Nelson Labs
A Sotera Health company

NELSON LABS ORIGINAL FINAL REPORT 1 (for Sponsor)

PROJECT NUMBER TE191866
STUDY NUMBER 19-B6959-G1

Sponsor	**Kraiburg TPE GmbH & Co. KG**
Contact	*Eugen Andert*
Address	Friedrich-Schmidt-Strasse 2 84478 Waldkraiburg Germany
Test Item Arrival Date	18 Jul 2019
Experimental Starting Date	23 Jul 2019
Experimental Completion Date	01 Aug 2019
PO Number	4500105895
Quotation	1907034
Study Plan	GEN/B-Microtox/18/0001 rev 00
Test Facility	Nelson Labs NV
Address	Romeinsestraat 12 3001 Leuven Belgium

MEM XTT-ASSAY:
IN-VITRO CYTOTOXICITY ASSAY ON L-929 MOUSE FIBROBLASTS OF HTM8510/207 (TM7APO)

GLP COMPLIANCE STATEMENT

This study meets the technical requirements of the study plan. The study described in this report was conducted in accordance with the OECD Principles of Good Laboratory Practice, except for the characterization of the test item. The study was performed under supervision of the Study Director. The study was conducted according to the procedures herein described and this report represents a true and accurate record of the results obtained.

Stefanie Roberfroid
Study Director

07 AUG 2019
Final Report Date

1.0 PURPOSE

The purpose of the test is to determine the biological reactivity of a mammalian cell line (L929 mouse fibroblast) in response to an extract of the test item. The test is based on the measurement of the viability of cells via mitochondrial dehydrogenase. XTT (2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino) carbonyl]-2H-tetrazolium hydroxide) is metabolically reduced in viable cells to a water-soluble formazan product. The number of viable cells correlates to the optical density determined by photometric measurements.

2.0 REFERENCES

- ISO 10993-5, 2009, Biological Evaluation of Medical Devices - Part 5: Tests for In Vitro Cytotoxicity.
- ISO 10993-12, 2012, Biological Evaluation of Medical Devices - Part 12: Sample Preparation and Reference Materials.
- SOP 0228, revision 14: MEM Elution Test
- SOP 0223, revision 10: Growth and Maintenance of Mammalian Cell Lines

3.0 LIST OF ANNEXES APPLICABLE FOR THIS STUDY

- Nelson Labs Study Report Annex : QA Statement for GLP Studies. D051053, current revision

4.0 EXPERIMENTAL DESIGN

All manipulations with sterile test items were conducted aseptically under a Laminar Flow Cabinet.

4.1. Equipment/Reagents/Disposables

Equipment	Reagents	Disposables
Laminar Flow Cabinet	Serum-supplemented Minimum Essential (MEM-Complete): - MEM - Penicillin/Streptomycin - Fetal Bovine Serum - L-Glutamine - Hepes Buffer	Disposable sterile pipettes
Automated pipettes		Extraction flasks, autoclaved
CO_2 incubator		96 well tissue culture plates
Inverted microscope		Sterile plastic Pasteur pipettes
Autoclave		Syringe filters 0.22 µm*
Laboratory balance		Sterile syringe*
Multi pipette		Multiwell dispenser unit (sterile)
Shaking incubator	Cell Proliferation kit (XTT)	
96-well plate photometer, equipped with 450 nm filter with WIN KQCL software		

Note (*):only used if extract was sterile filtered.

4.2. Identification of the test system and the reference (control) items

The test system was mouse fibroblast L929 cells. The cell line was obtained from Vircell (Article number: FTL9, Granada - Spain).
Silicone tubing was used as negative control item and natural rubber was used as positive control item. Control item extracts cannot be analyzed for concentration, homogeneity and stability.

4.3. Preparation of the extracts

Prior to extraction, the control items were autoclaved for 30 minutes at 121 ± 2°C. The test item and control items were extracted with MEM-complete in an extraction flask. 20 mL of MEM-complete was transferred to a sterile extraction flask and served as medium control. The test and control items were extracted dynamically. If the test item could not be steam sterilized prior to extraction, the extract was sterile filtered before being applied to the L929-cells.

