STUDY SPONSOR:	CBI
STUDY TYPE:	
TEST ITEM:	
PROJECT NUMBER:	

INTRODUCTION

A study was performed to assess the skin sensitization potential of the test item in the CBA/Ca strain mouse following topical application to the dorsal surface of the ear. This study was designed to be compatible with the procedures indicated by the following internationally accepted guidelines and recommendations:

- OECD Guideline for the Testing of Chemicals No. 429 "Skin Sensitization: Local Lymph Node Assay" (adopted 22 July 2010)
- Method B42 Skin Sensitization (Local Lymph Node Assay) of Commission Regulation (EC) No. 440/2008

The assay has undergone extensive inter-laboratory validation and has been shown to reliably detect test items that are moderate to strong sensitizers.

Experimental Starting Date:	18 June 2014
Experimental Completion Date:	08 July 2014

Test item characterization data are the responsibility of the Sponsor.

All raw data will be retained in CBI

METHODS

Following a preliminary screening test in which no clinical signs of toxicity were noted at a concentration of 100%, this concentration was selected as the highest dose investigated in the main test of the Local Lymph Node Assay. Three groups, each of four animals, were treated with 50 µL (25 µL per ear) of the undiluted test item or the test item as a solution in dimethyl sulphoxide at concentrations of 50% or 25% v/v. A further group of four animals was treated with dimethyl sulphoxide alone.

RESULTS

Preliminary Screening Test

Clinical observations, body weight and mortality data are given in Table 1 and local skin irritation is given in Table 2. The ear thickness measurements and mean ear thickness changes are given in Table 3.

Main Test

The radioactive disintegrations per minute per lymph node and the stimulation index are given in Table 4. Individual clinical observations and mortality data for test and control animals are given in Table 5 and the individual body weights and body weight change for test and control animals are given in Table 6.

The Stimulation Index expressed as the mean radioactive incorporation for each treatment group divided by the mean radioactive incorporation of the vehicle control group are as follows:

Concentration (% v/v) in dimethyl sulphoxide	Stimulation Index	Result
25	1.54	Negative
50	1.84	Negative
100	1.85	Negative

CONCLUSION

The test item was considered to be a non-sensitizer under the conditions of the test.

This study was conducted in a facility operating to Good Laboratory Practice within the UK national GLP monitoring programme, but the study report has not been audited by the QA Unit. No formal claim of GLP compliance is made for this study. This report is an accurate record of the study and its outcome.

A. Pooles ..... Date: 6/10/14 .....

A Pooles
Study Director

TABLES

Table 1 Clinical Observations, Body Weight and Mortality Data – Preliminary Screening Test

Concentration	Animal Number	Body Weight (g)		Day								
				1		2		3		4	5	6
		Day 1	Day 6	Pre-Dose	Post Dose	Pre-Dose	Post Dose	Pre-Dose	Post Dose			
100%	S-1	17	16	0	0	0	0	0	0	0	0	0

0 = No signs of systemic toxicity

Table 2 Local Skin Irritation – Preliminary Screening Test

Concentration	Animal Number	Local Skin Irritation											
		Day 1		Day 2		Day 3		Day 4		Day 5		Day 6	
		left	right	left	right	left	right	left	right	left	right	left	right
100%	S-1	0	0	0	0	0	0	0	0	0	0	0	0

Table 3 Measurement of Ear Thickness and Mean Ear Thickness Changes – Preliminary Screening Test

Concentration	Animal Number	Ear Thickness Measurement (mm)					
		Day 1		Day 3		Day 6	
		pre-dose		post dose			
		left	right	left	right	left	right
100%	S-1	0.21	0.20	0.23	0.22	0.22	0.20
overall mean (mm)		0.21		0.23		0.21	
overall mean ear thickness change (%)		na		9.76		2.44	

na = Not applicable

Table 4 Individual Disintegrations per Minute and Stimulation Indices

Concentration (% v/v) in dimethyl sulphoxide	dpm	dpm/Node [a]	Stimulation Index [b]	Result
Vehicle	26789.79	3348.72	na	na
25	41186.34	5148.29	1.54	Negative
50	49302.47	6162.81	1.84	Negative
100	49580.07	6197.51	1.85	Negative

dpm = Disintegrations per minute
a = Disintegrations per minute/node obtained by dividing the disintegrations per minute value by 8 (total number of lymph nodes)
b = Stimulation Index of 3.0 or greater indicates a positive result
na = Not applicable

Table 5 Individual Clinical Observations and Mortality Data

Concentration (% v/v) in dimethyl sulphoxide	Animal Number	Day 1		Day 2		Day 3		Day 4	Day 5	Day 6
		Pre-Dose	Post Dose	Pre-Dose	Post Dose	Pre-Dose	Post Dose			
Vehicle	1-1	0	0	0	0	0	0	0	0	0
	1-2	0	0	0	0	0	0	0	0	0
	1-3	0	0	0	0	0	0	0	0	0
	1-4	0	0	0	0	0	0	0	0	0
25	2-1	0	0	0	0	0	0	0	0	0
	2-2	0	0	0	0	0	0	0	0	0
	2-3	0	0	0	0	0	0	0	0	0
	2-4	0	0	0	0	0	0	0	0	0
50	3-1	0	0	0	0	0Fl	0Fl	0Fl	0Fl	0Fl
	3-2	0	0	0	0	0Fl	0Fl	0Fl	0Fl	0Fl
	3-3	0	0	0	0	0Fl	0Fl	0Fl	0Fl	0Fl
	3.4	0	0	0	0	0Fl	0Fl	0Fl	0Fl	0Fl
100	4-1	0	0	0	0	0Fl	0Fl	0Fl	0Fl	0Fl
	4-2	0	0	0	0	0Fl	0Fl	0Fl	0Fl	0Fl
	4-3	0	0	0	0	0Fl	0Fl	0Fl	0Fl	0Fl
	4-4	0	0	0	0	0Fl	0Fl	0Fl	0Fl	0Fl

0 = No signs of systemic toxicity
Fl = Fur loss

Table 6 Individual Body Weights and Body Weight Change

Concentration (% v/v) in dimethyl sulphoxide	Animal Number	Body Weight (g)		Body Weight Change (g)
		Day 1	Day 6	
Vehicle	1-1	17	18	1
	1-2	21	19	–2
	1-3	21	19	–2
	1-4	19	17	–2
25	2-1	19	21	2
	2-2	18	18	0
	2-3	18	19	1
	2-4	20	20	0
50	3-1	19	19	0
	3-2	18	19	1
	3-3	18	17	–1
	3.4	18	19	1
100	4-1	18	19	1
	4-2	18	20	2
	4-3	19	19	0
	4-4	18	18	0