

Final Report

Study Title	Evaluation of <i>in vitro</i> cytotoxicity on Balb/c 3T3 fibroblasts using the Neutral Red Uptake assay
Test Articles	PIP silicone gel breast implants: IMGHC-TX-H, batch 16306 IMGHC-TX-H, batch 02808 IMGHC-TX-H, batch 27609 IMGHC-TX-H, batch 41609 IMGHC-TX-S, batch 37908 [REDACTED] control silicone gel breast implant: [REDACTED]
Author	[REDACTED]
Sponsor	Medicines and Healthcare products Regulatory Agency 151 Buckingham Palace Road London SW1W 9SZ United Kingdom
Study Monitor	[REDACTED]
Test Facility	Covance Laboratories Ltd Otley Road, Harrogate North Yorkshire HG3 1PY, ENGLAND
Covance Client Identifier	1008167
Covance Study Number	8265940
Report Issued	December 2012
Page Number	1 of 39

**STUDY DIRECTOR AUTHENTICATION
AND GLP COMPLIANCE STATEMENT**

PIP silicone gel breast implants: Evaluation of *in vitro* cytotoxicity on Balb/c 3T3 fibroblasts using the Neutral Red Uptake assay

I, the undersigned, hereby declare that the work was performed under my supervision and that the findings provide a true and accurate record of the results obtained.

The study was performed in accordance with the agreed protocol and with Covance Laboratories Limited Standard Operating Procedures, unless otherwise stated, and the study objectives were achieved.

The study was conducted in compliance with the United Kingdom Good Laboratory Practice Regulations 1999, Statutory Instrument No. 3106 as amended by the Good Laboratory Practice (Codification Amendments Etc.) Regulations 2004 and the OECD Principles on Good Laboratory Practice (revised 1997, issued January 1998) ENV/MC/CHEM (98) 17.



Study Director

06 December 2012
Date

QUALITY ASSURANCE STATEMENT

PIP silicone gel breast implants: Evaluation of in vitro cytotoxicity on Balb/c 3T3 fibroblasts using the Neutral Red Uptake assay



This study has been reviewed by the GLP Quality Assurance Unit of Covance and the report accurately reflects the raw data. The following inspections were conducted and findings reported to the Study Director (SD) and associated management.

Critical procedures, which are performed routinely in an operational area, may be audited as part of a "process" inspection programme. This can be in addition to phases scheduled on an individual study basis. Selected process inspections conducted and considered applicable to this study are included below.

In addition to the inspection programmes detailed below, a facility inspection programme is also operated. Details of this programme, which covers all areas of the facility annually (at a minimum), are set out in standard operating procedures.

Inspection Dates		Phase	Date Reported to SD and SD Management
From	To		
29 May 2012	29 May 2012	Protocol Review	29 May 2012
06 Jun 2012	06 Jun 2012	Culture Establishment	06 Jun 2012
28 Jun 2012	28 Jun 2012	Protocol Amendment Review	28 Jun 2012
06 Jul 2012	10 Jul 2012	Extraction	10 Jul 2012
20 Aug 2012	21 Aug 2012	Draft Report and Data Review	21 Aug 2012
30 Nov 2012	30 Nov 2012	Final Report Review	30 Nov 2012

Inspection Dates		Phase	Date Reported to SD and SD Management
From	To		
06 Jun 2012	06 Jun 2012	Dose Preparation	06 Jun 2012
06 Jun 2012	06 Jun 2012	eArchiving Procedure	07 Jun 2012
04 Jul 2012	04 Jul 2012	Data Collation and Transfer	04 Jul 2012
09 Jul 2012	09 Jul 2012	Test Article Dilutions	09 Jul 2012



Quality Assurance Unit

6 Dec 2012
Date

REVIEWING SCIENTIST'S STATEMENT

PIP silicone gel breast implants: Evaluation of *in vitro* cytotoxicity on Balb/c 3T3 fibroblasts using the Neutral Red Uptake assay

I, the undersigned, hereby declare that I have reviewed this report in conjunction with the Study Director and that the interpretation and presentation of the data in the report are consistent with the results obtained.



Scientist

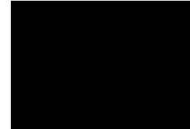
26 November 12
Date

RESPONSIBLE PERSONNEL

PIP silicone gel breast implants: Evaluation of *in vitro* cytotoxicity on Balb/c 3T3 fibroblasts using the Neutral Red Uptake assay

The following personnel were responsible for key elements of the study:

Study Director
Laboratory Supervisor
Study Monitor¹



¹ Located at Medicines and Healthcare products Regulatory Agency, London.

ARCHIVE STATEMENT

PIP silicone gel breast implants: Evaluation of *in vitro* cytotoxicity on Balb/c 3T3 fibroblasts using the Neutral Red Uptake assay

All primary data, or authenticated copies thereof, specimens and the final report will be retained in the Covance Laboratories Limited archives for one year after issue of the final report. At the end of the specified archive period the Sponsor will be contacted to determine whether the data should be returned, retained or destroyed on their behalf. Sponsors will be notified of the financial implications of each of these options at that time. One copy of the protocol and final report will be held in the Covance Laboratories Limited archives as per Covance company policy.

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SUMMARY

PIP silicone gel breast implants IMGHC-TX-H, batch 16306, IMGHC-TX-H, batch 02808, IMGHC-TX-H, batch 27609, IMGHC-TX-H, batch 41609, IMGHC-TX-S, batch 37908 and [REDACTED] control silicone gel breast implant [REDACTED] were assayed for cytotoxicity to Balb/c 3T3 fibroblast cells using the Neutral Red Uptake assay.

Balb/c 3T3 fibroblast cells seeded into 96 well microtitre plates were treated with a range of concentrations of five PIP silicone gel breast implants and one [REDACTED] control silicone breast implant or positive control chemical (Sodium Dodecyl Sulfate, SDS).

All silicone gel breast implant treatments in this study were performed using extractions of the insoluble silicone gel that comprised the inner part of each implant. Extracts were prepared in anhydrous analytical grade dimethyl sulphoxide (DMSO) or water for irrigation (purified water) at concentrations of 200 mg/mL (equivalent), with the extractions being conducted at 37°C for 72 hours, with shaking, in line with the principles of ISO Guideline 10993, Part 12, 2007. As all treatments were performed using extracts of the test articles, all treatment concentrations are expressed as equivalent concentrations.

Initial non-polar (organic) vehicle extractions were conducted in hexane (in order to be consistent with chemical analysis work previously conducted for the Sponsor), however under the extraction conditions employed for this study, when the hexane was evaporated to dryness prior to resuspension in DMSO (required to have extracts in a vehicle compatible with the assay system), it was clear that the hexane had dissolved some of the silicone gel, which remained in the extraction flasks and could not be removed during the evaporation and resuspension steps. This extraction methodology was therefore not considered appropriate to prepare non-polar (organic) vehicle test article extract samples for assaying in this biological assay system. It was therefore agreed with the Sponsor that non-polar (organic) extracts for use in this study would be prepared by direct extraction in DMSO.

Extracts were prepared in water for irrigation (purified water) for Experiment 1 and in anhydrous analytical grade dimethyl sulphoxide (DMSO) for Experiment 2.

Experiment 1 treatments with each silicone gel water extract were performed using final concentrations of PIP silicone gel breast implants models IMGHC-TX-H, batch 16306, IMGHC-TX-H, batch 02808, IMGHC-TX-H, batch 27609,

IMGHC-TX-H, batch 41609, IMGHC-TX-S, batch 37908 and [REDACTED] control silicone gel breast implant model [REDACTED] at 156.3, 312.5, 625, 1250, 2500, 5000, 10000 and 20000 µg/mL (equivalent), plus vehicle (blank extraction vehicle i.e. 10% extracted water in DMEM complete), negative (10% un-extracted water in DMEM complete), untreated (un-extracted DMEM complete) and positive controls.

Experiment 2 treatments with each silicone gel DMSO extract were performed using final concentrations of PIP silicone gel breast implants models IMGHC-TX-H, batch 16306, IMGHC-TX-H, batch 02808, IMGHC-TX-H, batch 27609, IMGHC-TX-H, batch 41609, IMGHC-TX-S, batch 37908 and [REDACTED] control silicone gel breast implant model [REDACTED] at 15.63, 31.25, 62.5, 125, 250, 500, 1000 and 2000 µg/mL (equivalent), plus vehicle (blank extraction vehicle i.e. 1% extracted DMSO in DMEM complete), negative (1% un-extracted DMSO in DMEM complete), untreated (un-extracted DMEM complete) and positive controls.

At the end of treatment, cytotoxicity was assessed by the Neutral Red Uptake assay.