4.4. Preparation of the L929 cell culture

Cell cultures were maintained as described in SOP 0223. Cell cultures were removed from the culture flasks by enzymatic digestion according to SOP 0223. The cells were re-suspended and diluted in MEM-complete until a concentration of approximately 10^5 cells/mL was obtained. 100 µL of MEM-complete was dispensed into the peripheral wells of a 96-well plate (= blank). In the remaining wells, approximately 10^4 cells per well in 100 µL of MEM-complete was seeded. The cells were incubated overnight (5 ± 1% CO_2, 37 ± 1°C)

4.5. Exposure of L929-cells to the test item extract

The subconfluency and the morphology of the cultures was verified under the microscope before starting the test. Each plate was examined under the microscope to ensure that cell growth is relatively even across the multi-well plate. The culture medium was aspirated from the cell-monolayers and replaced with either extracts of the test item, negative control, medium control (only MEM-complete) or positive control at 100 µL per well. The test and control extracts were tested in quadruplicate. The plate was incubated for 2 days at 37 ± 1°C in a 5 ± 1% CO_2 humidified atmosphere.

4.6. Evaluation

After examination of the plates, XTT-mix was added to the cells. The XTT mix was prepared as described in SOP 0228. The XTT-mix was thawed in an incubator away from the light at 37 ± 1°C until a clear solution was obtained. 50 µL of XTT mix was added into each well. The plates were incubated for 4 ± 1h at 37 ± 1°C in the dark. The plate was placed in the plate reader and absorption detected at 450 nm.

4.7. Data analysis

A decrease in number of living cells results in a decrease in the overall activity of mitochondrial dehydrogenase in the sample. This decrease directly correlates to the amount of orange formazan formed, as monitored by the optical density (OD) at 450 nm. To calculate the reduction of viability compared to the blank, the following

equation was used:

$$Viability\% = \frac{100\ x\ (OD_{450e} - OD_{450b})}{OD_{450m} - OD_{450b}}$$

OD_{450b} = mean OD of blank
OD_{450e} = mean OD of the extract of the test item, positive control or negative control
OD_{450m} = mean OD of medium control
The evaluation criteria are described in Table 1, and if these are not met for the controls, the test should be repeated.

Table 1 : Evaluation criteria

Identification	Criteria
Test item	If viability is reduced to < 70% of the medium control, the test item is considered to have cytotoxic potential.
Positive control	The viability of the positive control should be equal to or below 10%.
Negative control	The viability of the negative control should be equal to or above 90%.
Medium control	The mean OD_{450} of the medium controls should be ≥ 0.2. A test meets acceptance criteria if the left and right mean of the medium controls do not differ by more than 15% from the mean of all medium controls.

5.0 RECORDS

- All raw data and records, necessary to reconstruct the study are maintained in the archives of Nelson Labs NV in a binder coded with the unique TE-number. The study plan (including any amendments and TRF D040427), the raw data, one original final report (including any amendments) will be archived at Nelson Labs NV. The retention period will be at least 10 years covering three inspection cycles by the Belgian Monitoring Authorities.
- One original final report will be forwarded to the Sponsor.
- All unused test items are discarded after completion of the study.

6.0 DEVIATIONS AND CIRCUMSTANCES

Event	Present?	Impact on Results
Deviations from the study Plan	NO	Not Applicable
Any circumstances that may have affected the results	NO	Not Applicable

7.0 IDENTIFICATION AND EXTRACTION CONDITIONS OF THE TEST ITEM

Table 2: Identification of test item and extraction conditions

TEST ITEM	
Name	HTM8510/207 (TM7APO)
Lot/Batch #	1158853
Sterilization of test item	Steam Sterilisation
Extraction condition	24 ± 2 h at 37 ± 1°C

Test Item extracts cannot be analyzed for concentration, homogeneity and stability.

8.0 RESULTS

The mean OD_{450} of the medium control is ≥ 0.2. The mean OD of the medium controls differ not more than 15%. The viability of the positive and negative control is shown in Table 3. All controls pass the criteria of the test.
The viability of the extract of the test item is **93%** (see Table 4) and therefore considered as **not cytotoxic**.

Table 3: Viability of Control item extracts

Name	Extraction Ratio	Amount Tested/ Extraction Solvent	OD repeats				Viability
			# 1	# 2	# 3	# 4	
Negative Control: Silicone Tubing	0.2 g/mL	4 g /20 mL	2.758	2.897	2.925	2.926	92%
Positive Control: Natural Rubber	0.2 g/mL	4 g /20 mL	0.342	0.335	0.329	0.332	4%

Table 4: Viability of test item extracts

Name	Extraction Ratio	Amount Tested/ Extraction Solvent	Dilution	SUMMARY OF RESULTS				
				OD repeats				Viability
				# 1	# 2	# 3	# 4	
Test Item	1.25 cm^2/mL	1TI:315.41cm^2 /252.3mL	1:1	2.909	2.861	2.871	2.970	93%

9.0 REASON FOR RETEST

During the original execution of the assay, a wrong extraction volume was added to the test item. Therefore the first test was not performed according to an extraction ratio of 1.25cm^2/mL as requested. A retest was logged into our system with 19-B6959-G1R as retest number.
The content of this report included all information of the performed retest. The original data of the first test are not reported.

