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ACUTE DERMAL IRRITATION/CORROSION
OF
DECON SHIELD
IN
THE RABBIT (OECD404)

FINAL REPORT

Date: 07 October 2005

Submitted to: **Hypherion Corporation**
Suite 110, 1005 Terminal Way
Reno NV 89502
United States of America

Attn: Mr Anders Sundberg

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STUDY DIRECTOR'S STATEMENT

Acute dermal irritation/corrosion of Decon Shield in the rabbit

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

The study was performed in accordance with the agreed protocol and with ICP Firefly Pty Ltd Standard Operating Procedures, unless otherwise stated, and the study objectives were achieved.

GOOD LABORATORY PRACTICE COMPLIANCE

This document is issued in
Accordance with NATA's
GLP requirements.

Recognised for compliance with the
OECD Principles of Good Laboratory Practice

_____ _____

Emilia Rozinova Date
Study Director
ICP Firefly Pty Ltd
129 Queen Street
Beaconsfield NSW 2015
AUSTRALIA

QUALITY ASSURANCE STATEMENT

Acute dermal irritation/corrosion of Decon Shield in the rabbit

The study described in this report was subject to audit by the independent Quality Assurance Department. The findings of each audit were reported to the Study Director and Management as prescribed by the ICP Firefly Quality System.

The conduct of the audit serves to confirm that the methods, procedures, and observations are accurately described, and that the reported results accurately reflect the raw data of the study.

Type of Inspection	Inspection dates	Date reported to Study Director and Management	Auditor
Study plan/ Protocol review	-	10.05.2005	E. Ho
Experimental (Report No. IA722)	06 October 2005	06 October 2005	E. Ho
Data review	06 October 2005	06 October 2005	E. Ho
Data entry to report	07 October 2005	07 October 2005	E. Ho
Final report audit (Report No. IA723)	07 October 2005	07 October 2005	E. Ho

_____ _____

Edmund Ho Date
QA Manager
ICP Firefly Pty Ltd

REGULATORY STATEMENT

Acute dermal irritation/corrosion of Decon Shield in the rabbit

ICP Firefly Pty Ltd is accredited as an Animal Research Establishment (Ref. No: AW96/042) with the Animal Welfare Unit of New South Wales Agriculture. Operation of the test facility complies with the OECD Principles of GLP (NATA no: 14320), and AS/NZS ISO 9001:2000 (NCSI Certification No: 8116). The experimental protocol was approved by the ICP Firefly Animal Care and Ethics Committee. The facility operates in compliance with the New South Wales government regulations.

This report is considered to be an accurate presentation of the procedures and practices employed during the course of the study and an accurate presentation of the findings. There were no significant deviations from Good Laboratory Practice Regulations that could have affected the quality or integrity of the study data analysis.

All data supporting the study and a copy of the final report will be retained for a period of 5 years in accordance with the procedures defined in the ICP Firefly Quality System.

_____ _____

Dr Isabelle Meyer-Carrive Date
Managing Director
ICP Firefly Pty Ltd

ARCHIVES STATEMENT

Acute dermal irritation/corrosion of Decon Shield in the rabbit

All primary cage cards, raw data and copy of the final report will be retained in the ICP Firefly Pty Ltd archives for five years after the completion of the study. At this time, the sponsor or legal successor will be contacted to determine whether data should be returned or destroyed on their behalf.

Access to archives is restricted to authorised personnel.

PERSONNEL INVOLVED IN THE STUDY

Acute dermal irritation/corrosion of Decon Shield in the rabbit

The following personnel are aware of, and have adhered to GLP requirements and ICP Firefly Standard Operating Procedures.

Name	Job Title	Signature	Initials	Date
Ms F. Brook BSc	Senior Research Officer			
Dr E. Rozinova PhD	Senior Research Scientist/ Study Director			
Mrs P. Kempster Dip Med Tech	Laboratory Manager			
Mr E. Ho BAppSc, MBus	QA Manager			
Dr I. Meyer-Carrive BSc, MSc, PhD	Managing Director			

1.0. SUMMARY

The objective of this study was to evaluate the acute skin irritation/corrosion reactions of **Decon Shield** liquid**,** in 3 female New Zealand white rabbits using a primary dermal irritation/corrosion test (OECD Guidelines for the Testing of Chemicals, No. 404).

The test item was applied as supplied at a dose of 0.5 mL/site/animal under a semi-occlusive dressing to non-abraded shaved skin of 3 female rabbits. An initial single-animal test was carried out using patches removed at 3 minutes, 60 minutes and 4 hours after dosing. Two more were tested, the patches were removed after 4 hours and the area was assessed for irritation/corrosion at 60 minutes after patch removal. The sites were re-examined at 24, 48, 72 hours after patch removal.

