The first findings of CLVd in tomato in the UK were in 2007 and symptoms were detected early (April) in the cropping season. There were no findings in tomato in 2008, but in July 2009 a fruiting crop was found infected with CLVd; this is subject to eradication. There are no further details of this outbreak at present.

UK tomato fruit crops are surveyed annually by the Plant Health Service (PHS) for *Pepino mosaic virus* and *Potato spindle tuber viroid* and more frequently for those grown for propagation. Although CLVd is symptomatic in tomato, the 2007 and 2009 outbreaks were detected/reported several months apart. For this reason, it is not possible to state that the official inspections carried-out by the UK PHS would detect the presence of the viroid based upon symptoms alone. However, affected growers are likely to notice the symptoms and if sampled, testing would detect whether or not CLVd was present.

It is not known whether CLVd is established in ornamental hosts in the UK. There are four reported natural hosts but only one of them, tomato, is symptomatic. The three remaining hosts are all ornamental species and are asymptomatic.

9. What are the pest's host plants? List natural and experimental hosts.

Natural hosts are listed in Table 2 and the results of experimental transmission studies to other plant species are shown in Table 3; described below.

The viroid was first detected because seedlings of tomato (*L. esculentum*) inoculated with low molecular weight RNA preparations obtained from asymptomatic *C. erythropae* (lipstick vine) from a US nursery, developed symptoms (Owens *et al*., 1978). The viroid was further characterised by Hammond *et al*. (1989) who found it was transmissible to *Gynura aurantiaca* (purple velvet plant), *Cucumis sativus* (cucumber) as well as tomato (*L. esculentum*), but not to *Nicotiana tabacum* (tobacco plant). Unpublished results (Diener, Smith and Owens; cited by Hammond *et al*., 1989) showed transmission of the CLVd isolate from *C. erythrophae*, to potato (*S. tuberosum*).

CLVd-N, isolated from symptomatic *N. wettsteinii* (Goldfish plant) plants in Canada (Singh *et al*., 1992) (now CLVd-nem; ICTV, 2005) was experimentally transmissible to tomato, potato and the ornamental plant *Scopolia sinensis*.

CLVd-B, isolated from symptomless *B. undulata* (Jamaican raintree) in Germany (Spieker, 1996) (now CLVd-bru; ICTV, 2005) was found to be transmissible to tomato.

Thus, all of the isolates were shown to be transmissible to tomato (*L. esculentum*) and this was first confirmed as a natural host in 2004 (Verhoeven *et al.*, 2004).

Isolates from tomato were found to be transmissible to potato (*S. tuberosum*) and cucumber (*C. sativus*), Verhoeven *et al*. (2004).

Table 2. Natural hosts of *Columnea latent viroid*

Host (common name)	**Family**	**Symptom**	**Reference**
Columnea erythrophae (Lipstick vine)	Gesneriaceae	Asymptomatic.	Owens *et al*. (1978); Hammond *et al*. (1989).
Nematanthus wettsteinii (Goldfish plant)	Gesneriaceae	Asymptomatic.	Singh *et al*. (1992).
Brunfelsia undulata (Jamaican raintree)	Solanaceae	Asymptomatic.	Spieker (1996).
Lycopersicon esculentum (Tomato)	Solanaceae	Dutch outbreaks: Chlorosis, bronzing, leaf distortion and growth reduction.	Verhoeven *et al*. (2004).
		UK outbreaks: Leaf bronzing, necrosis and chlorosis; leaf distortion and growth reduction.	EPPO (2008); Matthews-Berry (2007); Nixon *et al*. (2009).
		French outbreaks: Severe leaf yellowing or reddening, distortion, stunting.	Steyer *et al*., 2009.

Table 3. Results of transmission experiments of isolates of *Columnea latent viroid* (grafted or manually inoculated) from known hosts to different plant species (experimental hosts)

Isolate source	**Experimental host species/family**		**Symptom**	**Reference**
Columnea erythropae (Lipstick vine)	*Lycopersicon esculentum* (Tomato)	Solanaceae	Leaf epinasty, plant stunting.	Owens *et al*. (1978).
Columnea erythropae (Lipstick vine)	*Gynura aurantiaca* (Purple velvet plant)	Asteraceae	Symptomatic (undescribed).	Hammond *et al.* (1989).
	Cucumis sativus (Cucumber)	Cucurbitaceae		
	Lycopersicon esculentum (Tomato)	Solanaceae		
	Nicotiana tabacum (Tobacco)	Solanaceae	Not transmitted.	
	Solanum tuberosum (Potato)	Solanaceae	Symptoms 'typical of PSTVd'.	
Nematanthus wettsteinii (Goldfish plant)	*Scopolia sinensis* (No common name - ornamental)	Solanaceae	Systemic necrotic spots and streaks plus premature leaf senescence.	Singh *et al.* (1992).
	Lycopersicon esculentum (Tomato)	Solanaceae	Leaf size reduction, leaf bunching at the top of the plant, occasional leaf mid-vein necrotic streaks, plant stunting.	
	Solanum tuberosum (Potato)	Solanaceae	Leaf size reduction, plant stunting.	
Brunfelsia undulata (Jamaican raintree)	*Lycopersicon esculentum* (Tomato)	Solanaceae	Leaf size reduction, leaf bunching at the top of the plant, occasional leaf mid-vein necrotic streaks, plant stunting.	Spieker, (1996).
Lycopersicon esculentum (Tomato)	*Solanum tuberosum* (Potato)	Solanaceae	Plant death in (one isolate); plant stunting; tuber symptoms: star cracking, stunting, malformation.	Verhoeven *et al*. (2004).
	Cucumis sativus (Cucumber)	Cucurbitaceae	No results or details given.	

10. Which hosts are of economic and/or environmental importance in the PRA area?

Tomato

The most important natural host of CLVd is tomato (*L. esculentum*). The most recent published UK annual production figure (provisional) for marketed fresh tomatoes in 2007 was 85.6 thousand tonnes, of which 4.3 thousand tonnes comprised exports (Defra, 2008b).

Ornamentals

There are only three other known natural hosts, all of which are grown as ornamental species in the UK (*B. undulata*, the Jamaican raintree; *C. erythrophae*, the lipstick vine; and, *N. wettsteini*, the goldfish plant). None of these are of major economic or environmental importance in the UK.

Brunfelsia species are native to the tropical Americas and are grown as ornamental plants (Mabberley, 1997); several UK suppliers are listed (RHS, undated). *Columnea* species are also native to the tropical Americas and grow as epiphytic plants in the wild (Mabberley, 1997); there are 27 records of varieties and suppliers in the UK (RHS, undated). *Nematanthus* species are native to South America and are cultivated as house plants (Mabberley, 1997), with three UK suppliers (RHS, undated).

Experimental hosts

The viroid has been shown by experiment to be transmissible to potato (*S. tuberosum*) and cucumber (*C. sativus*) as well as two other ornamental species which are grown as protected crops (*G. aurantica* and *S. sinensis*). *S. sinensis* could be grown outdoors. The most important of these experimental hosts are potato and cucumber.

The UK annual production figure for potato (including seed potatoes) in 2007 (provisional) was 5,635,000 tonnes of which 407,000 tonnes were exported (Defra, 2008).

Annual UK production for cucumber in 2007 (provisional) totalled 49.4 thousand tonnes, the export figure is unavailable (Defra, 2008a).

11. If the pest needs a vector, is it present in the PRA area?

The viroid is not known to be borne by any vector, but this has not been investigated to date.

12. Describe the pathway(s) considered by this PRA[4].

The main pathways of entry are plants for planting of known natural hosts from countries where CLVd is known to occur/has occurred (Canada and the USA; Belgium, France, the Netherlands and Germany). CLVd may occur in other countries (see 6.) but there are no official records and so these cannot

[4] A pathway description typically identifies a geographic origin, a host plant or plants and the intended use of the host. Other pathways including entry on other commodities or by natural means should be considered.

be considered to be pathways. The known natural hosts comprise three ornamental species in which CLVd is asymptomatic: *B. undulata*, *C. erythrophae*, and *N. wettsteinii* as well as plants of tomato (*L. esculentum*). In the UK outbreaks, seed of tomato was implicated as the source of infection and therefore is a potential pathway of entry. Although seed transmission has not yet been proven, tomato seed is considered further as a pathway in this PRA.

Experimentally-susceptible hosts may also represent pathways of entry but these have yet to be reported as natural hosts and so cannot be considered in this PRA.

The likelihood of entry is considered further for each pathway under section 13.

