FINAL REPORT

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

Study Title

ASSESSMENT OF ACUTE INHALATION TOXICITY WITH [redacted] PEPINO MOSAIC VIRUS, CH2 STRAIN, ISOLATE 1906 IN THE RAT

10.1.c wob juncto 63.2ter.d Vo 1107/2009 juncto 39sexies.2 Vo 178/2002

Author

[redacted]

Test Facility

[redacted]

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

Laboratory Project Identification

[redacted]

1. CONTENTS

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

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

[redacted] Pepino Mosaic Virus, CH2 strain, isolate 1906

[redacted]

2. STATEMENT OF GLP COMPLIANCE

NOTOX B.V., 's-Hertogenbosch, The Netherlands

The study described in this report has been correctly reported and was conducted in compliance with:

The Organization for Economic Cooperation and Development (OECD) Good Laboratory Practice Principles (1997).

Which essentially conform to:

The United States Food and Drug Administration Good Laboratory Practice Regulations.

The United States Environmental Protection Agency Good Laboratory Practice Regulations.

The sponsor is responsible for Good Laboratory Practice (GLP) compliance for all test substance information unless determined by [redacted]

Analysis of stability and homogeneity of the test substance under test conditions was not performed as part of this study.

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

The trial generations, performed in order to develop a method for the generation of a suitable test atmosphere, have a non-GLP status and were carried out in the quality assured environment of [redacted]

[redacted]

10.1.c Wob juncto 63.2ter.d Vo 1107/2009 juncto 39sexies.2 Vo 178/2002

[redacted]

[redacted]

[redacted]

Date: 26 January 2012

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

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

[redacted] Pepino Mosaic Virus, CH2 strain, isolate 1906

[redacted]

3. QUALITY ASSURANCE STATEMENT

[redacted]

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

This report was inspected by the [redacted] confirm that the methods and results accurately and completely reflect the raw data.

The dates of Quality Assurance inspections are given below. During the on-site process inspections, procedures applicable to this type of study were inspected.

Type of Inspections	Phase/Process	Start Inspection date	End Inspection date	Reporting date
Study	Protocol	24-Nov-11	24-Nov-11	24-Nov-11
	Report	16-Jan-12	16-Jan-12	16-Jan-12
Process	**Pathology**	08-Nov-11	14-Nov-11	15-Nov-11
	Observations/Measurements			
	Test substance Officers	19-Nov-11	23-Nov-11	23-Nov-11
	Test Substance Handling			
	SPF unit	28-Nov-11	07-Dec-11	09-Dec-11
	Test Substance Handling			
	Exposure			
	Observations/Measurements			
	Specimen Handling			

10.1.c wob juncto 63.2ter.d Vo 1107/2009 juncto 39sexies.2 Vo 178/2002

[redacted]

[redacted]

Date: 26-Jan-2012

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

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

[redacted] Pepino Mosaic Virus, CH2 strain, isolate 1906

[redacted]

4. SUMMARY

Assessment of acute inhalation toxicity with [redacted] Pepino Mosaic Virus, CH2 strain, isolate 1906 in the rat

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

The study was carried out based on the guidelines described in:
- OECD Guidelines, Section 4, Health Effects. No.403, "Acute Inhalation Toxicity", Sept 2009.
- Commission Regulation (EC) No 440/2008, B.2. Acute Toxicity (inhalation), L142, May 2008.
- EPA OPPTS 870.1300, Acute inhalation Toxicity. EPA 712-C-98-193, August 1998.
- JMAFF, 12 Nohsan, Notification No 8147, April 2011, including recent partial revisions.

[redacted], CH2 strain, isolate 1906 was administered as an aerosol/vapor mixture by inhalation for 4 hours to one group of five male and five female Wistar rats. Animals were subjected to daily observations and determination of body weights on Days 1, 2, 4, 8 and 15. Macroscopic examination was performed after terminal sacrifice (Day 15).

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

RESULTS

The mean total actual test substance concentration was 20.6 ± 2.0 mg/L. The time-weighted mean actual concentration for the aerosol droplets present in the test atmosphere was 0.11 ± 0.01 mg/L.

Based on the amount of test substance used, the exposure time and airflow used, a nominal concentration of 14.2 mg/L was calculated. This low nominal concentration may have been caused by uncertainties (multiple adjustments, discontinue monitoring and possible air loss) regarding the airflow used for the calculation of the nominal concentration. Since the air flow was not used to determine the actual test substance concentration, it was considered that the actual concentration was not affected. The generation efficiency (ratio of actual and nominal concentration) was 145%.

The concentration measurements equally distributed over time showed that the substance concentration was sufficiently stable

The Mass Median Aerodynamic Diameter (MMAD) and geometric standard deviation (gsd) was determined once. A second sample was not taken considering the very low concentration of the aerosol fraction (approximately 0.5%) and the long sample time needed. The MMAD was 1.3 µm (gsd 2.2).

