[redacted] [redacted]

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

FINAL REPORT

[redacted] Pepino Mosaic Virus, CH2 strain, isolate 1906 ACUTE DERMAL TOXICITY STUDY IN RATS

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

Study code: 11/236-002P

[redacted]

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

14 December 2011

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

STATEMENT OF THE STUDY DIRECTOR

This study has been performed in accordance with the study plan, OECD Guidelines for Testing of Chemicals (No.: 402, 24th Feb. 1987), Commission Regulation (EC) No 440/2008, B.3 (L142, 30 May 2008), OPPTS 870.1200 (EPA 712-C-98-192, August 1998),and the Principles of Good Laboratory Practice [redacted] which corresponds to the OECD GLP, ENV/MC/CHEM (98) 17.).

I, the undersigned, declare that this report constitutes a true record of the actions undertaken and the results obtained in the course of this study.

The acute dermal median lethal dose (LD_{50}) of the test item CH2 Mild 1906 was found to be higher than 2000 mg/kg body weight in male and female CRL:(WI) rats.

Signature: [redacted] Date: 14 December 2011

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

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

STATEMENT OF THE MANAGEMENT

According to the conditions of the research and development agreement De Ceuster n.v. (as Sponsor) [redacted] the study titled [redacted] Pepino Mosaic Virus, CH2 strain, isolate 1906 Acute Dermal Toxicity Study in Rats" was performed in compliance with the Principles of Good Laboratory Practice.

Signature: [redacted]

Date: 14 Dec 2011

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

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

QUALITY ASSURANCE STATEMENT

Study Code: 11/236-002P

Study title: [redacted] Pepino Mosaic Virus, CH2 strain, isolate 1906 Acute Dermal Toxicity Study in Rats

Test item: CH2 Mild 1906

This study has been inspected, and this report audited by the Quality Assurance Unit in compliance with the Principles of Good Laboratory Practice. As far as it can be reasonably established the methods described and the results incorporated in this report accurately reflect the raw data produced during this study.

All inspections, data reviews and the report audit were reported in written form to the study director and to management. The dates of such inspections and of the report audit are given below:

Date of Inspection	Phase(s) Inspected/Audited	Date of report to	
		Management	Study Director
04 October 2011	Study Plan	04 October 2011	04 October 2011
13 October 2011	Clinical observation	17 October 2011	17 October 2011
29 November 2011	Draft Report	29 November 2011	29 November 2011
14 December 2011	Final Report	14 December 2011	14 December 2011

Signature: [redacted]

Date: 14 December 2011

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

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

GENERAL INFORMATION

STUDY TITLE : [redacted] Pepino Mosaic Virus, CH2 strain, isolate 1906 Acute Dermal Toxicity Study in Rats

SPONSOR : De Ceuster n.v.

Fortsesteenweg 30, B-2860 Sint-Katelijne-Waver, Belgium
Phone: +32-14-86-16-55
Fax: +32-14-25-73-51

STUDY PERFORMED BY : [redacted]

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

STUDY DIRECTOR: [redacted]

QUALITY ASSURANCE: [redacted]

RESPONSIBLE PERSONS: [redacted]

CONTENTS

1. SUMMARY

An acute dermal toxicity study was performed with test item CH2 Mild 1906 in CRL:(WI) Wistar rats, in compliance with OECD Guideline No.: 402.

A limit test was carried out at 2000 mg/kg body weight (bw) in both sexes (5 rats/sex). The test item was applied as supplied as a single dermal 24-hour exposure followed by a 14-day observation period.

Clinical observations were performed on all animals at 1 and 5 hours after dosing and daily for 14 days thereafter. Body weight was measured prior to dosing on Day 0 and on Days 7 and 14. Rats were euthanized and a gross macroscopic examination performed at the end of the 2-week observation period (Day 14).

The results of the study were summarized as follows:

Mortality

No mortality occurred.

Systemic clinical signs

No clinical signs were observed after the treatment with the test item or during the 14-day observation period.

