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CENTRAL TOXICOLOGY LABORATORY

[redacted]

REPORT NO: CTL/P/6194

PERYLIMID F: LOCAL LYMPH NODE ASSAY

Number of pages in report: 19

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CENTRAL TOXICOLOGY LABORATORY

[redacted]

REPORT NO: CTL/P/6194

PERYLIMID F: LOCAL LYMPH NODE ASSAY

STUDY DETAILS

Sponsor:	BASF Aktiengesellschaft [redacted]
BASF Project Number:	45H0170/989121
BASF Monitoring Scientist:	[redacted]
Sponsor Reference:	CO8265
CTL Test Substance Reference Number:	Y10365/001
CTL Study Number:	GM7243

AUTHOR

[redacted]

DATE OF ISSUE

16 April 1999

STATEMENT OF DATA CONFIDENTIALITY CLAIM

THIS DOCUMENT CONTAINS INFORMATION CONFIDENTIAL AND TRADE SECRET TO BASF AKTIENGESELLSCHAFT.

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STATEMENT OF GLP COMPLIANCE AND AUTHENTICATION

I, the undersigned, declare that the objectives laid down in the protocol were achieved and that the data generated are valid. The report fully and accurately reflects the procedures used and the raw data generated in the above study.

The study was conducted in compliance with the UK Principles of Good Laboratory Practice (The United Kingdom GLP Regulations 1997) except for the deviations listed below. These Principles are in accordance with the OECD Principles of Good Laboratory Practice, revised 1997 (ENV/MC/CHEM(98)17).

The following GLP deviations are considered not to affect the integrity of the study or the validity of the conclusions drawn:

(i) the stability, homogeneity and achieved concentration of the test substance in the vehicle used were not determined by analysis

(ii) the animals were not uniquely identified but the cages were identified by cage cards.

[redacted]
Study Director

[redacted]

16 April 1999
Date

This page
may be required
by some
regulatory authorities.

QUALITY ASSURANCE STATEMENT

In accordance with CTL policy and QA procedures for Good Laboratory Practice, this report has been audited and the conduct of this study has been inspected as follows:

Date	Audit/Inspection	Date of QA Report
02 Mar 99	Draft report	05 Mar 99
16 Apr 99	Final report review	16 Apr 99

In addition, inspections associated with this type of study were made as follows:

20 Jan 99	Protocol	20 Jan 99
21 Jan 99	Dose preparation	21 Jan 99
22 Jan 99	Ear painting	22 Jan 99
25 Jan 99	Dose administration, radiolabelled preparation	25 Jan 99
25-26 Jan 99	Processing, removal of lymph nodes, scintillation counting	27 Jan 99

Facilities and process based procedures associated with this type of study were inspected in accordance with QA Standard Operating Procedures.

So far as can be reasonably established, the methods described and the results given in the final report accurately reflect the raw data produced during the study, GM7243.

[redacted] [redacted] 16 April 1999

(CTL Quality Assurance Unit)

STUDY CONTRIBUTORS

The following contributed to this report in the capacities indicated:

Name	Title
[redacted]	Study Director
[redacted]	Study Licensee
[redacted]	Report Writer

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TABLES

This page is provided for the Regulatory Authority Reviewer's notes.

1. SUMMARY

1.1 Study design

A sample of Perylimid F was assessed for its skin sensitisation potential using the mouse Local Lymph Node Assay. The assay determines the level of T lymphocyte proliferation in the lymph nodes draining the site of chemical application, by measuring the amount of radiolabelled thymidine incorporated into the dividing cells. The test substance was applied as 3%, 10% or 30% w/v preparations in propylene glycol.

1.2 Results

The test substance did not have the capacity to cause skin sensitisation when applied as 3%, 10% or 30% w/v preparations in propylene glycol.

In a positive control study, hexylcinnamaldehyde was shown to have the capacity to cause skin sensitisation when applied as a 10% w/v preparation in acetone, confirming the validity of the protocol used for this study.

1.3 Conclusion

In conclusion, Perylimid F is unlikely to be a moderate or strong skin sensitiser under the conditions of the test

2. INTRODUCTION

2.1 Purpose

The purpose of this study was to assess the skin sensitisation potential of Perylimid F, using the Local Lymph Node Assay (Kimber *et al* 1994).

