[redacted] [redacted]

10.1.c Wob juncto 63.2ter.d Vo 1107/2009 juncto 93e.2 Vo 178/2002

FINAL REPORT

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

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

10.1.c Wob juncto 63.2ter.d Vo 1107/2009 juncto 93e.2 Vo 178/2002

Study Code: 11/236-001P

Study Director: [redacted]

30 November 2011

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GENERAL INFORMATION

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

10.1.c Wob juncto 63.2ter.d Vo 1107/2009 juncto 93e.2 Vo 178/2002

SPONSOR: De Ceuster n.v.

Address: Fortsesteenweg 30, B-2860 Sint-Katelijne-Waver, Belgium

[redacted]

STUDY PERFORMED BY: [redacted]

STUDY DIRECTOR: [redacted]

RESPONSIBLE PERSONS: [redacted]

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STATEMENT OF THE STUDY DIRECTOR

This study has been performed in accordance with the study plan, OECD 423 (17th December 2001), Commission Regulation (EC) NO 440/2008 of 30 May 2008, B.1.Tris 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.

Under the conditions of this study, the acute oral LD_{50} value of the test item CH2 Mild 1906 was found to be above 2000 mg/kg bw in female CRL:(WI) rats.

According the GHS criteria, CH2 Mild 1906 can be ranked as "Unclassified" for acute oral exposure.

Signature: [redacted] Date: 30 November 2011

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STATEMENT OF THE MANAGEMENT

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

Signature: [redacted]

Date: 30 Nov 2011

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QUALITY ASSURANCE STATEMENT

Study Code: 11/236-001P

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

Test item: [redacted]Pepino Mosaic Virus, CH2 strain, isolate 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
17 October 2011	Study Plan	17 October 2011	17 October 2011
25 October 2011	Treatment	25 October 2011	25 October 2011
23 November 2011	Draft Report	23 November 2011	23 November 2011
30 November 2011	Final Report	30 November 2011	30 November 2011

Signature: [redacted] Date: 30 November 2011

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CONTENTS

1. SUMMARY

The single-dose oral toxicity of CH2 Mild 1906 was performed according to the acute toxic class method (OECD 423 and Commission Regulation (EC) NO 440/2008 of 30 May 2008, B.1.Tris) in CRL:(WI) rats.

Two groups of three female CRL:(WI) rats were treated with the test item at a dose level of 2000 mg/kg bw (Group 1 and Group 2).

Initially, three females (Group 1) were treated at a dose level of 2000 mg/kg bw. As no mortality was observed, a confirmatory group (Group 2) was treated at the same dose level. No mortality was observed in the confirmatory group, therefore no further testing was required according to OECD 423 and Commission Regulation (EC) NO 440/2008 of 30 May 2008, B.1.Tris.

A single oral treatment was carried out by gavage for each animal after an overnight food withdrawal. Food was made available again 3 hours after the treatment. The test item was administered formulated in Distilled water at a concentration of 200 mg/mL at a dosing volume of 10 mL/kg bw.

Clinical observations were performed at 30 minutes, 1, 2, 3, 4 and 6 hours after dosing and daily for 14 days thereafter. Body weight was measured on Days -1, 0 and 7 and before necropsy. All animals were subjected to a necropsy and a macroscopic examination.

Results

Mortality

CH2 Mild 1906 did not cause mortality at a dose level of 2000 mg/kg bw.

Clinical observations

Treatment with CH2 Mild 1906 did not cause any clinical signs during the 14 days observation period. (Appendix 1)

Body weight and body weight gain

Body weight gains of CH2 Mild 1906 treated animals during the study showed no indication of a test item-related effect. (Appendix 2)

Macroscopic Findings

No macroscopic observations were noted at a dose level of 2000 mg/kg bw. (Appendix 3 and 4)

Conclusion:

Under the conditions of this study, the acute oral LD_{50} value of the test item CH2 Mild 1906 was found to be above 2000 mg/kg bw in female CRL:(WI) rats.

According the GHS criteria, CH2 Mild 1906 can be ranked as "Unclassified" for acute oral exposure.

2. INTRODUCTION

The objective of the study was to assess the toxicity of test item CH2 Mild 1906 when administered as a single oral gavage dose to rats. The results of the study allow the test item to be ranked according to most classification systems currently used.

