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US Environmental Protection Agency
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 EPA East Room 6428
 Washington, DC 20460
 ATTN: Section 8(e) Coordinator



RE: Notification of Substantial Risk

Dear Sir or Madam:

In accordance with the provisions of Section 8(e) of the Toxic Substances Control Act (TSCA), Rhodia Inc. (Rhodia) is submitting the following information from a laboratory report entitled:

Skin Sensitization: Local Lymph Node Assay with Amgard TBEP¹

In this study three (3) groups of female mice (4 mice per group) were treated daily with the test material at concentrations of 10%, 25%, and 50% of test material (diluted in a mixture of acetone and olive oil). The compounds were applied to the ear lobes (left and right) of the mice for 3 consecutive days. A control group of 4 mice was treated with the vehicle only. Five days after the first topical application, the mice were injected intravenously with radio-labeled thymidine (³H-methyl thymidine). Approximately five hours after the intravenous injection, the mice were sacrificed, and the draining auricular lymph nodes excised and pooled per group. Single-cell suspensions of lymph node cells were prepared from the pooled nodes, washed and incubated with trichloroacetic acid for approximately 18 hours. The proliferation of pooled lymph node cells was determined by the incorporation of ³H-methyl thymidine, as measured by a scintillation counter.

Using this study design, a test compound is considered by the testing laboratory to be a potential sensitizer if the exposure to the test compound results in a 3-fold (or greater) increase in incorporation of ³H-methyl thymidine, as compared to the control animals and if the three doses result in a conventional dose-response curve. This thymidine incorporation endpoint is known as the "stimulation index". The stimulation indices for the tested concentrations are presented in the following table:

Concentration (%w/w) of Test Material in acetone/olive oil	Stimulation Index
10	1.6
25	3.1
50	5.5

The test substance was considered by the laboratory conducting the study to be a sensitizer under the conditions of the study because two of the three doses resulted in stimulation indices of 3.0 or greater and the three doses tested resulted in a conventional dose-response curve (increasing dose produced increasing stimulation index).

This study was communicated to Rhodia on February 16, 2010.

¹ Ethanol, 2-butoxy-, 1,1',1"-phosphate, CAS# 78-51-3

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CONTAINS NO CBI

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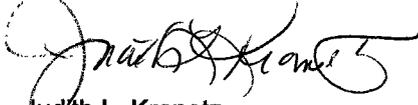
Although Rhodia Inc. does not believe that this substance presents an unreasonable hazard to workers or to the public when properly used in its intended applications, we believe the study meets the EPA's reporting criteria for TSCA Section 8(e).

In an earlier (Buehler "patch" design) dermal sensitization study on this chemical, using guinea pigs, the test material did not produce a sensitization reaction. Further, in decades of manufacture and use of this chemical, Rhodia is not aware of any reports of sensitization in individuals who work with this product.

Rhodia asserts that none of the information contained within this notice constitutes confidential business information.

Should you have any questions, or require any further information, please call (215) 369-9734. Thank you.

Very truly yours,
RHODIA INC.



Judith L. Kranetz
Manager, Regulatory Compliance & Product Stewardship

JLK/
Atts
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FINAL REPORT

**SKIN SENSITIZATION:
LOCAL LYMPH NODE ASSAY
WITH
AMGARD TBEP**

Date of Final Report: 18 January 2010

STUDY CODE: 09/276-037E

STATEMENT OF THE STUDY DIRECTOR

This study has been performed in accordance with the study plan, the OECD Guidelines for Testing of Chemicals No.: 429. Skin Sensitization: Local Lymph Node Assay. (Adopted: 24th April 2002) and the Principles of Good Laboratory Practice (Hungarian GLP Regulations: 9/2001. (III. 30.) EÜM-FVM joint decree of the Minister of Health and the Minister of Agriculture and Regional Development which corresponds to the OECD GLP, ENV/MC/CHEM (98) 17.).