There are no specific phytosanitary requirements for CLVd in the EC Plant Health Directive (Anon., 2000) that would directly affect entry of the pathogen into the UK. However, there are other EC phytosanitary requirements that will help reduce the risk of entry of CLVd to the UK. (See section 13.).

Tomato seed and plants are the first pathways of entry to be considered.

Tomato seed

Seed of tomato is allowed entry into the UK (EU) from third countries and can move from other EU countries to the UK, but only according to specific phytosanitary requirements. (See section 13).

Transmission of CLVd via tomato seed has not yet been proven, however, it is suspected, since all four UK outbreaks in tomato in 2007 were found to have been grown from seed from the same source. No details are available for the 2009 UK outbreak so the possible route of entry is unknown.

CLVd has only been reported in tomato (plants) in Belgium, France, the Netherlands and the UK, and is considered eradicated in the affected crops from the Netherlands; the 2007 UK outbreak is eradicated and the 2009 outbreak is subject to eradication; the status in France and Belgium is unknown.

In the UK, all seed used for propagation of tomato plants, to be planted as commercial tomato fruit crops, is imported from the Netherlands as well as from other countries (M. Lazenby, Fera PHSI, UK, *personal communication*, 2009). Tomato seed is often imported from third countries to the Netherlands where it can be legally repackaged and labelled 'repacked in Netherlands' (P. Reed, Fera, UK, *personal communication*, 2009).

Between 2006 and 2008 tomato seed was imported into the UK from countries outside the EU where CLVd has been reported, albeit not on tomato (Canada and the USA) (P. Kilby, Defra PHSI (now Fera), UK, *personal*

communication, 2008). In addition to the Netherlands, movement of tomato seed to the UK for the purpose of commercial growing-on can also occur from EU countries where CLVd has been previously reported; i.e. Germany (ornamental host), and Belgium (tomato), and, more recently France (tomato), as well as from third countries where the status of CLVd is unknown. The seed that was used to produce the infected tomato crops in the UK in 2007 entered the UK via France from Taiwan; the recent publication of records of CLVd in tomato crops in France confirms it's presence since at least 2007; however Taiwan has not yet reported CLVd.

Tomato plants

As previously stated, CLVd has been reported in tomato plants in Belgium, France, the Netherlands and the UK, it is considered eradicated in the affected crops from the Netherlands, the UK outbreaks in 2007 were eradicated and the 2009 outbreak is subject to eradication. The status in Belgium and France is unknown.

Most tomato seedlings used in the UK are grown in the UK, however some are introduced from the Netherlands (G. Hayman, British Tomato Growers Association, UK, *personal communication*, 2009). Tomato seedlings raised at UK propagators will have been produced from seed originating from EU or third countries. It is possible that CLVd may be present in tomato plants raised in other EU countries and so this is still a potential pathway of entry into the UK. However, import figures for tomato seedlings/plants from other EU countries to the UK are not collected.

Ornamental plants

A limiting factor for pathway analysis for specific CLVd ornamental hosts is that detailed (host and origin) trade statistics are not collected or not available/known. The UK imports ornamental plants for propagation, particularly from the Netherlands (Defra, 2007). Imports of indoor plants, saplings, shrubs and bushes and selected seeds from the Netherlands to the UK accounted for 38 million Euros in 2003 (Kamphuis, 2005). Some of these will have been originally imported from third countries and may pose a risk of entry of CLVd to the UK. More recent figures extracted from the Eurostat website by EPPO show the total value of imports of 'plants for planting' (presumed from this document to be ornamental plants) into the UK in 2007 was 205 billion Euros (EPPO, 2009).

13. How likely is the pest to enter the PRA area[5]?

Very unlikely		Unlikely		Moderately likely		Likely	✓	Very likely	

The pest has already been detected in the PRA area. Based upon the fact that outbreaks occurred in tomato crops in 2007 at four premises and again in 2009 at a separate premise there is a likelihood of further entry.

[5] Pest entry includes an assessment of the likelihood of transfer to a suitable host (ISPM No. 11, FAO, Rome)

No definitive centre of origin of the pathogen is known but it is likely to be associated with the Americas as this is the origin of all of the known host plants. CLVd is not listed in the EC Plant Health Directive (Anon., 2000) and so is currently not subject to phytosanitary measures. However, there are some EC phytosanitary requirements for tomato seed, tomato plants, and ornamental hosts that are members of the Solanaceae, described below. Likelihood of entry is described here for each pathway given at section12.

Tomato seed

Currently, seed of tomato can enter and move within the EU provided it has been subjected to acid-extraction or an equivalent measure (see below).

The common factor linking CLVd outbreaks in tomato at all four UK nurseries in 2007 was the same seed supply, cv. Santa, imported from Taiwan via France. It cannot be confirmed that this is the route by which the viroid entered the UK, however this is by far the most likely route. Outbreaks in French tomato crops in 2007 have only recently been reported (Steyer *et al*., 2009). Possible pathways of entry for the 2009 UK outbreak are unknown.

Although suspected, true seed transmission for tomato is not proven. Twenty seeds obtained from self-pollination of CLVd-N (CLVd-nem)-infected plants of tomato cv. Sheyenne were found not to be infected by CLVd when tested by R-PAGE (Singh *et al*., 1992). This negative result may reflect the very low number of seeds tested. One of the UK outbreaks of CLVd in tomato commenced with 3 out of 56,000 plants infected; to detect this level of infection (0.005%) with 95% confidence, it would be necessary to test 60,000 seeds (P. Reed, Fera, UK, *personal communication*, 2009). However, seed extracted from the fruit of naturally infected tomato plants from one UK outbreak was found to be CLVd positive by Scorpion assay (a molecular diagnostic technique) (CSL, 2008). It is possible that this assay, developed at CSL (now Fera), is more sensitive than previous assays utilised. Thus, other seed-testing laboratories may not have detected infected seed. It is not known whether tomato seed is infected internally, externally (seed coat), or just contaminated. An experimental transmission study from tomato seed (and seedlings) is in progress (HDC, 2008). Within this and other studies, various molecular methods of detection for CLVd in tomato seed (and foliar material) are being developed and tested for sensitivity at Fera, and will be ring-tested by the UK and other countries in due course (A. Fox, Fera, UK, *personal communication*, 2009).

Use of imported tomato seed by UK plant propagators is common practice because the UK does not have a tomato seed production industry. Some seed has originated from outside Europe including countries where CLVd has been previously reported (albeit not in tomato): Canada, the USA; as well as from Taiwan which was the source of seed for the UK outbreaks in tomato crops in 2007. Tomato seed is also introduced to the UK from within Europe including from countries where CLVd has been previously reported: the

Netherlands (tomato), as well as possibly from Germany (ornamental - *B. undulata*), Belgium and France (tomato). Seed originating in other EU Member States may also have been imported from third countries, repackaged and then shipped to the UK.

There are several phytosanitary requirements in place in the UK (EC requirements) for imports of tomato seed. These include a general requirement under Article 48, Annex, IVAI of the EC Plant Health Directive (Anon., 2000) that imports of tomato seed into the EU, from any third country are only permitted if "*seeds have been obtained by means of an appropriate acid extraction method or an equivalent method".* The same measures apply to tomato seed originating within the EU (Article 27, Annex IVAII), for which seed requires a Plant Passport to allow it to move between EU countries. Similar measures are in place under emergency EC legislation (Anon., 2004) for *Pepino mosaic virus* (PepMV); these apply to seed imports from third countries, as well as to seed originating within the EU. Thus, for seed originating outside of the EU in countries where CLVd occurs/has occurred (Canada, USA) and within the EU (Belgium, France, Germany and the Netherlands), this should be subject to acid-extraction (or equivalent) as it is a statutory requirement. The seed from Taiwan that was used to grow the affected tomato crops in the UK in 2007 should also therefore have been subject to such a treatment. The efficacy of this measure against CLVd is unknown but it would only be likely to have efficacy against surface-borne infection/contamination. If true seed transmission is confirmed (and this work is ongoing at Fera; A. Fox, Fera, UK, *personal communication*, 2009) it is likely that CLVd will continue to enter the PRA area on imports of tomato seed either from third countries or possibly from other EU Member States, as acid-extraction (or equivalent) will not eliminate systemic seed-borne infection (R. Mumford, Fera, UK, *personal communication*, 2009).