Upon patch removal, residual test item was removed with gauze moistened with warm tap water, and the area dried with gauze.

No erythema or oedema was observed in the single animal at 3 minutes or 1 hour after application.

Sixty minutes after the 4-hour patch removal, the test sites of all animals exhibited no signs of erythema or oedema. No erythema or oedema was observed in any animal at 24, 48, or 72 hours after patch removal.

No abnormal clinical signs or weight losses were observed throughout the 72-hour observation period.

The test item, **Decon Shield**, was classified as non irritant to skin according to the National Occupational Health and Safety Commission (NOHSC) "Approved Criteria for Classifying Hazardous Substances [NOHSC: 1008 (1999)]" under the conditions of this study.

2.0. INTRODUCTION

2.1. Sponsor

Hypherion Corporation
Suite 110, 1005 Terminal Way
Reno NV 89502
UNITED STATES of AMERICA.

2.2. Study number

ICPQN560
ICP Firefly protocol ICPQN560, Schedule 2.

2.3. Study Director

Emilia Rozinova, PhD.

2.4. Rationale for the study

Acute Dermal Irritation/Corrosion in the Rabbit. The experimental procedure was based on that recommended under the OECD Guidelines for the Testing of Chemicals No. 404 "Acute Dermal Irritation/Corrosion" [Adopted by the Council on 17th July 1992]. The aim of the study was to determine the potential for the test item to cause irritation to the skin. This data is currently unavailable. The irritation scores were classified as per Table 2.

2.5. Study timetable

Study initiation date	15 August 2005
Animal arrival date	21 September 2005
Experimental start date	26 September 2005
Experimental completion date	29 September 2005
Study completion date	07 October 2005

2.6. Study integrity

There are no known circumstances that might have affected the quality and integrity of the study.

2.7. Test Facility

ICP Firefly Pty Ltd
129 Queen Street
Beaconsfield NSW 2015
AUSTRALIA.

2.8. Definitions

2.8.1. Dermal irritation

Dermal irritation is the production of reversible inflammatory changes in the skin following application of a test item.

2.8.2. Dermal corrosion

Dermal corrosion is the production of irreversible tissue damage in the skin following the application of a test item.

2.8.3. Irritation index

The irritation index is the mean irritation score for all animals at each test site at each reading.

2.8.4. Irritation classification

Irritation is classified according to the National Occupational Health and Safety Commission (NOHSC) "Approved Criteria for Classifying Hazardous Substances [NOHSC: 1008(1999)]" (see Table 2).

2.8.5. Dose

Dose is the amount of the test item administered. Dose is expressed as weight (g, mg) or as weight/weight of test animal (e.g. mg/kg).

2.8.6. Test item

The test item is the article that is the subject of the study.

2.9. Principle of the test method

The test item was applied after preparation, 5%(v/v)A, 5%(v/v)B and 90% water, in a single dose of 0.5 mL/test site to the skin of three experimental animals. Untreated skin areas of the test animals served as controls. A single animal test was conducted initially for ethical reasons, since it was suspected the test item may be corrosive. The degree of irritation was read and scored at specific intervals and was further described to provide a complete evaluation of the effects.

3.0. MATERIALS AND METHODS

3.1. Regulatory references

3.1.1. Test guidelines

The experimental procedure was based on that recommended under the OECD Guidelines for the Testing of Chemicals No. 404 "Acute Dermal Irritation/Corrosion" [Adopted by the Council on 17th July 1992].

3.1.2. Good Laboratory Practice

This study was conducted in accordance with the Organization of Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice (as revised in 1997) ENV/MC/CHEM(98)17. The study was performed in accordance with the agreed protocol (ICPQN539) and with ICP Firefly Pty Ltd Standard Operating Procedures.

3.1.3. Animal Welfare Act compliance

ICP Firefly is accredited as an Animal Research Establishment with NSW Agriculture (the state of New South Wales, Australia) in compliance with the New South Wales Government legislation (the Animal Research Act 1985 and Regulation 1995). All work undertaken by ICP Firefly is approved by the Animal Ethics Committee (AEC) constituted according to the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, 1997 (National Health and Medical Research Council). It was granted Approval Number E100P.

3.2. Test item

3.2.1. Identification

ICPQN560.1: Decon Shield Part A Batch 222, manufacturing date 25/08/05.

ICPQN560.2: Decon Shield Part B Batch 222, manufacturing date 25/08/05.

3.2.2. Physical description

ICPQN560.1 – Viscous yellowish liquid.