I, the undersigned, declare that this report constitutes a true record of the actions undertaken and the results obtained in the course of this study. By virtue of my dated signature I accept the responsibility for the validity of the data and the following conclusion drawn from them:

In conclusion, under the conditions of the present assay AMGARD TBEP (Batch No.: JK20AHT021), tested in a suitable vehicle, was shown to have a sensitization potential (sensitizer) in the Local Lymph Node Assay.

Signature: *Dr. Török Magdolna*
Magdolna Török-Bathó, M.Sc.
Study Director

Date: *18 January 2010*

STATEMENT OF THE MANAGEMENT

According to the conditions of the research and development agreement between Rhodia UK Limited (as Sponsor) and LAB Research Ltd. the study titled "Skin Sensitization: Local Lymph Node Assay with AMGARD TBEP" has been performed on CBA/J@Rj mice, in accordance with the GLP requirements.

Signature: 
Christopher Banks, DABT
Managing Director

Date: 18 Jan 2010

QUALITY ASSURANCE STATEMENT

Study Code: 09/276-037E

Study Title: Skin Sensitization: Local Lymph Node Assay with AMGARD TBEP

Test Item: AMGARD TBEP

This study has been inspected, and this report audited by the Quality Assurance Unit in compliance with the Principles of Good Laboratory Practice. As far as it can be reasonably established the methods described and the results incorporated in this report accurately reflect the raw data produced during this study.

All inspections, data reviews and the report audit were reported in written form to the study director and to management. The dates of such inspections and of the report audit are given below:

Date of Inspection	Phase(s) Inspected/Audited	Date of report to	
		Management	Study Director
24 November 2009	Study Plan	24 November 2009	24 November 2009
15 December 2009	Determination of ³ HTdR incorporation	16 December 2009	16 December 2009
06 January 2010	Draft Report	07 January 2010	06 January 2010
18 January 2010	Final Report	18 January 2010	18 January 2010

Signature: Fabián Makovi Éva
 Éva Makovi-Fabián, B.Sc.
 On behalf of QA

Date: 18 January 2010

STUDY TITLE : Skin Sensitization: Local Lymph Node Assay with
AMGARD TBEP

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TEST ITEM : AMGARD TBEP

STUDY PERFORMED BY : LAB Research Ltd.
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Ágnes Rédl, M.Sc. – quality assurance unit
Ramóna Grób Heiderné, B.Sc. – quality assurance
unit
Éva Makovi-Fábián, B.Sc. – quality assurance unit
Vanda Gyimesi, M.Sc. – quality assurance unit

START OF EXPERIMENT : 09 December 2009
END OF EXPERIMENT : 15 December 2009
DATE OF DRAFT REPORT : 07 January 2010

BASIS OF STUDY : OECD Guidelines for Testing of Chemicals
No. 429. Skin Sensitisation: Local Lymph Node
Assay. Adopted: 24th April 2002

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1. SUMMARY

The aim of this study was to determine the skin sensitization potential of AMGARD TBEP following dermal exposure.

Based on the results of the Preliminary Compatibility Test and on the recommendations of the OECD Guideline [1], the test item was dissolved in Acetone: Olive oil 4:1 mixture (AOO). The maximum attainable concentration was 50 %.

The Preliminary Irritation/Toxicity Test was performed in CBA/J@Rj mice using two doses (test item concentrations of 50 and 25 (w/v) %) in the selected vehicle. The applicability and biocompatibility of the test item on the ears of animals at the maximum concentration of test item of 50 % was acceptable.

In the main assay, sixteen female CBA/J@Rj mice were allocated to four groups of four animals each:

- three groups received the appropriate formulation of AMGARD TBEP at concentrations of 50 %, 25 % and 10 %,
- the negative control group received AOO.

The test item solutions were applied on the dorsal surface of ears of experimental animals (25 µl/ear) for three consecutive days (Days 1, 2 and 3). There was no treatment on Days 4, 5 and 6. On Day 6, the cell proliferation in the local lymph nodes was measured by incorporation of tritiated methyl thymidine (³HTdR) and the values obtained were used to calculate stimulation indices (SI).