2.2 Regulatory guidelines

This study was conducted in accordance with the following Regulatory Guideline:

OECD guideline reference 406 (1992): Skin sensitisation.

2.3 Justification for test system selection

The CBA/Ca mouse is the species of choice because previous examination of strain difference in lymphocyte proliferation responses to skin sensitisers showed this strain of mouse to exhibit the most vigorous response (Kimber *et al* 1994).

2.4 Positive control study

A positive control study using hexylcinnamaldehyde is carried out at approximately 6 monthly intervals to ensure that the test system continues to respond to a known sensitising chemical. The positive control study closest in time to the main study is included in this report.

2.5 Study dates

The main study was initiated on 7 January 1999. The experimental phase started on 12 January 1999 and was completed on 19 January 1999.

For the positive control study, the experimental phase started on 18 August 1998 and was completed on 25 August 1998.

2.6 Data storage

An original report and all raw data pertaining to this study, and the raw data for the positive control study, are retained in the Archives, Central Toxicology Laboratory (CTL), A[redacted]

[redacted]

3. TEST SUBSTANCE, POSITIVE CONTROL SUBSTANCE AND VEHICLES

3.1 Test substance

Name:	Perylimid F
Source:	BASF Aktiengesellschaft
Colour:	Violet/red
Physical state:	Solid/powder
ZHT number:	98/170-1
Batch reference number:	Partie 18/CAS No. 81-33-4
CTL test substance reference number:	Y10365/001
Purity:	98.9%
Stability:	Guaranteed stable for the duration of the study by the Sponsor.
Date of Manufacture:	Second quarter 1996
Storage conditions:	Ambient temperature in the dark

3.2 Positive control substance

Name:	Hexylcinnamaldehyde
Source:	Aldrich Chemical Company
Colour:	Yellow
Physical state:	Liquid
CTL test substance reference number:	Y07859/001
Storage conditions:	Refrigerated, under an inert gas, in the dark

3.3 Vehicles

The vehicle for the test substance was propylene glycol (CTL reference: Y01015/038).

The vehicle for the positive control substance was acetone (CTL reference: Y00293/008)

4. EXPERIMENTAL PROCEDURES

4.1 Experimental design

4.1.1 Animals

Species:	Mouse
Strain:	CBA/Ca/Ola/Hsd
Source:	[redacted]
Sex:	Male
Number used:	4 per group
Specification:	Young adults

4.1.2 Accommodation and husbandry

A maximum of 4 mice was housed per cage, in cages suitable for animals of this strain and weight range.

The animal room was designed to give the environmental conditions shown as follows.

Temperature:	22±3°C
Relative humidity:	30-70%
Air:	A minimum of 15 changes per hour
Light cycle:	Artificial, giving 12 hours light, 12 hours dark

Both temperature and relative humidity were recorded daily. The recorded values were within the specified ranges.

Diet (R&M No.1), supplied by Special Diet Services Limited, Witham, Essex, UK, and mains water, supplied by an automatic system, were available *ad libitum*.

Each batch of diet is routinely analysed for composition and for the presence of contaminants. Water is also periodically analysed for the presence of contaminants. No contaminants were found to be present in the diet or water at levels considered to be capable of interfering with the purpose or outcome of the study. Certificates of analyses are retained in the CTL Archives.

4.1.3 Acclimatisation

The animals were housed under the experimental conditions for at least 5 days, prior to the start of the study.

4.1.4 Test method for the evaluation of Perylimid F

Groups of four male mice were used for this study. Approximately 25µl of a 3%, 10% or 30% w/v preparation of the test substance in propylene glycol was applied, using a variable volume micro-pipette, to the dorsal surface of each ear. A vehicle control group was similarly treated using propylene glycol alone. The procedure was repeated daily for 3 consecutive days. A concurrent naïve control group was not treated with the test substance or the vehicle.

