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Toxicity Report No. S.0055513-18, April 2018 - September 2018
Toxicology Directorate

***In Vitro* Dermal Corrosion Proficiency Demonstration, April 2018-September 2018**

Prepared by Emily May Lent

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ARIMS designation: 500C Toxicity Test

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14. ABSTRACT

Prior to routine use of any of the four validated in vitro dermal corrosion test methods specified in the OECD guideline, technical proficiency must be demonstrated using the twelve Proficiency Substances listed in the OECD guideline. This study was conducted as a proficiency demonstration. Proficiency substances were tested using the EPI-SCT-200 assay. All assay acceptance criteria were met and 100% of the proficiency substances were correctly classified, therefore the performing lab is technically proficient and EPI-SCT-200 may be routinely used for in vitro prediction of skin corrosion.

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Data Requirement

OECD Guidelines for the Testing of Chemicals, Section 4, Test No. 431: *In Vitro* Skin Corrosion: Reconstructed Human Epidermis (RhE) Test Method

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Good Laboratory Practice Compliance Statement

The study described in this report was conducted in compliance with Title 40, Code of Federal Regulations (CFR), Part 792, Good Laboratory Practice Standards, except for the following:

1. Manufacturer reported purity of the neat compounds was not verified analytically. This is not considered to have affected the outcome of the study as certificates of analyses were provided with each compound.

Submitted By:

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1 Summary

1.1 Purpose

The *in vitro* dermal corrosion test assesses the potential for chemicals to cause irreversible damage to the skin. This method uses tissue damage (i.e., viability) following topical exposure of reconstructed human epidermis (RhE) to neat test chemicals for 3 minutes or 1 hour to predict skin corrosion potential of chemicals. The following study was conducted to demonstrate technical proficiency using one of the four validated test methods, EpiDerm™ Skin Corrosion Test (EPI-SCT-200, MatTek, Ashland, Massachusetts).

1.2 Conclusions

The proficiency substances were tested using the EPI-SCT-200 assay as a demonstration of technical proficiency of the laboratory prior to routine use of this test method. All assay acceptance criteria were met and 100% of the proficiency substances were correctly classified, therefore the performing lab is technically proficient and EPI-SCT-200 may be routinely used for *in vitro* prediction of skin corrosion.

2 References

See Appendix A for a listing of references.

3 Authority

This study was sponsored by the U.S. Army Medical Command, Office of the Surgeon General and identified as WBS element S.0055513.

4 Background

Chemical-induced skin corrosion has historically been assessed in laboratory animals based on the manifestation of visible necrosis through the epidermis and into the dermis. The *in vitro* test system using reconstructed human epidermis (RhE), which closely mimics the biochemical and physiological properties of the upper parts of the human skin, i.e., the epidermis, is based on the premise that corrosive chemicals are able to penetrate the stratum corneum by diffusion or erosion and are cytotoxic to the cells in the underlying layers. Cell viability in RhE models is measured by dehydrogenase conversion of MTT [(3-4,5-Dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide, Thiazolyl blue], into a blue formazan salt that is quantitatively measured after extraction from tissues. Reduction in the viability of tissues exposed to chemicals in comparison with negative controls is used to predict skin corrosion potential. The RhE-based skin corrosion test methods have shown to

be predictive of *in vivo* skin corrosion effects [1-4] assessed in rabbits according to the OECD guideline 404 [5]. The RhE model tests allow discrimination between corrosives of United Nations (UN) Globally Harmonized System of Classification and Labeling of Chemicals (GHS) category 1 and non-corrosive. The tests do not provide information on irritation.

Prior to routine use of any of the four validated test methods specified in the OECD guideline, technical proficiency must be demonstrated using the twelve Proficiency Substances listed in the OECD guideline [6]. The present study was conducted as a technical proficiency demonstration.

5 Materials and Methods

5.1 Materials

5.1.1 Test Substances

The twelve proficiency substances listed in the OECD Guideline (Table 1) were purchased from Sigma Aldrich (St. Louis, MO).

Table 1. Proficiency Chemicals.

Substance	CASRN	Purity (%)	Chemical Class	UN GHS Cat. Based on *In Vivo* results	VRM Cat. Based on *In Vitro* results	MTT Reducer	Physical State
SUB-CATEGORY 1A *IN VIVO* CORROSIVES							
Bromoacetic acid	79-08-3	99.8	Organic acid	1A	1A		Solid
Boron trifluoride dihydrate	13319-75-0	96	Inorganic acid	1A	1A		Liquid
Phenol	108-95-2	100	Phenol	1A	1A		Solid
Dichloroacetyl chloride	79-36-7	99.2	Electrophile	1A	1A		Liquid
COMBINATION OF SUB-CATEGORIES 1B-AND-1C *IN VIVO* CORROSIVES							
Glyoxylic acid monohydrate	563-96-2	100.5	Organic acid	1B-and-1C	1B-and-1C		Solid
Lactic acid	598-82-3	88.6	Organic acid	1B-and-1C	1B-and-1C		Liquid
Ethanolamine	141-43-5	100	Organic acid	1B	1B-and-1C	Y	Viscous
Hydrochloric acid (14.4%)	7647-01-0	37.5	Inorganic acid	1B-and-1C	1B-and-1C		Liquid
***IN VIVO* NON CORROSIVES**							
Phenethyl bromide	103-63-9	99.1	Electrophile	NC	NC	Y	Liquid
4-Amino-1,2,4-triazole	584-13-4	97	Organic base	NC	NC		Solid
4-(methylthio)-benzaldehyde	3446-89-7	98.9	Electrophile	NC	NC	Y	Liquid
Lauric acid	143-07-7	100.4	Organic base	NC	NC		Solid