Local dermal signs

After treatment with CH2 Mild 1906 no local signs were observed after the treatment with the test item or during the 14-day observation period.

Body weight

The body weight and body weight gain of CH2 Mild 1906 treated animals did not show any test item-related effect.

Necropsy

There was no evidence of the observations at a dose level of 2000 mg/kg bw at necropsy.

Conclusions

The acute dermal median lethal dose (LD_{50}) of the test item CH2 Mild 1906 was found to be higher than 2000 mg/kg bw in male and female CRL:(WI) Wistar rats.

2. OBJECTIVE OF STUDY

The objective of the study was to assess the acute dermal toxicity of the test item CH2 Mild 1906 when administered as a single 24-hour dermal treatment in rats at one or more defined dose levels followed by a 14-day observation period.

2.1. STUDY SCHEDULE

	Study Day	Absolute Date
PRE-EXPERIMENTAL PERIOD		
Animal receipt:	Day [-6]	06 October 2011
Veterinary control:	Day [-5]	07 October 2011
Animal identification:	Day [-1]	11 October 2011
TREATMENT PERIOD		
The day of treatment:	Day 0	12 October 2011
Body weight measurement:	Day 0, 7, 14	12, 19, 26 October 2011
Clinical observation:	1 and 5 hours after treatment, then daily for 14 days	
Necropsy:	Day 14	26 October 2011

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

3. MATERIALS AND METHODS

3.1. TEST ITEM

Name:	[redacted] Pepino Mosaic Virus, CH2 strain, isolate 1906
Short Name:	CH2 Mild 1906
Batch/Lot number:	[redacted]
Appearance:	Yellow-brownish liquid
Manufacture date:	[redacted]
Expiry date:	
Storage conditions:	Freezer (≤-15 C), protected from light
Safety Precautions:	Routine safety precautions (gloves, goggles, face mask, lab coat) for unknown materials were applied to assure personnel health and safety.

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

3.1.1. Identification, Receipt

The CH2 Mild 1906 was a plant virus and was not harmful to humans or animals, the test item was a mild version of an endemic tomato virus. The laboratory was followed normal safety precautions, with disinfection or incineration of all potentially contaminated materials used in testing.

The test item of a suitable chemical purity together with all precautions required in the handling and disposal of the test item were supplied by the Sponsor. The identification of test item was made by its appearance and colour in the [redacted] on the basis of the information provided by Sponsor.

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

3.1.2. Formulation

The test item was administered as a single dose. The test item was placed onto a gauze pad. The gauze pad was fixed with a hypoallergenic plaster on the shaved skin of the animals. The entire trunk of the animal was then wrapped with semi occlusive plastic wrap for 24 hours.

At the end of the exposure period, the area of skin treated with the test item was washed with water of body temperature.

3.1.3. Other Materials

For treatment:

	Sterile gauze pad
Lot No.:	0920329
Expiry Date:	May 2014
Supplier:	[redacted]

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

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

	Silkplast
Lot No:	102/26
Expiry Date:	February 2015
Supplier:	[redacted]
For euthanasia:	
Name:	Euthasol® 40 %
Lot No.:	11B15 6
Expiry Date:	January 2014
Produced by:	[redacted]

3.2. EXPERIMENTAL ANIMALS

Species and strain:	CRL:(WI) Wistar rats
Source:	[redacted]
Hygienic level at arrival:	SPF
Hygienic level during the study:	Standard housing conditions
Justification of strain:	The Wistar rat is one of the standard rodent species used in acute toxicity studies
Number of animals:	5 animals/sex
Sex:	Male and female, female rats were nulliparous and non-pregnant.
Age of animals at study start:	Young adult rats
Body weight range at dosing:	Between 206 g and 261 g
Acclimatization time:	6 days

3.2.1. Husbandry

Animal health:	Only healthy animals were used for the study. The veterinarian certified the health status.
Room-Box:	242/5
Housing:	Individual caging
Cage type:	Type II. polypropylene/polycarbonate
Bedding:	Laboratory bedding: [redacted]
Light:	12 hours daily, from 6.00 a.m. to 6.00 p.m.
Temperature:	22 ± 3 °C
Relative humidity:	30 - 70 %
Ventilation:	15-20 air exchanges/hour
Enrichment:	Rodents were housed with deep wood sawdust bedding to allow digging and other normal rodent activities.