No mortality occurred.

During exposure, shallow respiration was seen in all animals. After exposure, lethargy, hunched posture, laboured respiration, rales, piloerection and/or ptosis were seen among the animals between Days 1 and 4. In one male, the rales persisted until Day 8.

Overall body weight gain in males and females was within the range expected for rats of this strain and age used in this type of study.

Macroscopic post mortem examination of the animals revealed pale discoloured kidneys in one male and one female and a reddish discoloured thymus in one male.

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

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

[redacted] Pepino Mosaic Virus, CH2 strain, isolate 1906 [redacted]

CONCLUSION

The inhalatory $LC_{50, 4h}$ value of [redacted] Pepino Mosaic Virus, CH2 strain, isolate 1906 in Wistar rats was established to exceed 20 mg/L.

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

Based on these results [redacted] Pepino Mosaic Virus, CH2 strain, isolate 1906 does not have to be classified and has no obligatory labelling requirement for acute inhalation toxicity according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) of the United Nations (2007) and Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures.

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

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

[redacted] Pepino Mosaic Virus, CH2 strain, isolate 1906

[redacted]

5. INTRODUCTION

5.1. Preface

Sponsor	De Ceuster N.V. Fortsesteenweg 30 2860 Sint-Katelijne Waver, Belgium
Study Monitor	[redacted]
Test Facility	[redacted]
Study Director	[redacted]
Study Plan (in-life phase)	Start: 28 November 2011 Completion: 12 December 2011

10.1.c Wob juncto 63.2ter.d Vo 1107/2009 juncto 39sexies.2 Vo 178/2002

5.2. Aims of study

The objective of this study was to assess the toxicity of the test substance in rats following inhalation exposure to one or more defined concentrations for a single period of 4 hours. The results of the study allow the test substance to be ranked according to most classification systems currently in use. This study should provide a rational basis for risk assessment in man. The inhalatory route was selected, as it is a possible route of human exposure during manufacture, handling or use of the test substance.

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

5.3. Guidelines

The protocol was reviewed and agreed by the [redacted] The study was conducted based on the following guidelines:

Organisation for Economic Co-operation and Development (OECD), OECD Guidelines for Testing of Chemicals, Section 4, Health Effects. No.403, "Acute Inhalation Toxicity", September 2009.

Commission Regulation (EC) No 440/2008 Part B: Methods for the Determination of Toxicity and other Health Effects; B.2. AcuteToxicity (inhalation). Official Journal of the European Communities No. L142, May 2008, including most recent amendments.

EPA Health Effects Test Guidelines OPPTS 870.1300, Acute inhalation Toxicity. EPA 712-C-98-193, August 1998.

Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF), 12 Nohsan, Notification No 8147, April 2011, including the most recent partial revisions.

5.4. Storage and retention of records and materials

Records and materials pertaining to the study including protocol, raw data and the final report are retained in the [redacted] archives for a period of at least 2 years after finalization of the report. After this period, the sponsor will be contacted to determine how the records and materials should be handled. [redacted] will retain information concerning decisions made.

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

[redacted] will retain a test substance sample until the expiry date, but no longer than 10 years after finalization of the report. After this period the sample will be destroyed.

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

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

[redacted] Pepino Mosaic Virus, CH2 strain, isolate 1906 [redacted]

6. MATERIALS AND METHODS

6.1. Test substance

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

6.1.1. Test substance information

Identification	[redacted] Pepino Mosaic Virus, CH2 strain, isolate 1906
Description	Brown suspension (determined at [redacted]
Batch	[redacted]
Purity	Not indicated by the sponsor; treated as 100% pure
Test substance storage	In freezer (≤ -15°C) in the dark
Stability under storage conditions	Stable
Expiry date	[redacted]

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

6.1.2. Study specific test substance information

Hygroscopic	No
Volatile	No
Stability at higher temperatures	Yes, maximum temperature: 50ºC

6.1.3. Test substance preparation

In consultation with the sponsor, the test substance was sieved (125 um) prior to use, in order to remove large particles that block the nebulizers used for the generation of the test atmosphere.