CASRN: Chemical Abstracts Service Registry Number, UN GHS: United Nations Globally Harmonized System, VRM: Validated Reference Method, NC: Not Corrosive, MTT: (3-4,5-Dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide, Thiazolyl blue, Y: yes

5.1.2 Test System, Controls, and Reagents

reagents The reconstructed human epidermal model EpiDerm™ was acquired from MatTek (EPI-200, MatTek, Ashland). The EpiDerm™ tissues are shipped as kits, containing 24 tissues on shipping agarose together with the necessary amount of culture media – Dulbeco's Modified Eagle's Medium (DMEM) based, Dulbeco's Phosphate Buffered Saline (DPBS), positive control (8N potassium hydroxide (KOH) solution), 6-well plates, and 24-well plates. In addition, the MTT kit (containing MTT concentrate, diluent, and extractant) was also purchased from MatTek. Additional DPBS without calcium, magnesium, or phenol red was purchased from Gibco, Inc. (a subsidiary of ThermoFisher, Waltham, MA). All test systems, and chemicals were stored according to the manufacturer's instructions.

5.2 Quality Assurance

5.2.1 Quality Control of Test System

The EpiDerm™ System is manufactured according to defined quality assurance procedures. All biological components of the epidermis and the culture medium are tested by the manufacturer for viral, bacterial, fungal, and mycoplasma contamination. MatTek determines the effective time for 50% viability (ET-50 value) following exposure to Triton X-100 (1%) for each EpiDerm™ lot. The ET-50 must fall within the range of the EpiDerm historical database of 4.77 – 8.72 hours. If tissue lots fail quality control (QC) or sterility testing, the manufacturer notifies the customer. All of the tissue lots used in this proficiency demonstration passed QC and sterility testing.

5.2.2 Assay Acceptance Criteria

The absolute optical density (OD) of the negative control (NC) tissues (treated with water) in the MTT-test is an indicator of tissue viability obtained in the testing laboratory after shipping and storing procedures and under specific conditions of use. The assay meets the acceptance criterion if the mean OD at 570 nanometers (OD_{570}) of the NC tissues is ≥ 0.8.

An 8N KOH solution is used as positive control (PC) and tested concurrently with the test chemicals. The assay meets the acceptance criterion if the mean viability of PC tissues exposed for 1 hour expressed as % of the negative control tissues is <15%.

Since in each test, skin corrosion potential is predicted from the mean viability determined on 2-3 single tissues per exposure time, the variability of tissue replicates should be acceptably low for tissues with viability in the range of 20-100%. The assay meets the acceptance criterion if the coefficient of variation (CV) calculated from individual % tissue viabilities of the 2-3 identically treated replicates does not exceed 0.3.

5.2.3 Quality Compliance

The APHC Quality Systems and Regulatory Compliance Office audited critical study phases. Appendix B provides the dates of these audits, the phases audited, and the dates the results were reported to the Study Director (SD) and Management.

5.3 Study Personnel

Appendix C lists the names of individuals contributing to the study performance.

5.4 Methods

All tests were conducted according to the manufacturer's instructions.

5.4.1 Test for Interference with MTT Endpoint

Because test substances may interfere with the MTT endpoint if they are colored and/or able to directly reduce MTT, tests for interference of chemicals with the MTT endpoint were conducted.

Some non-colored test materials may change into colored materials in wet or aqueous conditions and thus stain tissues during the 60 ± 5 minute exposure. Therefore, a functional check for this possibility should be performed. However, if the colored test material or the MTT reducing chemical is classified as corrosive by the assay, the correction procedures are not necessary.

5.4.1.1 Test for Colored Materials

To determine if non-colored test materials change into colored test materials, 50 µl (liquid) or 25 mg (solid - using a sharp spoon) of the test substance was added to 0.3 ml of deionized water in a glass vial and placed in the incubator (37±1°C, 5±1 % CO2, 95% RH) for 60 ± 5 minute. At the end of the exposure time, the mixture was evaluated for the presence and intensity of staining. If the solution changed color, a functional check on viable tissues was performed.

5.4.1.2 Test for Tissue Binding

To check the tissue-binding of a colored test substance (or a chemical that changes into a colored substance), one viable tissue was exposed to 50 µl of liquid test substance or 25 mg of solid test substance. In parallel, one tissue was exposed to water (negative control). Tissues were treated as described in section 5.4.2 except tissues were incubated for 3 hours ± 5 minutes in culture media without MTT (37±1°C, 5±1% CO_2, 95% RH) instead of media containing MTT.