10.0 SUMMARY

The observed cellular response obtained from the control extracts confirmed the suitability of the test system. 93% viability was observed for the L929 mammalian cells exposed to the test item extract at the 24 – 48 hours observation. The test item, HTM8510/207 (TM7APO) (Batch: 1158853), is considered to have **no cytotoxic** potential and **passes** the requirements of ISO 10993-5.

QUALITY ASSURANCE STATEMENT

(File Copy)[1] Study Plan Number: [GEN/B-MICROTOX/18/0001 rev 00]

TE n°: [TE191866] Study n°: [19-B6959-G1] [1]

Title: [In-vitro cytotoxicity assay on L929 mouse fibroblasts of HTM8510/207 (TM7APO)] [2]

The data contained in this report were inspected by the Quality Assurance Unit to assure compliance with the study plan, Standard Operating Procedures, the pertinent Good Laboratory Regulations of the OECD and the EEC Directives. The findings were reported to the Management and the Study Director. The inspections took place on the following dates:

Inspections	Date of Inspection	Date Reported to Study Director	Date Reported to Management
Review of the study plan (File copy)	15 MAY 2018	15 MAY 2018	15 MAY 2018
Review of the Study plan (D040427-Test Requisition Form)	07 AUG 2019	07 AUG 2019	07 AUG 2019
Review of Raw data & draft report	07 AUG 2019	07 AUG 2019	07 AUG 2019
Review of final report	07 AUG 2019	07 AUG 2019	07 AUG 2019

The critical phase of this study was inspected process-based. The last inspection of [the preparation of test and control item extracts] was performed on [25 JUN 2019].

This signed statement indicates that the report has been reviewed by the Quality Assurance Unit and accurately reflects the raw data developed during the study.

To the best of our knowledge, there is no significant deviation from applicable GLP-regulations that adversely affected the study quality or integrity.

[Johan Neys]
Quality Assurance

07 AUG 2019
Date

[1] In case of a study where a file copy study plan is used

[2] In case of a FCSP, the title of the study plan is extended with the test item name

Nelson Labs
A Sotera Health company

NELSON LABS ORIGINAL FINAL REPORT 2 (for Nelson Labs NV)

PROJECT NUMBER TE191866
STUDY NUMBER 19-B6959-G1

Sponsor	**Kraiburg TPE GmbH & Co. KG**
Contact	*Eugen Andert*
Address	Friedrich-Schmidt-Strasse 2 84478 Waldkraiburg Germany
Test Item Arrival Date	18 Jul 2019
Experimental Starting Date	23 Jul 2019
Experimental Completion Date	01 Aug 2019
PO Number	4500105895
Quotation	1907034
Study Plan	GEN/B-Microtox/18/0001 rev 00
Test Facility	Nelson Labs NV
Address	Romeinsestraat 12 3001 Leuven Belgium

MEM XTT-ASSAY:
IN-VITRO CYTOTOXICITY ASSAY ON L-929 MOUSE FIBROBLASTS OF HTM8510/207 (TM7APO)

GLP COMPLIANCE STATEMENT

This study meets the technical requirements of the study plan. The study described in this report was conducted in accordance with the OECD Principles of Good Laboratory Practice, except for the characterization of the test item. The study was performed under supervision of the Study Director. The study was conducted according to the procedures herein described and this report represents a true and accurate record of the results obtained.

Stefanie Roberfroid
Study Director

07 AUG 2019
Final Report Date

1.0 PURPOSE

The purpose of the test is to determine the biological reactivity of a mammalian cell line (L929 mouse fibroblast) in response to an extract of the test item. The test is based on the measurement of the viability of cells via mitochondrial dehydrogenase. XTT (2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino) carbonyl]-2H-tetrazolium hydroxide) is metabolically reduced in viable cells to a water-soluble formazan product. The number of viable cells correlates to the optical density determined by photometric measurements.

2.0 REFERENCES

- ISO 10993-5, 2009, Biological Evaluation of Medical Devices - Part 5: Tests for In Vitro Cytotoxicity.
- ISO 10993-12, 2012, Biological Evaluation of Medical Devices - Part 12: Sample Preparation and Reference Materials.
- SOP 0228, revision 14: MEM Elution Test
- SOP 0223, revision 10: Growth and Maintenance of Mammalian Cell Lines

3.0 LIST OF ANNEXES APPLICABLE FOR THIS STUDY

- Nelson Labs Study Report Annex : QA Statement for GLP Studies. D051053, current revision

4.0 EXPERIMENTAL DESIGN

All manipulations with sterile test items were conducted aseptically under a Laminar Flow Cabinet.