In experiments, sodium hypochlorite has shown some efficacy as a seed treatment on tomato seed against PepMV (R. Mumford, Fera, UK, *personal communication*, 2009). It is being used routinely by seed houses in the Netherlands and by the main UK propagators if seed used to raise plants has not been treated (M. Lazenby, Fera, PHSI, UK, *personal communication*, 2009). The efficacy of this treatment against CLVd is unknown, but like acid-extraction, it is only likely to be effective against surface contamination of the seed and not on systemic seed-borne infection (R. Mumford, Fera, UK, *personal communication*, 2009).

<u>Tomato plants</u>

The second most likely pathway of entry of CLVd into the PRA area is via imports of tomato seedlings.

Import of plants for planting of Solanaceae to the UK (EU) are <u>prohibited</u> from all countries outside of the EU except non-EU European or Mediterranean (Euro-Med) countries (Article 13, Annex IIIA; Anon., 2000), thus CLVd movement into the UK on imported tomato plants from sources other than EU

or Euro-Med countries should not occur. There are other phytosanitary requirements for specific pests and pathogens of tomato plants imported from non-EU Euro-Med countries and for movement of tomato plants between EU countries, but none of these pertain to CLVd, or would affect the risk of entry. Nevertheless, CLVd could be moved from other EU or Euro-Med countries to the UK with tomato seedlings (which may have been raised from seed imported from third countries). Movement of tomato plants is likely to occur at the beginning of the season and it is unlikely that symptoms would be apparent on very young plants. This is because symptoms in tomato have been shown by experiment to develop 3 to 5 weeks after inoculation (Verhoeven *et al*., 2004).

Ornamental plants and seeds

CLVd is likely to enter the PRA area undetected via asymptomatic ornamental hosts as there are no specific phytosanitary requirements in place in EC legislation for ornamental hosts of CLVd. Imported plants of the natural non-solanaceous ornamentals *N. wettsteinii* and *C. erythophae* could enter the UK from third countries, as well as from other EU countries. There is a risk that CLVd may enter the UK on these plants at least from Canada, the USA, Belgium, France, the Netherlands and Germany where outbreaks of CLVd have occurred, albeit the viroid has not been detected in ornamentals in the Netherlands (including in a 2006 survey of ornamental plants in the families Gesneriaceae and Solanaceae; Verhoeven *et al*., 2008) or in Belgium or France. Imports of plants of the solanaceous natural CLVd ornamental host *B. undulata* are prohibited from all countries outside of the EU or Euro-Med region (Article 13, Annex IIIA, Anon., 2000), as are all members of the Solanaceae. There are no reports of natural seed transmission for CLVd in ornamental hosts and there are no published studies to date, but this seed may be a possible route of entry.

14. How likely is the pest to establish outdoors in the PRA area?

Very unlikely		Unlikely	✓	Moderately likely		Likely		Very likely	

None of the four natural hosts of CLVd are grown commercially outdoors in the UK, thus establishment of CLVd outdoors in commercial crops of the known natural hosts is unlikely. However, tomato plants are grown outdoors in the UK in domestic gardens in the summer. These have potential to become infected. Establishment is unlikely because these plants are not kept over the winter period. Of the four known experimentally-susceptible hosts, potato (*S. tuberosum*) is the most important species that is grown commercially outdoors in the UK. It is possible that the pathogen could establish in potato crops if natural infection occurs, but this also depends upon the overwintering potential of CLVd outdoors in the UK. Viroid replication and symptom development is generally accepted to be enhanced as the temperature increases to above 20ºC to (at least) 35ºC (Hadidi *et al*., 2003); thus, should natural infection occur, disease development in UK potato crops is probably

only likely late in the cropping season. Repeated freezing and thawing of PSTVd in potato plants was found to result in loss of infectivity (Singh and Boucher, 1988). There is a possibility that CLVd may not overwinter in infected potato ground keepers left in the field in the UK where sub-zero temperatures occur. However, it is common practice for amateur growers and some farmers to use home/farm-saved potato seed. If infected, CLVd could survive in seed tubers stored overwinter which when planted could lead to systemic infection within the crop.

The experimental host *S. sinensis* could be grown outdoors, and possibly act as a symptomatic CLVd reservoir in the UK (symptoms developed after inoculation - see Table 3). However, transfer from this experimental host to at-risk crops such as tomato (under glass or in domestic gardens) or to potato crops outdoors would only occur if they were grown in close proximity.

15. How likely is the pest to establish in protected environments in the PRA area?

Very unlikely		Unlikely		Moderately likely		Likely		Very likely	✓

It is very likely that the pathogen could establish in protected environments in the PRA area. UK outbreaks of CLVd in protected tomato crops have already occurred. All of the natural CLVd hosts, and two experimental hosts require protected environments. These comprise the natural hosts: tomato, *C. erythophae*, *N. wettsteinii* and *B. undulata*, and the experimental hosts: cucumber (*C. sativus*) and *G. aurantiaca*.

The viroid is likely to be more prevalent in the warmer conditions that occur in protected environments.

Symptoms of CLVd were clearly evident in tomato plants at one of UK outbreak sites in April 2007, following introduction of plants to the glasshouses in December 2006 (it is not known how long symptoms were evident prior to April). The grower at one of the outbreak sites estimated that symptoms appeared in plants introduced into the glasshouse to replace those removed due to infection, in approximately 6 weeks. (Matthews-Berry, 2007). Thus, there is a period early in the growing season where infection in glasshouse-grown tomatoes is asymptomatic, thus facilitating cryptic spread. This is supported by experiments in which symptoms on tomato were first recorded from 3-5 weeks after inoculation with CLVd (Verhoeven *et al*., 2004).

<u>Details of the 2009 UK tomato outbreak are unknown.</u>
The natural ornamental hosts *C. erythophae*, *N. wettsteinii* and *B. undulata* are asymptomatic, thus where the viroid is present, establishment in these plants is very likely. Mechanical transmission to the most important host, tomato, has been experimentally demonstrated from all of the natural ornamental hosts; initially from asymptomatic infected *C. erythophae*, which is

how CLVd was first detected (Owens *et al*., 1978). Where glasshouses containing infected ornamental host plants are in proximity to tomato production or propagation glasshouses, asymptomatic infection could potentially spread CLVd by mechanical transmission (or by other means yet to be determined) from ornamentals to tomato crops. This scenario has not been found to date in the PRA area and is unlikely to occur on commercial nurseries. This is because tomato producers are generally well aware of the pest and disease risks associated with ornamental plants.

Hygiene measures to prevent the introduction of pathogens from other plants are implemented on tomato fruit production nurseries. For example, crop workers on most nurseries are required to wash and disinfect hands with an alcohol gel prior to starting work. They are not allowed to bring tomato fruit onto the site.

Tomato propagators tend to limit propagation of other plants while they are propagating tomatoes. Also, tomato propagation generally takes place at dedicated premises or in a separate area where strict hygiene measures are enforced.

Cucumber and *G. aurantiaca* are experimental hosts in which CLVd could theoretically establish in protected environments. Transmission from these experimental hosts to tomato has not been investigated.

16. How quickly could the pest spread[6] within the PRA area?

Very slowly		Slowly		Moderate pace	✓	Quickly		Very Quickly	

The pathogen is known to be transmitted at least by mechanical means (Hadidi *et al*., 2003). Other modes of transmission are being investigated for PSTVd (as a model for pospiviroids), including through feeding by thrips, movement in pollen by honeybees and through their feeding activities, as well as future work on mechanical transmission through cultural practices (EUPHRESCO Project 'Detection and Epidemiology of Pospiviroids' (DEP); R. Mumford, Fera, UK, *personal communication*, 2009).

Handling, direct plant-to-plant contact, use of contaminated tools and machinery or graft inoculation would facilitate spread on the affected premises. The UK findings in protected tomato in 2007 appeared to spread rapidly within the crops; this appeared to occur as a result of working on the crop, rather than direct plant-to-plant contact (Matthews-Berry, 2007). Dutch findings also indicate crop handling was the main route for transmission in tomato crops (Verhoeven *et al*. 2004).

[6] ISPM No 5. defines spread as the expansion of the geographic distribution of a pest within an area. Note that just because an organism can move or be transported quickly, does not mean that it will spread quickly, i.e. it also has to establish.

Spread between asymptomatic ornamental host plants at the same premises is likely to be rapid.

Long-distance spread is most likely via the movement of infected young tomato plants for planting as well as via asymptomatic ornamental plants and possibly via infected tomato seed stocks. Although seed-transmission in tomato is not proven, it is suspected that this was the route of introduction to the affected UK tomato crops in 2007. Seed transmission in asymptomatic ornamental plants has not been investigated.