5.4.1.3 Data Correction Procedure

The actual MTT OD (unaffected by interference with the colored test materials) is calculated using following formula:

$$OD = OD_{\text{colored tissue (MTT assay)}} - OD_{\text{colored tissue (no MTT assay)}}$$

5.4.1.4 Test for Direct MTT Reduction

To test if a material directly reduces MTT, 50 µl (liquid) or 25 mg (solid - using sharp spoon) of the test substance was added to 1 ml of the MTT medium and incubated in the incubator

(37±1°C, 5±1 CO2, 95% RH) for 60 ± 5 minute. Untreated MTT medium was used as control. If the MTT solution turned blue/purple, the test substance directly reduces MTT. The additional functional check was performed unless the extract from tissues treated by test substance had an OD <5% of the negative control tissue and the tissue viability (determined in MTT assay) was not close to the classification cut-off (50%).

5.4.1.5 Functional Test of Interference from Test Chemical

Each MTT reducing chemical was applied to two freeze-killed tissues. In addition, two freeze-killed tissues were left untreated. The entire assay protocol was performed on the frozen tissues.

5.4.1.6 Data Correction Procedure

True viability = Viability of treated tissue – Interference from test chemical
$= OD_{tvt} - OD_{kt}$

Where:
OD_{kt} = (mean OD_{tkt} – mean OD_{ukt})
tvt = treated viable tissue
kt = killed tissues
tkt = treated killed tissue
ukt = untreated killed tissue (NC treated tissue)

5.4.2 EPI-200-SCT Test

5.4.2.1 Day of Receipt

Upon receipt of assay kit, all components were stored according to the manufacturer's instructions. The EpiDerm tissues were maintained in the original packaging and stored at 4°C.

5.4.2.2 Day of Testing

The MTT concentrate was thawed and diluted with the MTT diluent then covered with foil and stored at 4 ± 2°C for same day use. The assay medium was brought to room temperature (20-25 °C) and 0.9 ml assay medium was pipetted into each well of 6, 6-well plates labelled according to treatment. The 24-well plates containing epidermal tissues were opened under sterile conditions in a biological safety cabinet (BSC) and quickly inspected for excess surface moisture or defects. The tissue surfaces were dried with a sterile cotton swab and tissue inserts transferred to the 6-well plates. Plates were placed in an incubator at (37±1°C, 5±1% CO2, 95% RH) for 60 ± 5 minutes. At the end of the 60 ± 5 minute pre-incubation, the inserts were transferred to new 6-well plates with 0.9 ml fresh medium. For the 1 hour application, tissues were dosed by applying 50 µl or 25 mg of undiluted test compound, negative control (NC), or positive control (PC) to 2 tissue replicates at 1 minute intervals. Liquid compounds were applied directly using a pipette. For solids, the surface was pre-wetted with 25 µl of water and then a level spoonful of compound was applied using a sharp application spoon (bone curette #1). For both

materials a glass rod was then used to gently distribute the material across the surface of the skin. For liquids that would not disperse evenly, a nylon mesh insert was placed on the surface as a spreading tool. After the 1 hour ± 1 minute application was complete, the plates were transferred to the incubator (37±1°C, 5±1% CO2, 95% RH) for the remainder of the 1 hour ± 1 minute exposure period.

During the 1 hour ± 1 minute incubation period, the 3 minute ± 10 seconds exposure was conducted. Tissues were dosed as described above for the 1 hour application. However, after 2 tissues were dosed and 3 minutes ± 10 seconds had elapsed from application of test compound to the first tissue, the first 3 tissues were rinsed at 1 minute intervals. Tissues were rinsed with DPBS by filling and emptying the tissue inserts 20X using a constant stream applied from a wash bottle at a distance of approximately 1.5 cm. The inserts were then submerged 3X in 150ml DPBS (separate clean beaker of DPBS per test chemical), shaken to remove test material then rinsed inside and out and blotted on sterile blotting paper. Tissues were transferred to 24-well plates pre-filled with 0.3 ml assay medium. Dosing was continued in this manner on tissues in groups of 3 until all tissues were dosed, rinsed, and transferred to the 24-well plate. Tissue surfaces were carefully dried with a sterile cotton swab and inserts were transferred to a new 24-well plate pre-filled with 0.3 ml MTT medium. The plate was placed in the incubator for 3 hours ± 5 minutes.

After the 60 ± 1 minute exposure was complete, tissues were rinsed and transferred to a holding plate and then a 24-well plate containing MTT medium as described above. The plate was then placed in the incubator for 3 hours ± 5 minutes.