ICPQN560.2 – Clear colourless liquid.

3.2.3. Storage

The test item was stored at room temperature, as per Sponsor requirements.

3.2.4. Purity and stability

A Certificate of Analysis for the test item was requested from the Sponsor but was not provided.

3.2.5. Retention

Any unused test item will be returned to the Sponsor after the final report has been issued, unless otherwise instructed by the Sponsor.

3.3. Animals

3.3.1. Justification for species selection

The rabbit was used since it is widely accepted and recognised by international guidelines as an appropriate experimental model for dermal irritation studies.

3.3.2. Species, strain, source and housing

Three (3) adult female New Zealand White albino rabbits were used for the test. The rabbits weighed between 3.0 and 3.5 kg at the beginning of the experiment. The animals were sourced from Merungora Rabbit Farm, Wauchope, NSW. The animals were acclimatised to the laboratory conditions for 5 days before commencement of the test. All animals were examined during the acclimation period to confirm suitability for the study. Observations were documented on ICPQF21 and included as a spreadsheets as an appendix to the report (Appendix D Raw Data Spreadsheets).

The rabbits were group housed in a polyethylene run (length 300cm, width 100cm, depth 120cm) on bedding of recycled paper pellets and shredded paper. The animals were identified individually on the inside of the ear using a waterproof colour marker. The run was labelled with the study number, animal numbers, gender, animal supplier, Study Director's name and animal ethics approval number.

3.3.3. Food and water

The rabbits were fed on Rabbit and Guinea pig pellets (Gordon's Specialty Stock Feeds, DOM: 26.07.2005) and were provided with tap water *ad libitum*.

Water is analysed routinely for bacterial contaminants and there were no contaminants, which could have interfered with the conduct of the study. Food and water were withheld during the 4-hour exposure period. Certificates of analysis for feed and water can be found in Appendix C Food and Water Certificate of Analysis.

3.3.4. Environment

Environmental controls for the animal room were set to maintain a temperature of 20 ± 3°C. The automated light/dark cycle was 12 hours light/12 hours dark.

3.4. Preparations

3.4.1. Animals

Approximately 24 hours before the test, fur was removed from the dorsal area of the trunk of the animals by shaving with electric clippers with fine cutter heads (0.5 mm). Care was taken not to abrade the skin. Only animals with healthy intact skin were used for the test.

3.4.2. Test item

The test item Decon Shield was mixed in accordance with the instructions from the Sponsor, 5%(v/v) A, 5%(v/v)B and 90% water) and applied undiluted. A dose of 0.5 mL per site was measured using a 1mL syringe and applied to the test site. An untreated shaved site, served as control.

3.5. Procedure

A dose of 0.5 mL of the test item was applied to the dorsal area of the rabbit. The test site was then covered with a gauze patch (2x3 cm Nu Gauze, Johnson & Johnson), which was held in place with non-irritating tape (3x4 cm Dermicel, Johnson & Johnson). The patch was held loosely in contact with the skin using Micropore hypoallergenic tape (3M) as a semi-occlusive dressing.

An initial test was conducted in one animal, with patch removal at 3 minutes, 60 minutes and 4 hours after application. Since this did not reveal any signs of irritation/corrosion, a full test was conducted using a 4-hour exposure period.

The animals were held restrained in polyethylene boxes (43x13x32 cm) for the 4-hour exposure period to prevent inhalation/ingestion of the test item, and to maximise contact of

the test item to the skin. At the end of the 4-hour exposure period, patch was removed. The residual test item was removed with gauze (Nu Gauze, Johnson & Johnson) moistened with warm tap water, and the area was dried with gauze. Animals were returned to group housing.

3.6. Observation of animals

3.6.1. Body weights

Body weights were determined at the beginning and at the end of the test [$T_{(o)}$ and $T_{(end)}$, respectively]. The weights were recorded on form ICPQF34 and included as spreadsheets as an appendix to the report (Appendix D Raw Data Spreadsheets).

3.6.2. Clinical observations

The animals were observed at least once daily for the 72-hour experimental period. Clinical signs were recorded on form ICPQF24 and included as a spreadsheet as an appendix to the report (Appendix D Raw Data Spreadsheets). Particular care was taken to look for signs of toxicity and abnormal behaviour. Skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behaviour patterns were monitored. Particular attention was paid to observation of tremor, convulsion, salivation, diarrhoea, lethargy, sleep and coma.

3.6.3. Skin irritation

The skin at the test site was assessed for signs of erythema and oedema. Irritation was scored at 60 minutes, 24, 48 and 72 hours after patch removal according to the scale in Table 1. Observations of the skin reactions were recorded on form ICPQF34 and included as spreadsheets as an appendix to the report (Appendix D Raw Data Spreadsheets).