The temperature and relative humidity was recorded twice daily during the study.

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

3.2.2. Food and Water Supply

Animals received ssniff® SM R/M-Z+H "Autoclavable complete feed for rats and mice – breeding and maintenance" produced by [redacted] *ad libitum*, and tap water from the municipal supply, as for human consumption from 500 ml bottle *ad libitum*. The food is not considered to contain any contaminants that could reasonably be expected to affect the purpose or integrity of the study.

For contents of the standard diet see Appendix 1. The supplier provided an analytical certificate for the batch used.

Water quality control analysis is performed once every three months and microbiological assessment is performed monthly, by [redacted] he quality control results are retained in the archives at [redacted]

3.2.3. Identification

The individual identification was performed using numbers written on the tail with a marker pen. The numbers were given on the basis of [redacted] for each animal allocated to the treatment groups. The cages were identified by cards containing information about study code, sex, dose group, cage number and individual animal numbers.

3.3. ADMINISTRATION OF THE TEST ITEM

3.3.1. Dosages

Justification of the doses:

The test item was not expected to be lethal at 2000 mg/kg bw. A limit test was therefore performed.

3.3.2. Experimental design

Dose Group	Number of cages	Number of animals
Male group 2000 mg/kg bw	Cages 1-5	5
Female group 2000 mg/kg bw	Cages 6-10	5

A single administration was performed by the dermal route and was followed by a fourteen-day observation period. The test item was applied as supplied.

3.3.3. Procedure

The back of each animal was shaved (approximately 10 % area of the total body surface) approximately 24 hours prior to treatment. The test item was applied as a single dose as supplied to the shaved skin and remained in contact with the skin for the 24- hour exposure period. Sterile gauze pads were placed on the skin of rats to cover the test item. These gauze pads were kept in contact with the skin by a patch with adhesive hypoallergenic plaster. The entire trunk of the animal was then wrapped with semi occlusive plastic wrap for 24 hours.

At the end of the exposure period, the area of skin treated with the test item was washed with water of body temperature.

3.4. OBSERVATIONS

3.4.1. Clinical Observations

Clinical observations were performed on the day of treatment at 1 and 5 hours after application of the test item and once each day for 14 days thereafter. Observations included the skin and fur, eyes and mucous membranes, the respiratory, circulatory, autonomic and central nervous system, somatomotor activity and behaviour pattern. Particular attention was directed to observation of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma.

3.4.2. Measurement of Body Weight

The body weights were recorded on Day 0 (before test item administration) and on Days 7 and 14.

3.5. NECROPSY

All animals were anaesthetised with Euthasol®40% (details in 3.1.3.) and exsanguinated. After examination of the external appearance, the cranial, thoracic and abdominal cavities were opened and the appearance of the tissues and organs was observed. All macroscopic changes were recorded.

3.6. EVALUATION

Body weight and body weight gain are summarized in tabular form. Clinical signs and necropsy findings are described and summarized in tabular form.

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

4. ARCHIVES

The study documents and samples:

- study plan,
- all raw data,
- sample of test item,
- one original of Final Report,
- correspondence

are stored according to the [redacted]

After the retention time agreed with the Sponsor has elapsed, all the archived materials listed above will be offered to the Sponsor or retained for a further period if agreed by a contract. Otherwise the materials will be discarded.

5. DEVIATIONS TO THE STUDY PLAN

The animals species and strain is **CRL:(WI) Wistar rats** instead of CRL:(WI)BR Wistar rats as it was indicated in the Study Plan.

This deviation have no pressumed impact on the outcome or integrity of the study.