Following treatment of cultures with PIP silicone gel breast implants models IMGHC-TX-H, batch 16306, IMGHC-TX-H, batch 02808, IMGHC-TX-H, batch 27609, IMGHC-TX-H, batch 41609, IMGHC-TX-S, batch 37908 and [REDACTED] control silicone gel breast implant model [REDACTED], no evidence of toxicity was noted and IC₅₀ values were not achieved for any of the test articles extracted in water or DMSO. The cell viability was >70% at all concentrations tested for each test article extracted in either water or DMSO.

It is concluded that PIP silicone gel breast implants models IMGHC-TX-H, batch 16306, IMGHC-TX-H, batch 02808, IMGHC-TX-H, batch 27609, IMGHC-TX-H, batch 41609, IMGHC-TX-S, batch 37908 and [REDACTED] control silicone gel breast implant model [REDACTED] were not cytotoxic in this *in vitro* test system when tested under the conditions of this study. These conditions included treatments with anhydrous analytical grade dimethyl sulphoxide (DMSO) and water extracts of silicone gel from each breast implant at concentrations up to 2000 or 20000 µg/mL (equivalent) respectively.

INTRODUCTION

The Neutral Red Uptake assay is a cell survival/viability chemosensitivity assay based on the ability of viable cells to incorporate and bind Neutral Red, a supravital dye. Neutral Red is a weak cationic dye that readily penetrates cell membranes by non-ionic diffusion and accumulates intracellularly in lysosomes (Nemes *et al.*, 1979; Borenfreund and Puerner, 1958). Thus, the Neutral Red Uptake assay examines the cellular membrane integrity as well as the cellular energy status since Neutral Red is taken up into lysosomes by an energy dependent process. Such changes brought about by the action of xenobiotics result in a decreased uptake and binding of Neutral Red. It is thus possible to distinguish between viable and damaged or dead cells, which is the basis of this assay.

Healthy Balb/c 3T3 cells, when maintained in culture, continuously divide and multiply over time. A toxic chemical, regardless of site or mechanism of action, will interfere with this process and result in a reduction of the growth rate as reflected by cell number. Cytotoxicity is expressed as a concentration-dependent reduction of the uptake of Neutral Red after 24 hours of chemical exposure, thus providing a sensitive, integrated signal of both cell integrity and growth inhibition.

Balb/c 3T3 cells are seeded into 96-well plates and maintained in culture for approximately 24 hours to form a semi-confluent monolayer. They are then exposed to the test articles over a range of eight concentrations. After a 24 hour exposure, Neutral Red Uptake is determined for each treatment concentration and compared to that in control cultures. For each concentration of the test article the percent inhibition of growth is calculated. The IC₅₀ (the concentration producing 50% reduction of Neutral Red uptake) is calculated from the concentration-response.

The silicone gel breast implants used for this study comprised an insoluble outer shell surrounding an insoluble silicone gel, the latter (inner) part of each implant being the subject of extraction and testing. As this material is insoluble in all commonly used vehicles, extracts of each test article were prepared according to the principles of ISO Guideline 10993, Part 3, 2003 and Part 12, 2007 and assessed in this study.

The objective of this study was to evaluate whether PIP silicone gel breast implants models IMGHC-TX-H, batch 16306, IMGHC-TX-H, batch 02808, IMGHC-TX-H, batch 27609, IMGHC-TX-H, batch 41609, IMGHC-TX-S, batch 37908 and [REDACTED] control silicone gel breast implant model [REDACTED] induced an *in vitro* cytotoxic effect when applied to Balb/c 3T3 fibroblasts using the Neutral Red Uptake assay as the endpoint. There is no specific guideline for this study type but the

study was performed based on guidance described in the ICCVAM: *in vitro* Cytotoxicity Test Methods for Estimating Starting Doses for Acute Oral Systemic Toxicity Testing (November 2006) and in accordance with the UK and OECD GLP guidelines.

The study was performed according to the protocol and one amendment.

The study was initiated on 28 May 2012. Experimental work started on 7 June 2012 and was completed on 11 July 2012. The study completion date is considered to be the date the Study Director signs the final report.

MATERIALS

Test articles

PIP silicone gel breast implants models IMGHC-TX-H, batch 16306, IMGHC-TX-H, batch 02808, IMGHC-TX-H, batch 27609, IMGHC-TX-H, batch 41609 and IMGHC-TX-S, batch 37908 and one [REDACTED] control silicone gel breast implant model [REDACTED], were received on 23 May 2012 (all PIP implants) or 25 May 2012 ([REDACTED]) and stored at 15-25°C, protected from light. All silicone gel breast implants were supplied as intact breast implants comprising an outer shell surrounding the inner silicone gel. The latter (inner) silicone gel components of each implant were clear white gels, and this part of the breast implant was that considered to be the subject of extraction and testing. All silicone gel samples taken for extraction throughout this study were from a single breast implant for each model/batch/manufacturer. The expiry dates of the breast implants were given as shown in the table below:

Test Article	Expiry Date
IMGHC-TX-H, batch 16306	April 2011*
IMGHC-TX-H, batch 02808	January 2013
IMGHC-TX-H, batch 27609	June 2014
IMGHC-TX-H, batch 41609	November 2014
IMGHC-TX-S, batch 37908	June 2013
[REDACTED]	October 2016

* The Sponsor confirmed that PIP silicone breast implant Model IMGHC-TX-H, Batch 16306 was suitable for use in this study at the time of assay, despite the package expiry date having already passed.

The test article information provided by the Sponsor is considered an adequate description of the characterisation and stability of the test articles. Determinations of stability and characteristics of the test articles were the responsibility of the Sponsor. Due to the nature of the test articles (prosthetic implants) specific purity values for these materials were not considered appropriate and no certificates of analysis were provided by the Sponsor.

As the test articles were insoluble in all commonly used vehicles, extracts of the test articles were prepared according to the principles of [ISO Guideline 10993, Part 3, 2003](#) and [Part 12, 2007](#). The test articles were extracted at 200 mg/mL for 72 hours at 37°C, with shaking, in one polar (aqueous) vehicle (sterile purified water) and one non-polar (organic) vehicle (dimethyl sulphoxide, DMSO). Maximum concentrations of 20000 µg/mL (equivalent) (water extract) and 2000 µg/mL (equivalent) (DMSO extract) were selected for the Cytotoxicity experiments, in order that treatments were performed up to the maximum recommended concentrations.

Initial non-polar (organic) vehicle extractions were conducted in hexane (in order to be consistent with chemical analysis work previously conducted for the Sponsor), however under the extraction conditions employed for this study, when the hexane was evaporated to dryness prior to resuspension in DMSO (required, to have extracts in a vehicle compatible with the assay system), it was clear that the hexane had dissolved some of the silicone gel, which remained in the extraction flasks and could not be removed during the evaporation and resuspension steps. This extraction methodology was therefore not considered appropriate to prepare non-polar (organic) vehicle test article extract samples for assaying in this biological assay system. It was therefore agreed with the Sponsor that non-polar (organic) extracts for use in this study would be prepared by direct extraction in DMSO.

Extracts were prepared in water for irrigation (purified water) for Experiment 1 and in anhydrous analytical grade dimethyl sulphoxide (DMSO) for Experiment 2.

For each test article, a silicone gel breast implant was initially weighed, then pierced and an appropriate aliquot of silicone gel for extraction was taken and cut into pieces (of approximately 1 cm³). Appropriate extraction vehicle was added such that each extraction was performed at 200 mg/mL, and then extracted for 72 hours at 37°C, with shaking, in order to provide extracts that were considered an appropriate exaggeration of product use ([ISO 10993-12:2007](#) Biological evaluation of medical devices – Part 12). The extractions conducted using water were membrane filter-sterilised (Pall Acrodisc filter, 0.2 µm pore size) prior to dilution or treatment. All operations were performed aseptically.

Subsequent dilutions of each test article extract were made using un-extracted DMSO/purified water (as appropriate).

Following this, a one hundred-fold dilution (DMSO extract solutions) or ten-fold dilution (water extract solutions) into DMEM complete (Dulbecco's Modified Eagle Medium, supplemented with 10% foetal calf serum, 4 mM L-glutamine and penicillin/streptomycin) of each test article extract formulation was performed to make the final treatment solutions and to achieve a maximum treatment concentration of 2000 µg/mL (equivalent) or 20000 µg/mL for DMSO and water, respectively .

The final test article extract treatment solutions were protected from light and used within approximately 3.5 hours of the end of the extraction period as follows:

Water extracts: 156.3, 312.5, 625.0, 1250, 2500, 5000, 10000 and 20000 µg/mL (equivalent).

DMSO extracts: 15.63, 31.25, 62.50, 125.0, 250.0, 500.0, 1000 and 2000 µg/mL (equivalent).