6.2. Exposure chamber

The design of the exposure chamber is based on the flow past nose-only inhalation chamber (Am. Ind. Hyg Assoc. J. 44(12): 923-928, 1983). The chamber consisted of three animal sections with eight animal ports each (Appendix 1, Figure 1). Each animal port had its own atmosphere inlet and exhaust outlet. The animals were placed in restraining tubes and connected to the animal ports. The number of animal sections and number of open inlets were adapted to the air flow in such a way that at each animal port the theoretical air flow was at least 1 L/min, which ensures an adequate oxygen supply to the animals. The main inlet of the test atmosphere was located at the top section and the main outlet was located at the bottom section. The direction of the flow of the test atmosphere guaranteed a freshly generated atmosphere for each individual animal.
The placement of the individual animals in the inhalation chamber is shown in Appendix 1 (Figure 2). All components of the exposure chamber in contact with the test material were made of stainless steel, glass, rubber or plastic. To avoid exposure of the personnel and contamination of the laboratory the exposure chamber was placed in a fume hood, which maintained at a slight negative pressure.

6.3. Test atmosphere generation

The test substance was placed on a magnetic stirrer and was transferred to a nebulizer (type 950, Hospitak Inc., Lindenhurst, NY, USA) by means of a rotating pump (type VL500 digit, VERDER Lab Tec GmbH & Co. KG, Haan, Germany). The concentration of the test substance in air was monitored using the relative humidity (RH) and temperature of the test atmosphere. For this reason, dry (RH approximately 2%) pressurized air was used to nebulize the test substance and both the pressurized air and generation equipment were warmed up to obtain a test atmosphere of approximately 30 ºC. The mean airflow used was 14 L/min. The test atmosphere was passed through the exposure chamber (Appendix 1, Figure 1).

From the exposure chamber the test atmosphere was passed through a filter before it was released to the exhaust of the fume hood.

6.4. Test atmosphere characterization

6.4.1. Nominal concentration

The nominal concentration was calculated by dividing the amount of test substance used by the volume of pressurized air (average air flow times exposure time) entering the exposure chamber used for exposure of the animals. Due to the small volume of the exposure chamber the equilibrium time was negligible. The volume of air was calculated from the average air flow (measured by means of thermal mass flow meters and was recorded regularly, preferably in 30 minute intervals) and the exposure time.

6.4.2. Actual concentration

Trial generations showed that it was not possible to determine the actual test substance concentration using the solid content of the test substance, since the test substance is a watery extract with a negligible small amount of solids. Therefore, dried air was used to nebulize the test substance which means that the water content of the test atmosphere completely originated from the test substance.

The water content was calculated using the saturated vapor pressure at the measured temperatures applying Magnus formula and the relative humidity's determined. For this, the temperature and relative humidity were measured with a humidity and temperature indicator (E+E Elektronik) and recorded approximately every 20 minutes. The probe of the indicator was inserted in a tube mounted on one of the free animal ports of the exposure chamber.

After correction for the slight water content of the dried pressurized air used for nebulization, the mean concentration and the standard deviation of the test substance concentration was calculated.

A very small fraction (approximately 0.5%) of the test atmosphere consisted of droplets. To determine the concentration of this fraction, samples were drawn at five occasions from the test atmosphere through a tube mounted in one of the free animal ports of the middle section of the exposure chamber. Samples were drawn through a glass fiber filter (type APFC04700, Millipore, Billerica, MA, USA). The collected amount of the test substance in the air sample was measured gravimetrically. Sample volumes were measured by means of a dry gas meter (type G 1.6, Actaris Meterfabriek B.V., Dordrecht, The Netherlands). Subsequently the time-weighted mean concentration with the standard deviation was calculated.

6.4.3. Stability monitoring

The opacity of the test atmosphere was too low to be monitored by means of a real time aerosol monitoring system (Microdust Pro, Casella, Amherst, NH, USA). An indication of the stability of the test atmosphere was obtained from the concentration measurements, which were equally distributed over time.

6.4.4. Droplet size characterization

The droplet size distribution was characterized during the exposure period. Considering the very low concentration of this fraction and the long sample time needed, the droplet size distribution was determined at one occasion. The samples were drawn at 5 L/min from the test atmosphere through a tube mounted in one of the free animal ports of the middle section of the exposure chamber (Appendix 1, Figures 1 and 2). The samples were collected with an 8 stage Marple personal cascade impactor containing fiber glass filters (SKC 225-713, fiber glass, SKC Omega Specialty Division, Chelmsford, MA, USA) and a fiber glass back-up filter (SEC-290-F1, Westech, Upper Stondon, Bedfordshire, England). Amounts of test substance collected were measured gravimetrically. Subsequently the Mass Median Aerodynamic Diameter (MMAD) and the Geometric Standard Deviation (GSD) were determined [1].

6.5. Test system

Species	Rat: Crl:WI(Han) (outbred, SPF-Quality) Recognised by international guidelines as the recommended test system (e.g. OECD, EC). Source: Charles River Deutschland, Sulzfeld, Germany.
Number of animals	5 males and 5 females (females were nulliparous and non-pregnant) per exposure level.
Age and body weight	Young adult animals were selected (approximately 9 weeks old). Animals used within the study were of approximately the same age and body weight variation did not exceed +/- 20% of the sex mean.
Identification	Tailmark
Health inspection	A health inspection was performed prior to commencement of treatment, to ensure that the animals were in a good state of health.