2.1. STUDY SCHEDULE

Experimental Starting Date	25 October 2011
Experimental Completion Date	09 November 2011
Reception of Animals	13 October 2011
Treatment	25 October 2011 (females no. 9389, 9390, 9391) 26 October 2011 (females no. 9392, 9393, 9394)
Observation	25 October – 08 November 2011 (females no. 9389, 9390, 9391) 26 October – 09 November 2011 (females no. 9392, 9393, 9394)
Necropsy	08 November 2011 (females no. 9389, 9390, 9391) 09 November 2011 (females no. 9392, 9393, 9394)

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3. MATERIALS AND METHODS

3.1. TEST ITEM

Information supplied by the Sponsor:

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.
Vehicle:	Distilled water
Lot number:	2190511
Expiry Date:	31 May 2014
Dose volume:	10 mL/kg bw

*: *Note that the test item "CH2 Mild 1906" was used to describe this test item in raw data and in the Report.*

3.1.1. Identification, Receipt

The CH2 Mild 1906 is a plant virus and is not harmful to humans or animals, the test item is 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 the test item was made in the [redacted]. on the basis of the information provided by Sponsor. (For Copy of the Certificate of Analyses see Appendix 6.)

3.1.2. Formulation

Test item was freshly formulated at a concentration of 200 mg/mL in the vehicle, in [redacted] on the day of administration. The formulation was stirred with a magnetic stirrer up to finishing the treatment.

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3.2. EXPERIMENTAL ANIMALS

Species and strain:	CRL:(WI) rats
Source:	[redacted]
Hygienic level at arrival:	SPF
Hygienic level during the study:	Standard housing conditions
Number of animals:	6 animals, 3 animals/group
Sex:	Female, nulliparous and non-pregnant.
Age of animals at dosing:	Young healthy adult rats, 9 weeks old
Body weight at treatment:	204 – 216 g
Acclimation period:	At least 12 days

3.2.1. Husbandry

Animal health:	Only healthy animals were used for the test. The veterinarian certified health status.
Number of animal room:	522/9
Housing:	3 animals / cage
Cage type:	Type II polypropylene/polycarbonate
Bedding:	Lignocel Bedding for Laboratory Animals was available to animals during the study. [redacted].
Lighting period:	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:	Animals were housed by group to allow social interaction and with deep wood sawdust bedding to allow digging and other normal rodent activities.

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

3.2.2. Food and Water Supply

Animals received [redacted] "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 considered not to contain any contaminants that could reasonably be expected to affect the purpose or integrity of the study.

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For contents of the diet see Appendix 5. 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 [redacted] The quality control results are retained in the archives at [redacted]

3.2.3. Animal Identification

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

3.3. ADMINISTRATION OF THE TEST ITEM

3.3.1. Doses

Justification of the dose:

The initial dose level was selected by the study director to be that which is most likely to produce mortality in some of the dosed animals. In the lack of any preliminary toxicological information, 2000 mg/kg bw was selected to be the starting dose.

Initially, three female animals were treated with 2000 mg/kg bw of CH2 Mild 1906. No mortality was observed, therefore further 3 animals were treated at the dose level of 2000 mg/kg bw. As no mortality was observed in this second dose group, further testing was not required according to the test guidelines (OECD 423, Commission Regulation (EC) NO 440/2008 of 30 May 2008, B.1.Tris).

3.3.2. Procedure

A single oral gavage administration was followed by a fourteen-day observation period. On the day before treatment, the animals were fasted. The food but not water was withheld during an overnight period. Animals were weighed just before treatment. The test item was administered by oral gavage in the morning. The food was returned 3 hours after the treatment.

3.4. OBSERVATIONS

3.4.1. Clinical Observations

Clinical observations were performed on all animals at 30 minutes, 1, 2, 3, 4 and 6 hours after dosing and daily for 14 days thereafter. Individual observations were performed on the skin, fur, eyes, mucous membranes, 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. Body Weight Measurement

The body weight was recorded on the day before treatment (Day -1), on the day of the treatment (Day 0) and weekly after.

3.5. NECROPSY

Macroscopic examination was performed on all animals. The animals were sacrificed by exsanguination under pentobarbital anaesthesia (Euthasol® 40 %; *Lot: 11B15 6; Expiry date: January 2014; Produced by: AST Beheer B.V. Oudewater Netherlands (Produlab Pharma, Raamsdonksveer)*). After examination of the external appearance, the cranial, thoracic and the abdominal cavities were opened and the organs and the tissues were observed. Macroscopic abnormalities were recorded.

3.6. EVALUATION OF THE RESULTS

The method used was not intended to allow the calculation of a precise LD_{50} value.

The test item was ranked into categories of Globally Harmonized Classification System (GHS) described in the OECD Guideline No. 423.

Clinical signs, body weight, body weight gain and gross macroscopic data were tabulated.