Analysis of achieved concentration

Duplicate samples (approximately 1 mL) of each test article extract and blank extraction vehicle on each extraction occasion were retained for analysis of achieved concentration. Samples were taken from the same bulk extraction as were used for treatment. Samples were stored at 15-25°C, protected from light, prior to dispatch of one replicate of each to the following address for analysis at the end of the experimental phase.

FAO [REDACTED]
LGC
The Health Business Park
Runcorn
Cheshire
WA7 4QX.

The remaining portion of [REDACTED] control silicone gel breast implant model [REDACTED], was also dispatched with the samples.

Data generated from analysis of the extraction samples will be reported separately and does not constitute part of this report.

Controls

Vehicle controls comprised treatments with the blank extraction vehicle i.e. DMEM complete containing 10% extracted purified water or 1% extracted DMSO, as appropriate, carried through the same procedures used to extract the test article (including filter sterilisation of water extracts prior to dilution or treatment). Volume additions used were the same as for the respective test article extractions.

Negative controls were also tested, which comprised of either un-extracted DMSO or purified water, added at 1% or 10% volume additions in DMEM complete as appropriate. Volume additions used were the same as for the respective test article extractions.

Untreated controls consisting of un-extracted DMEM complete were also included. Volume additions used were the same as for the respective test article extractions.

The positive control was 100 µg/mL Sodium Dodecyl Sulphate (SDS) in DMEM complete.

Vehicle, negative, untreated and positive control treatments were performed for each test article.

Cell cultures

Mouse fibroblasts (Balb/c 3T3 clone A31), supplied by the European Collection of Cell Cultures are maintained at Covance Laboratories Limited in DMEM complete at $37\pm 1^{\circ}\text{C}$ in an atmosphere of $5\pm 1\%$ CO_2 in air. Stocks of cells preserved in liquid nitrogen are reconstituted for each study. The cells are routinely screened for mycoplasma contamination. Cultures are refed with the appropriate culture medium and subcultured whenever required to avoid overgrowth. For the assay, at least one vial was removed from storage and grown in tissue culture medium as described above.

METHODS

Cell culture preparation

Balb/c 3T3 mouse fibroblasts were maintained in DMEM complete with appropriate re-feeding and subculturing until required for assay treatment. Cultures were incubated at $37\pm 1^{\circ}\text{C}$ in a humidified atmosphere of 5% (v/v) CO_2 in air.

Near confluent cultures were trypsinized and the cells resuspended in supplemented DMEM. The cell number in the suspension prepared was determined using a haemocytometer. Aliquots (100 μL) of DMEM complete were dispensed into the top and bottom line of peripheral wells (blanks) of an appropriate number of 96-well plates. The cell suspension was diluted to give a final concentration of 1×10^5 cells/mL and 100 μL pipetted into the appropriate number of wells (i.e. 10^4 cells/well). One plate per test article was set up. One column of six wells was used for each concentration of test article, negative control, vehicle control, untreated control, or positive control. Plates were incubated at $37\pm 1^{\circ}\text{C}$ for approximately 24 hours in a humidified atmosphere of $5\pm 1\%$ (v/v) CO_2 in air.

Treatment

Final treatment concentrations in DMEM complete were prepared as detailed in the "Test articles" section. Media was removed from appropriate wells of the 96 well plates; wells were washed once with 150 μL phosphate buffered saline (PBS) and then 100 μL of test article, negative, vehicle, untreated or positive control solutions were added to the appropriate wells. All plates were incubated at $37\pm 1^{\circ}\text{C}$ for approximately 24 hours in a humidified atmosphere of 5% (v/v) CO_2 in air.

Evaluation of cytotoxic effects

Microscopic evaluation

At the end of the incubation period, cells were briefly examined microscopically for signs of cytotoxicity.

Evaluation of cytotoxic effects – Neutral Red Uptake assay

Immediately following the visual assessment, the cells were washed with 150 μL of PBS. This was removed, and 100 μL of Neutral Red solution (50 $\mu\text{g}/\text{mL}$ in DMEM) was added to each well. The plates were incubated at $37\pm 1^{\circ}\text{C}$ in a humidified atmosphere of $5\pm 1\%$ (v/v) CO_2 in air for approximately 3 hours.

Following the incubation, the Neutral Red solution was removed and the cells washed twice with 150 μ L PBS. PBS was removed and 150 μ L of Neutral Red destain solution (ethanol:acetic acid:distilled water, 50:1:49) was added. Plates were shaken for approximately 40 minutes to allow extraction of Neutral Red from the cells.

Optical densities (OD) of each well were read on a VERSAmax™ plate reader, at a wavelength of 540 nm. Neutral Red absorbances were expressed in terms of absolute optical density (OD₅₄₀).

Analysis of results

IC₅₀ value

A calculation of cell viability expressed as Neutral Red Uptake was made for each concentration of test article, negative, vehicle, untreated and positive control, using the mean Neutral Red absorbance of the six replicate values. The mean OD₅₄₀ values for each test article treatment concentration were compared with the mean OD₅₄₀ value for the vehicle control. Relative cell viability was expressed as a percentage of the vehicle control.

The mean OD₅₄₀ for the positive and negative controls were compared with the mean OD₅₄₀ value for the untreated control. Relative cell viability was expressed as a percentage of the untreated control.

The concentration (μ g/mL) of each test article inducing a 50% inhibition of Neutral Red Uptake (IC₅₀ value) was estimated, together with variance for the estimate, using validated software from OECD (Phototox version 2). The data from each experiment was analysed separately.

Acceptance criteria

The assay was considered valid if all the following criteria were met:

1. For negative, untreated and vehicle control treatments, and treatments with cell survival > 50%, there is a low variability in OD₅₄₀ values between all treatment replicates (coefficient of variance < 15%).
2. The positive control treatment causes > 50% decrease in Neutral Red Uptake relative to the untreated control.
3. The mean OD₅₄₀ of the vehicle control is > 0.2.

Evaluation criteria

For valid data, the following evaluation criteria were applied for each test article in each extraction vehicle. Each test article was assessed separately according to the following criteria:

If the cell viability of the highest concentration of the extracted test article was >70% of the vehicle control, the test article was considered to be non-cytotoxic.

If the cell viability of the highest concentration of the extracted test article was <70% of the vehicle control, the test article was considered to be cytotoxic.

Computer Systems

The major computer systems used on this study were as follows:

Activity	Computer system
Scheduling	CMS (Covance Management System)
Formulations	Pristima
Data generation and collation	Phototox, Softmax Pro
Report generation	Microsoft Office/Adobe Acrobat

Version numbers of the systems are held on file at Covance.

RESULTS

The plate layout, the plate optical density reading and effect (survival) data for each plate are presented in [Appendix 1](#) and [Appendix 2](#). The cell survival graphs are presented in [Appendix 3](#).

Eight concentrations of PIP silicone gel breast implants models IMGHC-TX-H, batch 16306, IMGHC-TX-H, batch 02808, IMGHC-TX-H, batch 27609, IMGHC-TX-H, batch 41609, IMGHC-TX-S, batch 37908 and [REDACTED] control silicone gel breast implant model [REDACTED], ranging from 156.3 to 20000 µg/mL (water extractions) or 15.63 to 2000 µg/mL (DMSO extractions), were used for the Cytotoxicity experiments, in order that treatments were performed up to the maximum recommended concentrations.

Concentrations

All test article treatment concentrations tested are detailed in the “Test articles” section.

Visual evaluation

Approximately 24 hours after test article exposure, cell monolayers were examined microscopically. In both experiments, good cell coverage was seen in all wells containing test article concentrations, vehicle controls, negative controls and untreated controls for both extraction vehicles. There was clear evidence of toxicity in all positive control wells.

Neutral Red Uptake assay

Following treatment of cultures with PIP silicone gel breast implants models IMGHC-TX-H, batch 16306, IMGHC-TX-H, batch 02808, IMGHC-TX-H, batch 27609, IMGHC-TX-H, batch 41609, IMGHC-TX-S, batch 37908 and [REDACTED] control silicone gel breast implant model [REDACTED], no evidence of toxicity was noted and IC₅₀ values were not achieved (and therefore variance was not estimated) for any of the test articles extracted in water and DMSO. The cell viability was >70% at all concentrations tested for each test article extracted in either water or DMSO.

The survival data and the coefficient of variance data from each test article are shown in [Table 1](#) to [Table 4](#).

