ANNEX 2
first draft SOP

CONTENTS:

1 RATIONALE

Phototoxicity (photoirritation) is here defined as acute toxic response that is elicited after the first exposure of skin to certain chemicals and subsequent exposure to light, or that is induced similarly by skin irradiation after systemic administration of a chemical substance.

The present assay is designed to detect the phototoxic potential of a chemical by using a three dimensional human epidermis model*. Since the assay allows application of test materials to the air exposed surface (stratum corneum), it mimics the *in vivo* situation and thus may allow to predict phototoxic potency of test materials applied in usage concentrations. The test is based upon a comparison of the cytotoxicity of a chemical when tested with and without additional exposure to a non toxic dose of UVA+visible light. Cytotoxicity is expressed as reduction of mitochondrial conversion of MTT to formazan [1], determined one day after chemical treatment and UVA exposure.

** MatTek´s EpiDerm System [2, 3] consists of normal, human-derived epidermal keratinocytes which have been cultured to form a multilayered, highly differentiated model of the human epidermis. It consists of organised basal, spinous and granular layers, and a multi-layered stratum corneum containing intercellular lamellar lipid layers arranged in patterns analogous to those found in vivo. The EpiDerm tissues (surface 0.6 cm²) are cultured on specially prepared cell culture inserts (Millicells®, 10 mm ∅) and shipped worldwide as kits, containing 24 tissues on shipping agarose.*

2 NEED FOR THE ASSAY

It has been shown in a joint EU/COLIPA validation project [6, 7], that the phototoxic potential of chemicals can be correctly predicted by using cell culture monolayers in a specially designed cytotoxicity assay, the 3T3-NRU-photoirritation test. Since the phototoxic potential of a chemical predicted using a cellular system may not be relevant when topically applied to the skin at low concentrations (e.g. in a formulation) there is a need for adjunct tests, which allow for the assessment of safe usage concentrations on a dose per area basis. Reconstituted skin models and epidermis models have shown to be able to predict both, photoirritancy [4, 5, 8], as well as the photoprotective action of sunscreens [5]. Thus, in a testing strategy which is based purely on *in vitro* tests, there is a need to combine the basic 3T3 NRU PIT with other *in vitro* tests, which may allow a ranking of relative phototoxic potency, as well as a safety assessment of formulations. In addition, tests involving skin models could allow risk benefit analysis of dermal pharmaceuticals.

3 BASIC PROCEDURE

On day of receipt (e.g. Tuesday afternoon) EpiDerm™ tissues are stored over night in a refrigerator. Next day, one hour before starting the assay, tissues are transferred to 6-well plates with assay medium. Then, five concentrations of the test material (suspended in oil or water/oil) are topically applied onto 2 tissues per concentration (vehicle control + 5 concentrations = 12 tissues). A second set of 12 tissues is treated identically. Plates are incubated over night for 21 hrs. Then, one set of tissues is exposed to a UVA dose of 6 J/cm² (= **phototoxicity assay**), whereas the other set is kept in the dark, during irradiation period (= **cytotoxicity assay**). After irradiation all tissues are thoroughly rinsed with PBS to remove test material, transferred to new 6 well plates with fresh assay medium and incubated overnight for 21 hrs. The assay medium is then replaced by MTT-solution and tissues are incubated for 3 hours. Tissues are thoroughly rinsed with PBS, and the formazan is extracted with Isopropanol. Optical density is determined at 570 nm in a plate spectrophotometer. Cell viabil-

ity is calculated for each tissue as % of the corresponding irradiated or unirradiated vehicle control, respectively.

4 MATERIALS

4.1 Materials, not provided with the Kits:

4.1.1 Laboratory aids

Sterile, blunt-edged forceps	*For transferring inserts from agarose to 6 well plates*
6-well tissue culture plate (in addition to those provided)	*Multilab GmbH, Reichenberger Str.154 10999 Berlin For replacing assay medium*
Sterile disposable pipettes, pipette tips and pipetters	*For diluting, adding, and removing media and test materials. For topically applying test materials to tissues*
Mortar and pestle	*For grinding granulars and preparing an oil/ test material suspension*
5 Beakers á 50 ml	*For preparing and handling distilled water (di-H_2O)., oil-suspension, assay medium, extractant solution, PBS solution*
15 ml and 50 ml sterile, capped glass test tubes	*For preparing the concentration series*
Repeat pipetter (1ml)	*For adding assay medium*
Repeat pipetter (2ml)	*For adding the extractant solution*
Positive displacement Pipet (50µl)	*For application of viscous test materials*
Pipet (100 µl)	*For pipetting the concentration series*
Pipet (200 µl)	*For pipetting the concentration series*
Pipet (1000 µl)	*For rinsing with PBS*

4.1.2 Technical Equipment

Bunsen burner or autoclave	*For sterilising forceps*
37 °C incubator with 5% CO_2, 90 % humidity	*For incubating tissues prior to and during assays*
Vacuum source/trap	*For aspirating solutions*
Laminar flow hood	*For transferring tissues under sterile conditions and for application of test materials.*
37 °C water bath	*For warming Serum-Free Assay Medium.*

4.1.2 continued

Laboratory balance .	*For preparing an oil vehicle or water/oil vehicle*
96-well Spectrophotometer (Plate-Reader)	*For reading optical density at 570 nm, using 630 nm as reference*
Shaker for cell culture plates	*For extraction of formazan*
Laboratory Centrifuge 1500 x g	*For centrifugation of MTT medium*
Vortex mixer	*For keeping test suspensions homogeneous during preparation of the concentration series*