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3.7. DEVIATION FROM THE STUDY PLAN

There was no deviation during the study.

3.8. 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 th [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.

3.9. THE PERMISSION OF THE [redacted]

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

3.10. 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

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4. REFERENCES

OECD Guidelines for Testing of Chemicals No. 423. Acute Oral Toxicity – Acute Toxic Class Method. Adopted: 17 December 2001

[redacted]

Commission Regulation (EC) NO 440/2008 of 30 May 2008, B.1.Tris

5. RESULTS

Mortality

CH2 Mild 1906 did not cause mortality at a dose level of 2000 mg/kg bw.

Clinical observations

Treatment with CH2 Mild 1906 did not cause any clinical signs during the 14 days observation period. (Appendix 1)

Body weight and body weight gain

Body weight gains of CH2 Mild 1906 treated animals during the study showed no indication of a test item-related effect. (Appendix 2)

Macroscopic Findings

No macroscopic observations were noted at a dose level of 2000 mg/kg bw. (Appendix 3 and 4)

6. CONCLUSION

Under the conditions of this study, the acute oral LD_{50} value of the test item CH2 Mild 1906 was found to be above 2000 mg/kg bw in female CRL:(WI) rats.

According the GHS criteria, CH2 Mild 1906 can be ranked as "Unclassified" for acute oral exposure.

APPENDICES

APPENDIX 1:

CLINICAL OBSERVATIONS

DOSE LEVEL: 2000 mg/kg bw, Treatment on Day 0 **SEX: FEMALE**

Cage No.	Animal Number	Observations	Observation days							Frequency
			0						1-14	
			30'	1h	2h	3h	4h	6h		
1	9389	Symptom Free	+	+	+	+	+	+	+	20/20
	9390	Symptom Free	+	+	+	+	+	+	+	20/20
	9391	Symptom Free	+	+	+	+	+	+	+	20/20
2	9392	Symptom Free	+	+	+	+	+	+	+	20/20
	9393	Symptom Free	+	+	+	+	+	+	+	20/20
	9394	Symptom Free	+	+	+	+	+	+	+	20/20

Remarks: + = present

h = hour (s) ' = second

Frequency of observation = number of occurence of observation / total number of observations

APPENDIX 2:

BODY WEIGHT DATA

DOSE LEVEL: 2000 mg/kg bw, Treatment on Day 0 **SEX: FEMALE**

Cage No.	Animal No.	Body weight (g) Days				Body Weight Gain (g)			
		-1	0	7	14	-1-0	0-7	7- 14	-1 - 14
1	9389	237	216	258	265	-21	42	7	28
	9390	233	216	247	247	-17	31	0	14
	9391	234	212	241	259	-22	29	18	25
2	9392	211	204	233	236	-7	29	3	25
	9393	218	205	234	254	-13	29	20	36
	9394	225	208	244	262	-17	36	18	37
Mean:		226.3	210.2	242.8	253.8	-16.2	32.7	11.0	27.5
Standard deviation:		10.2	5.3	9.2	10.8	5.5	5.3	8.7	8.5

APPENDIX 3:

NECROPSY FINDINGS

DOSE LEVEL: 2000 mg/kg bw, Treatment on Day 0 **SEX: FEMALE**

Cage No.	Animal ID	Necropsy Day	External Observations	Internal Observations	Organ/Tissue
1	9389	Day 14	No external observations recorded	No internal observations recorded	Not applicable
	9390	Day 14	No external observations recorded	No internal observations recorded	Not applicable
	9391	Day 14	No external observations recorded	No internal observations recorded	Not applicable
2	9392	Day 14	No external observations recorded	No internal observations recorded	Not applicable
	9393	Day 14	No external observations recorded	No internal observations recorded	Not applicable
	9394	Day 14	No external observations recorded	No internal observations recorded	Not applicable

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APPENDIX 4:

COPY OF THE PATHOLOGY REPORT

PATHOLOGY REPORT

INTRODUCTION

The objective of the study was to assess the acute oral toxicity of CH2 Mild 1906 when administered in a single dose to rats at a dose level of 2000 mg/kg bw. The results of the study allow the test item to be ranked according to most classification systems currently in use.

RESULTS AND DISCUSSION

All rats survived until the scheduled termination of the study.

All animals were euthanized upon completion of the observation 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

No macroscopic observations were noted at a dose level of 2000 mg/kg bw.

CONCLUSION

A single oral gavage of CH2 Mild 1906 to the CRL: (WI) female rat at a dose level of 2000 mg/kg bw followed by a 14 day of observation period, was not associated with any gross findings.