After 3 hour ± 5 minutes incubation, the MTT medium was gently aspirated from all wells. All wells were rinsed twice by refilling with DPBS and aspirating. Tissue surfaces were dried with sterile cotton swabs and inserts blotted on sterile paper and transferred to a new 24-well plate. Isopropanol/MTT extractant solution (2ml) was pipetted into each insert. Plates were sealed with adhesive plate covers and/or parafilm and placed on a plate shaker (120 rpm) for at least 2 hours at room temperature. Inserts were pierced with forceps to release solution into the well and the solution mixed by pipetting up and down. Two aliquots (200 µl) were transferred to a 96-well flat bottom plate according to the plate layout. The OD was read at 570 nm (540-595nm acceptable) without a reference filter, using isopropanol/MTT extractant as a blank.

5.5 Data Calculations, Analyses, and Interpretation

Experimental data generated during the course of this study were recorded by hand and tabulated, summarized, and/or analyzed using Microsoft® Excel.

The mean OD of the blank (MTT extractant solution) aliquots was subtracted from the OD reading for each well (blank corrected OD). Mean ODs were calculated by aliquot then by tissue replicate for each test substance. For each individual tissues treated with a test substance (TS), the positive control (PC), and the negative control (NC), the individual relative tissue viability was calculated according to the following formulas:

Relative viability TS (%) = [OD_{TS} / mean of OD_{NC}] x 100
Relative viability NC (%) = [OD_{NC} / mean of OD_{NC}] x 100
Relative viability PC (%) = [OD_{PC} / mean of OD_{NC}] x 100

Results were compared to the assay acceptance criteria (see Section 5.2.2) and the prediction model for skin corrosion (Table 2).

Table 2. Prediction Model for Skin Corrosion.

In vitro result mean tissue viability	In vivo prediction
3 min < 50%	Corrosive (C), GHS category 1
3 min ≥ 50% and 1 hour < 15%	Corrosive (C), GHS category 1
3 min ≥ 50% and 1 hour ≥ 15%	Non-corrosive

6 Results and Discussion

6.1 Test for Interference with MTT Endpoint

6.1.1 Test for Colored Materials

None of the test materials were colored or changed color significantly, therefore the functional check on viable tissues indicated in the manufacturer's instructions was not necessary.

6.1.2 Test for Direct MTT Reduction

Direct MTT reduction (i.e., MTT solution turned blue/purple) was observed for phenol, ethanolamine, and 4-(methylthio)-benzaldehyde.

6.1.3 Functional Test of Interference from Test Chemical

Because phenol and ethanolamine were classified as corrosive by the skin corrosion test (SCT), the functional check was not performed with these test chemicals. A functional check of interference from the test chemical in the assay was performed for 4-(methylthio)-benzaldehyde.

6.1.4 Data Correction Procedure

True viability = Viability of treated tissue – Interference from test chemical
$= OD_{tvt} - OD_{kt}$

Where:
OD_{kt} = (mean OD_{tkt} – mean OD_{ukt})
tvt = treated viable tissue

kt = killed tissues
tkt = treated killed tissue
ukt = untreated killed tissue (NC treated tissue)

True viability =2.01 – (0.155-0.152)
=2.01-(0.003)
=2.007

The OD_{ukt} and OD_{kt} values were very similar and correction of ODtvt values for direct MTT reduction did not alter the resulting percent viability. Therefore, direct reduction of MTT by 4-(methylthio)-benzaldehyde did not interfere with the MTT assay.

6.2 EPI SCT-200 Test

As recommended by the manufacturer, proficiency test substances were tested in groups of 4 and tested concurrently with the NC and PC. Solid test substances were ground with a mortar and pestle to a fine powder, as necessary, and tested in neat form. Liquid test substances were tested undiluted.

Three EPI-SCT-200 assays were conducted with the proficiency chemicals. The mean OD and percent viability for each test substance, NC, and PC were within expected limits (Table 3). Data for each run, from individual replicate tissues can be found in Appendix D.

Table 3. EPI-SCT-200 assay results. Mean optical density and relative viability (%) for proficiency chemicals, negative control (NC), and positive control (PC).