4.1.3 UVA-vis Irradiation equipment

UV-sun simulator, type SOL 500, Dr. Hönle	*Dr. K. Hönle GmbH, Frauenhoferstr. 5, D-82152 Martinsried, Germany ☎: +49-89-856 08-0 Contact: Dr. G. Schmid Fax: +49-89-856 08-48*
Any appropriate, adjustable and stable tripod	*For the fixation of the SOL 500*
UVA-meter, type No. 37, Dr. Hönle	*For everyday check of calibration*
UVA-meter, type No. 37, Dr. Hönle	*Use only as a reference in case of unexpected readings with the everyday radiometer*
Filter, type H1, Dr. Hönle	*Use to cut-off emitted UVB*

4.1.4 Solutions, Reagents

Sesame oil (purity: pharmacopeia grade, e.g. according to USP, BP, EP, DAB)	*source: any pharmacy* *Vehicle for test materials*
H_2O Aqua Pur (Millipore®), or Aqua destillata	*Vehicle for test materials*
PBS with Ca^{++} and Mg^{++}: ~500ml per test (e.g. Gibco # 14040)	*For rinsing-off test materials after irradiation*

4.1.5 Computer software

KOWIRA for Windows*	*For data communication with the 96-well plate photometer and recording of raw data*
MS Excel 5.0	*For and analysis of data in the Data Spreadsheet*

* KOWIRA (= Konzentrations Wirkungs Analyse) is a German Software, which was specially developed for ZEBET in 1993 for analysing Concentration-Response-Curves obtained with cytotoxicity assays, which make use of a 96-well spectrophotometer. KOWIRA is able to operate several spectrophotometers from SLT and DYNATEC. In the present assay it is only used to pick up the ODs from the photometer and to export these data into the MS EXCEL spreadsheet.

4.2 Epi-200 Kit Components

Examine all kit components for integrity. If there is a concern call MatTek Corporation immediately (Mitch Klausner, ☎ +1-508-881-6771, Fax +1-508-879-1532).

1	Sealed 24-well plate	*Contains 24 inserts with tissues on agarose*
2	24-well plates	*Use for MTT viability assay*
4	6-well plates	*Use for storing inserts, or for topically applying test agents*
1 bottle	Serum-Free Assay Medium	*DMEM-based medium*
1 bottle	PBS Rinse Solution (100ml)	*Use for rinsing the inserts in MTT assay*
1 vial	1% Triton X-100 Solution (10ml)	*Skin irritant reference chemical* ***Do not use in present assay***
1	MTT Assay Protocol	*MatTek Corporation: steps are included in the present protocol*

4.3 MTT-100 Assay Kit Components

1 vial, *2 ml*	MTT concentrate	
1 vial, *8 ml*	MTT diluent	*For diluting MTT concentrate prior to use in the MTT assay*
1 bottle, *60ml*	Extractant Solution (Isopropanol)*	*For extraction of formazan crystals*

** To avoid shipping delays due to dangerous good transport kits can be ordered <u>without</u> isopropanol*

5 METHODS

5.1 Expiration and Kit Storage

Epi-200 kits are shipped from Boston on Monday; make sure that they are arriving in the laboratory on Tuesday. Upon receipt of the EpiDerm tissues, place the sealed 24 well plates and the assay medium into the refrigerator (4°C). Place the MTT concentrate containing vial in the freezer (- 20°C) and the MTT diluent in the refrigerator (4°C).

part#	description	conditions	shelf life
EPI-200	EpiDerm cultures	refrigerate (4°C)	96 hours
EPI-100	assay medium	refrigerate (4°C)	7 days
MTT-099	MTT diluent	refrigerate (4°C)	7 days
MTT-100	MTT concentrate	freeze (- 20°C)	2 month

Record lot numbers of all components and transfer lot/production label on sealed tray onto the Methods Documentation Sheet (see **ANNEX** B).

Note:
Since testing starts on **Wednesday**, irradiation on **Thursday**, MTT assay on **Friday**, do not order more Epi-200 kits per week than can be dosed or irradiated on **one** day, respectively.

5.2 Calibration of the Solar Simulator

1. Mount the SOL 500, equipped with a H1-filter, on any appropriate stable tripod allowing fine-adjustment of the exposure distance.
2. ***Note:*** *New metal halide burners should be run for about 100 hrs prior to first use to achieve a stable emittance of energy.*
3. Adjust SOL 500 to a distance of about 60 cm.
4. Measure energy using the calibrated UV radiometer (type 37, Dr. Hönle), which is equipped with an UVA-sensor of the same serial number.
5. Adjust distance of SOL 500 to achieve a UVA irradiance of **1.7 mW/cm²**
   (The resulting dose will be 1 J/cm² per 10 min. exposure time)
6. According to the number of plates to be exposed concurrently, check the exposure area for equal distribution of irradiance:
   A range of **1.6 - 1.8 mW/cm²** is acceptable. ***Important:*** A maximum difference of **1.5 and 1.9 mW/cm²** can be accepted, if the positions of the plates with low and high irradiance are changed of position after half time of the irradiation (30 minutes) is reached (like chess castling).

The SOL 500 shall be calibrated as described above before performing a phototoxicity assay.

In case measurements with the UV radiometer reveal unexpected results, either the metal halide burner may have reached the end of it's shelflife, or the radiometer is de-calibrated due to various reasons. In this case, a second reference radiometer of the same type and calibration, which is has not been handled every day and kept in the dark and may be used for cross check.

5.3 Quality Controls

5.3.1 UVA Sensitivity of the Epi-200 Tissues

A UVA sensitivity experiment should be performed once the test is set up in the laboratory for the first time. If UVA sensitivity of the tissues is within the acceptance range this type of experiment should be repeated in greater periods (e.g. once every 6 month). Untreated tissues are irradiated with a dose series of UVA-vis:

1. Adjust irradiance of the Sol 500 to 1.7 mW/cm².
2. For the experiment use 24 tissues, previously incubated for 1 hour (37°C, 5 % CO_2) in assay medium after transfer from shipping agarose.
3. Place three tissues (= 3 millicell inserts) each into a well of a 6 well plate filled with 1 ml assay medium. Use a total of 21 tissues (= 7 wells).