No mortality or systemic clinical signs were observed during the study. No treatment related effects were observed on animal body weights in any treated groups. No cutaneous reactions were observed at the site of the treatment in any of the treated groups.

Stimulation index values of the test item were 5.5, 3.1 and 1.6 at treatment concentrations of 50 %, 25 % and 10 %, respectively.

The result of the latest reliability check (performed within an interval of no longer than six months, Study code: 09/188-037E) was used to demonstrate the appropriate performance of the assay in accordance with the OECD guideline 429 [1]. The positive control substance α -Hexylcinnamaldehyde (HCA) was examined at a concentration of 25 % in the relevant vehicle. A significant lymphoproliferative response (SI \geq 3) was noted for HCA with stimulation index value of 4.9, the result confirms the validity of the LLNA in this laboratory.

In conclusion, under the conditions of the present assay AMGARD TBEP (Batch No.: JK20AHT021), tested in a suitable vehicle, was shown to have a sensitization potential (sensitizer) in the Local Lymph Node Assay.

2. INTRODUCTION

The basic principle underlying the LLNA is that skin sensitizers induce proliferation of lymphocytes in the lymph nodes draining the site of chemical application.

Generally, under appropriate test conditions, this proliferation is proportional to the concentration applied, and provides a means of obtaining an objective, quantitative measurement of sensitisation potential. The test measures cellular proliferation as a function of *in vivo* radioisotope incorporation into the DNA of dividing lymphocytes. The LLNA assesses proliferation in the draining auricular lymph nodes located in the cervical region at the bifurcation of the jugular vein. Lymphocyte proliferation in test groups is compared to that in the vehicle treated control. The ratio of the proliferation in test groups to that in the control, termed Stimulation Index (SI), is determined and must be at least equal or greater than three, for a test substance to classify as a potential skin sensitizer.

The purpose of this study was to determine the skin sensitization potential of the test item following dermal exposure in the Local Lymph Node Assay.

3. MATERIALS AND METHODS

3.1. TEST ITEM

Name:	AMGARD TBEP
Batch No.:	JK20AHT021
Chemical name:	TRIBUTOXYETHYL PHOSPHATE
Synonyms:	TBEP
CAS number:	78-51-3
Molecular formula:	C ₁₈ H ₃₉ O ₇ P
Molecular weight:	398.54
Active component:	TRIBUTOXYETHYL PHOSPHATE: 97.4 %
Other components:	Dibutoxyethyl phosphate: 1.5 % Butoxyethanol: 0.6 %
Appearance:	Clear colourless liquid with characteristic odour
Manufacturer:	See Sponsor
Manufacture date:	October 2009
Expiry date:	16 November 2010
Storage condition:	Room temperature 15-25 °C (humidity 50% ± 20)
Safety Precautions:	Routine safety precautions (lab coat, gloves, goggles, face mask) for unknown materials were applied to assure personnel health and safety.

Any remaining test substance will be destroyed after finalisation of all studies with this compound or 3 months after sending the (draft) reports.

3.1.1. Identification, Receipt

The test item of a suitable chemical purity was provided by the Sponsor. All precautions required in the handling and disposal of the test item were outlined by the Sponsor. Identification of the test item was performed in the Central Dispensary Unit of LAB Research Ltd. on the basis of name, batch number, density, appearance and colour.

3.1.2. Formulation

During the Preliminary Compatibility Test the solubility in Acetone: Olive oil 4:1 mixture (AOO) of the test item was examined. Since the solubility was greater than 50 %, AOO was chosen as vehicle. The test item was weighed and formulations prepared daily on a weight: volume basis in the Central Dispensary Unit of LAB Research Ltd.

3.2. CONTROLS

3.2.1. Negative Control

Based on the results of the Preliminary Compatibility Test, Acetone: Olive oil 4:1 mixture was chosen as the vehicle for the Study. The abbreviation used for the vehicle in the Study Report is AOO.