Potato (an experimental host) is vegetatively propagated. If the pathogen became established in seed stocks of potato it could be distributed relatively quickly throughout the PRA area.

17. Which part of the PRA area is the endangered area?[7]

The endangered area is the protected tomato growing area of the UK, possibly the protected cucumber cropping area but only if natural infection occurs, and, potentially locations where the known ornamental hosts are grown under protection. Ornamentals that are grown under protection that may harbour CLVd are the natural asymptomatic CLVd hosts within the Gesneriaceae (*C. erythropae*, *N. wettsteinii*) and the Solanaceae (*B. undulata*), and potentially the experimental (symptomatic) host *G. aurantica* (Asteraceae). These species may facilitate CLVd spread to UK tomato crops where grown in close proximity; but this is unlikely to happen in commercial crops or at propagation nurseries. Cucumber (*C. sativus*, a member of the *Cucurbitaceae*) is experimentally-susceptible and is potentially at risk from infection via asymptomatic ornamental species grown under protection, or more likely from infected tomato crops where grown in close proximity.

The UK potato crop area (*S. tuberosum* is an experimentally-susceptible host) is potentially endangered via infected planting material, or contact with other hosts. Currently the only plant that is known to be a potential host that may be grown outdoors is *S. sinensis* (also a member of the Solanaceae). However, as CLVd has not been found naturally in potatoes or *S. sinensis* and contact between potato crops and natural hosts outdoors is currently limited to tomatoes grown in private gardens, the risk to UK potato production under normal growing conditions is probably quite low.

18. What is the pest's economic, environmental or social impact within its existing distribution?

Very small		Small		Medium	✓	Large		Very large	

The current geographic distribution of the pathogen is uncertain, however

[7] An **area** where ecological factors favour the **establishment** of a **pest** whose presence in the **area** will result in economically important loss (see Glossary Supplement No. 2) [FAO, 1995]

records exist for CLVd in asymptomatic ornamental hosts in the USA, Canada and Germany with a CLVd-related viroid in selected *Columnea* cultivars from 'European nurseries' (Hammond *et al*., 1989; no further details). See section 6. There are no reports of economic damage to ornamentals presumably because those that have been reported to be natural hosts are asymptomatic.

Published records of CLVd in tomato are known for the Netherlands (eradicated), Belgium and France (status unknown) and the UK (eradicated 2007). The 2009 UK outbreak in the UK is subject to eradication. There are no experimental data available for the impact that CLVd has on tomato. Yield losses for the crops from which the affected tomato plants in the Netherlands, Belgium or France were derived were not described. However, the collective description of symptoms caused by four viroids that were detected in tomato, including CLVd, were: chlorosis, bronzing, leaf distortion and growth reduction, with infection rates varying from a limited number of plants to 100% infection (Verhoeven *et al*., 2004).

For UK outbreaks of CLVd in tomato crops in 2007, symptoms were similar to those described by Verhoeven *et al*. (2004) (see Table 2). At one outbreak site, 50-60% of tomato plants appeared to be infected at the end of the season and the grower estimated financial losses at £250,000. Approximately 19,000 plants were removed and destroyed from one glasshouse containing 56,000 plants. As a result, yield losses were estimated to be down from an average of 20 kg/m^2 in 2006 to 16 kg/m^2 in 2007, although some of this may be attributed to seasonal differences. Statutory controls were put in place on the affected nurseries. (Matthews-Berry, 2007). Potential losses for the 2009 outbreak have not been estimated to date.

Potato and cucumber are currently experimental hosts only, thus there are no recorded impacts for these crops at present.

19. What is the pest's potential to cause economic, environmental or social impacts in the PRA area?

Very small		Small		Medium		Large	✓	Very large	

The potential economic impact will vary with the crop as described below.

<u>Tomato</u>
If CLVd becomes established in the UK, it has the potential to cause significant yield losses in tomato crops.

Observations at one of the UK outbreaks of CLVd in tomato crops in 2007 showed that CLVd has the potential to cause significant losses (based upon one affected UK grower comparing 2006 yields to 2007). No experimental work has been undertaken to support this however. There were no symptoms observed on the fruit, so there were no obvious negative effects on quality

(Matthews-Berry, 2007). There have been no experiments investigating the effect of CLVd on tomato fruit quality. There are no details of the effect that CLVd has had on the UK tomato crop found infected with CLVd in the summer of 2009.

UK (2007) and Dutch (Verhoeven *et al*., 2004) findings have shown that the viroid can spread easily and rapidly within tomato crops. Compared to a UK outbreak of PSTVd at a tomato production nursery in 2003 (Mumford *et al*., 2004), CLVd is considered potentially more damaging, spreading more rapidly within the crop in the glasshouse than PSTVd did, and apparently causing greater losses (Matthews-Berry, 2007).

If CLVd became listed as a quarantine pest by any country to which the UK exports tomato fruit or plants (there is no tomato seed industry), there is the potential for loss of exports, and/or, additional costs being incurred to meet the importing countries phytosanitary requirements.

Ornamental hosts

Natural ornamental hosts of CLVd are asymptomatic (Owens *et al.*, 1978; Hammond *et al*., 1989; Singh *et al.*, 1992; Spieker, 1996) and so there are no reports of quality losses for these hosts. If the pathogen becomes established in the UK and becomes listed as a quarantine pest by other countries with requirements for pest-freedom on exported plants (or seeds) there may be economic implications associated with importing country requirements.

Potato

Potato has the potential to become infected by CLVd but this is yet to occur naturally. Published experimental work suggests that there is a risk of yield and quality reduction should CLVd infect field-grown potato crops as described below:

Experimental transmission (by various methods) of CLVd-nem to potato plants of cultivars Katahdin, Kennebec, and Red Pontiac led to ‘mild’ symptoms of leaf size reduction and stunting. Symptoms appeared 3-4 weeks post-inoculation. (Singh *et al*. 1992).

Experimental inoculation of CLVd isolates from tomato to the first leaves of potato plants (cv. Nicola) under glasshouse conditions did not lead to symptomatic leaves. However, progeny tubers that developed from these artificially-infected plants, exhibited symptoms comprising stunting, star cracking and malformation. The same CLVd isolates were used to inoculate potato plants in the field (under quarantine conditions). These plants were tested for the presence of CLVd two months after inoculation, proving negative, possibly due to cool growing conditions, or low viroid titre, preventing detection using an R-PAGE assay. Tubers forming on these plants, as well as the plants tested initially in the glasshouse, were planted in the field. Plants arising from the tubers harvested from the glasshouse experiments were severely stunted and themselves produced malformed

tubers; one isolate led to death of plants a couple of weeks after emergence. Tubers arising from the field-infected plants were small and often malformed and the plants arising from them had reduced growth. One isolate led to yield losses in tubers of *ca.* 82%. Field conditions were not optimal for potato however; thus the authors warn that the implications for yield are only indicative. Verhoeven *et al*. (2004).

Cucumber

Cucumber has the potential to become a natural host as it is experimentally-susceptible.

CLVd isolates from *C. erythropae* were found to replicate and cause symptoms of disease (undescribed) in the cucumber cv. Suyo (Hammond *et al*. 1989), and CLVd tomato isolates were found to infect cucumber (Verhoeven *et al*. 2004). No further details were given. Thus, the potential impact to cucumber is not possible to estimate.

Experimentally-susceptible ornamental hosts

The experimental hosts *G. aurantiaca* and *S. sinensis* did develop symptoms post-inoculation and so as they are ornamental plants there is potential for quality losses. These species could potentially act as CLVd reservoirs in the PRA area. There are currently no data to confirm transmission from these experimental hosts to more economically important hosts such as tomato and potato. However, the EUPHRESCO DEP project will investigate some elements of this (R. Mumford, Fera, UK, *personal communication*, 2009).

20. What is the pest's potential as a vector of plant pathogens?

None.

STAGE 3: PEST RISK MANAGEMENT

21. How likely is the pest to continue to be excluded from the PRA area?

	Very likely	Likely	Moderately likely	Unlikely	Very unlikely
Under protection	☐	☐	☐	☑	☐
Outdoors	☐	☐	☑	☐	☐

CLVd has already entered the UK. The route of entry that led to the outbreaks in UK tomato crops in 2007 is suspected to be tomato seed, but this has not been proven. There were no outbreaks detected in the UK in 2008. However, there was a single outbreak in a tomato crop in 2009; the route of entry for which is unknown. CLVd may also have been brought in to the UK on asymptomatic ornamental hosts.