4. Prepare two 6 well plates with 1 ml assay medium per well. Place these plates in a dark box at room temperature and transfer 3 tissues (= dark controls) in the first well.
5. Start irradiation of the 21 tissues. Every 30 minutes (= 3 J/cm^2) transfer 3 tissues from the irradiation site to the dark box. The resulting dose series is **3, 6, 9, 12, 15, 18, 21** J/cm^2.
6. Incubate tissues over night (16 - 21 hrs.) at 37°C, 5 % CO_2, 90 % humidity
7. Determine tissue viability according to **5.5**. (third day of testing). Compared to the non irradiated tissues (100 % viability) up to 6 J/cm^2 (= 60 minutes) there should be no significant reduction of viability. The approximated ID_{50} of UV-radiation shall be in the range between 12 - 18 J/cm^2.

5.3.2 Quality Check of the Assay: Tissue Viability

Prior to release to the customers, each lot of EpiDerm is checked at MatTek for viability and sensitivity. In the laboratory, the **absolute $OD_{540\ MTT}$** of the negative control tissues in the MTT-test is an indicator of tissue viability obtained in the testing laboratory after the shipping procedure and under specific conditions of the assay. The confidence interval of historical negative controls data has not been properly determined.

According to a preliminary analysis at ZEBET, the absolute **OD_{540}** of negative controls should be at ≥0.8 for EPI-200 respectively.

5.3.3 Quality Check of the Assay: Positive Control

It is not necessary to include a positive control into each phototoxicity assay. Once the laboratory is experienced with the assay, the positive control should be run at least twice a year: Apply 50µl **Chlorpromazine** disolved in water, in five concentrations ranging from 0.001% up to 0.1%. A dose dependent reduction of cell viability occurring only in the UVA-irradiated tissues, should be observed between 0.005% and 0.05% (see **ANNEX D**).

5.4 Sample Preparation and Test Concentrations

According to their solubility, chemicals are applied as solution in **water**, solution or suspension in **oil**, or as a **water in oil** suspension.

DESCRIPTIVE TERM	RANGE OF SOLUBILITY	% (w/v)	CATEGORY
very soluble	> 1 g/ml	>100.00	1
freely soluble	> 100 mg/ml - 1000 mg/ml	>10.00	2
soluble	> 30 mg/ml - 100 mg/ml	>3.00	3
sparingly soluble	> 10 mg/ml - 30 mg/ml	>1.00	4
slightly soluble	> 1 mg/ml - 10 mg/ml	>0.10	5
very slightly soluble	> 0.1 mg/ml - 1 mg/ml	>0.01	6
practically insoluble	0.1 mg/ml and lower	<0.01	7

As a basic recommendation, poorly water soluble test materials (category 5-7) should be tested in oil. Water soluble test materials (category 1-4) may be tested disolved (!) in water, or dispersed in a water/oil suspension (1 part H_2O + 4 parts oil). Use appropriate techniques

for preparing the suspensions, e.g. a mortar and pestle, or, if necessary a homogenise (Potter, Vortex or Sonicator). Use sesame oil (pharmaceutical grade) and di-H_2O.

Prepare five concentrations of the test material, and apply **50µL** per tissue. Where possible, the highest concentration of a test material should show cytotoxicity in non-irradiated tissues. The highest test concentration should **not exceed 10%** (w/v).

If you have no information on the test material, start with the following concentration series:

vehicle	**%** (w/v)	**%** (w/v)	**%** (w/v)	**%** (w/v)	**%** (w/v)
oil	10	3.16	1	0.316	0.1
water in oil (1+4)	1	0.316	0.1	0.0316	0.01
water	1	0.316	0.1	0.0316	0.01

Note: According to ZEBET's experience, the series for oil or water/oil may be sufficient for many test materials. Materials solved in water pass the stratum corneum more quickly. If they are, in addition highly cytotoxic the concentration series may have to be shifted to a lower range in a second experiment.

5.5 Experimental Procedure

Day before testing (e.g. Tuesday)

1. Upon receipt of the EpiDerm Skin Model, place the sealed 24 well plates containing the tissues and the assay medium into the refrigerator (4°C +/- 2°C). Place the vial containing the MTT concentrate in the freezer (-20°C ± 5°C).

First day of testing (e.g. Wednesday)

1. Before treatment pre-warm the assay medium in a 37°C waterbath
2. Pipet 1 ml of the assay medium into each well of the sterile 6 well plates
3. One hour before dosing, remove the EpiDerm tissues from the refrigerator. Under sterile conditions using sterile forceps, transfer the inserts into the 6 well plates containing the pre-warmed assay medium. Note: Care should be taken to remove all adherent agarose sticking to the outside of the inserts. Label the 6 well plates (lid and bottom) outside the irradiation area indicating the test material and the corresponded test concentrations to be run.
4. Place the 6 well plates containing the tissues into a humidified 37°C, 5% CO_2, incubator for 1 hour prior to dosing (pre-incubation).
5. Prepare the series of concentrations (5 concentrations + 1 vehicle). Per EpiDerm kit (=24 tissues) twelve tissues are dosed in the **cytotoxicity assay** and twelve tissues are dosed as duplicates in the same way to be used in the **phototoxicity assay**. Apply the vehicle control and 5 concentrations of the test chemical, each in 2 replicates. Using this dosing scheme, one chemical can be tested per Epi-200 kit.