Table 1: Survival Data - Experiment 1 (water extract)

Experiment 1	IMGHC-TX-H, batch 16306	IMGHC-TX-H, batch 02808	IMGHC-TX-H, batch 27609	IMGHC-TX-H, batch 41609	IMGHC-TX-S, batch 37908	██████████
Conc (µg/mL)	% Survival	% Survival	% Survival	% Survival	% Survival	% Survival
0	100	100	100	100	100	100
156.3	99	98	97	97	92	93
312.5	93	96	97	92	92	93
625	101	96	98	87	86	90
1250	103	107	98	97	95	94
2500	103	106	101	95	93	94
5000	103	102	95	98	94	95
10000	106	101	98	96	98	96
20000	104	96	95	91	95	97
SDS 100	1	0	2	0	1	1

Table 2: Coefficient of variance (%CV) data - Experiment 1 (water extract)

Experiment 1	IMGHC-TX-H, batch 16306	IMGHC-TX-H, batch 02808	IMGHC-TX-H, batch 27609	IMGHC-TX-H, batch 41609	IMGHC-TX-S, batch 37908	
Conc (µg/mL)	% CV	% CV	% CV	% CV	% CV	% CV
0	4	6	3	8	7	3
156.3	7	9	2	8	7	2
312.5	3	3	5	7	7	4
625	3	4	5	7	7	5
1250	7	5	3	6	8	8
2500	4	6	2	5	8	4
5000	6	5	3	7	6	4
10000	6	6	5	9	7	3
20000	3	4	6	5	5	5
SDS 100	361	0	201	0	181	308

In all cases where %CV is >15% the survival at the corresponding concentration is ≤50%

Table 3: Survival Data - Experiment 2 (DMSO extract)

Experiment 2	IMGHC-TX-H, batch 16306	IMGHC-TX-H, batch 02808	IMGHC-TX-H, batch 27609	IMGHC-TX-H, batch 41609	IMGHC-TX-S, batch 37908	██████████
Conc (µg/mL)	% Survival	% Survival	% Survival	% Survival	% Survival	% Survival
0	100	100	100	100	100	100
15.63	97	101	98	96	105	104
31.25	104	99	92	103	102	102
62.5	98	99	94	103	109	99
125	103	109	98	101	112	106
250	106	102	101	102	113	107
500	104	104	98	105	119	109
1000	102	105	105	104	119	125
2000	97	104	103	107	125	117
SDS 100	0	0	0	0	0	0

Table 4: Coefficient of variance (%CV) data - Experiment 2 (DMSO extract)

Experiment 2	IMGHC-TX-H, batch 16306	IMGHC-TX-H, batch 02808	IMGHC-TX-H, batch 27609	IMGHC-TX-H, batch 41609	IMGHC-TX-S, batch 37908	██████████
Conc (µg/mL)	% CV	% CV	% CV	% CV	% CV	% CV
0	12	8	6	7	6	6
15.63	4	8	7	7	6	6
31.25	11	6	5	4	4	5
62.5	3	7	7	6	7	6
125	5	7	4	7	12	4
250	3	8	6	7	8	5
500	5	8	5	8	10	9
1000	5	6	2	6	13	8
2000	3	6	6	3	3	3
SDS 100	0	0	0	0	0	0

CONCLUSION

It is concluded that PIP silicone gel breast implants models IMGHC-TX-H, batch 16306, IMGHC-TX-H, batch 02808, IMGHC-TX-H, batch 27609, IMGHC-TX-H, batch 41609, IMGHC-TX-S, batch 37908 and [REDACTED] control silicone gel breast implant model [REDACTED] were not cytotoxic in this *in vitro* test system when tested under the conditions of this study. These conditions included treatments with anhydrous analytical grade dimethyl sulphoxide (DMSO) and water extracts of silicone gel from each breast implant at concentrations up to 2000 or 20000 µg/mL (equivalent) respectively.

REFERENCES

1. Borenfreund E, Puerner J. (1985) Toxicity determination in vitro by morphological alterations and Neutral Red absorption. *Toxicology Letters* 24: 119-124
2. ICCVAM (November 2006). *in vitro* Cytotoxicity Test Methods for Estimating Starting Doses for Acute Oral Systemic Toxicity Testing
3. ISO 10993-3:2003 Biological evaluation of medical devices – Part 3: Tests for genotoxicity, carcinogenicity and reproductive toxicity. International Organization for Standardization
4. ISO 10993-12:2007 Biological evaluation of medical devices – Part 12: Sample preparation and reference materials. International Organization for Standardization
5. Nemes A et al. (1979) The pharmacological relevance of vital staining with Neutral Red. *Experimentia* 35: 1475-1477.

APPENDICES

Appendix 1 Tables of results – Water Extractions

Table 5: Raw data – Experiment 1 (water extract) - IMGHC-TX-H, batch 16306

Phototox Project Dataset											
Project: 8265940 E1-Water.PXP Date: June 09, 2012											
Dataset# 1 Lab: Covance Chem: 16306 Run: 1											
Plate Layout											
Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
Source Data											
-0.005	-0.002	-0.003	-0.004	0.0095	-0.002	-0.003	-0.004	-0.005	0.0269	-0.001	-0.005
0.7759	0.8554	0.7659	0.8429	0.7228	0.8031	0.7574	0.7936	0.7705	0.8217	0.8000	0.00
0.7023	0.8018	0.7698	0.7014	0.6878	0.7685	0.7551	0.7652	0.7267	0.7650	0.7756	-0.001
0.7915	0.9187	0.7350	0.7142	0.7406	0.7854	0.9034	0.8097	0.8715	0.8478	0.8080	0.0380
0.8466	0.7957	0.8148	0.7921	0.6934	0.7406	0.7611	0.8456	0.7793	0.7596	0.7934	-0.004
0.8350	0.8516	0.7806	0.7283	0.7154	0.7727	0.7682	0.7478	0.8035	0.8028	0.7738	-0.002
0.7082	0.8213	0.7229	0.7703	0.7005	0.7484	0.7596	0.7767	0.7864	0.8768	0.8424	-0.003
0.0004	-0.002	0.0011	-0.003	-0.003	-0.002	0.0023	0.0083	0.0037	-0.002	-0.002	-0.005
Effect Data											
		100.1	110.2	94.5	105.0	99.0	103.8	100.7	107.4	104.6	
		100.6	91.7	89.9	100.5	98.7	100.0	95.0	100.0	101.4	
		96.1	93.4	96.8	102.7	118.1	105.9	113.9	110.8	105.6	
		106.5	103.6	90.7	96.8	99.5	110.6	101.9	99.3	103.7	
		102.1	95.2	93.5	101.0	100.4	97.8	105.1	105.0	101.2	
		94.5	100.7	91.6	97.9	99.3	101.6	102.8	114.6	110.1	
Dose Data in columns											
			156.3	312.5	625	1250	2500	5000	10000	20000	

Scan of the printout (from Phototox version 2.0 software) for this treatment is shown above. The full test article name is IMGHC-TX-H, batch 16306.

Plate layout: Shows the treatment plan of the 96 well plates used in this treatment. 'Control' refers to the vehicle controls. Hyphen (-) on column 1 represents Untreated Control (UTC). Hyphen (-) on column 2 represents the Negative Control. Hyphen (-) on column 12 represents the Positive Control.

Source data: Shows the optical density (OD) for each well. Where a value of 0.00 is presented in the table above, this indicates that the value is between 0 and -0.00049.

Effect data: Shows the percentage survival for each well, compared to the mean survival in solvent controls.

Dose data in columns: Concentration in µg/mL.

Table 6: Raw data – Experiment 1 (water extract) – IMGHC-TX-H, batch 02808

Phototox Project Dataset											
Project: 8265940 E1-Water.PXP Date: June 09, 2012											
Dataset# 2 Lab: Covance Chem: 02808 Run: 1											
Plate Layout											
Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
Source Data											
0.0230	-0.006	-0.006	-0.007	0.0034	-0.004	0.0185	0.0053	0.0192	-0.005	-0.002	-0.003
0.7476	0.8511	0.8128	0.8675	0.8486	0.8558	0.8905	0.8893	0.8482	0.8389	0.8150	-0.006
0.8009	0.7620	0.7810	0.7910	0.7876	0.7886	0.8904	0.8412	0.8772	0.7729	0.7585	-0.006
0.7631	0.8206	0.8433	0.9505	0.8108	0.8193	0.9496	0.9673	0.8760	0.8545	0.8512	-0.004
0.7465	0.8025	0.9148	0.7364	0.7807	0.8036	0.8814	0.8393	0.7837	0.8177	0.8011	-0.006
0.7250	0.8464	0.8088	0.7811	0.8325	0.7850	0.9335	0.8589	0.8157	0.8588	0.7592	0.0051
0.7151	0.7805	0.8605	0.7910	0.7845	0.7720	0.8305	0.9080	0.9117	0.9223	0.8118	-0.005
-0.007	-0.007	-0.007	-0.006	-0.006	-0.006	-0.006	-0.006	0.0227	0.00	-0.005	-0.001
Effect Data											
		97.1	103.7	101.4	102.3	106.4	106.3	101.4	100.2	97.4	
		93.3	94.5	94.1	94.2	106.4	100.5	104.8	92.4	90.6	
		100.8	113.6	96.9	97.9	113.5	115.6	104.7	102.1	101.7	
		109.3	88.0	93.3	96.0	105.3	100.3	93.6	97.7	95.7	
		96.6	93.3	99.5	93.8	111.5	102.6	97.5	102.6	90.7	
		102.8	94.5	93.7	92.2	99.2	108.5	108.9	110.2	97.0	
Dose Data in columns											
			156.3	312.5	625	1250	2500	5000	10000	20000	

Scan of the printout (from Phototox version 2.0 software) for this treatment is shown above. The full test article name is IMGHC-TX-H, batch 02808.

