Appendix to:

EFSA (European Food Safety Authority), 2016. Conclusion on the peer review of the pesticide risk assessment of the active substance Mild Pepino mosaic virus isolate VX1. EFSA Journal 2016;14(14):4650, 16 pp. doi:10.2903/j.efsa.2016.4650

Appendix A – List of end points for the active substance and the representative formulation

Chapter 1 Identity, Biological properties, Details of Uses, Further information

Active micro-organism	Mild Pepino mosaic virus isolate VX 1
Function (e.g. control of fungi)	Elicitor (Control of aggressive Pepino mosaic viruses)

Identity of the micro-organism (Annex IIM 1)

Name of the organism	*Pepino mosaic virus*
Taxonomy	Potex viruses
Species, subspecies, strain:	Genus: *Potexvirus* Family: *Alphaflexiviridae* Order: *Tymovirales*
Identification	ELISA qRT-PCR bioassay (infectivity on tomato)
Culture collection	German Collection of Micro-organisms and Cell Cultures (DSMZ) Reference no. DSM 26974.
Minimum and maximum concentration of the MPCA used for manufacturing of the formulated product (cfu; g/kg):	10-50 mg/L $1.5x10^{11}$ to $7.5x10^{11}$ virus particles/mL
Identity and content of relevant impurities, additives, contaminating organisms in the technical grade of MPCA:	Nicotine < 0.1 mg/L
Is the MCPA genetically modified; if so provide type of modification:	no

Biological properties of the micro-organism (Annex IIM 2)

Origin and natural occurrence	*Pepino mosaic virus* is first reported in Peru but probably present world-wide. Mild Pepino mosaic virus VX 1 is a mild variant of the Peruvian (LP) strain. Mild Pepino mosaic virus VX 1 has been isolated from a tomato plant in a greenhouse in the Westland (NL) in 2004. Background levels are unknown but the virus is present and replicates efficiently on *Solanaceae* and can survive for short times on plants of other botanical families as well.
Target organism(s)	*Pepino mosaic virus* targets*, Solanum lycopersicum* (tomato)*, Solanum muricatum* (pepino). The isolate VX1, prevents infection with virulent strains.
Mode of action	Mild *Pepino mosaic virus* induces plant resistance, most likely through induction of a RNA silencing process which destroys RNAs of all closely related *Pepino mosaic virus* strains
Host specificity	Besides the target hosts tomato and pepino the *Pepino mosaic virus* can survive for short times on plants of other botanical families as well.
Life cycle	*Pepino mosaic virus* propagates on viable host tissue. After transmission the virus enters tissue through (damaged) epidermis. Viral single stranded RNA is copied and integrated in host genome. The proposed mode of action is that host cells synthesise small interfering RNA which are incorporated in a RNA–induced silencing complex. This complex destroys RNA of invading (closely related variants of) *Pepino mosaic virus*.
Infectivity, dispersal and colonisation ability	*Pepino mosaic virus* is highly infective for plants belonging to the *Solanaceae. Pepino mosaic virus* might survive or replicate also on plants of other botanical families but without causing adverse effects.
Relationships to known plant, animal or human pathogens	None. Potex viruses are plant viruses not known to interfere with animal or human systems.
Genetic stability	As with all RNA viruses genetic mutation may occur. Risk management procedures are in place to prevent the occurrence of virulent strains in the end product.
Production of relevant metabolites/toxins	Viruses do not produce metabolites.
Resistance/sensitivity to antibiotics used in human or veterinary medicine	Not relevant for plant viruses.