Plate A

NC	A1	A2	A3	A3	A5
NC	A1	A2	A3	A4	A5

+UVA

Plate B

NC	A1	A2	A3	A4	A5
NC	A1	A2	A3	A4	A5

-UVA

NC = negative control (vehicle)
A1 -A5 = test concentrations of chemical A

6. Following the 1 hour pre-incubation, aspirate off the assay medium and replace with 1 ml (per well) of per-warmed, fresh assay medium. (Any air bubbles trapped underneath the inserts should be released).
7. Add 50 µl of test material, (oil or water/oil-suspension as a vehicle), into the insert on top of the tissue (2 tissues per concentration).
8. Add 50 µl oil or water/oil-suspension onto the tissues of the two vehicle controls
9. Once the tissues have been dosed, cover the plates with the lids and incubate for 21 hours at 37°C, 5% CO_2, 90% humidity.

Second day of testing (Thursday)

1. Remove the plates from the incubator and expose one set of plates for **60 min.** to UVA at an energy of 1.67 mW/cm^2 (= **6 J/cm^2**) at room temperature (phototoxicity assay) and leave the duplicate set of plates in the dark at room temperature (= cytotoxicity assay).
2. After irradiation, prepare a set of new 6 well plates. Pipet 1 ml of fresh assay medium into the appropriate number of wells.
3. Since sesame oil cannot be removed easily, use a wash bottle and rinse each insert with an amount of at least 0.5 ml PBS each. Repeat the rinsing procedure at least **4** times, aspirate off to remove any residual test material and PBS and transfer the inserts into the new plates.
4. Incubate the plates for 21 hours at 37°C, 5 % CO_2, 90 % humidity.

Third day of testing (Friday)

1. Prepare MTT solution: 1 hours before incubation is complete, thaw the MTT concentrate and dilute with the MTT diluent. Spin down (300 g for 5 min.) the MTT solution to remove any precipate present. Pre-warm the solution in a water bath at 37°C.

2. Per test material prepare a 24 well plate with MTT solution. Pipet 300 µl of the MTT solution into the appropriate number of wells. Label the 24 well plate to indicate to which wells the samples will be transferred (lid and bottom).
3. Under sterile conditions transfer the inserts into the 24 well plate containing the MTT solution. Any air bubbles trapped underneath the insert should be released.
4. Incubate the 24 well plate 3 hours at 37°C, 5 % CO_2, 90 % humidity. Deviations from 3 hour time for MTT incubation will result in different MTT readings and for consistency it is recommended that 3 hour MTT incubation time be adhered very strictly.
5. After incubation period is complete, remove each insert individually and gentle rinse with PBS to remove any residual MTT solution (for rinsing procedure see step 3, second day of testing). Remove excess PBS by gently aspirating the inserts.
6. Per test material prepare a 24 well extraction plate. Place the inserts into the pre-labelled plate. Immerse the inserts using 2.0 ml of the extractant solution per well, completely covering the tissues. Cover the extraction plate to reduce evaporation of extractant.
7. With shaking (125 r/min), allow the extraction to proceed for 2 hours at room temperature.

7a. Alternative: Incubate the 24 well plate without shaking over night at room temperature in the dark.

8. After the extraction period is complete, decant the liquid within each insert back into the well from which it was taken. The inserts can be discarded. Pipet the extractant solution up and down at least 3 times to insure that the extraction solutions are well mixed.
9. Per tissue (= per well) pipet 3 x 200 µl (aliquots) of the mixed extraction solution into each well of a 96 well microtiter plate. Determine the optical density of the extracted samples at **570 nm** (reference filter 630 nm) with a 96 well plate reader.

6 DATA COLLECTION AND DOCUMENTATION

Principle: *Raw data (OD values) produced by the Reader-Software (e.g. KOWIRA used at ZEBET) are transferred into a MS EXCEL spreadsheet in the format 8 x 12 value matrix. Before transferring data into the MS EXCEL spreadsheet they can be saved as a *.ASC file (ASCII format). Data are transferred to MS EXCEL by using the WINDOWS Clipboard.*

A MS EXCEL spreadsheet **EPIPHOT.XLS**, is provided to the participating laboratories by ZEBET. This spreadsheet consists of two maps, named **import** and **spread**.

The spreadsheet will perform all calculations after the 96 OD values derived from one test of a single chemical have been pasted into the map **import** from Clipboard. The user has to place the cursor in a marked field in the **import** map and then to copy the data from the

Clipboard. Clicking to the map **spread** afterwards, he will find the calculated values in 4 small tables and a diagram.

If concentrations deviating from the default values are used, the concentrations have to be corrected by hand in the tables and in the diagram in the map **spread**. In addition , fill in also all informations requested in the map **spread** and .save the new file with a reasonable new name. Use **EPIPHOT.XLS** as the primary spreadsheet. An example of the map **spread** is given in **ANNEX E**

7 EVALUATION, PREDICTION MODEL

OD_{570} of each tissue are determined under test and the % viability is calculated in comparison to the mean OD_{570} of the three untreated vehicle controls. These calculations are done for the set of UVA-irradiated tissues as well as for the set of non-irradiated tissues.

Means ### SD are calculated of all tissue viabilities as percentage compared to untreated vehicle controls.

A chemical is considered to have a phototoxic potential if one or more test concentrations with UVA irradiation reveal a decrease in viability **exceeding 30%** when compared with dark controls of the same test concentrations. This classification is supported, if the phototoxic effect shows a dose relationship within the five concentrations tested.