6.6. Animal husbandry

Conditions
Animals were housed in a controlled environment, in which optimal conditions were considered to be approximately 15 air changes per hour, a temperature of 21.0 ± 3.0°C (actual range: 19.8 – 21.5°C), a relative humidity of 40-70% (actual range: 30 - 56%) and 12 hours artificial fluorescent light and 12 hours darkness per day.

Accommodation
Before exposure
Group housing of five animals per sex per cage in labelled Makrolon cages (type IV; height 18 cm) containing sterilised sawdust as bedding material (Litalabo, S.P.P.S., Argenteuil, France) and paper as cage-enrichment (Enviro-dri, Wm. Lillico & Son (Wonham Mill Ltd), Surrey, United Kingdom).
Acclimatisation period was at least 5 days before start of treatment under laboratory conditions.

After exposure
Group housing as described above, except that a paper sheet was introduced into the cage covering the bedding and cage enrichment to prevent suffocation in case of bad health condition. At the end of the Day of exposure the paper sheet was removed.

[1] Graphs of the cumulative mass of test substance collected (percentage of total collected) against the cut points of the impactor stages were drawn on log-normal paper. When drawing the graphs more weight was given to the cut points where the cumulative mass sampled was within the range of 5 to 95%. In case a linear relationship was found, the Mass Median Aerodynamic Diameter (MMAD), the $\sigma_{84\%}$ were read from the graph. The geometric standard deviation (gsd) was calculated as $\sigma_{84\%}$ / MMAD. The MMAD is the particle size where 50% of the particle mass is borne by particles smaller than the MMAD, the $\sigma_{84\%}$ is the particle size where 84% of the particle mass is borne by particles smaller than the $\sigma_{84\%}$.

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

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

[redacted] Pepino Mosaic Virus, CH2 strain, isolate 1906

[redacted]

Diet
Free access to pelleted rodent diet (SM R/M-Z from SSNIFF® Spezialdiäten GmbH, Soest, Germany) except during exposure to the test substance.

Water
Free access to tap water except during exposure to the test substance.

Results of analysis for each batch of diet (nutrients and contaminants), sawdust, paper and water were assessed and did not reveal any findings that were considered to have affected the study integrity. All certificates and results of analysis are retained in the [redacted] archives.

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

6.7. Study design

Target concentrations were based on the cut off concentration values specified in the UN and EC classification guidelines. Five animals of each sex were exposed in a limit test for 4 hours to a target concentration of the test substance of 20 mg/L.

6.8. Treatment

Prior to exposure the animals were restrained in polycarbonate restraining tubes; these tubes were connected to the exposure chamber. Fifteen minutes after the last animal was placed the generation of the test atmosphere was started. The exposure time was 4 hours.

6.9. Observations

Mortality/Viability	Twice daily.
Clinical signs	*During exposure* Three times during exposure for mortality, behavioural signs of distress and effects on respiration.
Clinical signs	*After exposure* On Day 1, one and three hours after exposure and once daily thereafter until Day 15. The symptoms were graded according to fixed scales and the time of onset, degree and duration were recorded: Maximum grade 4: grading slight (1) to very severe (4) Maximum grade 3: grading slight (1) to severe (3) Maximum grade 1: presence is scored (1).
Body weights	Days 1 (pre-administration), 2, 4, 8 and 15.
Necropsy	All animals were sacrificed at the end of the observation period by an intraperitoneal injection with Euthasol ® (AST Farma BV, Oudewater, The Netherlands). All animals assigned to the study were subjected to necropsy and descriptions of all internal macroscopic abnormalities were recorded. Particular attention was given to any changes in the respiratory tract.

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

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

[redacted] Pepino Mosaic Virus, CH2 strain, isolate 1906 [redacted]

6.10. Electronic data capture

Observations/measurements in the study were recorded electronically using the following programme(s):

- REES Centron Environmental Monitoring system version SQL 2.0 (REES Scientific, Trenton, NJ, USA): Environmental monitoring.
- TOXDATA version 8.0 [redacted] Mortality / Clinical signs / Body weights. Clinical signs during exposure or not defined in [redacted] and body weights of decedent animals were recorded manually.

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

6.11. Interpretation

The $LC_{50,4h}$ value of the test substance was ranked within the following ranges:

0 – 0.5; 0.5 – 2.0; 2.0 – 10; 10 – 20 or as exceeding 20 mg/L.

No statistical analysis was performed since only the results of one exposure group were available.

The results were evaluated according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) of the United Nations and Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures.