4.1. Equipment/Reagents/Disposables

Equipment	Reagents	Disposables
Laminar Flow Cabinet	Serum-supplemented Minimum Essential (MEM-Complete): - MEM - Penicillin/Streptomycin - Fetal Bovine Serum - L-Glutamine - Hepes Buffer	Disposable sterile pipettes
Automated pipettes		Extraction flasks, autoclaved
CO_2 incubator		96 well tissue culture plates
Inverted microscope		Sterile plastic Pasteur pipettes
Autoclave		Syringe filters 0.22 µm*
Laboratory balance		Sterile syringe*
Multi pipette		Multiwell dispenser unit (sterile)
Shaking incubator	Cell Proliferation kit (XTT)	
96-well plate photometer, equipped with 450 nm filter with WIN KQCL software		

Note (*):only used if extract was sterile filtered.

4.2. Identification of the test system and the reference (control) items

The test system was mouse fibroblast L929 cells. The cell line was obtained from Vircell (Article number: FTL9, Granada - Spain).
Silicone tubing was used as negative control item and natural rubber was used as positive control item. Control item extracts cannot be analyzed for concentration, homogeneity and stability.

4.3. Preparation of the extracts

Prior to extraction, the control items were autoclaved for 30 minutes at 121 ± 2°C. The test item and control items were extracted with MEM-complete in an extraction flask. 20 mL of MEM-complete was transferred to a sterile extraction flask and served as medium control. The test and control items were extracted dynamically. If the test item could not be steam sterilized prior to extraction, the extract was sterile filtered before being applied to the L929-cells.

4.4. Preparation of the L929 cell culture

Cell cultures were maintained as described in SOP 0223. Cell cultures were removed from the culture flasks by enzymatic digestion according to SOP 0223. The cells were re-suspended and diluted in MEM-complete until a concentration of approximately 10^5 cells/mL was obtained. 100 µL of MEM-complete was dispensed into the peripheral wells of a 96-well plate (= blank). In the remaining wells, approximately 10^4 cells per well in 100 µL of MEM-complete was seeded. The cells were incubated overnight (5 ± 1% CO_2, 37 ± 1°C)

4.5. Exposure of L929-cells to the test item extract

The subconfluency and the morphology of the cultures was verified under the microscope before starting the test. Each plate was examined under the microscope to ensure that cell growth is relatively even across the multi-well plate. The culture medium was aspirated from the cell-monolayers and replaced with either extracts of the test item, negative control, medium control (only MEM-complete) or positive control at 100 µL per well. The test and control extracts were tested in quadruplicate. The plate was incubated for 2 days at 37 ± 1°C in a 5 ± 1% CO_2 humidified atmosphere.

4.6. Evaluation

After examination of the plates, XTT-mix was added to the cells. The XTT mix was prepared as described in SOP 0228. The XTT-mix was thawed in an incubator away from the light at 37 ± 1°C until a clear solution was obtained. 50 µL of XTT mix was added into each well. The plates were incubated for 4 ± 1h at 37 ± 1°C in the dark. The plate was placed in the plate reader and absorption detected at 450 nm.

4.7. Data analysis

A decrease in number of living cells results in a decrease in the overall activity of mitochondrial dehydrogenase in the sample. This decrease directly correlates to the amount of orange formazan formed, as monitored by the optical density (OD) at 450 nm. To calculate the reduction of viability compared to the blank, the following

equation was used:

$$Viability\% = \frac{100\ x\ (OD_{450e} - OD_{450b})}{OD_{450m} - OD_{450b}}$$

OD_{450b} = mean OD of blank
OD_{450e} = mean OD of the extract of the test item, positive control or negative control
OD_{450m} = mean OD of medium control

The evaluation criteria are described in Table 1, and if these are not met for the controls, the test should be repeated.

Table 1 : Evaluation criteria

Identification	Criteria
Test item	If viability is reduced to < 70% of the medium control, the test item is considered to have cytotoxic potential.
Positive control	The viability of the positive control should be equal to or below 10%.
Negative control	The viability of the negative control should be equal to or above 90%.
Medium control	The mean OD_{450} of the medium controls should be ≥ 0.2. A test meets acceptance criteria if the left and right mean of the medium controls do not differ by more than 15% from the mean of all medium controls.