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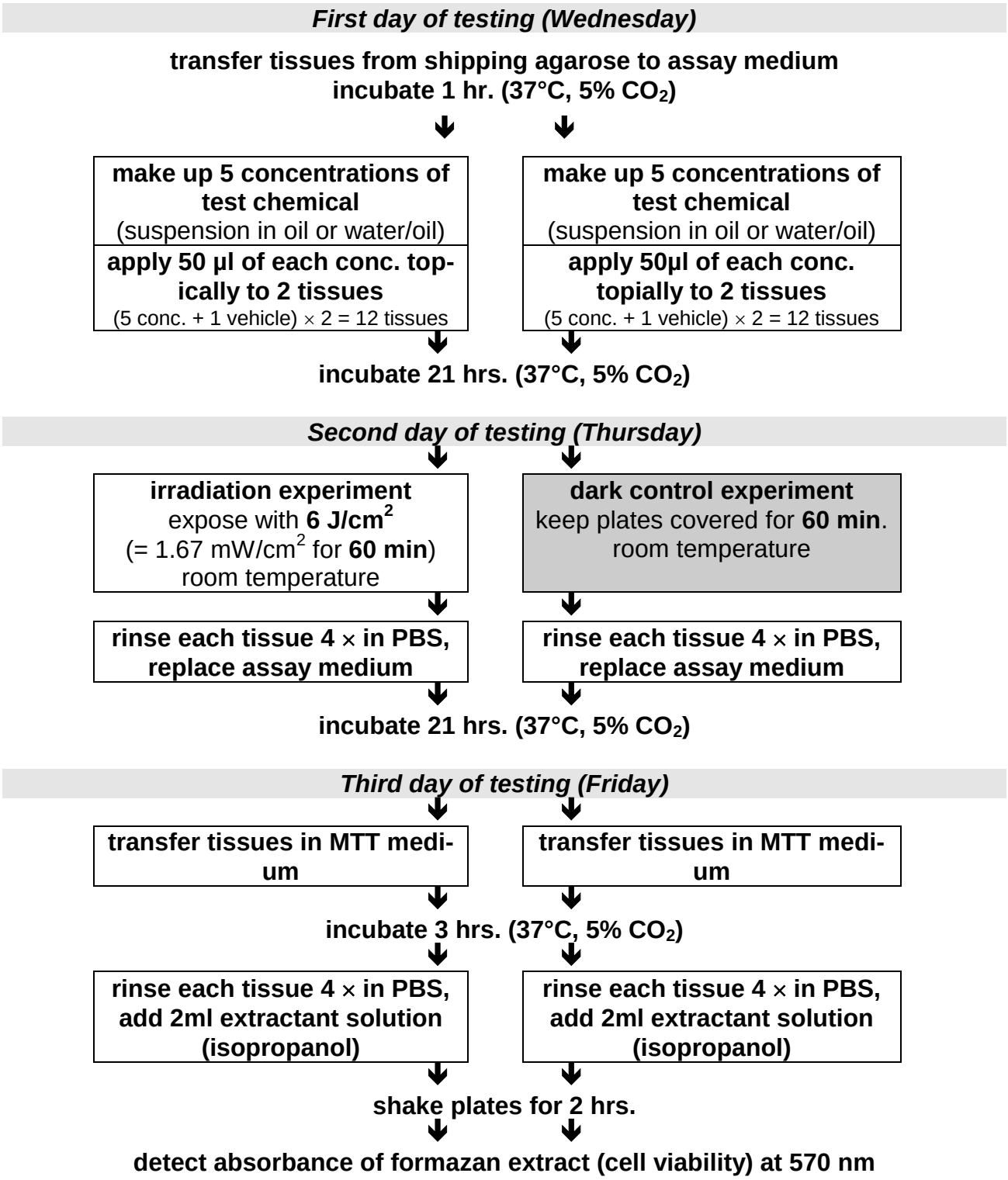
9 ANNEX A: Phototoxicity Test Procedure



10 ANNEX B: Methods Documentation Sheet

Day prior to testing (Tuesday)

TEST MATERIAL: diluted in:

Operator ID _____
Date started _____

Tissue Lot#
EpiDerm Lot # _____ Prod. date _____

First day of testing (Wednesday)

Incubator verification

CO_2 (%)	Temperature (°C)	Check water in reservoir (ü)	Operator ID / date

1 hour Incubation: Start:
Stop:

Preparation of test concentration series

stock preparation:	
1. dilution:	
2. dilution:	
3. dilution:	
4. dilution:	
5. dilution:	

Fill in weights and volumes used

Concentration (%)										
0.0001	0.0005	0.001	0.005	0.01	0.05	0.1	0.5	1.0	5.0	10.0

Assign dilution number (Table above) to concentrations used.

Incubation: Start:
(over night) Stop:

Second day of testing (Thursday)

Incubator verification

CO_2 (%)	Temperature (°C)	Check water in reservoir (ü)	Operator ID / date

Check UVA Irradiance

SOL 500 (Dr. Höhnle)	mW/cm^2

Fill in irradiance measured at plate positions (range from min. to max.), see **4.2.6**

Irradiation: Start:
Stop:

Incubation: Start:
(over night) Stop:

Third day of testing (Friday)

Incubator verification

CO_2 (%)	Temperature (°C)	Check water in reservoir (ü)	Operator ID /date

Incubation: Start:
(MTT) Stop:

Formazan-extraction: Start:
Stop:

Check plate photometer (✓)

reading filter 570 nm	
reference-Filter 630 nm	

Operator ID / date:

11 ANNEX C: Plate configurations

6 well-plates *(chemical treatment, irradiation and incubation)*

plate A
UVA +

Vehicle-control	1	2
Vehicle-control	1	2

plate A
UVA -

Vehicle-control	1	2
Vehicle-control	1	2

plate B
UVA +

3	4	5
3	4	5

plate B
UVA -

3	4	5
3	4	5

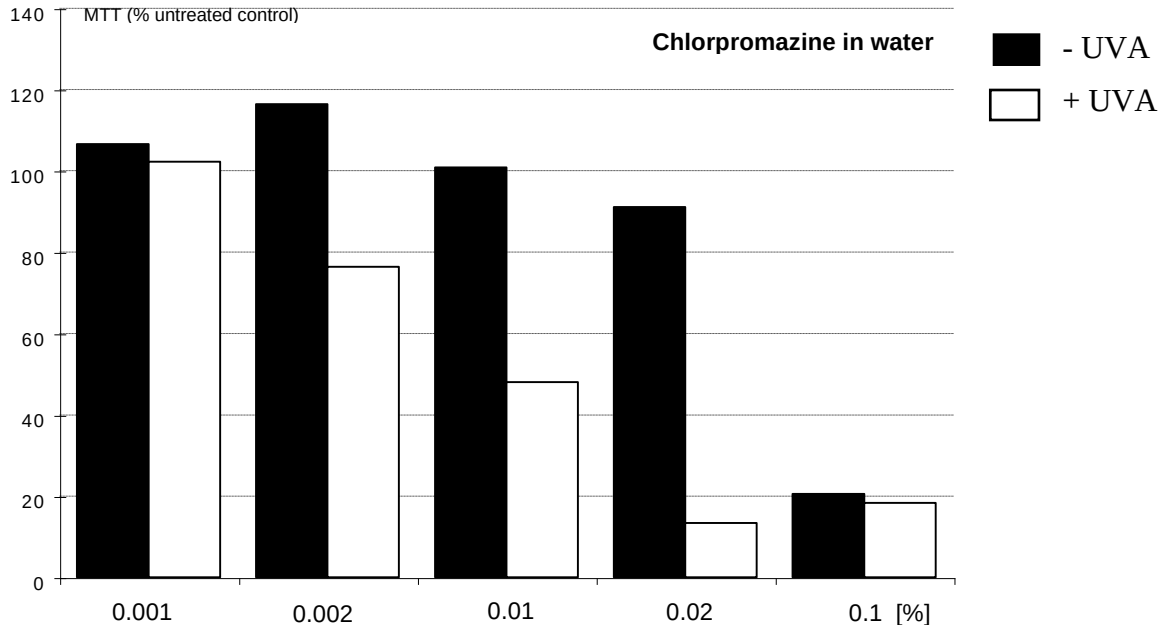
24 well-plates (MTT assay)

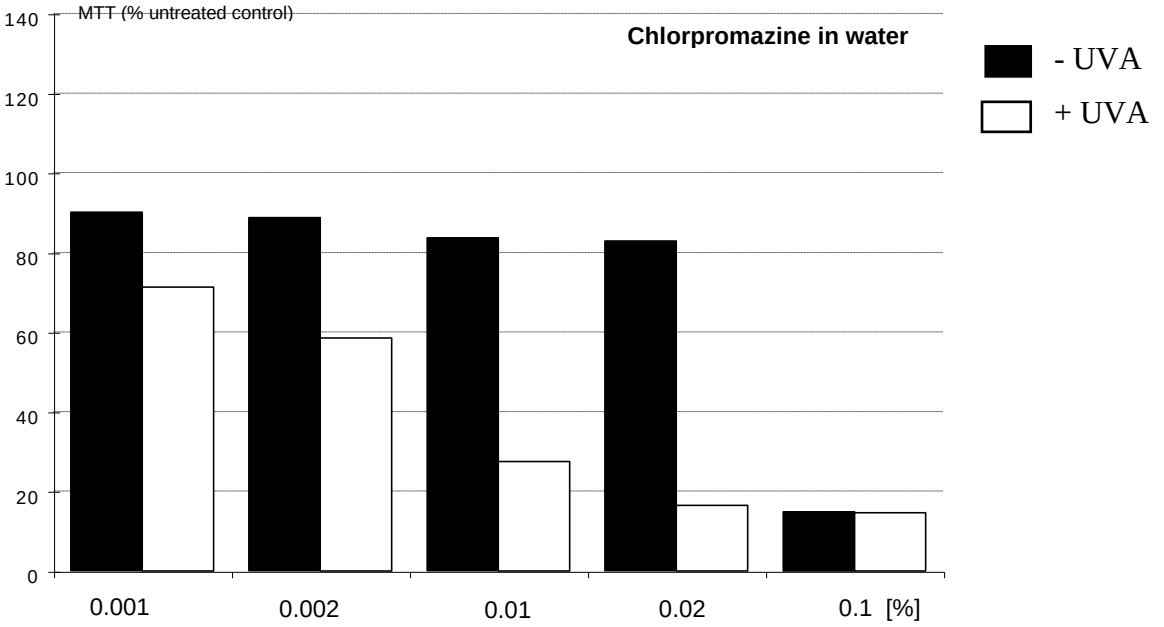
Vehicle-control	1	2	3	4	5	plate A
Vehicle-control	1	2	3	4	5	UVA +
Vehicle-control	1	2	3	4	5	plate B
Vehicle-control	1	2	3	4	5	UVA -