In the absence of suitable phytosanitary measures, CLVd is unlikely to

continue to be excluded from entering the UK. Exclusion from susceptible crops grown under protection is unlikely. However, given the currently-limited natural host range it is moderately likely to be excluded from those plants grown outdoors (see below).

Existing phytosanitary measures that may help prevent entry are described under section 13.

Detection of CLVd in seeds or planting material is a prerequisite for prevention of entry. Effective methods of detection are needed because of the asymptomatic nature of infection in the three known ornamental hosts, and to confirm the viroid in tomato seed or seedlings at the earliest possible stage of infection. Current diagnostic methods employed by laboratories in the EU comprise R-PAGE, nucleic acid hybridisation (Singh, 1983), or RT-PCR (Verhoeven *et al*. 2004). Robust molecular assays incorporating improved sensitivity of detection of CLVd (Scorpion and TaqMan assays) are currently being used at Fera and are subject to further development and sensitivity-testing for seeds and foliar material (A. Fox, Fera, UK, *personal communication*, 2009). As tomato seed is extremely expensive it would be prudent to await the results of these studies as well as the seed transmission studies/growing-on tests that are being undertaken at Fera/in the EU, before determining the best approach to a) excluding CLVd from the PRA area and b) preventing further entry/spread within the EU. One option may be testing of mother plants (J. Chard, SASA, *personal communication*, 2009) but the reliability of this would depend upon a) the rate of infection in seed and, b) the rate of transmission from seed to seedlings. Because of this it may be that a tiered screening approach including inspection/testing of mother plants, testing of seed, and inspection and testing of progeny would be required (A. Fox, Fera, *personal communication*, 2009). The details of this cannot be determined until all experimental work is completed. In the meantime the risk management options for each pathway of entry are discussed separately in turn:

<u>Tomato seed</u>

Entry into tomato crops grown under protection seems likely on tomato seed from third countries as well as possibly from seed produced within the EU. It is not confirmed, but highly suspected that CLVd is transmitted by seed as described in section 13. Acid-extraction or equivalent measures that are required for entry of tomato seed into the UK (EU) and for movement of seed within the EU are unlikely to be effective against anything other than external contamination of seed by CLVd. In the absence of an effective seed treatment, the alternative is for seed to be tested for CLVd, but this depends upon the availability of a robust and sensitive detection method, preferably using non-destructive techniques due to the potentially large numbers of expensive seed that may be required to be tested, depending upon the sensitivity of the test. This will be investigated if seed transmission is proven (A. Fox, Fera, *personal communication*, 2009).

Until all countries exporting tomato seed to the PRA area are able/willing to test tomato seed for CLVd, and phytosanitary measures regarding detection of CLVd in tomato seed from exporting countries are in place, it is suspected that CLVd will enter the PRA area in tomato seed. The viroid could be specifically tested for when adequate diagnostic tools are more widely-available. Seed treatments (e.g. heat) may also help prevent entry and will be investigated further if seed transmission is proven (HDC, 2008).

Tomato plants

Potential entry on tomato seedlings to the UK is only legally possible on plants from within the EU and Euro-Med countries. This is because third country imports of Solanaceous plants for planting are prohibited under EC legislation (Anon., 2000).

CLVd has already been reported as being present on tomatoes in the EU (the Netherlands – eradicated; Belgium, and France); on an ornamental host in Germany; and in the PRA area (eradicated on tomatoes in 2007, new outbreak 2009). The full status of CLVd in the EU and non-EU Euro-Med countries is not known, as it has not been subject to surveys. Very young tomato plants are likely to be asymptomatic so there is still potential for symptomless infected tomato plants (seedlings) to enter the UK from other EU and Euro-Med countries. Older plants (being grown-on at production nurseries) are likely to be symptomatic.

Prevention of further entry of CLVd on tomato plants into the UK requires surveillance and testing to determine the distribution of CLVd at tomato propagation nurseries within the EU and Euro-Med countries. Where CLVd is known to occur, pest-free places of production would be required to limit further movement. Robust methodology to test tomato seedlings prior to movement from the place of production would need to be implemented. Experimentally-infected tomato plants can be detected by Scorpion assay from three weeks post-inoculation which may be helpful for future diagnostic testing of seedlings at places of production (A. Skelton, CSL (now Fera), UK, *personal communication,* 2008). Fera is continuing to develop the molecular methods that can be used to test foliar material for CLVd and it is hoped that these will ultimately become more widely-available (A. Fox, Fera, UK, *personal communication*, 2009).

Ornamental hosts

All three known ornamental hosts are asymptomatic. CLVd-infected, asymptomatic ornamental plants could therefore potentially enter the PRA area.

Entry on asymptomatic natural hosts that are not solanaceous can occur from third countries as well as from countries within the EU. Currently those that are known are *C. erythrophae* (reported from the USA) and *N. wettsteinii* (reported from Canada). Testing of *Columnea* cultivars from nurseries in unspecified European countries have been reported to be positive for a CLVd-

related viroid so the pathogen may already be present on at least this host genus in the EU.

Those ornamental hosts that are solanaceous can enter from EU countries and non-EU Euro-Med countries. Currently, the only known solanaceous ornamental natural host is *B. undulata* reported from Germany.

A Dutch survey of ornamentals in the families Gesneriaceae and Solanaceae in 2006 did not detect CLVd (Verhoeven *et al*., 2008).

Measures to ensure freedom of the viroid from plants, and seed (if found to be seed-borne) would be required to prevent entry to the PRA area and this would again rely on robust detection methodology such as that being developed at Fera (A. Fox, Fera, UK, *personal communication*, 2009) as well as the development of seed treatments if appropriate.

Experimental hosts.
Although experimentally susceptible, there are no records of CLVd in potato and cucumber and so there are no specific measures that can be implemented without an identifiable origin. Similarly, the few ornamental species that have been tested and found to be experimentally-susceptible cannot be subject to phytosanitary measures to prevent further entry to the UK.

22. If the pest enters or has entered the PRA area how likely are outbreaks to be eradicated?

	Very likely	Likely	Moderately likely	Unlikely	Very unlikely
Under protection	☐	☑	☐	☐	☐
Outdoors	☐	☐	☑	☐	☐

It is likely that outbreaks could be successfully eradicated in protected crops and moderately likely outdoors. The four 2007 UK outbreaks in tomato have not shown any re-occurrence on the affected premises to date and have been declared eradicated. The 2009 UK outbreak in tomato is still subject to eradication.

Success would depend on the host, location (protected environment or outdoors) and how early the outbreak was discovered.

Outbreaks in protected crops would be contained and an eradication programme could be implemented during and at the end of the cropping season.

The outbreak may be more difficult to eradicate should it occur in an outdoor crop such as potato (an experimental host), but this depends firstly upon

when symptoms develop, and on the overwintering potential in volunteer potatoes and in weeds, which has not been studied, but may be limited. These could be destroyed once symptoms are detected and infection by CLVd is proven. However, elimination of volunteer potatoes from a field can take many years in an arable rotation.

Survival in seed potato tubers stored over the winter months may protract the problem, depending upon whether the viroid is systemic and causes symptoms in emerging/emerged plants in the first year after planting. If symptom development does not occur in the first year after planting, or goes undetected, this has potential to disseminate the viroid in second generation seed.

There is also potential for CLVd to be spread between seed potato crops on sap contaminating machinery or the clothing and hands of seed potato inspectors or growers. This infection would only become apparent if symptoms of infection developed in the same growing season. If not, then spread and further establishment may occur in harvested seed tubers.

Survival in ware potatoes would only be a problem where they are kept as home-saved seed over winter and the viroid is systemic and causes symptoms in the emerging crop. Ware potatoes are only likely to be used as farm-saved seed in the first year after harvest and so if symptoms develop, destruction of the crop and control of volunteers and weeds that may harbour the viroid should eradicate the viroid.

One further problem is whether or not the viroid is spread by insects, a topic which is being studied for PSTVd in the EUPHRESCO DEP Project (R. Mumford, Fera, UK, *personal communication*, 2009). If this is the case it would be more difficult to eradicate the viroid in outdoor-grown crops as it may have spread more widely than the affected crop.

The first step in eradicating the viroid is to destroy infected plant material and any uninfected hosts in the vicinity of the affected plants and to dispose of the material safely.

For protected crops, growing media should be safely disposed of and the glasshouse/polytunnel etc thoroughly cleaned and decontaminated.