Summary of intended uses

PPP (product name/code)	**V10**	**Formulation: Type:**	**SC**
active substance 1	**Mild PepMV isolate VC1**	**Conc. of as 1:**	**5-25 mg/L**
active substance 2	**Mild PepMV isolate VX1**	**Conc. of as 2:**	**5-25 mg/L**
Zone(s): EU		**professional use** ☒	**non professional use** ☐

1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*10*	*11*	*12*	*13*	*14*
Use-No.	**Member state(s)**	**Crop and/ or situation (crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**			**Application rate**			**PHI** (days)	**Remarks:**
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	kg, L product / ha a) max. rate per appl. b) max. total rate per crop/season	g, kg a.s./ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		e.g. g safener/synergist per ha
1	All	*Solanum lycopersicum* (tomato)	G	*Pepino mosaic virus*	Downward Spraying (12-15 bar)	Young tomato plants (BBCH 13-51, 10-30 cm high) Jan-Dec	a) 1 per crop cycle b)* 8 per 12 months (8 crop cycles per year)	a) 70 L product / ha per appl. b) 560 L product / ha per season	a) 1.75 g a.s.1/ha per application 1.75 g a.s.2/ha per crop cycle b) 14 g a.s.1/ha per season 14 g a.s.2/ha per season	3500 L/ha per appl.	-	1-8 crop cycles per 12 months V10 is applied in combination with 800 grams of carborundum per 100 litres of spray liquid
2	All	*Solanum lycopersicum* (tomato)	G	*Pepino mosaic virus*	Rubbing individual plants	Young tomato plants (BBCH 13-61) Jan-Dec	a) 1 per crop cycle b)* 8 per 12 months (8 crop cycles per year)	a) 0.8 L product / ha per appl. b) 6.4 L product / ha per 12 months	a) 0.02 g a.s.1/ha per application 0.02 g a.s.2/ha per crop cycle b) 0.16 g a.s.1/ha per season 0.16 g a.s.2/ha per season	8 L/ha per appl.	-	1-8 crop cycles per 12 months V10 is applied in combination with 15 grams of carborundum per 1 litre of liquid

*V10 is applied once per tomato plant. The product can be applied at tomato plant propagation or after transplant to tomato production greenhouses, as long as the application takes place before BBCH 51. Plant propagation companies can have up to 8 productions per 12 months. Tomato producing companies have 1-3 crops per year.

Further information

Proposal for classification and labelling	'Micro-organisms may have the potential to provoke sensitising reactions' P280c Wear protective gloves and protective clothing.

Chapter 2 Analytical Methods

Analytical methods for the micro-organism (Annex IIM 4.2.8; 4.3; IIIM 5.3)

Manufactured micro-organism (principle of method)	DAS-ELISA; qRT-PCR;
Impurities and contaminating micro-organisms in manufactured material (principle of method)	Nicotine: GC-MS (validation report due in September 2016) Additives: IC Contaminating micro-organisms: ISO methods
Plant protection product (principle of method)	DAS-ELISA; qRT-PCR;

Analytical methods for residues (viable and non-viable) in exposed compartments and organisms (Annex IIM 4.5)

of the active micro-organism (principle of method)	Not necessary.
of relevant metabolites/toxins (principle of method)	Not necessary (no metabolites).

Chapter 3 Effects on Human Health

Effects on human health (Annex IIM 5; IIIM 7)

Medical data and direct observation, e.g. clinical cases (Annex IIM 5.2)	No known reports of adverse effects.
Medical surveillance on manufacturing plant personnel	No known reports of adverse effects.
Sensitisation/allergenicity observations, if appropriate	No reports of allergenic observations. Based on common knowledge on micro-organisms repeated exposure may lead to provocation of sensitising reactions.
Acute toxicity, pathogenicity and infectiveness	
Acute oral toxicity, pathogenicity and infectiveness	LD50, oral > 2000 mg/kg bw
Acute inhalation toxicity, pathogenicity and infectiveness	LD50, inhalation >5.04 mg/L
Acute dermal toxicity:	LD50, dermal > 2000 mg/kg bw
Intraperitoneal/subcutaneous single dose	In a literature study it was found that after intravenous application in mice plant virus particles were found in a wide variety of tissues, but no adverse effects were seen and a rapid clearance from the body was observed.
Genotoxicity (Annex IIM 5.3.5)	Not genotoxic in Ames test with and without metabolic activation with S9.
Cell culture studies (Annex IIM, point 5.3.6)	No data (not necessary).
Information on short term toxicity and pathogenicity (Annex IIM, point 5.3.7)	No data (not necessary).
First aid measures, medical treatment (Annex IIM, point 5.2.5)	AFTER INHALATION: In case of respiratory problems, provide victim with fresh air and place in resting position that facilitates breathing. In case of respiratory symptoms: Contact an ANTI-POISON CENTRE or a doctor. AFTER SKIN CONTACT: wash with abundant water and soap. In case of skin irritation or rash: Contact a doctor
Specific toxicity, pathogenicity and infectiveness studies (Annex IIM, point 5.5)	*In vitro* skin corrosion: non-corrosive