6. THE PERMISSION OF [redacted]

[redacted] reviewed the study plan and authorised the conduct of the study.

7. DISTRIBUTION OF THE FINAL REPORT

Sponsor:	A PDF document sent by email, and 1 bound certified copy, 1 unbound certified copy sent by courier.
Archive:	1 original

8. RESULTS AND CONCLUSION

8.1. MORTALITY

No mortality occurred after a 24-hour dermal exposure to CH2 Mild 1906 administered at 2000 mg/kg bw to CRL:(WI) Wistar rats followed by a 14-day observation period.

8.2. SYSTEMIC CLINICAL SIGNS

Tables for individual clinical observations are listed in Appendix 2, page 19

No clinical signs were observed after the treatment with the test item or during the 14-day observation period.

8.3. LOCAL DERMAL SIGNS

Tables for individual clinical observations are listed in Appendix 2, page 19

After treatment with CH2 Mild 1906 no local signs were observed after the treatment with the test item or during the 14-day observation period.

8.4. BODY WEIGHT

Tables for individual body weight and body weight gain are listed in Appendix 3, page 20

The body weight and body weight gain of CH2 Mild 1906 treated animals did not show any test item-related effect.

8.5. MACROSCOPIC FINDINGS

Tables for macroscopic findings are listed in Appendix 4, page 21

There was no evidence of the observations at a dose level of 2000 mg/kg bw at necropsy.

CONCLUSIONS

The acute dermal median lethal dose (LD_{50}) of the test item CH2 Mild 1906 was found to be higher than 2000 mg/kg body weight in male and female CRL:(WI) Wistar rats.

APPENDICES

APPENDIX 1:

CONTENTS OF THE DIET

SSNIFF® SM R/M-Z+H, AUTOCLAVABLE
Complete feed for rats and mice – breeding and maintenance

Batch number:	**683 5949**
Expiry date:	**January 2012**

NUTRIENTS

Crude protein	19.00%
Crude fat	3.50%
Crude fibre	3.60%
Ash	6.50%
Lysine	1.10%
Methionine	0.56%
Calcium	1.00%
Sodium	0.20%
Magnesium	0.22%
Phosphorus	0.70%

VITAMINS

Vitamin A	25000 IU
Vitamin D_3	1000 IU
Vitamin E	125 mg/kg

These data are standard and guaranteed values which were provided by the supplier.

APPENDIX 2:

CLINICAL OBSERVATIONS

INDIVIDUAL CLINICAL OBSERVATIONS
STUDY CODE: 11/236-002P
TEST SYSTEM: CRL: (WI) Wistar Rats
TEST ITEM: CH2 Mild 1906

DOSE LEVEL: 2000 mg/kg bw **SEX: MALE**

Cage No.	Animal No.	Observations	Observation days																Frequency
			0		1	2	3	4	5	6	7	8	9	10	11	12	13	14	
			1h	5h															
1	9237	Symptom Free	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	16/16
2	9238	Symptom Free	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	16/16
3	9239	Symptom Free	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	16/16
4	9240	Symptom Free	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	16/16
5	9241	Symptom Free	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	16/16

DOSE LEVEL: 2000 mg/kg bw **SEX: FEMALE**

Cage No.	Animal No.	Observations	Observation days																Frequency
			0		1	2	3	4	5	6	7	8	9	10	11	12	13	14	
			1h	5h															
6	9242	Symptom Free	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	16/16
7	9243	Symptom Free	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	16/16
8	9244	Symptom Free	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	16/16
9	9245	Symptom Free	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	16/16
10	9246	Symptom Free	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	16/16

Remarks: + = present
h = hour (s) Treatment day = Day 0
Frequency of observation = number of occurence of observation / total number of observations

APPENDIX 3:

BODY WEIGHT DATA

INDIVIDUAL BODY WEIGHT AND BODY WEIGHT GAIN
STUDY CODE: 11/236-002P
TEST SYSTEM: CRL: (WI) Wistar Rats
TEST ITEM: CH2 Mild 1906