Plate layout: Shows the treatment plan of the 96 well plates used in this treatment. 'Control' refers to the vehicle controls. Hyphen (-) on column 1 represents Untreated Control (UTC). Hyphen (-) on column 2 represents the Negative Control. Hyphen (-) on column 12 represents the Positive Control.

Source data: Shows the optical density (OD) for each well. Where a value of 0.00 is presented in the table above, this indicates that the value is between 0 and -0.00049.

Effect data: Shows the percentage survival for each well, compared to the mean survival in solvent controls.

Dose data in columns: Concentration in µg/mL.

Table 7: Raw data – Experiment 1 (water extract) – IMGHC-TX-H, batch 27609

Phototox Project Dataset											
Project: 8265940 E1-Water.PXP						Date: June 09, 2012					
Dataset# 3				Lab: Covance				Chem: 27609			
Plate Layout											
Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
Source Data											
0.0267	-0.004	-0.003	-0.006	-0.005	-0.005	-0.005	-0.002	-0.004	0.0044	-0.005	-0.005
0.7452	0.8217	0.8931	0.8299	0.8865	0.8021	0.8666	0.8737	0.8324	0.8878	0.8720	0.0588
0.8432	0.8203	0.8538	0.8221	0.7952	0.7896	0.7996	0.8600	0.8003	0.8220	0.8440	-0.005
0.8000	0.8439	0.8561	0.8017	0.8194	0.8109	0.8308	0.8294	0.7938	0.8192	0.7614	-0.005
0.8130	0.8308	0.8488	0.8370	0.7882	0.8405	0.8403	0.8598	0.7783	0.7962	0.7910	0.0073
0.8018	0.8734	0.8075	0.8497	0.7999	0.8573	0.8382	0.8715	0.8207	0.8025	0.7531	0.0242
0.7476	0.8367	0.8601	0.8055	0.8667	0.9130	0.8665	0.8669	0.8440	0.8742	0.8653	-0.005
-0.001	0.0035	0.0015	0.0098	-0.005	-0.004	-0.003	0.0110	-0.004	-0.005	0.0112	-0.003
Effect Data											
		104.7	97.3	103.9	94.0	101.6	102.4	97.6	104.1	102.2	
		100.1	96.4	93.2	92.5	93.7	100.8	93.8	96.3	98.9	
		100.3	94.0	96.0	95.0	97.4	97.2	93.0	96.0	89.2	
		99.5	98.1	92.4	98.5	98.5	100.8	91.2	93.3	92.7	
		94.6	99.6	93.7	100.5	98.2	102.1	96.2	94.1	88.3	
		100.8	94.4	101.6	107.0	101.6	101.6	98.9	102.5	101.4	
Dose Data in columns											
			156.3	312.5	625	1250	2500	5000	10000	20000	

Scan of the printout (from Phototox version 2.0 software) for this treatment is shown above. The full test article name is IMGHC-TX-H, batch 27609.

Plate layout: Shows the treatment plan of the 96 well plates used in this treatment. 'Control' refers to the vehicle controls. Hyphen (-) on column 1 represents Untreated Control (UTC). Hyphen (-) on column 2 represents the Negative Control. Hyphen (-) on column 12 represents the Positive Control.

Source data: Shows the optical density (OD) for each well. Where a value of 0.00 is presented in the table above, this indicates that the value is between 0 and -0.00049.

Effect data: Shows the percentage survival for each well, compared to the mean survival in solvent controls.

Dose data in columns: Concentration in µg/mL.

Table 8: Raw data – Experiment 1 (water extract) – IMGHC-TX-H, batch 41609

Phototox Project Dataset											
Project: 8265940 E1-Water.PXP Date: June 09, 2012											
Dataset# 4 Lab: Covance Chem: 41609 Run: 1											
Plate Layout											
Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
Source Data											
-0.004	-0.003	-0.004	-0.003	-0.004	0.0218	-0.004	-0.004	-0.005	-0.004	-0.003	-0.004
0.7293	0.9180	0.8612	0.8590	0.8436	0.7770	0.7930	0.8015	0.8185	0.7871	0.7943	-0.004
0.6980	0.8406	0.8059	0.7189	0.6996	0.6825	0.8818	0.7331	0.7473	0.7616	0.6806	-0.004
0.7034	0.8694	0.9089	0.7859	0.7586	0.6844	0.7650	0.8249	0.8675	0.9038	0.7462	-0.002
0.6719	0.7558	0.7494	0.7414	0.7001	0.6715	0.7429	0.7228	0.7539	0.7022	0.7566	-0.004
0.7095	0.8332	0.7467	0.7642	0.7361	0.6850	0.7847	0.7833	0.7500	0.7387	0.7181	-0.004
0.7500	0.7950	0.8358	0.8728	0.7760	0.7812	0.7727	0.7828	0.8585	0.8414	0.7637	-0.004
0.0320	-0.003	-0.003	0.0027	0.0052	-0.001	-0.003	-0.003	0.0053	-0.004	-0.003	-0.004
Effect Data											
		105.3	105.0	103.1	95.0	96.9	98.0	100.1	96.2	97.1	
		98.5	87.9	85.5	83.4	107.8	89.6	91.4	93.1	83.2	
		111.1	96.1	92.7	83.7	93.5	100.8	106.1	110.5	91.2	
		91.6	90.6	85.6	82.1	90.8	88.4	92.2	85.8	92.5	
		91.3	93.4	90.0	83.7	95.9	95.8	91.7	90.3	87.8	
		102.2	106.7	94.9	95.5	94.5	95.7	105.0	102.9	93.4	
Dose Data in columns											
			156.3	312.5	625	1250	2500	5000	10000	20000	

Scan of the printout (from Phototox version 2.0 software) for this treatment is shown above. The full test article name is IMGHC-TX-H, batch 41609.

Plate layout: Shows the treatment plan of the 96 well plates used in this treatment. 'Control' refers to the vehicle controls. Hyphen (-) on column 1 represents Untreated Control (UTC). Hyphen (-) on column 2 represents the Negative Control. Hyphen (-) on column 12 represents the Positive Control.

Source data: Shows the optical density (OD) for each well. Where a value of 0.00 is presented in the table above, this indicates that the value is between 0 and -0.00049.

Effect data: Shows the percentage survival for each well, compared to the mean survival in solvent controls.

Dose data in columns: Concentration in µg/mL.

Table 9: Raw data – Experiment 1 (water extract) – IMGHC-TX-S, batch 37908

Phototox Project Dataset											
Project: 8265940 E1-Water.PXP						Date: June 09, 2012					
Dataset# 5				Lab: Covance				Chem: 37908			
Plate Layout											
Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
Source Data											
-0.002	0.0159	-0.001	-0.002	-0.002	-0.001	-0.002	-0.001	-0.003	-0.001	-0.002	0.0128
0.7689	0.7745	0.8809	0.7904	0.8081	0.7355	0.8073	0.8052	0.8050	0.8641	0.8374	0.0287
0.7609	0.7225	0.7648	0.7185	0.7308	0.7108	0.8365	0.8030	0.7493	0.7230	0.7635	-0.002
0.7334	0.8122	0.8662	0.7750	0.7554	0.6432	0.6883	0.6937	0.8263	0.8600	0.7771	0.0001
0.6174	0.7663	0.8722	0.6817	0.6980	0.6712	0.7071	0.7141	0.7095	0.7737	0.7831	-0.003
0.8346	0.8043	0.7610	0.7423	0.7016	0.6985	0.8171	0.7133	0.7259	0.7566	0.7330	0.0296
0.7669	0.7790	0.7772	0.8327	0.8317	0.7813	0.8269	0.8459	0.7987	0.8224	0.7585	-0.002
-0.001	-0.001	0.0016	0.00	0.0014	-0.001	-0.002	-0.003	-0.002	-0.001	0.00	-0.003
Effect Data											
		107.4	96.3	98.5	89.7	98.4	98.1	98.1	105.3	102.1	
		93.2	87.6	89.1	86.6	102.0	97.9	91.3	88.1	93.1	
		105.6	94.5	92.1	78.4	83.9	84.6	100.7	104.8	94.7	
		106.3	83.1	85.1	81.8	86.2	87.0	86.5	94.3	95.5	
		92.8	90.5	85.5	85.1	99.6	86.9	88.5	92.2	89.3	
		94.7	101.5	101.4	95.2	100.8	103.1	97.4	100.2	92.5	
Dose Data in columns											
			156.3	312.5	625	1250	2500	5000	10000	20000	

Scan of the printout (from Phototox version 2.0 software) for this treatment is shown above. The full test article name is IMGHC-TX-S, batch 37908.