Sodium hypochlorite (1-3%) is effective at preventing mechanical transmission via sap even when high viroid inoculum levels are present (Hadidi *et al*., 2003). It has been shown to have efficacy as a disinfectant against PSTVd at >0.5% (HDC, 2006). However, sodium hypochlorite is corrosive to the structure of the glasshouse and has to be rinsed off thoroughly after use. A number of other products that have been shown to be effective when tested as disinfectants on various surfaces against PSTVd (HDC, 2006) may be effective against CLVd, but the use of disinfectant products needs to be coupled with thorough cleaning.

The viroid is readily transmitted by contact. Measures to limit contact with infected plants (e.g. the use of disposable gloves and coats etc. when working in the crop) and spread to uninfected plants and surfaces (e.g. disinfection of tools and boots etc.) would have to be implemented.

Control of volunteer tomato seedlings in the following season and Solanaceous weeds in and around glasshouses/polytunnels should be undertaken to prevent them being reservoirs of inoculum.

Separation of ornamental species, especially in the Solanaceae, from tomato fruit crops and propagation material is highly advisable. This is good practice at propagation nurseries in the UK.

Separation of foreign tomato fruit brought into tomato production nurseries would reduce/minimise the risk of sap transfer. Most UK packing sites have strict controls on this.

Outbreaks of CLVd in tomato crops have already occurred in the PRA area for which eradication measures have been implemented. These included recommendations regarding restrictions on the marketing of tomato fruit and the movements of staff, together with hygiene measures. Measures to prevent re-infection have included: landfill disposal of all infected and associated plants; landfill disposal or recycling for non-horticultural use of rockwool slabs; disinfection of irrigation pipes; pressure-washing and disinfection of flooring in glasshouses; cleaning and disinfection of the glasshouse structure; and, elimination of volunteer tomato seedlings (Matthews-Berry, 2007). The 2009 UK outbreak is still subject to eradication.

Chemical methods of control aimed at eradicating infection from plants and preventing spread have not been evaluated for effectiveness and cost-effectiveness for CLVd. In one experiment on PSTVd, chitosan reduced infection rates in tomato by 50-75% when sprayed within 3 hours of inoculation (no field evaluation data available), (Hadidi *et al*., 2003), but this approach would not be practical on a commercial scale.

Some success has been reported for the eradication of viroids affecting tomatoes. For example, CLVd was eradicated from tomato crops in the Netherlands, albeit details of measures were not described) (Verhoeven *et al*. 2004). PSTVd was eradicated from a UK outbreak in a tomato crop in 2003. Recommendations for eradication of CLVd in the UK in 2007 were in line with action undertaken for this outbreak (Matthews-Berry, 2007).

23. If eradication is not possible, what management options are available for containment and/or non-statutory control?
Containment and/or non-statutory control relies firstly on detection of the viroid in plants and seed of known natural hosts.

The three known ornamental hosts were all detected because of molecular detection/surveys for viroids and not because they were symptomatic; thus, detection in these hosts will rely on robust molecular methods. Tomato is symptomatic but experimental work has shown symptoms developing 3 to 5 weeks after inoculation (Verhoeven *et al*., 2004). The viroid can be detected in tomato by molecular methods 3 weeks post-inoculation (A. Skelton, CSL, (now Fera), UK, *personal communication*, 2008) and detection methodology for plants and seed of tomato is available and is being further developed at Fera; methodology for detection in ornamental plant and seeds will also be developed (A. Fox, Fera, UK, *personal communication*, 2009).

Following on from detection of the viroid, removal and destruction of known infected plant material would be necessary.

The use of disinfectants for tools, equipment and surfaces, such as those recommended for PSTVd (HDC, 2006) following thorough removal of infected plant material and crop debris would also help prevent spread of CLVd.

CLVd is spread in sap and possibly by seed transmission. Thus, strict hygiene measures for tools, equipment and manual handling of plants by workers on affected premises would be needed to limit further spread. Such measures should ideally be implemented as a matter of course, before infection is detected due to the delay between infection and symptom expression and the potential for cryptic spread. Transmission by insect vectors and pollen is being investigated for PSTVd in the EUPHRESCO DEP Project (R. Mumford, Fera, UK, *personal communication*, 2009); depending upon the results there may need to be additional measures to contain or manage an outbreak of CLVd.

Further exclusion of the viroid from planting material relies on accurate diagnosis both in plants and seeds; this is being developed at Fera. Effective seed sampling procedures, and proficiency of laboratories responsible for seed testing and testing of plants for planting using molecular methods would help prevent movement of the pathogen in commercially-traded material. Seed treatments for tomato seed (including heat treatment) will be investigated if seed transmission is proven (HDC, 2008).

If no statutory phytosanitary controls are implemented, the viroid could be specifically included in domestic certification schemes. However, neither tomato or the known natural ornamental hosts of CLVd currently have an EPPO or a national certification scheme in the PRA area. Practical procedures for certification again rely on adequate methods of detection. Most certification schemes rely on the initial starting material for propagation (nuclear stock) having been tested and found free from a list of important pathogens – the plants are then visually inspected during further multiplication to check that they remain free from infection. Maintenance of high standards of plant health in ornamental crops may require initiatives to make exporters and importers of ornamentals aware of the risks. Methods are still required to

detect and eliminate viroids from infected material.

Tissue culture methods could play an important part of a non-statutory control programme. Work with another *Pospiviroid*, PSTVd, in potato showed that meristem culture of infected plants yielded 53% of PSTVd-free potato plantlets following a 6-month cold treatment (6-8^{o}C) of infected plants (Lizaragga *et al*. 1980).

There have been no investigations of potential sources of resistance to CLVd in tomato or the known natural hosts.

Transgenic approaches to produce CLVd resistant plants have not yet been examined and are very unlikely to be accepted by consumers in the UK/Europe. Where transgenic approaches have been explored for a *Pospivioid* (PSTVd) some success has been achieved in experiments with potato but the resistance was inefficient in tomato (Yang *et al*. 1997). Field trials of transgenic potato lines for PSTVd resistance have been inconsistent (Hadidi *et al*., 2003).

24. Conclusion and recommendations

CLVd has been reported from asymptomatic ornamentals in Canada, the USA and Germany; and, on symptomatic tomato crops in Belgium, the Netherlands (eradicated), France and the UK.

The first findings in tomato were reported retrospectively on diagnostic samples tested in the Netherlands, from the Netherlands and Belgium, in 2004. No data were given for losses experienced in the Netherlands or Belgium but the affected plants were symptomatic and showed reduction in growth. CLVd was detected for the first time in the UK in 2007 in four tomato crops and was subject to eradication which was successful. One of the affected growers reported a 20% reduction in yield compared to 2006; this did not account for seasonal differences. No experimental work has been published regarding the effect on yield. No symptoms were reported on tomato fruit; however, like yield, there are no experimental data reported in the literature regarding the effect of CLVd on tomato fruit quality. I In July 2009, a further outbreak occurred in the UK at a different site to those affected in 2007 and this is subject to eradication; no details on the potential quality or yield losses were reported. In September 2009, a report of the first findings of CLVd in tomato crops in France, dating back to 2007, was made (Steyer *et al*., 2009), again with no details of the potential impact on the crop. From observations of the 2007 UK outbreak it seems likely that CLVd has the potential to cause significant reductions in yield of tomato crops.

The viroid was previously reported infecting asymptomatic ornamental hosts (*C. erythropae* - USA, reported 1978, and *N. wettsteinii* - Canada - reported 1992; both members of the Gesneriaceae; and, *B. undulata* - Germany - reported 1996, a member of the Solanaceae). Sampling of *Columnea* cultivars from European nurseries (country/countries of origin not described)

indicated the presence of a CLVd-related viroid. However, no further information is available for these findings. It is possible that CLVd may be present in European countries in asymptomatic ornamental plants. These are potential reservoirs of infection for tomato fruit crops and plants for planting. However, the risk of spread from these plants to tomato in the UK is low where good nursery practice is deployed, especially avoiding growing ornamental plants adjacent to tomato fruit crops or at propagation nurseries.

Prior to the first outbreaks in tomato in the Netherlands, CLVd had been shown by experiment to be transmissible to tomato, as well as to potato (Solanaceae) in which it was symptomatic and showed potential for yield and quality reductions. Experimental transmission was shown to cucumber (Cucurbitaceae) as well as to two ornamental species (*G. aurantica* - Asteraceae and *S. sinensis* - Solanaceae) all of which developed symptoms as a result.

The status of CLVd in ornamental plants in the UK is unknown but it is considered to have been introduced to tomato for the first time in 2007, possibly on infected tomato seed which was imported from Taiwan via France. Outbreaks of CLVd occurring in tomato crops in France in 2007 were recently-published but the route of entry for these outbreaks is unknown; similarly the route of entry for the 2009 UK outbreak in tomato crops is unknown. Seed transmission has not yet been proven but is suspected and is subject to investigation as part of an HDC-funded research project at CSL (now Fera) (HDC, 2008).