Test Substance	Time Period	Date	Mean of OD	SD of OD	Mean of Viabilities %	SD of viabilities	CV
NC	3 min	4/18/2018	2.27	0.13	100.0	5.9	0.1
PC	3 min	4/18/2018	0.35	0.03	15.5	1.3	0.1
boron trifluoride dihydrate	3 min	4/18/2018	0.23	0.03	10.1	1.4	0.1
dichloroacetyl chloride	3 min	4/18/2018	0.04	0.00	1.9	0.1	0.1
lactic acid	3 min	4/18/2018	1.89	0.12	83.4	5.4	0.1
Phenethyl bromide	3 min	4/18/2018	2.40	0.06	106.0	2.7	0.0
NC	1 hour	4/18/2018	2.24	0.00	100.0	0.0	0.0
PC	1 hour	4/18/2018	0.06	0.00	2.5	0.0	0.0
boron trifluoride dihydrate	1 hour	4/18/2018	0.17	0.01	7.5	0.2	0.0
dichloroacetyl chloride	1 hour	4/18/2018	0.04	0.00	1.9	0.0	0.0
lactic acid	1 hour	4/18/2018	0.09	0.05	4.1	2.0	0.5
Phenethyl bromide	1 hour	4/18/2018	2.03	0.10	90.6	4.5	0.1
NC	3 min	4/25/2018	2.25	0.15	100.0	6.7	0.1
PC	3 min	4/25/2018	0.24	0.01	10.8	0.2	0.0
bromo acetic acid	3 min	4/25/2018	0.10	0.00	4.3	0.0	0.0
phenol	3 min	4/25/2018	0.17	0.01	7.6	0.5	0.1
glyoxylic acid monohydrate	3 min	4/25/2018	1.77	0.11	78.6	5.1	0.1
lauric acid	3 min	4/25/2018	2.09	0.01	93.0	0.5	0.0
NC	1 hour	4/25/2018	2.21	0.06	100.0	2.7	0.0
PC	1 hour	4/25/2018	0.10	0.01	4.6	0.3	0.1
bromo acetic acid	1 hour	4/25/2018	0.10	0.00	4.7	0.1	0.0
phenol	1 hour	4/25/2018	0.14	0.00	6.2	0.2	0.0
glyoxylic acid monohydrate	1 hour	4/25/2018	0.12	0.01	5.4	0.5	0.1
lauric acid	1 hour	4/25/2018	0.99	0.02	44.8	0.9	0.0
NC	3 min	5/2/2018	2.01	0.07	100.0	3.4	0.0
PC	3 min	5/2/2018	0.28	0.02	13.7	0.8	0.1
ethanolamine	3 min	5/2/2018	1.38	0.66	68.9	32.8	0.5
hydrochloric acid (14.4%)	3 min	5/2/2018	1.56	0.10	77.6	5.0	0.1
4-(methylthio)-benzaldehyde	3 min	5/2/2018	2.01	0.03	100.0	1.4	0.0
4-amino-1,2,4-triazole	3 min	5/2/2018	1.97	0.09	97.9	4.7	0.0
NC	1 hour	5/2/2018	1.96	0.02	100.0	1.2	0.0
PC	1 hour	5/2/2018	0.13	0.03	6.4	1.6	0.2
ethanolamine	1 hour	5/2/2018	0.17	0.00	8.6	0.2	0.0
hydrochloric acid (14.4%)	1 hour	5/2/2018	0.18	0.01	9.0	0.5	0.1
4-(methylthio)-benzaldehyde	1 hour	5/2/2018	2.02	0.02	101.9	0.9	0.0
4-amino-1,2,4-triazole	1 hour	5/2/2018	1.87	0.04	95.5	2.2	0.0

SD: standard deviation; OD: optical density; CV: coefficient of variation; NC: negative control; PC: positive control

6.3 Criteria for Valid Assay

6.3.1 Test Acceptance Criterion for the NC

Tissue viability of the water-treated tissues was determined in the MTT assay. In the 3 assays conducted, the mean OD_{570} of the NC tissues were 2.27, 2.25, and 2.01 for the 3

minute exposure and 2.24, 2.21, and 1.96 for the 1 hour exposure. This meets the acceptance criterion of mean $OD_{570} \geq 0.8$.

6.3.2 Test Acceptance Criterion for the PC

In the 3 assays, 2 replicate tissues were treated with an 8N KOH solution as a positive control in the 1 hour exposure. The relative tissue viabilities were 2.5, 4.6, and 6.4%. The assay meets the acceptance criterion of mean viability of PC being < 15% of the negative control tissues.

6.3.3 Variability of the Tests

To determine if the 2 replicate tissues reacted similarly, the tissue viability coefficients of variation were examined for test substances with tissue viabilities in the range of 20-100%. All test substances met the acceptance criteria.

6.4 Classification of Proficiency Chemicals

The *in vitro* classification of the test substances was determined by comparing the mean relative viability to the prediction model for skin corrosion. According to the EU and GHS classification, a corrosive is predicted if the mean relative tissue viability of three individual tissues exposed for 3 minutes to the test substance is reduced below 50% of the mean viability of the negative controls. In addition, those chemicals classified as non-corrosive after 3 minutes are classified as corrosive if the relative tissue viability after 1 hour of treatment with a test material is below 15%. The *in vitro* classification was then compared to the GHS *in vivo* classification to determine whether the test substances were correctly classified in the assay (Table 4). All test chemicals were appropriately classified.

Therefore, the sensitivity (prediction of corrosives), specificity (prediction of non-corrosives), and accuracy (overall concordance) were all 100%.

Table 4. Classification of Proficiency Chemicals. In vitro classification using EPI-SCT-200 compared with GHS in vivo classification.