5.0 RECORDS

- All raw data and records, necessary to reconstruct the study are maintained in the archives of Nelson Labs NV in a binder coded with the unique TE-number. The study plan (including any amendments and TRF D040427), the raw data, one original final report (including any amendments) will be archived at Nelson Labs NV. The retention period will be at least 10 years covering three inspection cycles by the Belgian Monitoring Authorities.
- One original final report will be forwarded to the Sponsor.
- All unused test items are discarded after completion of the study.

6.0 DEVIATIONS AND CIRCUMSTANCES

Event	Present?	Impact on Results
Deviations from the study Plan	NO	Not Applicable
Any circumstances that may have affected the results	NO	Not Applicable

7.0 IDENTIFICATION AND EXTRACTION CONDITIONS OF THE TEST ITEM

Table 2: Identification of test item and extraction conditions

TEST ITEM	
Name	HTM8510/207 (TM7APO)
Lot/Batch #	1158853
Sterilization of test item	Steam Sterilisation
Extraction condition	24 ± 2 h at 37 ± 1°C

Test Item extracts cannot be analyzed for concentration, homogeneity and stability.

8.0 RESULTS

The mean OD_{450} of the medium control is ≥ 0.2. The mean OD of the medium controls differ not more than 15%. The viability of the positive and negative control is shown in Table 3. All controls pass the criteria of the test.
The viability of the extract of the test item is **93%** (see Table 4) and therefore considered as **not cytotoxic**.

Table 3: Viability of Control item extracts

Name	Extraction Ratio	Amount Tested/ Extraction Solvent	OD repeats				Viability
			# 1	# 2	# 3	# 4	
Negative Control: Silicone Tubing	0.2 g/mL	4 g /20 mL	2.758	2.897	2.925	2.926	92%
Positive Control: Natural Rubber	0.2 g/mL	4 g /20 mL	0.342	0.335	0.329	0.332	4%

Table 4: Viability of test item extracts

Name	Extraction Ratio	Amount Tested/ Extraction Solvent	Dilution	SUMMARY OF RESULTS				
				OD repeats				Viability
				# 1	# 2	# 3	# 4	
Test Item	1.25 cm^2/mL	1TI:315.41cm^2 /252.3mL	1:1	2.909	2.861	2.871	2.970	93%

9.0 REASON FOR RETEST

During the original execution of the assay, a wrong extraction volume was added to the test item. Therefore the first test was not performed according to an extraction ratio of 1.25cm^2/mL as requested. A retest was logged into our system with 19-B6959-G1R as retest number.
The content of this report included all information of the performed retest. The original data of the first test are not reported.

10.0 SUMMARY

The observed cellular response obtained from the control extracts confirmed the suitability of the test system. 93% viability was observed for the L929 mammalian cells exposed to the test item extract at the 24 – 48 hours observation. The test item, HTM8510/207 (TM7APO) (Batch: 1158853), is considered to have **no cytotoxic** potential and **passes** the requirements of ISO 10993-5.

QUALITY ASSURANCE STATEMENT

(File Copy)[1] Study Plan Number: [GEN/B-MICROTOX/18/0001 rev 00]

TE n°: [TE191866] Study n°: [19-B6959-G1] [1]

Title: [In-vitro cytotoxicity assay on L929 mouse fibroblasts of HTM8510/207 (TM7APO)] [2]

The data contained in this report were inspected by the Quality Assurance Unit to assure compliance with the study plan, Standard Operating Procedures, the pertinent Good Laboratory Regulations of the OECD and the EEC Directives. The findings were reported to the Management and the Study Director. The inspections took place on the following dates:

Inspections	Date of Inspection	Date Reported to Study Director	Date Reported to Management
Review of the study plan (File copy)	15 MAY 2018	15 MAY 2018	15 MAY 2018
Review of the Study plan (D040427-Test Requisition Form)	07 AUG 2019	07 AUG 2019	07 AUG 2019
Review of Raw data & draft report	07 AUG 2019	07 AUG 2019	07 AUG 2019
Review of final report	07 AUG 2019	07 AUG 2019	07 AUG 2019

The critical phase of this study was inspected process-based. The last inspection of [the preparation of test and control item extracts] was performed on [25 JUN 2019].

This signed statement indicates that the report has been reviewed by the Quality Assurance Unit and accurately reflects the raw data developed during the study.

To the best of our knowledge, there is no significant deviation from applicable GLP-regulations that adversely affected the study quality or integrity.

[Johan Neys]
Quality Assurance

07 AUG 2019
Date

[1] In case of a study where a file copy study plan is used

[2] In case of a FCSP, the title of the study plan is extended with the test item name