Plate layout: Shows the treatment plan of the 96 well plates used in this treatment. 'Control' refers to the vehicle controls. Hyphen (-) on column 1 represents Untreated Control (UTC). Hyphen (-) on column 2 represents the Negative Control. Hyphen (-) on column 12 represents the Positive Control.

Source data: Shows the optical density (OD) for each well. Where a value of 0.00 is presented in the table above, this indicates that the value is between 0 and -0.00049.

Effect data: Shows the percentage survival for each well, compared to the mean survival in solvent controls.

Dose data in columns: Concentration in µg/mL.

Table 10: Raw data – Experiment 1 (water extract) –

Phototox Project Dataset											
Project: 8265940 E1-Water.PXP Date: June 09, 2012											
Dataset# 6 Lab: Covance Chem: 2187001 Run: 1											
Plate Layout											
Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
Source Data											
-0.002	-0.003	-0.004	-0.005	-0.005	0.0039	0.0224	-0.007	-0.006	-0.004	0.0014	-0.006
0.6866	0.8433	0.7859	0.7849	0.8285	0.8081	0.8715	0.7922	0.7992	0.7917	0.8068	-0.005
0.7708	0.8234	0.8541	0.7753	0.7924	0.7249	0.7392	0.7723	0.7660	0.7753	0.7806	0.0190
0.7315	0.8428	0.8371	0.7750	0.7590	0.7745	0.7370	0.8128	0.8411	0.8249	0.8299	-0.005
0.7908	0.7968	0.8274	0.8021	0.7763	0.6970	0.7253	0.7347	0.7627	0.7724	0.7578	-0.005
0.7656	0.8301	0.8295	0.7619	0.7427	0.7503	0.8095	0.7761	0.7814	0.8216	0.7784	-0.003
0.7424	0.8747	0.8569	0.7662	0.7532	0.7571	0.8204	0.8273	0.8084	0.8056	0.8664	0.0253
-0.003	-0.002	-0.005	0.0020	-0.001	-0.005	-0.004	-0.005	-0.003	0.0337	0.0090	-0.005
Effect Data											
		94.5	94.4	99.6	97.1	104.8	95.2	96.1	95.2	97.0	
		102.7	93.2	95.3	87.1	88.9	92.8	92.1	93.2	93.8	
		100.6	93.2	91.2	93.1	88.6	97.7	101.1	99.2	99.8	
		99.5	96.4	93.3	83.8	87.2	88.3	91.7	92.9	91.1	
		99.7	91.6	89.3	90.2	97.3	93.3	93.9	98.8	93.6	
		103.0	92.1	90.5	91.0	98.6	99.5	97.2	96.8	104.2	
Dose Data in columns											
			156.3	312.5	625	1250	2500	5000	10000	20000	

Scan of the printout (from Phototox version 2.0 software) for this treatment is shown above. The full test article name is

Plate layout: Shows the treatment plan of the 96 well plates used in this treatment. 'Control' refers to the vehicle controls. Hyphen (-) on column 1 represents Untreated Control (UTC). Hyphen (-) on column 2 represents the Negative Control. Hyphen (-) on column 12 represents the Positive Control.

Source data: Shows the optical density (OD) for each well. Where a value of 0.00 is presented in the table above, this indicates that the value is between 0 and -0.00049.

Effect data: Shows the percentage survival for each well, compared to the mean survival in solvent controls.

Dose data in columns: Concentration in µg/mL.

Appendix 2
Tables of results– DMSO Extractions

Table 11: Raw data – Experiment 2 (DMSO extract) –IMGHC-TX-H, batch 16306

Phototox Project Dataset											
Project: 8265940 E1 DMSO.PXP						Date: July 11, 2012					
Dataset# 1			Lab: Covance			Chem: 16306			Run: 1		
Plate Layout											
Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
Source Data											
0.0005	0.0001	0.0002	0.0002	0.0004	0.00	0.0003	-0.001	-0.001	-0.001	-0.001	-0.001
0.6000	0.6097	0.5417	0.5357	0.5241	0.6126	0.6246	0.6646	0.6461	0.6551	0.5917	-0.001
0.5268	0.5001	0.5202	0.5984	0.6067	0.5628	0.6477	0.6505	0.6517	0.5936	0.5896	-0.001
0.4688	0.6204	0.6257	0.5992	0.6264	0.5915	0.6630	0.6613	0.6406	0.6505	0.6043	-0.001
0.5002	0.6186	0.7040	0.5907	0.6127	0.5886	0.5746	0.6235	0.6096	0.6264	0.5496	0.00
0.4674	0.5718	0.6656	0.6053	0.6659	0.6022	0.6219	0.6101	0.5796	0.5917	0.5926	-0.001
0.4556	0.5645	0.5728	0.5859	0.7270	0.5874	0.5905	0.6499	0.6603	0.6000	0.5780	-0.001
0.0000	0.00	0.00	0.0005	0.0001	0.0000	0.0004	0.0001	0.0002	0.0012	0.0016	-0.001
Effect Data											
		89.5	88.5	86.6	101.3	103.2	109.9	106.8	108.3	97.8	
		86.0	98.9	100.3	93.0	107.1	107.5	107.7	98.1	97.5	
		103.4	99.0	103.5	97.8	109.6	109.3	105.9	107.5	99.9	
		116.4	97.6	101.3	97.3	95.0	103.1	100.8	103.5	90.8	
		110.0	100.0	110.1	99.5	102.8	100.8	95.8	97.8	98.0	
		94.7	96.8	120.2	97.1	97.6	107.4	109.1	99.2	95.5	
Dose Data in columns											
			15.63	31.25	62.5	125	250	500	1000	2000	

Scan of the printout (from Phototox version 2.0 software) for this treatment is shown above. The full test article name is IMGHC-TX-H, batch 16306.

Plate layout: Shows the treatment plan of the 96 well plates used in this treatment. 'Control' refers to the vehicle controls. Hyphen (-) on column 1 represents Untreated Control (UTC). Hyphen (-) on column 2 represents the Negative Control. Hyphen (-) on column 12 represents the Positive Control.

Source data: Shows the optical density (OD) for each well. Where a value of 0.00 is presented in the table above, this indicates that the value is between 0 and -0.00049.

Effect data: Shows the percentage survival for each well, compared to the mean survival in solvent controls.

Dose data in columns: Concentration in µg/mL.

Table 12: Raw data - Experiment 2 (DMSO extract) – IMGHC-TX-H, batch 02808

Phototox Project Dataset											
Project: 8265940 E1 DMSO.PXP						Date: July 11, 2012					
Dataset# 2				Lab: Covance				Chem: 02808			
Plate Layout											
Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
Source Data											
-0.002	-0.002	-0.002	-0.002	-0.002	-0.003	-0.003	0.0054	-0.003	-0.002	-0.003	-0.003
0.6044	0.5838	0.5377	0.6052	0.6034	0.6074	0.7122	0.7045	0.7053	0.6557	0.6577	-0.003
0.6382	0.6203	0.6345	0.6300	0.6497	0.5788	0.6882	0.6136	0.6102	0.5647	0.6397	-0.003
0.5724	0.6156	0.6041	0.5791	0.5879	0.6484	0.6306	0.6094	0.6396	0.6548	0.6506	-0.003
0.4745	0.6222	0.6190	0.7057	0.6178	0.5347	0.6226	0.6504	0.6328	0.6596	0.6754	-0.003
0.5518	0.4704	0.6742	0.6019	0.6069	0.5987	0.7182	0.5935	0.6520	0.6422	0.5955	-0.002
0.5177	0.6178	0.5880	0.5696	0.5430	0.6519	0.6099	0.5592	0.5577	0.6699	0.5754	-0.002
-0.001	0.0007	-0.002	-0.002	-0.002	-0.002	-0.001	-0.002	-0.003	0.0400	-0.002	-0.003
Effect Data											
		88.2	99.3	99.0	99.6	116.8	115.6	115.7	107.6	107.9	
		104.1	103.3	106.6	95.0	112.9	100.7	100.1	92.6	104.9	
		99.1	95.0	96.4	106.4	103.4	100.0	104.9	107.4	106.7	
		101.5	115.8	101.3	87.7	102.1	106.7	103.8	108.2	110.8	
		110.6	98.7	99.6	98.2	117.8	97.4	107.0	105.4	97.7	
		96.5	93.4	89.1	106.9	100.1	91.7	91.5	109.9	94.4	
Dose Data in columns											
			15.63	31.25	62.5	125	250	500	1000	2000	

Scan of the printout (from Phototox version 2.0 software) for this treatment is shown above. The full test article name is IMGHC-TX-H, batch 02808.