Test Substance	Date	GHS *in vivo*	*in vitro*	Concordance
Bromoacetic acid	4/25/2018	1A	C	yes
Boron trifluoride dihydrate	4/18/2018	1A	C	yes
Phenol	4/25/2018	1A	C	yes
Dichloroacetyl chloride	4/18/2018	1A	C	yes
Glyoxylic acid monohydrate	4/25/2018	1B - 1C	C	yes
Lactic acid	4/18/2018	1B	C	yes
Ethanolamine	5/2/2018	1B - 1C	C	yes
Hydrochloric acid (14.4%)	5/2/2018	1B - 1C	C	yes
Phenethyl bromide	4/18/2018	NC	NC	yes
4-Amino-1,2,4-triazole	5/2/2018	NC	NC	yes
4-(methylthio)-benzaldehyde	5/2/2018	NC	NC	yes
Lauric acid	4/25/2018	NC	NC	yes

GHS: Globally Harmonized System, NC: non-corrosive; C: corrosive

7 Conclusions

The proficiency substances were tested using the EPI-SCT-200 assay as a demonstration of technical proficiency of the laboratory prior to routine use of this test method. All assay acceptance criteria were met and 100% of the proficiency substances were correctly classified, therefore the performing lab is technically proficient and EPI-SCT-200 may be routinely used for *in vitro* prediction of skin corrosion.

8 Point of Contact

Questions pertaining to this report should be referred to Emily May Lent at DSN 584-3980, commercial 410-436-3980, or by e-mail: usarmy.apg.medcom-aphc.mbx.tox-info@mail.mil.

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EMILY MAY LENT
Toxicologist
Toxicity Evaluation Division (TEV)

Date

Toxicity Report No. S.0055513-18, April 2018-September 2018

Approved By:

ONEILL.ARTHUR.J.III.1299508443 Digitally signed by ONEILL.ARTHUR.J.III.1299508443 Date: 2019.02.12 11:41:07 -05'00'

ARTHUR J. O'NEILL
Division Chief, TEV

Date

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MARK S. JOHNSON
Director, Toxicology

Date

Appendix A

References

1. Eskes, C., et al., *The ECVAM international validation study on in vitro tests for acute skin irritation: selection of test chemicals.* Altern Lab Anim, 2007. **35**(6): p. 603-19.

2. Fentem, J.H., et al., *The ECVAM International Validation Study on In Vitro Tests for Skin Corrosivity. 2. Results and Evaluation by the Management Team.* Toxicol In Vitro, 1998. **12**(4): p. 483-524.

3. Fentem, J.H. and P.A. Botham, *Update on the validation and regulatory acceptance of alternative tests for skin corrosion and irritation.* Altern Lab Anim, 2004. **32 Suppl 1B**: p. 683-8.

4. Liebsch, M., et al., *The ECVAM Prevalidation Study on the Use of EpiDerm for Skin Corrosivity Testing.* Altern Lab Anim, 2000. **28**(3): p. 371-401.

5. OECD, *Test No. 404: Acute Dermal Irritation/Corrosion*. 2015, OECD Publishing: Paris.

6. OECD, *Test No. 431: In Vitro Skin Corrosion: Reconstructed Human Epidermis (RHE) Test Method*. 2016, OECD: Paris.

Appendix B

QUALITY ASSURANCE STATEMENT

For: Toxicology Study No. S.0055513-18, Protocol No. 69-iv18-04-01, entitled "*In vitro* dermal corrosion proficiency demonstration, April 2018-May 2018", the following Good Laboratory Practice Standard Inspections were conducted:

Study Specific Critical Phase Inspected/Audited	Date Inspected /Audited	Date Reported to Management/SD
Type Protocol Good Laboratory Practice Standard Review	3/21/2018	3/21/2018
Test System, Test Article and Reagents facilities, control, handling and storage	9/21/2018	10/29/2018
Maintenance and Calibration of Equipment and Good Documentation Practices	9/21/2018	10/29/2018
Compliance with Type Protocol and OECD test guideline 431 demonstration of proficiency requirements	9/21/2018	10/29/2018
Study Raw Data Good Laboratory Practice Standard Review	10/18/2018	10/19/2018
Final Study Good Laboratory Practice Standard Report Review	10/18/2018	10/19/2018

Note 1: *All findings were made known to the Study Director and the Program Manager at the time of the audit/inspection. If there were no findings during the inspection, the inspection was reported to Management and the Study Director on the date shown in the table.*

Note 2: *This report has been audited by the Quality Assurance Unit (QSARC), and is considered to be an accurate account of the data generated and of the procedures conducted.*

Note 3: *In addition to the study specific critical phase inspections listed here, general facility and process based inspection not specifically related to this study are done monthly or annually in accordance with QA Standard Operating Procedure.*

Michael P. Kefauver
Good Laboratory Practice Standards
Quality Assurance Specialist, QSARC

08 FEB 2019
Date

Appendix C

Archives and Study Personnel

C-1 Archives

All raw data, documentation, records, protocol, and a copy of the final report generated as a result of this study will be archived in room 1026, building E-2100, APHC, for a minimum of ten (10) years following submission of the final report to the Sponsor. If the report is used to support a regulatory action, it shall, along with all supporting data, be retained indefinitely.

Some ancillary records pertaining to this study, such as instrument maintenance logs will not be archived until those logbooks have been completed. Once complete they will be archived in room 1026, building E-2100, APHC.