Reference values

ADI, ARfD and AOEL	Not needed for *Mild Pepino Mosaic* virus

Exposure scenarios

Operators	Pepino mosaic virus: No AOEL set for the TGAI. Operators wear standard respiratory and dermal protection. No further operator assessment is necessary for Pepino Mosaic Virus isolate VX1. Nicotine: Spray application: No unacceptable risk. Rubbing on individual plants: No unacceptable risk when using appropriate protective equipment (gloves).
Workers	No AOEL set for the TGAI. No further operator assessment is necessary for Pepino Mosaic Virus isolate VX1. Nicotine No unacceptable risk for the unprotected worker.
Bystanders and residents	Not relevant (greenhouse use only).

Chapter 4 Residues

Residues in or on treated products, food and feed (Annex IIM 6; IIIM 8)

Viable residues	Considering all the available information, it would not be necessary to derive reference values for Mild Pepino Mosaic virus isolate VX1, and therefore consumer exposure calculations are not necessary. No MRL is required for Mild Pepino Mosaic virus isolate VX1.
Non-viable residues	Residues of nicotine in tomato are expected to be below the default MRL of 0.01 mg/kg. The assessment shows that the exposure to the impurity nicotine is well below the ADI and ARfD. A risk assessment considering exposure to nicotine from other dietary sources was not conducted in the remit of the peer review.

Chapter 5 Fate and Behaviour in the Environment (Annex IIM 7; IIIM 9)

Persistence and multiplication in soil	*Pepino mosaic virus* can only reproduce inside its host. Multiplication in soil is not relevant. Persistence in soil has not been quantified. Nicotine: The concentration of nicotine in the product is ≤ 0.1 mg/L. This value is used for the risk assessment. DT_{50}: 0.5, 1 day (n=2) (30 days used in risk assessment according to ECHA (2014)) $PIEC_{max,soil}$: 0.007 µg/kg
Persistence and multiplication in water	*Pepino mosaic virus* can only reproduce inside its host. Multiplication in water is not relevant. Persistence in nutrient solution up to 3 weeks (20ºC) Nicotine: DT50 water: 15 days can be used in risk assessment according to ECHA (2015) Ready biodegradable: yes (OECD301B) $PIEC_{max,water:}$ $1.9x10^{-11}$ g/L (18.6 pg/L)
Persistence and multiplication in air	DT_{50}: 1.4 hours (AOPWIN v1.92) 12h irradiation per day and 1.5 10^6 OH·/cm3
Mobility	It is unlikely that *Pepino mosaic virus* will be mobile in the environment via soil, or air. Nicotine: Koc: estimated 48.23 L/kg (from logKow)

Chapter 6 Effects on Non-target Species (Annex IIM 8; IIIM 10)