6.12. List of deviations

6.12.1. List of protocol deviations

1. Temporary deviations from the minimum level of relative humidity occurred.
   Evaluation: Laboratory historical data do not indicate an effect of the deviations.

The study integrity was not adversely affected by the deviation.

6.12.2. List of standard operating procedures deviations

There were no deviations from standard operating procedures during the study.

7. RESULTS

7.1. Test atmosphere characterization

7.1.1. Concentration (APPENDIX 1, Table 1, Table 2 and Figure 3)

The mean total actual test substance concentration was 20.6 ± 2.0 mg/L. The time-weighted mean actual concentration for the aerosol droplets present in the test atmosphere was 0.11 ± 0.01 mg/L.

Based on the amount of test substance used, the exposure time and airflow used, a nominal concentration of 14.2 mg/L was calculated. This low nominal concentration may have been caused by uncertainties (multiple adjustments, discontinue monitoring and possible air loss) regarding the airflow used for the calculation of the nominal concentration. Since the air flow was not used to determine the actual test substance concentration, it was considered that the actual concentration was not affected. The generation efficiency (ratio of actual and nominal concentration) was 145%.

The concentration measurements equally distributed over time showed that the substance concentration was sufficiently stable.

7.1.2. Droplet size (APPENDIX 1, Table 3)

The Mass Median Aerodynamic Diameter (MMAD) and geometric standard deviation (gsd) was determined once. A second sample was not taken considering the very low concentration of the aerosol fraction (approximately 0.5%) and the long sample time needed. The MMAD was 1.3 µm (gsd 2.2).

7.2. Observations

7.2.1. Mortality (APPENDIX 1, Table 4)

No mortality occurred.

7.2.2. Clinical Signs (APPENDIX 1, Table 5)

During exposure, shallow respiration was seen in all animals. After exposure, lethargy, hunched posture, laboured respiration, rales, piloerection and/or ptosis were seen among the animals between Days 1 and 4. In one male, the rales persisted until Day 8.

7.2.3. Body Weights (APPENDIX 1, Table 6)

Overall body weight gain in males and females was within the range expected for rats of this strain and age used in this type of study.

7.3. Macroscopic Findings (APPENDIX 1, Table 7)

Macroscopic post mortem examination of the animals revealed pale discoloured kidneys in one male and one female and a reddish discoloured thymus in one male.

Pelvic dilation of the kidneys as noted in one male is occasionally seen among rats of this age and strain and was therefore considered not related to treatment.

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

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

[redacted] Pepino Mosaic Virus, CH2 strain, isolate 1906 [redacted]

8. CONCLUSION

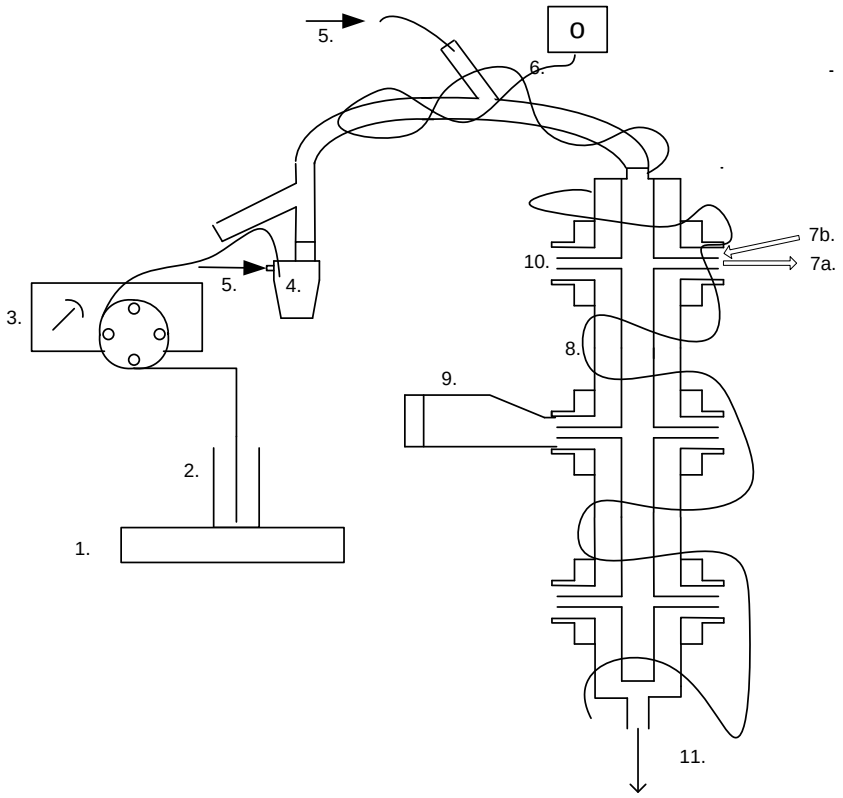
The inhalatory $LC_{50, 4h}$ value of [redacted] Pepino Mosaic Virus, CH2 strain, isolate 1906 in Wistar rats was established to exceed 20 mg/L.