CLVd has the potential to establish in protected tomato crops as well as in the three known ornamental hosts which are grown under protection. The fundamental problem is that CLVd is asymptomatic in these ornamental plants and because it is (at least) mechanically transmitted it has the potential to be spread by humans, tools and machinery from these to tomato fruit crops and possibly to tomato plants for planting where these are grown on the same premises. Nevertheless, UK tomato growers are well-versed in the potential for spread of viruses and viroids to their crops, particularly based upon their experiences with PepMV and PSTVd and the industry has provided evidence-based guidance (HDC, 2006) on how to limit the potential for these and viroids such as CLVd to spread to their crops.

Other modes of transmission (including by insects, as well as further work on mechanical transmission through cultural practices) are being investigated using PSTVd (as a model for CLVd) in the EUPHRESCO DEP Project (R. Mumford, Fera, UK, *personal communication*, 2009).

CLVd is not specifically regulated by the UK/EC or by any national or regional plant protection organisation and is thus not subject to specific phytosanitary requirements. However, there are UK/EC requirements for solanaceous hosts as described in section 13 which may have some impact on preventing entry of CLVd to the UK (and EU).

Considering the known natural hosts as well as the existing phytosanitary requirements the potential pathways of entry for CLVd and the recommendations for measures for individual pathways are:

a) Tomato seed from outside or within Europe. The EC requirements for acid extraction of seed or equivalent measures will only potentially affect external contamination and not systemic or possibly seed-coat infection. If CLVd is confirmed to be truly seed-borne in tomatoes, measures to facilitate freedom of the pathogen on seed from exporting countries, particularly from those countries where the viroid is suspected to originate, are required. Official testing of seed will however rely on the technology available at official seed testing laboratories. Good detection methodology for CLVd is available at Fera and is being ring-tested; seed treatments will also be investigated if transmission is proven (HDC, 2008). Measures will also require visual inspection of the seed crop in the producer country.
It is recommended to consider CLVd for listing as a IAI quarantine pathogen. It is recommended to include CLVd in the tomato seed requirements within Annex IVAI and Annex IVAII with specific requirements for inspection of seed crops and testing of seed. Seed treatments may need to be part of the requirements depending upon the outcome of HDC (2008).

b) Tomato plants which are permitted entry from within the EU or the European or Mediterranean area. Symptoms of CLVd infection are only likely to be detected at least several weeks after infection has occurred. Detection methodology is available at Fera. It is clear from UK and Dutch outbreaks that CLVd poses a significant risk to tomato crops in the PRA area. Therefore continuing statutory action under Article 16(2) of the 2000/29/EC Directive against future outbreaks of the viroid in tomato crops is recommended with a view to securing EC listing. ***It is recommended to consider CLVd for listing as a IAI quarantine pathogen with specific requirements for plants for planting of tomato in Annex IVAI as well as IVAII.***

c) Non-solanaceous ornamental plants from within the EU, and at least from North America. Known hosts are *C. erythrophae* and *N. wettsteinii.* These are asymptomatic. Sampling of selected *Columnea* cultivars obtained from European nurseries (country/countries of origin not described) indicated the presence of a CLVd related viroid, so CLVd may already be present in the EU in *Columnea* spp. There may be other natural hosts which are asymptomatic and this information would only emerge based upon surveys using molecular detection methods. The Netherlands has undertaken a survey for viroids in a range of ornamental plants from the Gesneriaceae (and Solanaceae) and CLVd was not found, although other viroids were (Verhoeven *et al*., 2008). ***A UK/EU survey of the distribution of the viroid in ornamental hosts could be considered. This will require testing of asymptomatic material using a robust diagnostic technique (likely to be developed at Fera).***

d) Solanaceous ornamental plants from within the EU and the European-Mediterranean area. The Netherlands has undertaken a survey for viroids in a range of ornamental plants including those in the Solanaceae and CLVd was not found, although other viroids were (Verhoeven *et al*., 2008). ***A UK/EU survey of the distribution of the viroid in ornamental hosts could be considered. This will require testing of asymptomatic material using a robust diagnostic technique (likely to be developed at Fera).***

e) Seed of solanaceous and non-solanaceous ornamental hosts. There are no experimental data to show that this is a pathway but based upon the potential entry of CLVd on tomato seed to the UK it is a strong possibility. ***A UK survey of the distribution of the viroid in seed of ornamental hosts could be considered. This will require testing of seed using a robust diagnostic technique (likely to be developed at Fera).***

Pending the results of experimental work being carried-out at Fera and in the EU on seed-transmission studies (seed to seedling, seedling to plant, plant to seed) and the numbers of seed that need to be sampled to detect CLVd, it might be appropriate to have a multi-layered approach to determining pest-freedom in seed, the aim being to reduce the quantity of seed that would need to be tested. The details of this can only be determined when the experimental work is completed, most likely in 2010. (A. Fox, Fera, personal communication, 2009).

CLVd has been shown to be transmissible to potato and cucumber but there are no records of CLVd in these species. Imports of tubers of potato from outside of the EU are prohibited except from Switzerland (Annex IIIA, Article 10, Anon., 2000) and plants of Solanaceae are prohibited from third countries except those in the non-EU European and Mediterranean area (Annex IIIA, Article 13, Anon., 2000). There are no EC prohibitions on imports of cucumber plants or seed. Nevertheless these pathways of entry are hypothetical whilst no records of CLVd have been reported for these experimentally-susceptible hosts.

There are no official records of CLVd in any other country but there is some information that suggests it may be present in other locations, particularly Taiwan, the source of seed for the UK outbreaks in tomato in 2007. This seed was imported via France – a country which is now known to have experienced outbreaks of CLVd in tomato crops in 2007. CLVd may originate in the Americas as it was first reported in *Columnea erythrophae* which originates from Central America. NCBI accessions exist for tomato plants in Portugal but with no provenance stated. Accessions also exist for two tomato isolates which were detected on seed imported into Thailand which was subsequently destroyed. However, the origin of the seed is unknown. Until official reports of CLVd are made no specific pathways of entry can be identified and no recommendations for regulation specific to these countries can be made.

Whilst these recommendations are considered it is advised that commercial

growers of tomato fruit and plants for propagation be vigilant and report unusual symptoms to their local Plant Health and Seeds Inspector. Plants should be tested for CLVd and where confirmed, measures aimed at eradication should be implemented.

It is advised that the finalisation of the proposed measures be determined once the experimental work on detection methodology and seed transmission is completed.

Further work that would help reduce uncertainties:

Area of PRA	Uncertainties	Further work that would reduce uncertainty
Taxonomy	Relationship of tomato isolates of CLVd to CLVd-bru and CLVd-nem.	A study to generate sequence data for CLVd isolates from UK tomato outbreaks and to analyse the relationship with ornamental isolates and tomato isolates from the Netherlands as well as accessions in the NCBI database.
Distribution	The geographic distribution is uncertain because of the asymptomatic nature of infection in the ornamental hosts that are currently known, as well as the lack of information on the full potential host-range of the viroid and its variants. Detection methodology for CLVd varies in its efficiency so determining distribution may be difficult.	Detection methodology needs to be refined for asymptomatic ornamental hosts (this is proposed at Fera) and the outputs from the detection methodology study for tomato seed and seedlings needs to be made available once completed (2010, HDC Project PC 294). Surveys of tomato crops in EU countries and testing of (at least) the three known ornamental hosts would help determine distribution. Host-range testing to determine what other asymptomatic hosts may be acting as reservoirs would be helpful.
Hosts	The full host range of CLVd-nem, CLVd-bru and CLVd from tomatoes and *Columnea* is not known.	Host range testing for each isolate.
Pathways	Full geographic distribution is unknown.	Detection methodology, host-range testing and surveys.
Establishment	The ability of CLVd to survive outdoors in the PRA area is unknown.	Quarantine contained experiments to determine the ability of the viroid to survive over winter in UK conditions in infected plant material (e.g. inoculated potato plants and tubers).
Spread	Seed transmission is uncertain for tomato although suspected. Detection methodology for CLVd varies in its efficiency, thus seed-testing laboratories for tomato may not detect CLVd. Spread may also be possible in very young seedlings of tomato, which can be asymptomatic for up to 3-5 weeks post-inoculation; spread will also occur in asymptomatic ornamental hosts. Ornamental seed transmission has not been examined. Epidemiology of the disease is uncertain, including	Experiments should be conducted to conclusively determine whether the pathogen is transmitted in seed (especially tomato), also for ornamental and possibly cucumber seed. Determination of whether CLVd is vectored by aphids (as some other viroids e.g. PSTVd are known to be aphid-borne.). HDC Project PC 294 should help elucidate aspects related to tomato seed and seedling detection and transmission in seed. The