4.0. RESULTS

The raw data sheets providing the results of irritation reactions, animal weights and general clinical observation are presented as spreadsheets as an appendix to the report - Appendix D Raw Data Spreadsheets and Tables 3 and 4.

4.1. Body weights

No weight losses were observed in any animal throughout the 72-hour observation period.

4.2. Clinical observations

All animals survived until the end of the study. No clinical abnormalities were observed in any animal throughout the 72-hour observation period.

4.3. Skin irritation

The test site was examined for skin irritation at 60 minutes, 24, 48 and 72 hours after patch removal.

4.3.1. Sixty minutes

Sixty (60) minutes after patch removal, test sites showed no signs of erythema or oedema (scores of 0).

4.3.2. Twenty-four hours

Twenty-four (24) hours after patch removal, test sites showed no signs of erythema or oedema (scores of 0).

4.3.3. Forty-eight hours

Forty-eight (48) hours after patch removal, test sites showed no signs of erythema or oedema (scores of 0).

4.3.4. Seventy-two hours

Seventy-two (72) hours after patch removal, formation of eschar was present on all test sites (scores of 4) with no accompanying oedema (scores of 0).

4.3.5. Discussion

The test item can be classified as a non-skin irritant according to the National Occupational Health and Safety Commission (NOHSC) "Approved Criteria for Classifying Hazardous Substances [NOHSC: 1008 (1999)]" (as per Table 2).

5.0. CONCLUSION

Test item, **Decon Shield**, produced no erythema or oedema in all of the three rabbits at 60 minutes, 24, 48, and 72 hours, following a 4-hour exposure period at 0.5 mL test item/test site/animal.

Mean scores of 0 and 0 were obtained for erythema and oedema respectively over all animals at each observation time.

The test item, **Decon Shield**, is classified as non-irritant to skin according to the criteria of the National Occupational Health and Safety Commission (NOHSC) "Approved Criteria for Classifying Hazardous Substances [NOHSC: 1008(1999)]" under the conditions of this study.

TABLE **1** **SCALES FOR GRADING SKIN REACTIONS**

ERYTHEMA FORMATION	Score
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to eschar formation preventing of grading of erythema	4
Maximum score for Erythema	**4**
OEDEMA FORMATION	
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well defined by definite raising)	2
Moderate oedema (area raised approx. 1 mm)	3
Severe oedema (raised more than 1 mm and extending beyond area of exposure)	4
Maximum Score for Oedema	**4**
Maximum Score for Primary Irritation	**8**

TABLE **2** **CLASSIFICATION OF DERMAL IRRITATION**

Classification	**Criteria**
Non-irritant	Mean score < 2 for either erythema and eschar formation or oedema formation
Irritant	A mean score of 2 or more for either erythema and eschar formation or oedema formation, calculated over all animals tested; or For either erythema, eschar or oedema formation, a mean score of 2 or more, calculated for each animal separately, in two or more animals where three animals are used in the test. Inflammation persists at the end of the observation time

Assessment against the NOHSC "Approved Criteria for Classifying Hazardous Substances [NOHSC: 1008(1999)]".
All scores at each of the reading times (24, 48 and 72 hours) for an effect are used in calculating the respective mean values.

TABLE **3** **ACUTE DERMAL IRRITATION REACTIONS IN RABBITS**

Rabbit	**Weight** (kg)	**Skin irritation readings**		**Clinical observations**	**Time of Evaluation**
		Erythema	Oedema		
1	$T_{(o)}$:	0	0	NA	60 mins
		0	0	NA	24 hrs
		0	0	NA	48 hrs
	$T_{(end)}$:	0	0	NA	72 hrs
2	$T_{(o)}$:	0	0	NA	60 mins
		0	0	NA	24 hrs
		0	0	NA	48 hrs
	$T_{(end)}$:	0	0	NA	72 hrs
3	$T_{(o)}$:	0	0	NA	60 mins
		0	0	NA	24 hrs
		0	0	NA	48 hrs
	$T_{(end)}$:	0	0	NA	72 hrs

Notes: $T_{(o)}$ and $T_{(end)}$, at the beginning and at the end of the test; **NA**, no abnormalities.

TABLE **4 SUMMARISED SKIN IRRITATION SCORES**

Rabbit No	**Skin irritation scores after patch removal**						**Mean irritation scores**	
	24 hrs		48 hrs		72 hrs			
	Erythema	Oedema	Erythema	Oedema	Erythema	Oedema	Erythema	Oedema
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
Mean irritation scores	0	0	0	0	0	0	0	0