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

Based on these results [redacted] Pepino Mosaic Virus, CH2 strain, isolate 1906 does not have to be classified and has no obligatory labelling requirement for acute inhalation toxicity according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) of the United Nations (2007) and Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures.

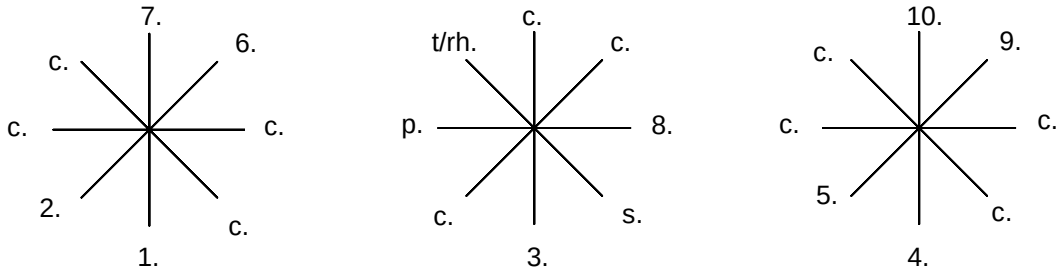
APPENDIX 1 FIGURES AND TABLES

Figure 1: Schematic presentation of the experimental set-up used for exposure



1. Magnetic stirrer.
2. Test substance container.
3. Peristaltic pump.
4. Nebulizer.
5. Pressurized air (dried and warmed) inlet (→).
6. Heating ribbon.
7a. Test atmosphere inlet to animal.
7b. Exhaust outlet from animal.
8. Exposure chamber, three levels (No 1 at the top, No 3 at the bottom).
9. Animal restrainer.
10. Openings used for concentration, temperature and relative humidity. measurements.
11. Main exhaust outlet of exposure chamber to vacuum pump.

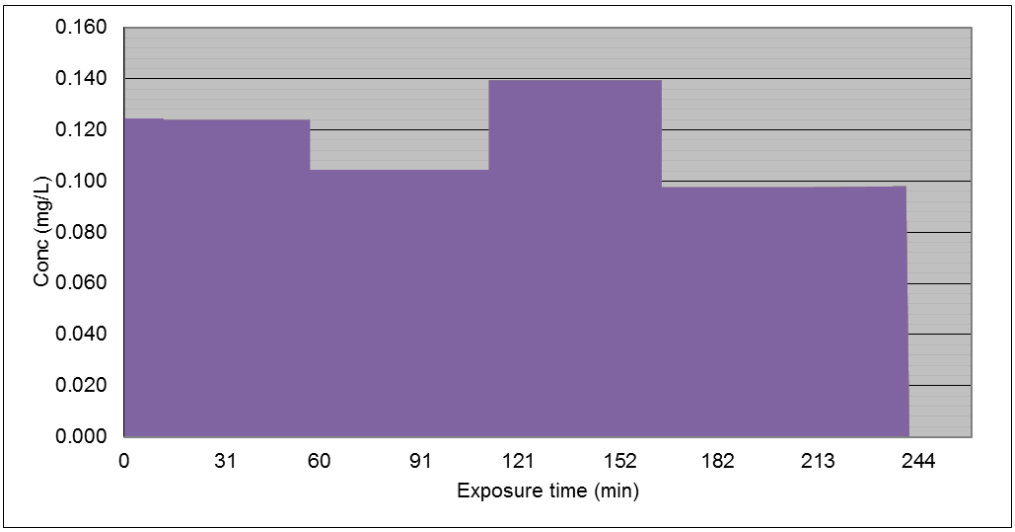
Figure 2: Schematic presentation of the placement of the animals and the location of the sampling point during exposure



Level 1 (top) Level 2 Level 3

1.-10. animal number.
c. closed atmosphere inlet to animal port.
o. open atmosphere inlet to animal port.
p. pressure gauge (inlet closed).
s total concentration measurement and particle sizing (inlet open).
t/rh temperature and relative humidity (inlet open).

Figure 3: Stability of the test atmosphere



The concentration of the aerosol droplets during the exposure time showed that the concentration of the test atmosphere was sufficiently stable.