[redacted]

29 NOV 2011
Date

APPENDIX 5:

CONTENTS OF THE DIET

SSNIFF® SM R/M-Z+H COMPLETE DIET FOR RATS AND MICE

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

Crude protein	19.00	%
Crude fat	3.50	%
Crude fibre	3.60	%
Crude Ash	6.50	%
Lysine	1.10	%
Methionine	0.56	%
Calcium	1.00	%
Sodium	0.20	%
Magnesium	0.22	%
Phosphorus	0.70	%
Vitamin A	25000 IU	
Vitamin D_3	1000 IU	
Vitamin E	125 mg/kg	

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

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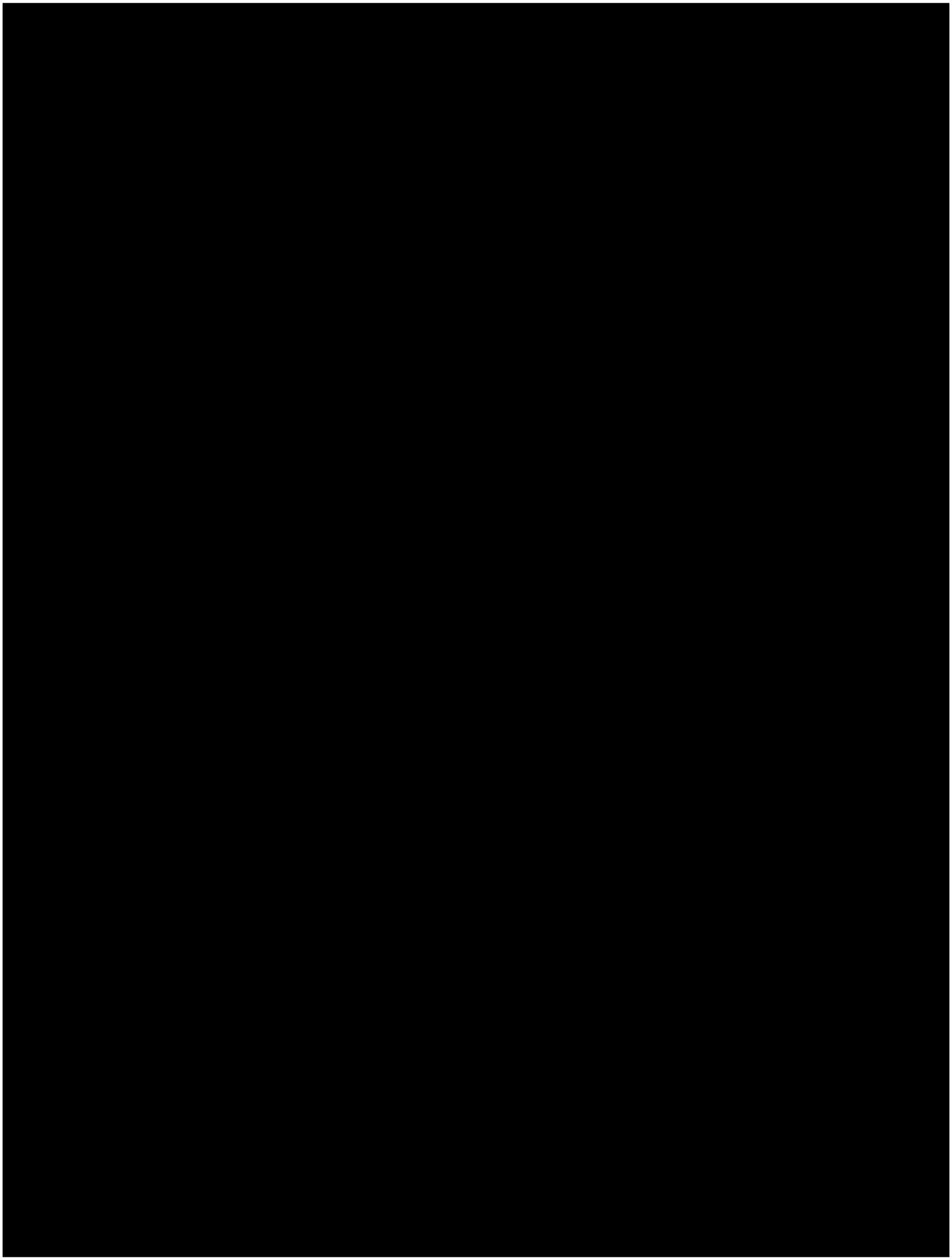
APPENDIX 6:

COPY OF THE CERTIFICATE OF ANALYSES



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APPENDIX 7:

[redacted]