Plate layout: Shows the treatment plan of the 96 well plates used in this treatment. 'Control' refers to the vehicle controls. Hyphen (-) on column 1 represents Untreated Control (UTC). Hyphen (-) on column 2 represents the Negative Control. Hyphen (-) on column 12 represents the Positive Control.

Source data: Shows the optical density (OD) for each well. Where a value of 0.00 is presented in the table above, this indicates that the value is between 0 and -0.00049.

Effect data: Shows the percentage survival for each well, compared to the mean survival in solvent controls.

Dose data in columns: Concentration in µg/mL.

Table 13: Raw data - Experiment 2 (DMSO extract) – IMGHC-TX-H, batch 27609

Phototox Project Dataset											
Project: 8265940 E1 DMSO.PXP Date: July 11, 2012											
Dataset# 3 Lab: Covance Chem: 27609 Run: 1											
Plate Layout											
Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
Source Data											
0.0005	-0.001	-0.001	-0.001	0.00	-0.001	0.0003	0.00	0.00	0.0001	-0.001	-0.001
0.5751	0.6717	0.5495	0.5499	0.5433	0.5746	0.6413	0.6348	0.5664	0.6409	0.6623	0.00
0.5050	0.6024	0.6317	0.5859	0.5364	0.5210	0.5921	0.5513	0.5907	0.6452	0.6447	-0.001
0.4951	0.6206	0.6028	0.5556	0.5576	0.5563	0.5791	0.6432	0.6235	0.6251	0.6034	0.00
0.5405	0.6194	0.6465	0.6221	0.5996	0.5595	0.5997	0.6537	0.6197	0.6570	0.6320	0
0.5573	0.6476	0.6227	0.6134	0.5964	0.6044	0.5853	0.6358	0.6202	0.6270	0.6649	-0.001
0.4972	0.5854	0.6087	0.6606	0.5300	0.6339	0.5801	0.5907	0.5499	0.6360	0.5648	-0.001
0.00	-0.001	-0.001	0.00	0.0004	-0.001	0.0006	-0.001	-0.001	-0.001	0.0119	-0.001
Effect Data											
		90.0	90.1	89.0	94.1	105.1	104.0	92.8	105.0	108.5	
		103.5	96.0	87.9	85.4	97.0	90.3	96.8	105.7	105.6	
		98.8	91.0	91.4	91.1	94.9	105.4	102.2	102.4	98.9	
		105.9	101.9	98.2	91.7	98.3	107.1	101.5	107.6	103.6	
		102.0	100.5	97.7	99.0	95.9	104.2	101.6	102.7	108.9	
		99.7	108.2	86.8	103.9	95.0	96.8	90.1	104.2	92.5	
Dose Data in columns											
			15.63	31.25	62.5	125	250	500	1000	2000	

Scan of the printout (from Phototox version 2.0 software) for this treatment is shown above. The full test article name is IMGHC-TX-H, batch 27609.

Plate layout: Shows the treatment plan of the 96 well plates used in this treatment. 'Control' refers to the vehicle controls. Hyphen (-) on column 1 represents Untreated Control (UTC). Hyphen (-) on column 2 represents the Negative Control. Hyphen (-) on column 12 represents the Positive Control.

Source data: Shows the optical density (OD) for each well. Where a value of 0.00 is presented in the table above, this indicates that the value is between 0 and -0.00049.

Effect data: Shows the percentage survival for each well, compared to the mean survival in solvent controls.

Dose data in columns: Concentration in µg/mL.

Table 14: Raw data - Experiment 2 (DMSO extract) – IMGHC-TX-H, batch 41609

Phototox Project Dataset											
Project: 8265940 E1 DMSO.PXP						Date: July 11, 2012					
Dataset# 4				Lab: Covance				Chem: 41609			
Plate Layout											
Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
Source Data											
0.0032	0..00	0..00	-0.001	-0.001	0.0003	0.0002	0.0006	-0.001	0.0002	-0.001	-0.001
0.4608	0.5423	0.5349	0.6280	0.6510	0.6596	0.6893	0.6753	0.7053	0.6897	0.6929	0.0005
0.5797	0.5284	0.6089	0.5838	0.6390	0.6749	0.6377	0.6380	0.7233	0.6644	0.6590	-0.001
0.5614	0.6154	0.6054	0.6198	0.6062	0.5674	0.6010	0.5618	0.6017	0.5820	0.6402	0..00
0.5882	0.6860	0.6418	0.5967	0.5879	0.6461	0.5580	0.5900	0.6191	0.6203	0.6288	0..00
0.5871	0.6323	0.6545	0.5122	0.6419	0.6118	0.6301	0.6298	0.6296	0.6615	0.6511	-0.001
0.5374	0.6320	0.6361	0.6113	0.6516	0.6487	0.6046	0.6557	0.6007	0.6134	0.6707	-0.001
0.0006	0..00	0..00	-0.002	-0.001	0..00	0.0004	0.0001	0..00	0.0021	0.0007	0..00
Effect Data											
		87.2	102.3	106.1	107.5	112.3	110.1	114.9	112.4	112.9	
		99.2	95.1	104.1	110.0	103.9	104.0	117.9	108.3	107.4	
		98.7	101.0	98.8	92.5	97.9	91.6	98.1	94.8	104.3	
		104.6	97.2	95.8	105.3	90.9	96.2	100.9	101.1	102.5	
		106.7	83.5	104.6	99.7	102.7	102.6	102.6	107.8	106.1	
		103.7	99.6	106.2	105.7	98.5	106.9	97.9	100.0	109.3	
Dose Data in columns											
			15.63	31.25	62.5	125	250	500	1000	2000	

Scan of the printout (from Phototox version 2.0 software) for this treatment is shown above. The full test article name is IMGHC-TX-H, batch 41609.

Plate layout: Shows the treatment plan of the 96 well plates used in this treatment. 'Control' refers to the vehicle controls. Hyphen (-) on column 1 represents Untreated Control (UTC). Hyphen (-) on column 2 represents the Negative Control. Hyphen (-) on column 12 represents the Positive Control.

Source data: Shows the optical density (OD) for each well. Where a value of 0..00 is presented in the table above, this indicates that the value is between 0 and -0.00049.

Effect data: Shows the percentage survival for each well, compared to the mean survival in solvent controls.

Dose data in columns: Concentration in µg/mL.

Table 15: Raw data - Experiment 2 (DMSO extract) – IMGHC-TX-S, batch 37908

Phototox Project Dataset											
Project: 8265940 E1 DMSO.PXP Date: July 11, 2012											
Dataset# 5 Lab: Covance Chem: 37908 Run: 1											
Plate Layout											
Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
Source Data											
0.0005	-0.001	-0.001	0.00	0.0001	-0.002	0.00	-0.001	-0.002	-0.001	-0.001	-0.002
0.5224	0.5507	0.5227	0.5369	0.5654	0.5731	0.7070	0.6660	0.7629	0.7857	0.7167	-0.001
0.4838	0.5163	0.4913	0.5579	0.5435	0.6217	0.6932	0.6918	0.6653	0.7238	0.6850	-0.002
0.4762	0.5145	0.5750	0.5920	0.5524	0.5156	0.5405	0.5738	0.6088	0.6039	0.6754	-0.002
0.4642	0.5592	0.5657	0.6186	0.5752	0.6363	0.5798	0.5770	0.5817	0.5909	0.6620	0.00
0.4854	0.4761	0.5470	0.5406	0.5217	0.6129	0.5437	0.5773	0.6261	0.5907	0.6589	-0.001
0.4330	0.4923	0.5521	0.5563	0.5742	0.5974	0.5805	0.5976	0.6213	0.5835	0.6651	-0.001
0.0003	-0.001	0.0001	0.00	-0.001	-0.001	0.00	-0.001	-0.001	-0.001	0.0031	0.0120
Effect Data											
		96.4	99.0	104.3	105.7	130.4	122.8	140.7	144.9	132.2	
		90.6	102.9	100.2	114.6	127.8	127.6	122.7	133.5	126.3	
		106.0	109.2	101.9	95.1	99.7	105.8	112.3	111.4	124.5	
		104.3	114.1	106.1	117.3	106.9	106.4	107.3	109.0	122.1	
		100.9	99.7	96.2	113.0	100.3	106.5	115.5	108.9	121.5	
		101.8	102.6	105.9	110.2	107.0	110.2	114.6	107.6	122.6	
Dose Data in columns											
			15.63	31.25	62.5	125	250	500	1000	2000	

Scan of the printout (from Phototox version 2.0 software) for this treatment is shown above. The full test article name is IMGHC-TX-S, batch 37908.