Effects on birds	Plant pathogenic viruses are generally considered to be pathogenic towards plant species only and not towards other organisms. Limited exposure for glasshouse use. Nicotine: Limited exposure for glasshouse use.
Effect on aquatic organisms	Plant pathogenic viruses are generally considered to be pathogenic towards plant species only and not towards other organisms. Nicotine – data from the public literature: *Nandus nandus* 168 h EC50: 2.21 mg/L *Daphnia pulex* 48h EC50 0.24 mg/L *Selenastrum capricornutum* 96h E_bC50 115 mg/L E_rC50 72.9 mg/L TER-values using PECsw of $1.9x10^{-11}$ g nicotine/L TER for fish: $>1x10^8$ TER for invertebrates: $>1x10^7$ TER for algae: $>1x10^9$
Effect on plants other than algae	The host range of *Pepino mosaic virus* is mainly restricted to plant species of the Solanaceae family cotinine (metabolite of nicotine) *Lemna gibba* 7d EC50 >1000µg/L
Effects on bees and other arthropods	Plant pathogenic viruses are generally considered to be pathogenic towards plant species only and not towards other organisms. Bumblebees can transmit mechanically. Any adverse effects on bumblebees are not reported. Nicotine: Bees: reduced larval survival 5 µg/g diet NTA: adverse effects 0.1 mg/kg diet Estimated exposure rates are 0.0012 mg/tomato plant. Naturally occurring nicotine concentrations in tomatoes: 0.01 mg/kg fruits and in flowers of *Nicotiana* spp. and *Tilia cordata* 0.1 – 5 mg/kg (public literature)
Effects on earthworms	Plant pathogenic viruses are generally considered to be pathogenic towards plant species only and not towards other organisms. Exposure to soil organisms is limited for glasshouse use. Nicotine: Exposure to soil organisms is limited for glasshouse use.
Effects on non-target soil micro-organisms	Plant pathogenic viruses are generally considered to be pathogenic towards plant species only and not towards other organisms. Exposure to soil

	organisms is limited for glasshouse use. Nicotine: Exposure to soil organisms is limited for glasshouse use.
Additional studies	None submitted

Abbreviations

1/*n*	slope of Freundlich isotherm
λ	wavelength
ε	decadic molar extinction coefficient
a.s.	active substance
AChE	acetylcholinesterase
ADE	actual dermal exposure
ADI	acceptable daily intake
AF	assessment factor
AAOEL	acute acceptable operator exposure level
AOEL	acceptable operator exposure level
AP	alkaline phosphatase
AR	applied radioactivity
ARfD	acute reference dose
AST	aspartate aminotransferase (SGOT)
AUC	area under the blood concentration/time curve
AV	avoidance factor
BCF	bioconcentration factor
BUN	blood urea nitrogen
bw	body weight
CAS	Chemical Abstracts Service
CFU	colony-forming units
ChE	cholinesterase
CI	confidence interval
CIPAC	Collaborative International Pesticides Analytical Council Limited
CL	confidence limits
Cmax	concentration achieved at peak blood level
DAA	days after application
DAT	days after treatment
DDD	daily dietary dose
DM	dry matter
DT_{50}	period required for 50% dissipation (define method of estimation)
DT_{90}	period required for 90% dissipation (define method of estimation)
dw	dry weight
EbC_{50}	effective concentration (biomass)
EC_{50}	effective concentration
ECHA	European Chemicals Agency
EEC	European Economic Community