C-2 Personnel

Management: Dr. Mark S. Johnson, Director, Toxicology; MAJ Jarod Hanson, Executive Officer, Toxicology; Mr. Arthur J. O'Neill, Chief, Toxicity Evaluation Division (TEV); Dr. Michael J. Quinn, Chief, Health Effects Research Division (HEF).

Study Director: Dr. Emily May Lent, Toxicologist, TEV.

Quality Assurance: Michael P. Kefauver, Quality Assurance Specialist, Quality Systems and Regulatory Compliance Office.

Archivist: Lee C.B. Crouse, Biologist, TEV.

Appendix D

EPI-SCT-200 Data

Toxicity Report No. S.0055513-18, April 2018-September 2018

Exp. No.:	1
Tissue Lot No.:	28326
Date:	18-Apr-18
Operator:	Emily Lent

						mean			
Blanks:	0.033	0.032	0.034	0.032	0.032	0.03	0.033		
Code	Tissue	Raw Data			Blank Corrected			Mean of Aliquots	% Viability
		Aliquot 1	Aliquot 2	Aliquot 3	Aliquot 1	Aliquot 2	Aliquot 3		
NC 3 min	1	2.274	2.148	2.192	2.24	2.12	2.16	2.17	95.81
	2	2.4021	2.3867	2.3955	2.37	2.35	2.36	2.36	104.19
PC 3 min	1	0.373	0.3694	0.3689	0.340	0.33665	0.33615	0.37	16.34
	2	0.365	0.3611	0.3624	0.33265	0.32835	0.32965	0.33	14.57
C1-boron trifluoride dehydrate 3 min	1	0.211	0.2032	0.2029	0.17845	0.17045	0.17015	0.21	9.08
	2	0.253	0.2504	0.2482	0.21985	0.21765	0.21545	0.25	11.05
C2-dichloroacetyl chloride 3 min	1	0.044	0.0514	0.0414	0.01085	0.01865	0.00865	0.05	2.01
	2	0.042	0.0424	0.0408	0.00875	0.00965	0.00805	0.04	1.83
C3-lactic acid 3 min	1	1.836	1.8024	1.7764	1.80275	1.76965	1.74365	1.80	79.61
	2	2.040	1.9368	1.9524	2.00745	1.90405	1.91965	1.98	87.18
C4-phenethyl bromide 3 min	1	2.463	2.4346	2.4456	2.43065	2.40185	2.41285	2.45	107.98
	2	2.406	2.3622	2.3127	2.37345	2.32945	2.27995	2.36	104.12
NC 1 hour	1	2.313	2.190	2.232	2.281	2.157	2.199	2.25	100.01
	2	2.2867	2.2274	2.2191	2.25395	2.19465	2.18635	2.24	99.99
PC 1 hour	1	0.057	0.056	0.0574	0.02395	0.02325	0.02465	0.06	2.53
	2	0.056	0.0551	0.0567	0.02365	0.02235	0.02395	0.06	2.50
C1 1 hour	1	0.177	0.1699	0.1681	0.14445	0.13715	0.13535	0.17	7.65
	2	0.167	0.1614	0.1627	0.13465	0.12865	0.12995	0.16	7.30
C2 1 hour	1	0.043	0.0417	0.0436	0.01015	0.00895	0.01085	0.04	1.90
	2	0.045	0.0424	0.0425	0.01235	0.00965	0.00975	0.04	1.93
C3 1 hour	1	0.063	0.0604	0.0625	0.02995	0.02765	0.02975	0.06	2.76
	2	0.127	0.1237	0.1261	0.09445	0.09095	0.09335	0.13	5.54
C4 1 hour	1	1.989	1.9636	1.9286	1.95665	1.93085	1.89585	1.96	87.34
	2	2.130	2.0984	2.0862	2.09695	2.06565	2.05345	2.10	93.77