	whether it is vector borne or not.	EUPHRESCO DEP project may elucidate the potential for transmission (based upon PSTVd) by insect vectors either through feeding or through spread in pollen by bees.
Impact	Few data are available on the impact of viroids on crop plants. Observations suggest severe yield losses in tomato and the potential for yield and quality loss in potato. No comparative data are available for the different variants of CLVd.	Comparative trials conducted to determine yield and quality losses in cultivars of the major crop plants (tomato, and the experimental hosts potato and cucumber) using CLVd from tomato, *Columnea* and CLVd-bru and CLVd-nem.
Management	Destruction of infected plants is the primary means of managing the pathogen. Efficacy of chemical disinfectants is uncertain for CLVd. Efficacy of acid extraction and sodium hypochlorite treatments currently used for other pathogens of tomato seed is unknown but is only likely to be effective against surface contamination. No data on cultivar resistance for tomato. No data on the efficacy of tissue culture, heat and cold treatments or transgenics for tomato or other natural or experimental hosts.	Experiments to investigate these different elements are required. HDC Project PC 294 includes an investigation of tomato seed treatments for CLVd if seed transmission is proven.

References

Anon, 2000 (*as amended*). 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. *Official Journal of the European Communities.* **43 no. L 169**, pp. 1 - 112.

Anon., 2004. Commission Decision 2004/200/EC of 27 February 2004 on measures to prevent the introduction and spread within the Community of *Pepino mosaic virus*. *Official Journal of the European Union*. **L64, 43-44.**

CSL, 2008. Central Science Laboratory Internal Report. Pathogen Diary, CSL ref: 20720666.

Defra, 2007. Responsibility and Cost sharing options for Quarantine Plant Health.
http://www.fera.defra.gov.uk/plants/plantHealth/documents/strategy05.pdf

Defra, 2008. Agriculture in the UK 2007, Tables & Charts. Potatoes in the UK. Table 5.11. Update of 27 March 2008.
https://statistics.defra.gov.uk/esg/publications/auk/2007/excel.asp

Defra, 2008a. Basic Horticultural Statistics 2008**.** Protected vegetables: planted area, marketed yield per planted hectare and home production marketed (hpm) for the calendar year in the UK. Table 14
https://statistics.defra.gov.uk/esg/publications/bhs/2008/vegetable%20details.pdf

Defra, 2008b. Basic Horticultural Statistics 2008. Vegetables: Suppliers of cabbages, cauliflowers, carrots, mushrooms, lettuce & tomatoes for the calendar year in the UK. Table 20.
https://statistics.defra.gov.uk/esg/publications/bhs/2008/vegetable%20details.pdf

EPPO, 2008. European and Mediterranean Plant Protection Organisation website. First report of *Columnea latent viroid* on tomatoes in the United Kingdom. http://archives.eppo.org/EPPOReporting/2008/Rse-0801.pdf

EPPO, 2009. Pest Risk Analysis for *Metamasius hemipterus.* Version of 28 April 2009. 75pp.

Hadidi A, Flores R, Randles JW, Semancik JS (Eds.), 2003. Viroids. CSIRO Publishing, Victoria, Australia. 370 pp.

Hammond R, Smith DR, Diener TO, 1989. Nucleotide sequence and proposed secondary structure of *Columnea latent viroid*: a natural mosaic of viroid sequences. *Nucleic Acids Research,* **17**, 10083-10093.

HDC, 2006. *Potato spindle tuber viroid* in tomato and new viroid reports. Factsheet 09/06, Tomatoes, Project no. PC 212. Horticultural Development Council, UK. 8PP.

HDC, 2008. Detection and elimination of solanaceous viroids in tomato seeds and seedlings. Horticultural Development Company, Project PC294. (Period covered 1 October 2008 to 30 September 2010).

ICTV, 2005. Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA, 2005. Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, 1259pp.

Kamphuis BM, 2005. The seed sector in the Netherlands - an overview of production, trade and related institutions. Report of the Agricultural Economics Research Institute (LEI).

Lizaragga R.E, Salazar S.F, Roca W.M, and Schilde-Rentschler L . 1980. Elimination of *Potato spindle tuber viroid* by low temperature and meristem culture. *Phytopathology,* **70**, 754-755.

Mabberley DJ, 1997. The Plant-Book. Second Edition. Cambridge University Press. 858pp.

Matthews-Berry, 2007. Report of a visit to a UK CLVd-outbreak in tomato. Internal CSL Report. 7pp.

Mumford RA, Jarvis B, Skelton A , 2004. The first report of *Potato spindle tuber viroid* (PSTVd) in commercial tomatoes in the UK. *Plant Pathology,* ***53,*** 242-242.

NCBI, undated. National Center for Biotechnology Information website. http://www.ncbi.nlm.nih.gov/

Nixon T, Glover R, Mathews-Berry S, Daly M, Hobden E, Lambourne C, Harju V, Skelton A, 2009. *Columnea latent viroid* (CLVd) in tomato: the first report in the United Kingdom. *New Disease Reports* [http://www.bspp.org.uk/publications/new-disease-reports/volumes.php] Volume 19.

Owens R, Smith DR and Diener, TO, 1978. Measurement of viroid sequence homology by hybridisation with complementary DNA prepared *in vitro. Virology,* **89***,* 388-394*.*

RHS, undated. Royal Horticultural Society Plant Finder website. *http://www.rhs.org.uk/rhsplantfinder/plantfinder.asp.*

Singh RP 1983. Viroids and their potential danger to potatoes in hot climates.

Canadian Plant Disease Survey, **63**, 13-18.

Singh RP, Boucher A, 1988. Loss of *Potato Spindle Tuber Viroid* from tuber tissues after repeated freezing. *American Potato Journal,* **65**, 283-287.

Singh RP, Lakshman DK, Boucher A, Tavantzis SM, 1992. A viroid from *Nematanthus wettsteinii* plants closely related to the *Columnea latent viroid. Journal of General Virology,* **73**, 2769-2774.

Spieker RL, 1996. A viroid from *Brunfelsia undulata* closely related to the *Columnea latent viroid*. *Archives of Virology,* **141**, 1823-1832.

Steyer S, Olivier T, Skelton A, Nixon T, Hobden E, 2009. *Columnea latent viroid* (CLVd): first report in tomato in France. *New Disease Reports* [http://www.bspp.org.uk/publications/new-disease-reports/volumes.php]. Volume 20.

Verhoeven J Th J, Jansen CCC, Willemen TM, Kox LFF, Owens RA, Roenhorst JW, 2004. Natural infection of tomato by *Citrus exocortis viroid*, *Columnea latent viroid*, *Potato spindle tuber viroid* and *Tomato chlorotic dwarf viroid*. *European Journal of Plant Pathology*, **110**, 823-831.

Verhoeven J Th J, Jansen CCC, Roenhorst JW, 2008. First report of pospiviroids infecting ornamentals in the Netherlands: *Citrus exocortis viroid* in *Verbena* sp., *Potato spindle tuber viroid* in *Brugmansia suaveolens* and *Solanum jasminoides*, and *Tomato apical stunt viroid* in *Cestrum* sp. *Plant Pathology*, **57**, 399-399.

Yang X, Yie Y, Zhu F, Liu Y, Kang L, Wang, X, and Tien P, 1997. Ribozyme-mediated high resistance against *Potato spindle tuber viroid* in transgenic potatoes. *Proceedings of the National Academy of Science*, *USA,* **94**, 4861-4865.

Version 1:

Pest Risk Analysts:
Claire Sansford, Jane Morris.

Reviewers and/or Advice: Neil Giltrap, Peter Reed, Adrian Fox, Sharon Matthews-Berry, Rick Mumford, Marcus Lazenby.

Date: 15th May 2009

Version 2:

Revision of Version 1: Claire Sansford
Reviewer/advice: Adrian Fox

Address: Food and Environment Research Agency, Sand Hutton, York, YO41 1LZ UK.

Date: 13th October 2009

Version 3:

Minor revision of Version 2: Claire Sansford

Address: Food and Environment Research Agency, Sand Hutton, York, YO41 1LZ UK.

Date: 21st January 2010