EMDI	estimated maximum daily intake
ER_{50}	emergence rate/effective rate, median
ErC_{50}	effective concentration (growth rate)
ETR	exposure toxicity ratio
ETR_{acute}	exposure toxicity ratio for acute exposure
ETR_{larvae}	exposure toxicity ratio for chronic exposure
ETR_{larvae}	exposure toxicity ratio for larvae
ETR_{HPG}	exposure toxicity ratio for effects on honeybee hypopharygeal glands
EU	European Union
EUROPOEM	European Predictive Operator Exposure Model
f(twa)	Time-weighted average factor
FAO	Food and Agriculture Organization of the United Nations
FID	flame ionisation detector
FIR	food intake rate
FOB	functional observation battery
FOCUS	Forum for the Co-ordination of Pesticide Fate Models and their Use
GAP	Good Agricultural Practice
GC	gas chromatography
GCPF	Global Crop Protection Federation (formerly known as International Group of National Associations of Manufacturers of Agrochemical Products; GIFAP)
GGT	gamma glutamyl transferase
GM	geometric mean
GS	growth stage
GSH	glutathione
Hb	haemoglobin
Hct	haematocrit
HPLC	high-pressure liquid chromatography or high-performance liquid chromatography
HPLC-MS	high-pressure liquid chromatography–mass spectrometry
HPG	hypopharygeal glands
HQ	hazard quotient
$HQ_{contact}$	hazard quotient for contact exposure
HR	hazard rate
IEDI	international estimated daily intake
IESTI	international estimated short-term intake
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
iv	intravenous

JMPR	Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues (Joint Meeting on Pesticide Residues)
K_{doc}	organic carbon linear adsorption coefficient
K_{Foc}	Freundlich organic carbon adsorption coefficient
LC	liquid chromatography
LC_{50}	lethal concentration, median
LC-MS	liquid chromatography–mass spectrometry
LC-MS-MS	liquid chromatography with tandem mass spectrometry
LD_{50}	lethal dose, median; dosis letalis media
LDD_{50}	lethal dietary dose; median
LDH	lactate dehydrogenase
LOAEL	lowest observable adverse effect level
LOD	limit of detection
LOQ	limit of quantification
M/L	mixing and loading
MAF	multiple application factor
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
mm	millimetre (also used for mean measured concentrations)
mN	milli-newton
MRL	maximum residue level
MS	mass spectrometry
MSDS	material safety data sheet
MTD	maximum tolerated dose
MWHC	maximum water-holding capacity
NESTI	national estimated short-term intake
NOAEC	no observed adverse effect concentration
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
NOEL	no observed effect level
NPD	nitrogen–phosphorus detector
OECD	Organisation for Economic Co-operation and Development
OM	organic matter content
Pa	pascal
PD	proportion of different food types
PEC	predicted environmental concentration

PEC_{air}	predicted environmental concentration in air
PEC_{gw}	predicted environmental concentration in groundwater
PEC_{sed}	predicted environmental concentration in sediment
PEC_{soil}	predicted environmental concentration in soil
PEC_{sw}	predicted environmental concentration in surface water
PHED	pesticide handler's exposure data
PHI	pre-harvest interval
PIE	potential inhalation exposure
pK_a	negative logarithm (to the base 10) of the dissociation constant
P_{ow}	partition coefficient between *n*-octanol and water
PPE	personal protective equipment
ppm	parts per million (10^{-6})
PT	proportion of diet obtained in the treated area
PTT	partial thromboplastin time
QSAR	quantitative structure–activity relationship
r^2	coefficient of determination
RPE	respiratory protective equipment
RUD	residue per unit dose
SC	suspension concentrate
SD	standard deviation
SFO	single first-order
SMILES	simplified molecular-input line-entry system
SPG	specific protection goal
SSD	species sensitivity distribution
STMR	supervised trials median residue
$t_{1/2}$	half-life (define method of estimation)
TER	toxicity exposure ratio
TER_A	toxicity exposure ratio for acute exposure
TER_{LT}	toxicity exposure ratio following chronic exposure
TER_{ST}	toxicity exposure ratio following repeated exposure
TK	technical concentrate
TLV	threshold limit value
Tmax	time until peak blood levels achieved
TMDI	theoretical maximum daily intake
TRR	total radioactive residue
TSH	thyroid-stimulating hormone (thyrotropin)
TWA	time-weighted average
UDS	unscheduled DNA synthesis

UF	uncertainty factor
UV	ultraviolet
W/S	water/sediment
w/v	weight per unit volume
w/w	weight per unit weight
WBC	white blood cell
WG	water-dispersible granule
WHO	World Health Organization