Toxicity Report No. S.0055513-18, April 2018-September 2018

Exp. No.:	2								
Tissue Lot No.:	28335								
Date:	25-Apr-18								
Operator:	Emily Lent								
						mean			
Blanks:	0.034	0.035	0.034			0.03275	0.033		
Code	Tissue	Raw Data				Blank Corrected		Mean of Aliquots	% Viability
		Aliquot 1	Aliquot 2	Aliquot 3	Aliquot 1	Aliquot 2	Aliquot 3		
NC 3 min	1	2.399	2.376	2.382	2.37	2.34	2.35	2.35	104.74
	2	2.165	2.193	2.1604	2.13	2.16	2.13	2.14	95.26
PC 3 min	1	0.239	0.2406	0.2385	0.206	0.20785	0.20575	0.24	10.65
	2	0.279	0.2794	0.2806	0.24635	0.24665	0.24785	0.25	10.99
C1-bromo acetic acid 3 min	1	0.097	0.0965	0.0958	0.06405	0.06375	0.06305	0.10	4.29
	2	0.097	0.0966	0.0945	0.06415	0.06385	0.06175	0.10	4.27
C2-phenol 3 min	1	0.178	0.1784	0.1769	0.14475	0.14565	0.14415	0.18	7.91
	2	0.162	0.1638	0.1627	0.12955	0.13105	0.12995	0.16	7.25
C3-glyoxylic acid monohydrate 3 min	1	1.873	1.8365	1.8332	1.83975	1.80375	1.80045	1.85	82.23
	2	1.693	1.6669	1.6978	1.66005	1.63415	1.66505	1.69	75.04
C4-lauric acid 3 min	1	2.099	2.0778	2.0665	2.06615	2.04505	2.03375	2.08	92.63
	2	2.112	2.0904	2.0918	2.07895	2.05765	2.05905	2.10	93.38
NC 1 hour	1	2.300	2.211	2.245	2.268	2.178	2.212	2.25	101.89
	2	2.2229	2.1498	2.1325	2.19015	2.11705	2.09975	2.17	98.11
PC 1 hour	1	0.102	0.0944	0.0953	0.06915	0.06165	0.06255	0.10	4.40
	2	0.109	0.1074	0.1077	0.07605	0.07465	0.07495	0.11	4.88
C1 1 hour	1	0.103	0.1017	0.1027	0.07055	0.06895	0.06995	0.10	4.64
	2	0.106	0.1024	0.1044	0.07335	0.06965	0.07165	0.10	4.72
C2 1 hour	1	0.145	0.1367	0.1415	0.11225	0.10395	0.10875	0.14	6.38
	2	0.135	0.1334	0.1363	0.10205	0.10065	0.10355	0.13	6.10
C3 1 hour	1	0.127	0.1254	0.127	0.09465	0.09265	0.09425	0.13	5.73
	2	0.114	0.113	0.1125	0.08165	0.08025	0.07975	0.11	5.04
C4 1 hour	1	1.024	0.9943	0.9926	0.99105	0.96155	0.95985	1.00	45.41
	2	0.991	0.9709	0.9666	0.95865	0.93815	0.93385	0.98	44.17

Toxicity Report No. S.0055513-18, April 2018-September 2018

Exp. No.:	3								
Tissue Lot No.:	283352								
Date:	2-May-18								
Operator:	Emily Lent								
						mean			
Blanks:	0.0344	0.0346	0.0336			0.03275	0.033		
Code	Tissue	Raw Data				Blank Corrected		Mean of Aliquots	% Viability
		Aliquot 1	Aliquot 2	Aliquot 3	Aliquot 1	Aliquot 2	Aliquot 3		
NC 3 min	1	2.014	2.007	1.954	1.98	1.97	1.92	1.96	97.56
	2	2.0976	2.0876	2.083	2.06	2.05	2.05	2.06	102.44
PC 3 min	1	0.263	0.2664	0.262	0.230	0.23365	0.22925	0.26	13.13
	2	0.322	0.3191	0.3189	0.28875	0.28635	0.28615	0.29	14.30
C1-ethanolamine 3 min	1	0.932	0.9041	0.9158	0.89925	0.87135	0.88305	0.92	45.69
	2	1.845	1.8542	1.8485	1.81175	1.82145	1.81575	1.85	92.10
C2-hydrochloric acid (14.4%) 3 min	1	1.517	1.46	1.4836	1.48415	1.42725	1.45085	1.49	74.05
	2	1.670	1.6033	1.6118	1.63755	1.57055	1.57905	1.63	81.11
C3-4-(methylthio)-benzaldehyde 3 min	1	2.026	1.971	1.9679	1.99305	1.93825	1.93515	1.99	99.03
	2	2.017	2.0096	2.0589	1.98395	1.97685	2.02615	2.03	101.03
C4-4-amino-1,2,3-triazole 3 min	1	1.938	1.8872	1.8712	1.90525	1.85445	1.83845	1.90	94.57
	2	2.094	2.0095	1.9915	2.06135	1.97675	1.95875	2.03	101.19
NC 1 hour	1	1.982	1.948	1.906	1.949	1.916	1.873	1.95	99.17
	2	2.0388	1.942	1.953	2.00605	1.90925	1.92025	1.98	100.83
PC 1 hour	1	0.103	0.1036	0.1042	0.06995	0.07085	0.07145	0.10	5.28
	2	0.147	0.1442	0.151	0.11435	0.11145	0.11825	0.15	7.52
C1 1 hour	1	0.164	0.1685	0.1632	0.13155	0.13575	0.13045	0.17	8.43
	2	0.173	0.1707	0.1683	0.14035	0.13795	0.13555	0.17	8.70
C2 1 hour	1	0.168	0.1674	0.17	0.13565	0.13465	0.13725	0.17	8.59
	2	0.189	0.181	0.1792	0.15605	0.14825	0.14645	0.18	9.33
C3 1 hour	1	2.042	1.9821	2.013	2.00955	1.94935	1.98025	2.01	102.59
	2	2.023	2.0507	2.0277	1.99015	2.01795	1.99495	2.03	101.30
C4 1 hour	1	1.878	1.8134	1.8335	1.84515	1.78065	1.80075	1.84	93.88
	2	1.898	1.8997	1.9125	1.86525	1.86695	1.87975	1.90	97.03