96 well-plate *(OD reading in plate photometer, 3 aliquots per tissue)*

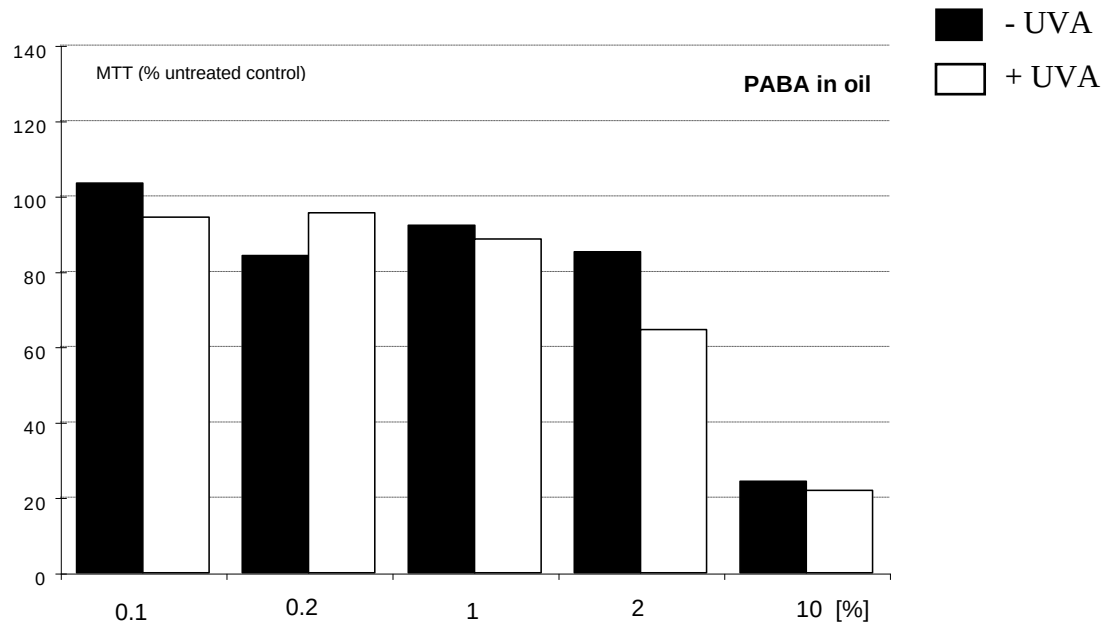
VC	VC	1	1	2	2	3	3	4	4	5	5	
VC	VC	1	1	2	2	3	3	4	4	5	5	UVA+
VC	VC	1	1	2	2	3	3	4	4	5	5	
VC	VC	1	1	2	2	3	3	4	4	5	5	
VC	VC	1	1	2	2	3	3	4	4	5	5	UVA-
VC	VC	1	1	2	2	3	3	4	4	5	5	