Table 1: Total concentration of test substance

Start of generation of test atmosphere: 10:02
End of generation of test atmosphere: 14:02

Time	Temperature (°C)	Relative Humidity (%)	Water content (mg/L)	Test substance concentration 1) (mg/L)
10:05	27.9	71	18.3	17.7
10:23	28.6	71	19.3	18.7
10:35	29.4	70	20.1	19.5
10:50	30.7	72	21.8	21.2
11:05	29.6	73	21	20.4
11:20	26.5	72	17.5	16.9
11:36	30.7	78	23.7	23.1
11:50	30.7	70	21.2	20.6
12:05	30.2	77	23.4	22.8
12:20	30.1	73	22.1	21.5
12:35	29.9	83	23.8	23.2
12:51	27	80	20.6	20
13:05	30.4	71	21.5	20.9
13:20	31.2	75	24	23.4
13:35	27	72	18.5	17.9
13:50	30.8	73	22.1	21.5
14:00	30.8	70	21.2	20.6
			Mean	20.6
			Stdev	2.0

1) corrected with -0.6 mg/L for the relative humidity of 2% for the pressurized air used for nebulization.

Note: The water content in air from 30 degrees C and Relative Humidity of 100% at atmospheric pressure is 30 mg/L. For this study, the water content in air was calculated with a humidity calculator. The calculation is based on the saturated vapor pressure at the measured temperatures using the Magnus formula and the relative humidity's determined.

Table 2: Gravimetrical concentration of the aerosol fraction

Start of generation of test atmosphere: 10:02
End of generation of test atmosphere: 14:02

Time (hh:mm)	Action	Sample volume (L)	Mass sampled (mg)	Concentration (mg/L)	% of total exposure time	Weight concentration (mg/L)
10:02	Start of exposure	n.a.	n.a.	n.a.	0.0	n.a.
10:15	Start sampling	20	2.49	0.125	5.4	0.007
11:00	Start sampling	20	2.48	0.124	18.8	0.023
11:55	Start sampling	20	2.09	0.105	22.9	0.024
12:48	Start sampling	20	2.79	0.140	22.1	0.031
13:30	Start sampling	21	2.05	0.098	17.5	0.017
14:02	End of exposure	n.a.	n.a.	0.098	13.3 1)	0.013
				Mean weight concentration		0.115
				Standard deviation		0.009
				n		5

1) Assumed concentration, based on the last sample.
2) n.a.= not applicable

Table 3: Aerodynamic particle size distribution in the test atmosphere

Start of generation of test atmosphere: 10:02
End of generation of test atmosphere: 14:02
Sampling speed (L/min): 4.9

measurement 1:
Sampling time: 11:08
Sample volume (L): 100

Stage	Cut point (µm)	Mass sampled (mg)	Relative mass (%)	Cumulative mass (% of total sampled)
1	13.3	0.00	0.00	100.00
2	9.5	0.00	0.00	100.00
3	6.3	0.03	0.31	99.69
4	3.8	0.51	5.22	94.47
5	2.2	2.91	29.79	64.69
6	1.3	4.30	44.01	20.68
7	0.6	1.00	10.24	10.44
8	0.3	0.76	7.78	2.66
Back up	0.16	0.26	2.66	0.00
MMAD[1] (µm):	1.3			
gsd[2]:	2.2			

[1] Mass Median Aerodynamic Diameter
[2] Geometric standard deviation

Table 4: Mortality

TEST DAY	1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
HOURS AFTER TREATMENT	1	3														
MALES 20 MG/LITER	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
FEMALES 20 MG/LITER	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 5: Clinical signs

TEST DAY		1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
HOURS AFTER TREATMENT	MAX GRADE	1	3														
MALES 20 MG/LITER																	
ANIMAL 1																	
Behavior																	
Lethargy	(3)	2	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Posture																	
Hunched posture	(1)	1	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-
Breathing																	
Laboured respiration	(3)	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rales	(3)	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Skin / fur																	
Piloerection	(1)	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Various																	
Ptosis	(3)	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ANIMAL 2																	
Behavior																	
Lethargy	(3)	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Posture																	
Hunched posture	(1)	1	1	1	1	1	-	-	-	-	-	-	-	-	-	-	-
Breathing																	
Laboured respiration	(3)	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rales	(3)	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Skin / fur																	
Piloerection	(1)	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Various																	
Ptosis	(3)	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ANIMAL 3																	
Behavior																	
Lethargy	(3)	2	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Posture																	
Hunched posture	(1)	1	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-
Breathing																	
Laboured respiration	(3)	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rales	(3)	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Skin / fur																	
Piloerection	(1)	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Various																	
Ptosis	(3)	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ANIMAL 4																	
Behavior																	
Lethargy	(3)	2	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Posture																	
Hunched posture	(1)	1	1	1	1	1	-	-	-	-	-	-	-	-	-	-	-
Breathing																	
Laboured respiration	(3)	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rales	(3)	-	-	1	1	1	1	1	1	1	-	-	-	-	-	-	-
Skin / fur																	
Piloerection	(1)	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Various	(3)	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ptosis																	