DOSE LEVEL: 2000 mg/kg bw **SEX: MALE**

Cage No.	Animal No.	Body weight (g) Days			Body Weight Gain (g)		
		0	7	14	0-7	7-14	0-14
1	9237	236	277	326	41	49	90
2	9238	241	284	336	43	52	95
3	9239	261	313	355	52	42	94
4	9240	242	297	344	55	47	102
5	9241	238	271	315	33	44	77
Mean:		243.6	288.4	335.2	44.8	46.8	91.6
Standard deviation:		10.0	16.8	15.5	8.8	4.0	9.2

DOSE LEVEL: 2000 mg/kg bw **SEX: FEMALE**

Cage No.	Animal No.	Body weight (g) Days			Body Weight Gain (g)		
		0	7	14	0-7	7-14	0-14
6	9242	226	228	236	2	8	10
7	9243	215	238	252	23	14	37
8	9244	243	257	272	14	15	29
9	9245	228	243	260	15	17	32
10	9246	206	209	228	3	19	22
Mean:		223.6	235.0	249.6	11.4	14.6	26.0
Standard deviation:		14.0	17.9	17.8	8.8	4.2	10.5

Remark: Treatment day = Day 0

APPENDIX 4:

MACROSCOPIC FINDINGS

INDIVIDUAL INTERNAL AND EXTERNAL MACROSCOPIC OBSERVATIONS
STUDY CODE: 11/236-002P
TEST SYSTEM: CRL: (WI) Wistar Rats
TEST ITEM: CH2 Mild 1906

DOSE LEVEL: 2000 mg/kg bw **SEX: MALE**

Cage No.	Animal No.	Necropsy Date	External Observations	Internal Observations	Organ/ Tissue
1	9237	26 October 2011	No external observations	No internal observations	Not applicable
2	9238	26 October 2011	No external observations	No internal observations	Not applicable
3	9239	26 October 2011	No external observations	No internal observations	Not applicable
4	9240	26 October 2011	No external observations	No internal observations	Not applicable
5	9241	26 October 2011	No external observations	No internal observations	Not applicable

DOSE LEVEL: 2000 mg/kg bw **SEX: FEMALE**

Cage No.	Animal No.	Necropsy Date	External Observations	Internal Observations	Organ/ Tissue
6	9242	26 October 2011	No external observations	No internal observations	Not applicable
7	9243	26 October 2011	No external observations	No internal observations	Not applicable
8	9244	26 October 2011	No external observations	No internal observations	Not applicable
9	9245	26 October 2011	No external observations	No internal observations	Not applicable
10	9246	26 October 2011	No external observations	No internal observations	Not applicable

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

APPENDIX 5:

PATHOLOGY REPORT

PATHOLOGY REPORT

INTRODUCTION

The objective of the study was to assess the acute dermal toxicity of CH2 Mild 1906 when administered in a single 24 hour dermal application to rats at one or more defined dose levels followed by 14 days observation.

RESULTS AND DISCUSSION

All rats survived until the scheduled termination of the study.

All animals were euthanized upon completion of the treatment period on Day 14. Rats were anesthetized with pentobarbital, followed by exsanguination. Gross pathology consisted of an external examination, including identification of all clinically-recorded lesions, as well as a detailed internal examination. Histopathological examination was not performed.

TERMINAL (DAY 14)

Macroscopic Findings

There was no evidence of the observations at a dose level of 2000 mg/kg bw at necropsy.

CONCLUSION

A single 24 hour dermal application of CH2 Mild 1906 to CRL: (WI) Wistar rat at a dose level of 2000 mg/kg bw was not associated with any macroscopic findings.