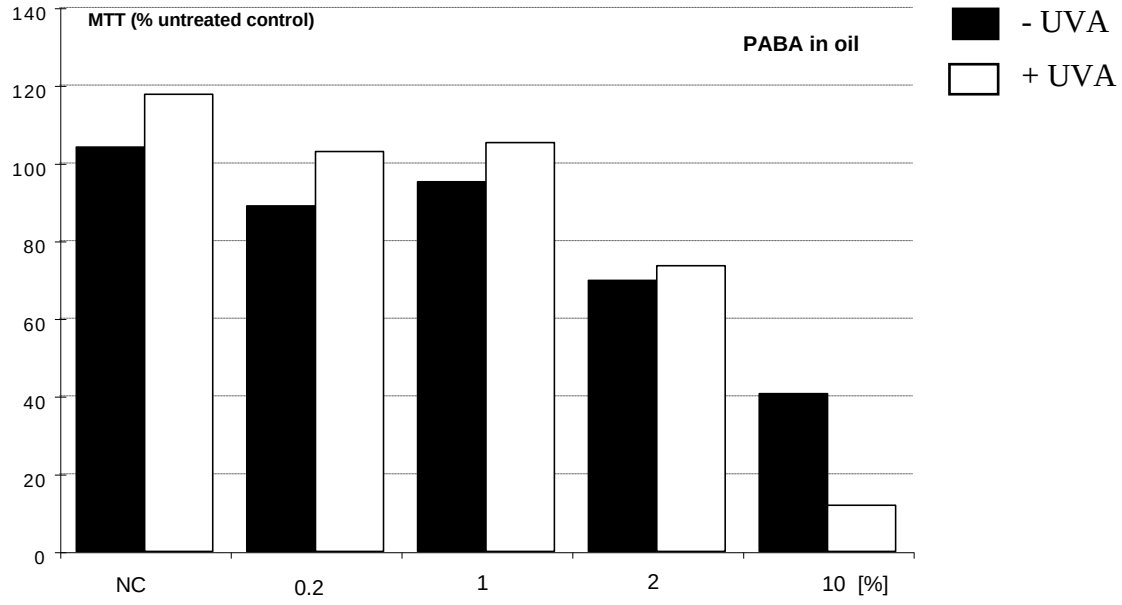
12 ANNEX D: Positive Reference Data





13 ANNEX D: Negative Reference Data

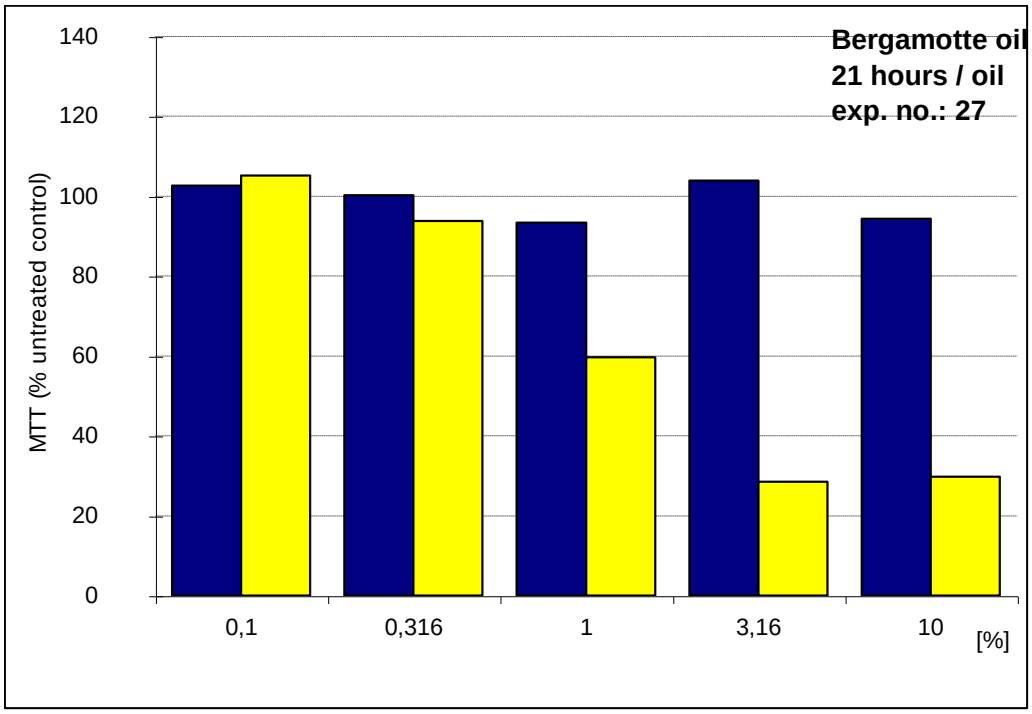




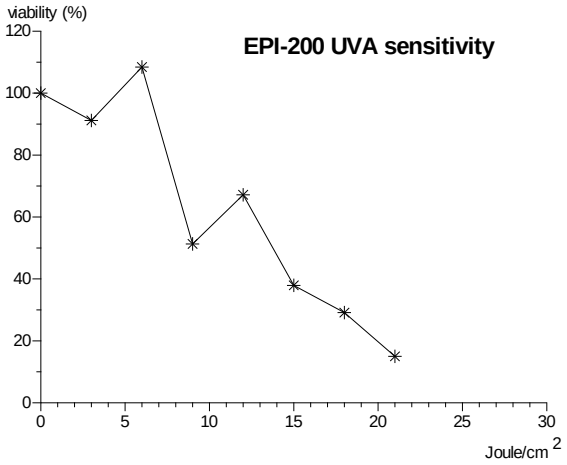
14 ANNEX E: Template Spreadsheet (Map: 2 spread)

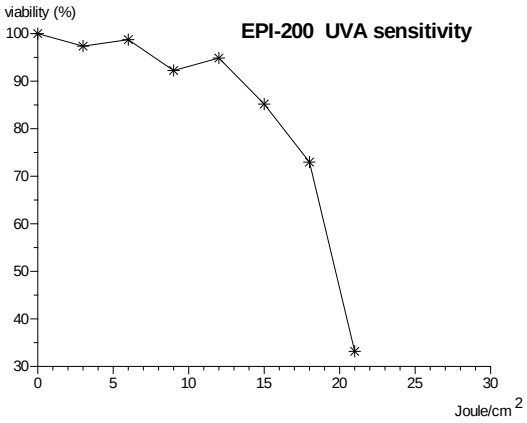
	chemical:			**Bergamotteoil**			solvent:		oil	
	tissue-lot no.:			1230 C			date of start:		07.05.97	
	exp. no.:			27			Irradiation:		6 J/cm²	
							application time:		21 hours	
				mean						mean
control	0,920	0,902	0,887	0,903		control	1,117	1,168	1,185	1,157
	1,058	1,052	1,030	1,047			0,884	0,884	0,896	0,888
0,1	1,045	1,041	1,038	1,041		0,1	1,079	1,076	1,093	1,083
	0,982	0,951	0,943	0,959			1,060	1,054	1,088	1,067
0,316	1,051	1,055	1,012	1,039		0,316	0,975	0,970	0,979	0,975
	0,936	0,902	0,907	0,915			0,949	0,930	0,949	0,943
1	0,972	0,961	0,948	0,960		1	0,594	0,595	0,600	0,596
	0,867	0,861	0,854	0,861			0,622	0,625	0,625	0,624
3,16	0,969	0,969	0,958	0,965		3,16	0,312	0,310	0,313	0,312
	1,063	1,069	1,051	1,061			0,271	0,268	0,271	0,270
10	0,936	0,917	0,904	0,919		10	0,342	0,325	0,321	0,329
	0,917	0,933	0,912	0,921			0,281	0,280	0,280	0,280
	w/out UVA						*with UVA*			

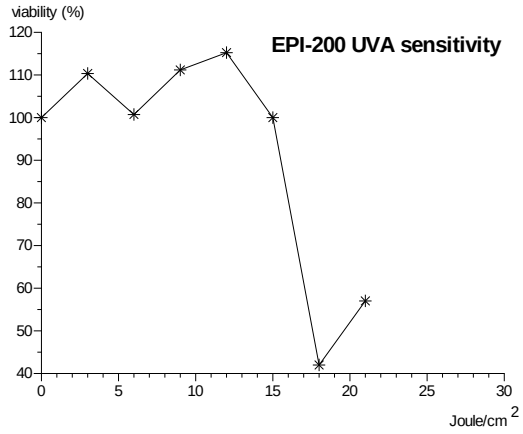
	mean	Δ tissue [%]	% untreated control			mean	Δ tissue [%]	% untreated control
control	0,975	14,74	100		control	1,022	26,28	100
0,1	1,000	8,27	103		0,1	1,075	1,43	105
0,316	0,977	12,72	100		0,316	0,959	3,34	94
1	0,911	10,95	93		1	0,610	4,53	60
3,16	1,013	9,44	104		3,16	0,291	14,33	28
10	0,920	0,18	94		10	0,305	16,07	30

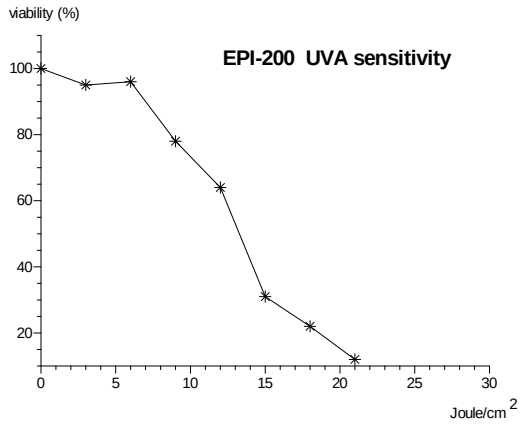


15 ANNEX F: EPI-200 UVA-Sensitivity









The figures show 4 independent experiments performed according to **5.3.1.** The dose of 6 J/cm^2 used in the Phototoxicity Assay is not cytotoxic in any of the single experiments.