Plate layout: Shows the treatment plan of the 96 well plates used in this treatment. 'Control' refers to the vehicle controls. Hyphen (-) on column 1 represents Untreated Control (UTC). Hyphen (-) on column 2 represents the Negative Control. Hyphen (-) on column 12 represents the Positive Control.

Source data: Shows the optical density (OD) for each well. Where a value of 0.00 is presented in the table above, this indicates that the value is between 0 and -0.00049.

Effect data: Shows the percentage survival for each well, compared to the mean survival in solvent controls.

Dose data in columns: Concentration in µg/mL.

Table 16: Raw data - Experiment 2 (DMSO extract) –

Phototox Project Dataset											
Project: 8265940 E1 DMSO.PXP						Date: July 11, 2012					
Dataset# 6				Lab: Covance		Chem: 2187001		Run: 1			
Plate Layout											
Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
-	-	Control	Dose	Dose	Dose	Dose	Dose	Dose	Dose	Dose	-
Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
Source Data											
0.0004	-0.001	-0.001	0.0002	0.0013	-0.002	0.0002	0.00	0.0079	-0.001	0.0003	-0.003
0.5899	0.5150	0.5088	0.5321	0.5919	0.6050	0.5908	0.5934	0.6622	0.7469	0.6707	-0.001
0.4909	0.4851	0.5618	0.5770	0.5804	0.5307	0.5881	0.5892	0.6469	0.7311	0.6246	-0.002
0.5077	0.5841	0.5514	0.5335	0.5806	0.5176	0.5624	0.5754	0.5602	0.6154	0.6329	-0.001
0.4410	0.5883	0.5948	0.6043	0.5193	0.5255	0.5800	0.5529	0.5215	0.6312	0.6590	0.00
0.4748	0.5916	0.5077	0.5533	0.5397	0.5483	0.6061	0.6432	0.6062	0.6875	0.6284	0.0011
0.4549	0.5876	0.5569	0.6011	0.5332	0.5189	0.5425	0.5695	0.5946	0.6764	0.6259	-0.001
0.0020	0.0005	0.0005	-0.001	0.0025	-0.001	-0.001	-0.001	0.0000	-0.001	-0.001	-0.002
Effect Data											
		93.0	97.3	108.2	110.6	108.0	108.5	121.1	136.6	122.6	
		102.7	105.5	106.1	97.0	107.5	107.7	118.3	133.7	114.2	
		100.8	97.5	106.2	94.6	102.8	105.2	102.4	112.5	115.7	
		108.8	110.5	95.0	96.1	106.1	101.1	95.4	115.4	120.5	
		92.8	101.2	98.7	100.3	110.8	117.6	110.8	125.7	114.9	
		101.8	109.9	97.5	94.9	99.2	104.1	108.7	123.7	114.4	
Dose Data in columns											
			15.63	31.25	62.5	125	250	500	1000	2000	

Scan of the printout (from Phototox version 2.0 software) for this treatment is shown above. The full test article name is [REDACTED]

Plate layout: Shows the treatment plan of the 96 well plates used in this treatment. 'Control' refers to the vehicle controls. Hyphen (-) on column 1 represents Untreated Control (UTC). Hyphen (-) on column 2 represents the Negative Control. Hyphen (-) on column 12 represents the Positive Control.

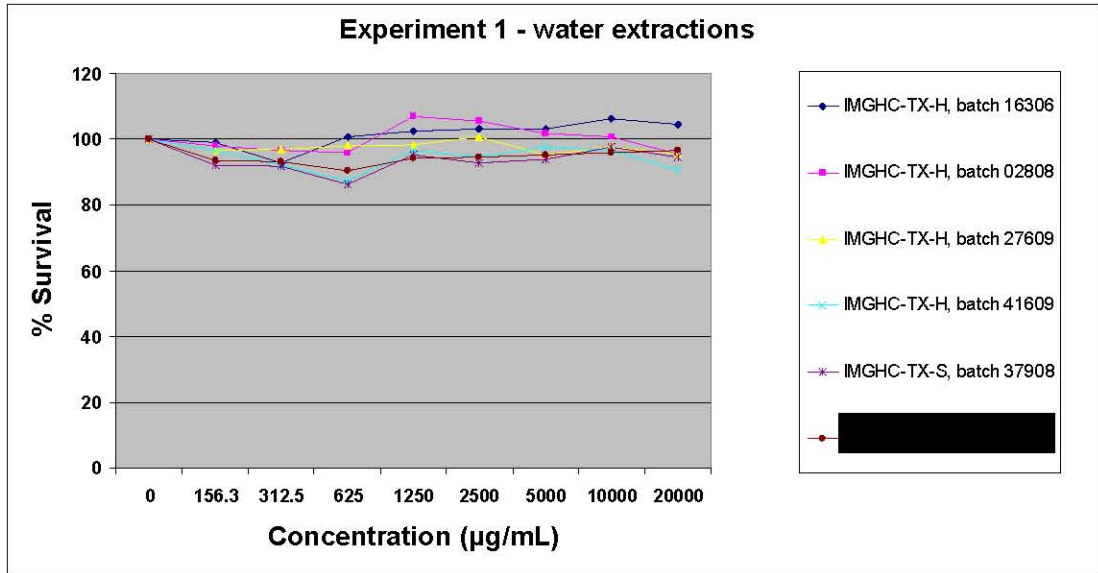
Source data: Shows the optical density (OD) for each well. Where a value of 0.00 is presented in the table above, this indicates that the value is between 0 and -0.00049.

Effect data: Shows the percentage survival for each well, compared to the mean survival in solvent controls.

Dose data in columns: Concentration in µg/mL.

Appendix 3 Survival curves

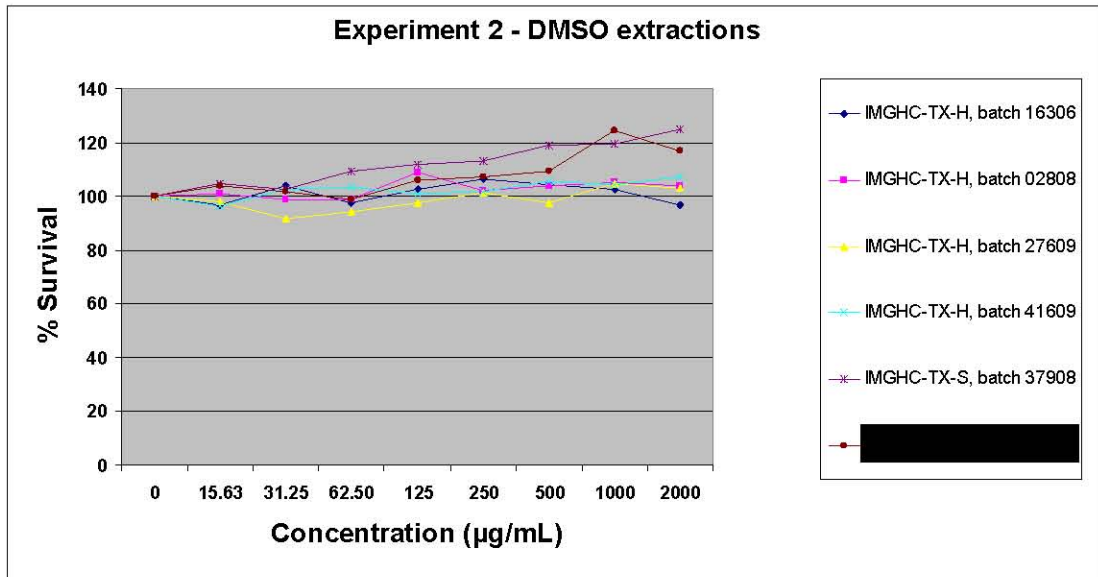
Figure 1 – Survival curve - Experiment 1 (Water Extractions):



Y-axis Percentage survival compared to vehicle controls

X-axis Concentration in µg/mL

Figure 2 - Survival curve: - Experiment 2 (DMSO Extractions):



Y-axis Percentage survival compared to vehicle controls
X-axis Concentration in µg/mL