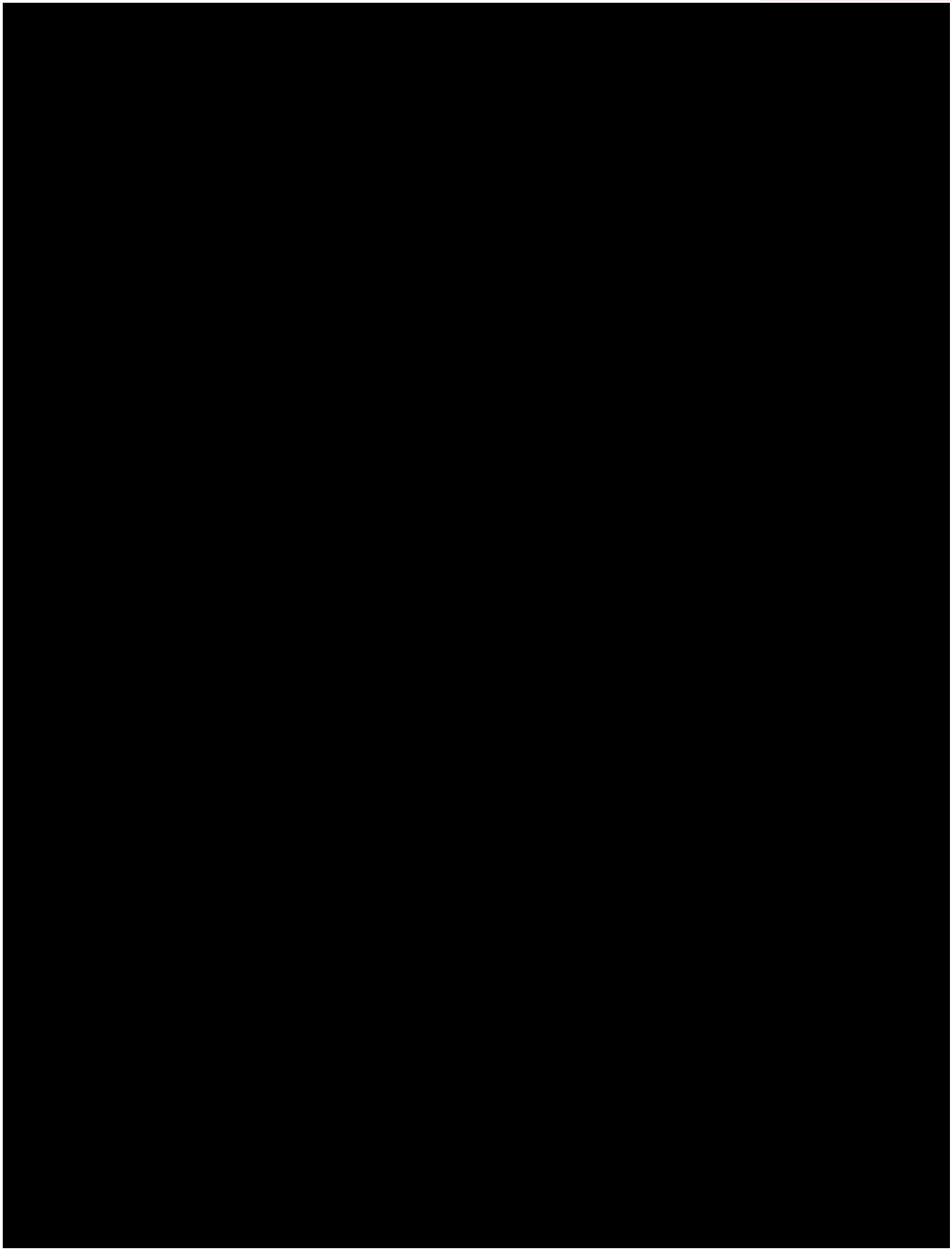
[redacted]

14 Dec 2011
Date

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

APPENDIX 6:

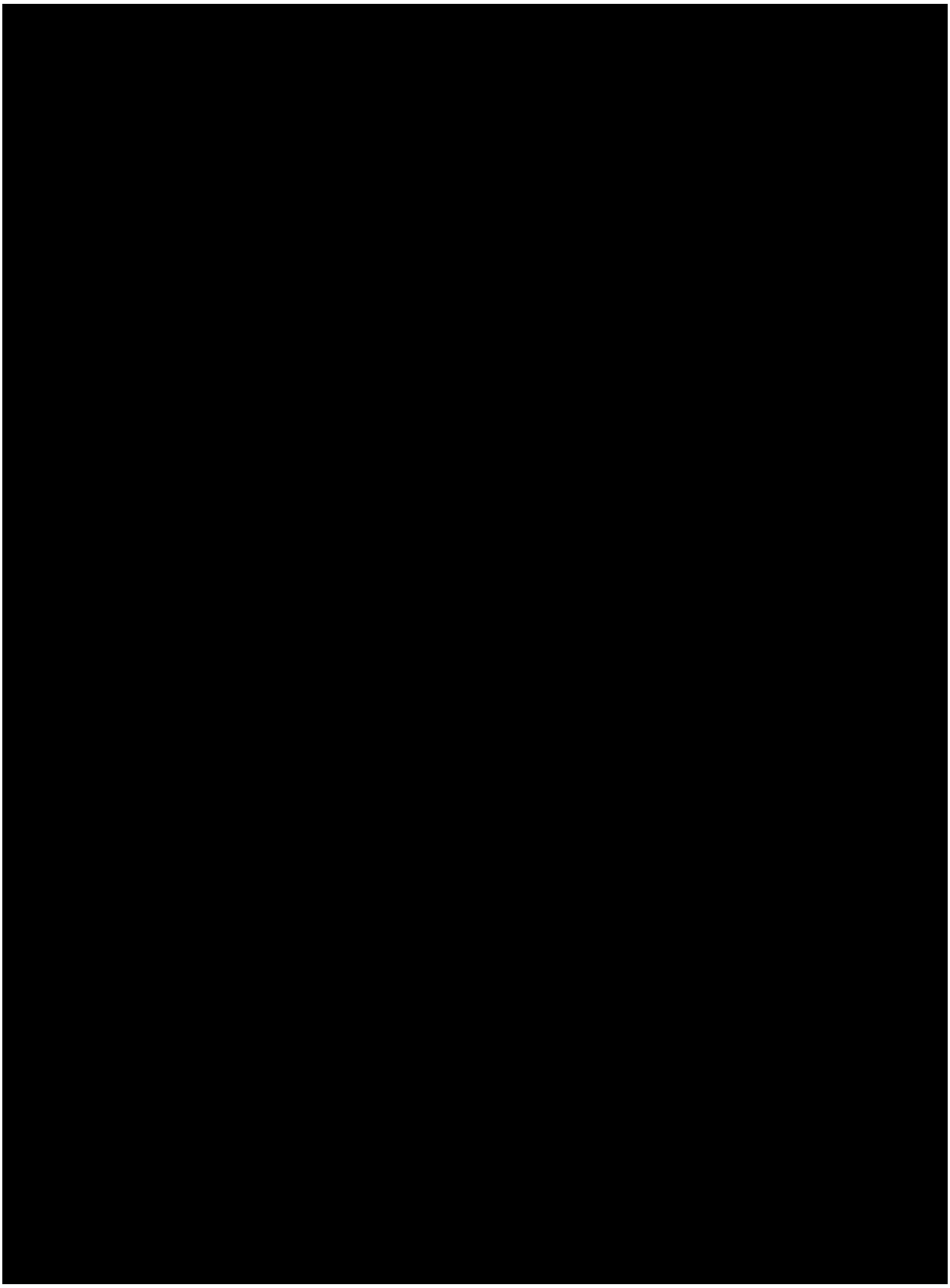
COPY OF THE CERTIFICATE OF ANALYSIS



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

APPENDIX 7:

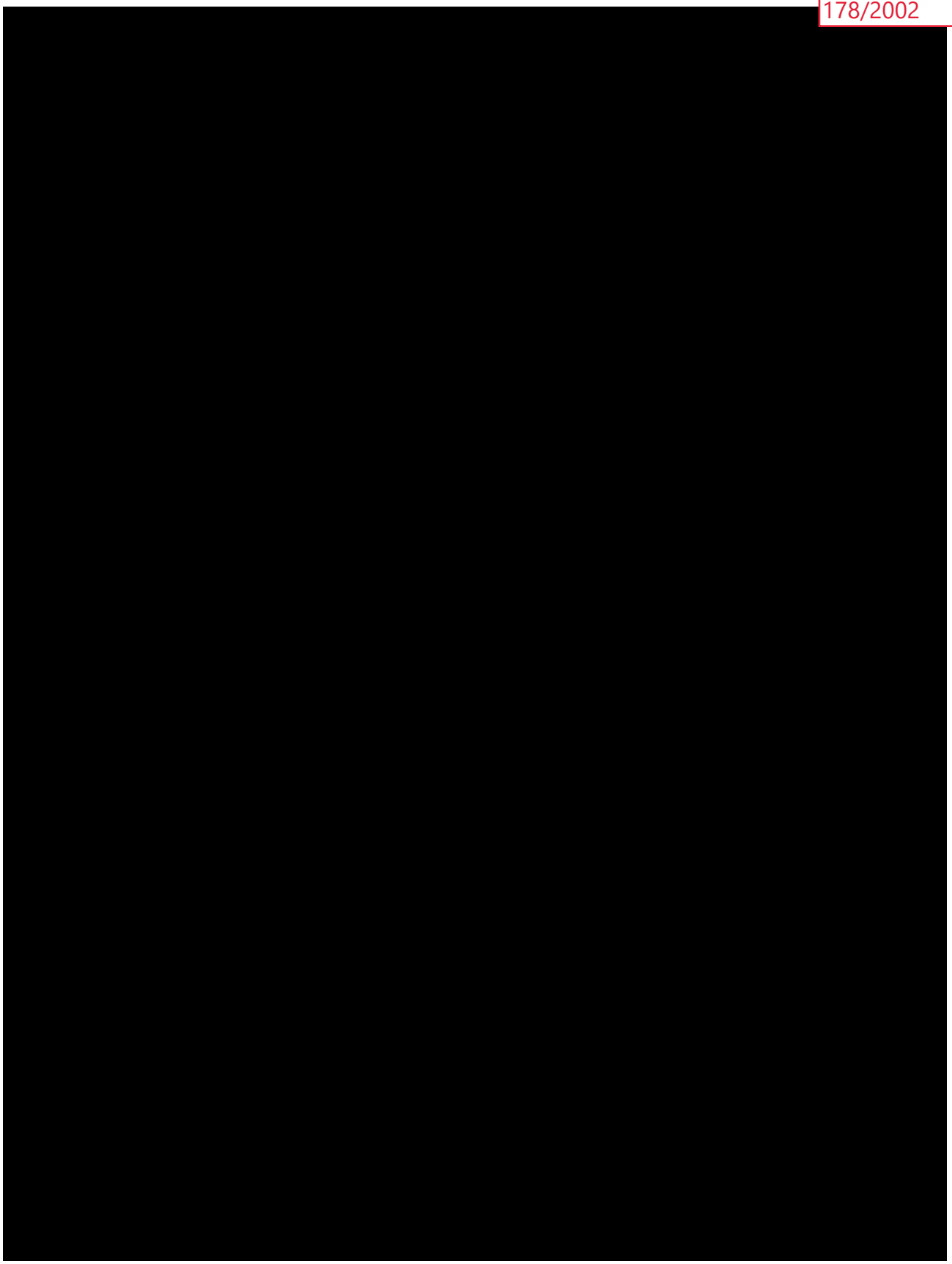
COPY OF THE TEST ITEM DATA SHEET



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

APPENDIX 7:

COPY OF THE TEST ITEM DATA SHEET



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

APPENDIX 8:

COPY OF THE GLP CERTIFICATE