- = SIGN NOT OBSERVED / . = OBSERVATION NOT PERFORMED / + = ANIMAL DEAD

Table 5: Clinical signs (cont'd)

TEST DAY		1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
HOURS AFTER TREATMENT	MAX GRADE	1	3														
MALES 20 MG/LITER																	
ANIMAL 5																	
Behavior	(3)	2	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lethargy																	
Posture	(1)	1	1	1	1	1	-	-	-	-	-	-	-	-	-	-	-
Hunched posture																	
Breathing	(3)	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Laboured respiration	(3)	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Rales																	
Skin / fur	(1)	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Piloerection																	
Various	(3)	2	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ptosis																	
FEMALES 20 MG/LITER																	
ANIMAL 6																	
Posture	(1)	1	1	-	1	1	-	-	-	-	-	-	-	-	-	-	-
Hunched posture																	
Breathing	(3)	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Rales																	
Skin / fur	(1)	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Piloerection																	
ANIMAL 7																	
Behavior	(3)	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lethargy																	
Posture	(1)	1	1	1	1	1	-	-	-	-	-	-	-	-	-	-	-
Hunched posture																	
Breathing	(3)	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Rales																	
Skin / fur	(1)	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Piloerection																	
Various	(3)	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ptosis																	
ANIMAL 8																	
Behavior	(3)	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lethargy																	
Posture	(1)	1	1	1	1	1	-	-	-	-	-	-	-	-	-	-	-
Hunched posture																	
Breathing	(3)	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Laboured respiration	(3)	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Rales																	
Skin / fur	(1)	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Piloerection																	
Various	(3)	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ptosis																	
ANIMAL 9																	
Behavior	(3)	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lethargy																	
Posture	(1)	1	1	1	1	1	-	-	-	-	-	-	-	-	-	-	-
Hunched posture																	
Breathing	(3)	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Laboured respiration	(3)	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Rales																	
Skin / fur	(1)	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Piloerection																	
Various	(3)	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ptosis	(3)	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

- = SIGN NOT OBSERVED / . = OBSERVATION NOT PERFORMED / + = ANIMAL DEAD

Table 5: Clinical signs (cont'd)

TEST DAY HOURS AFTER TREATMENT	MAX GRADE	1 1	1 3	2	3	4	5	6	7	8	9	10	11	12	13	14	15
FEMALES 20 MG/LITER																	
ANIMAL 10																	
Behavior Lethargy	(3)	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Posture Hunched posture	(1)	1	1	1	1	1	-	-	-	-	-	-	-	-	-	-	-
Breathing Rales	(3)	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Skin / fur Piloerection	(1)	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-

- = SIGN NOT OBSERVED / . = OBSERVATION NOT PERFORMED / + = ANIMAL DEAD

Table 6: Body weights (gram)

SEX/DOSE LEVEL	ANIMAL	DAY 1	DAY 2	DAY 4	DAY 8	DAY 15
MALES 20 MG/LITER						
	1	248	224	234	250	275
	2	257	244	257	275	310
	3	249	232	243	262	289
	4	257	229	249	270	298
	5	266	239	255	273	301
	MEAN	255	234	248	266	295
	ST.DEV.	7	8	9	10	13
	N	5	5	5	5	5
FEMALES 20 MG/LITER						
	6	175	174	179	185	204
	7	189	180	185	190	208
	8	182	174	181	190	202
	9	171	158	167	171	187
	10	171	168	164	171	186
	MEAN	178	171	175	181	197
	ST.DEV.	8	8	9	10	10
	N	5	5	5	5	5

Table 7: Macroscopic findings

ANIMAL	ORGAN	FINDING	DAY OF DEATH
MALES 20 MG/LITER			
1		No findings noted	Scheduled necropsy Day 15 after treatment
2	Kidneys	Both sides: pelvic dilation.	Scheduled necropsy
	Thymus	Discolouration, reddish.	Day 15 after treatment
3		No findings noted	Scheduled necropsy Day 15 after treatment
4	Kidneys	Both sides: discolouration, pale.	Scheduled necropsy Day 15 after treatment
5		No findings noted	Scheduled necropsy Day 15 after treatment

Table 7: Macroscopic findings (cont'd)

ANIMAL	ORGAN	FINDING	DAY OF DEATH
FEMALES 20 MG/LITER			
6		No findings noted	Scheduled necropsy Day 15 after treatment
7		No findings noted	Scheduled necropsy Day 15 after treatment
8		No findings noted	Scheduled necropsy Day 15 after treatment
9	Kidneys	Both sides: discolouration, pale.	Scheduled necropsy Day 15 after treatment
10		No findings noted	Scheduled necropsy Day 15 after treatment