Three days after the third application, all the animals were injected, via the tail vein, with approximately 250µl of phosphate buffered saline (PBS) containing approximately 20µCi of a 2.0Ci/mmol specific activity ^{3}H-methyl thymidine. Approximately 5 hours later, the animals were humanely killed by inhalation of halothane vapour followed by cervical dislocation. The draining auricular lymph nodes were removed from each animal and, together with the nodes from the other animals in the group, were placed in a container of PBS.

A single cell suspension was prepared by mechanical disaggregation of lymph nodes through a 200-mesh stainless steel gauze. The cell suspensions were then washed three times by centrifugation with approximately 10ml of PBS. Approximately 3ml of 5% w/v trichloroacetic acid (TCA) was added and after overnight precipitation at 4°C, the samples were pelleted by centrifugation and the supernatant was discarded. The cells were then resuspended in approximately 1ml of TCA.

The lymph node suspensions were transferred to scintillation vials and 10ml of scintillant (Optiphase) was added prior to β-scintillation counting using a Packard Tri-Carb 2500TR Liquid Scintillation Counter.

4.1.5 Clinical observations

Animals were checked at least once daily for signs of systemic toxicity.

4.1.6 Positive control study

The sensitisation potential of hexylcinnamaldehyde was assessed using the method described above in 4.1.4.

Approximately 25µl of a 1%, 3% or 10% w/v preparation of hexylcinnamaldehyde in acetone was applied, and a vehicle control group was similarly treated using acetone.

5. DATA EVALUATION

The results are expressed as a disintegrations per minute (dpm) value per lymph node for each group. The activity of each test group is then divided by the activity of the vehicle control group to give a test:control ratio for each concentration.

The criterion for a positive response is that one or more concentrations of the test substance should elicit a 3-fold or greater increase in isotope incorporation relative to the vehicle control group. The assay is able to identify those materials that elicit moderate or greater responses in standard guinea pig tests for skin sensitisation (Kimber *et al* 1994). Consequently, a test substance which does not fulfil the above criterion is designated as unlikely to be a moderate or strong sensitiser.

6. RESULTS

6.1 Evaluation of test substance (Table 1)

The application of the test substance at concentrations of 3%, 10% and 30% w/v in propylene glycol resulted in an increase in isotope incorporation which was less than 3-fold at all three concentrations. Consequently, the test substance is designated as unlikely to be a moderate or strong sensitiser under the conditions of the test.

6.2 Positive control (Table 2)

The application of hexylcinnamaldehyde at concentrations of 1%, 3% and 10% w/v in acetone resulted in a greater than 3-fold increase in isotope incorporation at the 10% w/v concentration. Therefore, hexylcinnamaldehyde was shown to be a skin sensitiser, confirming the validity of the protocol used for the study.

7. CONCLUSION

In conclusion, Perylimid F is unlikely to be a moderate or strong skin sensitiser under the conditions of the test.

8. REFERENCE

Kimber I, Dearman RJ, Scholes EW and Basketter DA (1994). The Local Lymph Node Assay: Developments and Applications. Toxicology, **93**, 13-31.

TABLE 1 - SKIN SENSITISATION POTENTIAL OF PERYLIMID F

Concentration of test substance (% w/v)	Number of lymph nodes assayed	Disintegrations per minute (dpm)	dpm per lymph node ($x10^{-2}$)	Test:control ratio
Naïve control	8	2301	2.88	N/A
0 (vehicle only)	8	1989	2.49	N/A
3	8	2322	2.90	1.16
10	8	3216	4.02	1.61
30	8	2613	3.27	1.31

N/A - not applicable

TABLE 2 - SKIN SENSITISATION POTENTIAL OF THE POSITIVE CONTROL SUBSTANCE (HEXYLCINNAMALDEHYDE)

Current CTL Study No.: GM7176

Concentration of hexylcinnamaldehyde (% w/v)	Number of lymph nodes assayed	Counts per minute (cpm)	cpm per lymph node ($x10^{-2}$)	Test:control ratio
Naïve control	8	4057	5.07	N/A
0 (vehicle only)	8	2293	2.87	N/A
1	8	3318	4.15	1.45
3	8	4752	5.94	2.07
10	8	9851	12.31	4.29

N/A - not applicable