Chemicals used for the preparation of the vehicle:

Name:	Acetone
Batch No.:	KBR52355
Manufacturer:	Reanal Co.
Expiry:	January 2014
Storage condition:	15-25 °C
Name:	Olive oil
Batch No.:	058K0684
Manufacturer:	Sigma-Aldrich Co.
Expiry:	30 July 2010
Storage condition:	Room temperature

3.2.2. Positive Control

The results of the latest reliability check (performed using the positive control substance α -Hexylcinnamaldehyde (HCA) with the same vehicle within an interval of no longer than six months) was used to demonstrate the appropriate performance of the assay. (Study Code: 09/188-037E, Start of Experiment: 16 September 2009, End of Experiment: 20 October 2009). The α -Hexylcinnamaldehyde was examined at a concentration of 25%. Results of this study and historical control data for the positive and negative control substances are appended in a tabulated form (Appendices 5 and 6).

Name:	α -Hexylcinnamaldehyde, technical grade
CAS Number:	101-86-0
Lot No. :	02002DH
Manufacturer:	Sigma-Aldrich Co.
Nominal purity:	85 %
Purity:	99 %
Expiry:	30 December 2009
Storage condition:	Room temperature, 15-25 °C
Safety Precautions:	Routine safety precautions (lab coat, gloves, goggles, face mask and lab coat) were applied to assure personnel health and safety.

3.3. OTHER CHEMICALS USED IN THE STUDY

The chemicals used are summarized in the following table:

Table 1: Chemicals Used in the Experiments

Chemical	Supplier	Batch Number	Expiry date
Distilled water	Gyógyszerkutató Intézet	490791	08 February 2010
Phosphate Buffered Saline, 10X Concentrate	Sigma-Aldrich Co.	098K0394	30 May 2010
Trichloroacetic acid (abbreviation: TCA)	Sigma-Aldrich Co.	038K0679	30 December 2009
[Methyl-3-H]-Thymidine	ARC Inc.	PP 10082/090910	-
Optiphase HiSafe 3	PerkinElmer	152-090701	01 February 2011

3.4. INSTRUMENT SYSTEM

Name: Tri-Carb 2810 Liquid Scintillation Analyzer
Serial Number: DG10084483
IQ / OQ Protocol #: 1593646-1
Date of IQ / OQ: 25 November 2008
Manufacturer: PerkinElmer

3.5. EXPERIMENTAL ANIMALS

Species and strain: CBA/J@Rj mice
Source: ELEVAGE JANVIER
Route des Chènes Secs B.P. 4105
53940 LE GENEST-ST-ISLE, France
Hygienic level at arrival: SPF
Hygienic level during the study: Standard housing conditions
Justification of strain: On the basis of OECD Guideline, mice of CBA/Ca or CBA/J strain can be used. Females are used because the existing database is predominantly based on females.
Number of animals: 4 animals / treatment group
Sex: female, nulliparous, non pregnant
Age of animals at starting: 9 - 10 weeks old
Body weight range at starting: 20.7 - 21.7 grams (The weight variation in animals involved in the study did not exceed $\pm 20\%$ of the mean weight)
Acclimatization time: 26 days

3.5.1. Husbandry

Animal health: Only healthy animals were used for the study. Health status was certified by the veterinarian.
Housing / Enrichment: Individual caging / mice were provided with glass tunnel-tubes
Cage type: Type II. polypropylene/ polycarbonate
Bedding: Bedding was available to animals during the study
Light: 12 hours daily, from 6.00 a.m. to 6.00 p.m.
Temperature: 22 ± 3 °C
Relative humidity: 30 - 70 %
Ventilation: 15-20 air exchange/hour

The temperature and relative humidity were recorded twice every day during the acclimatisation and experimental phases.

Room/Cabinet (non-radioactive phase): 244/8
Room/Cabinet (radioactive phase): 139 - 140

3.5.2. Food and feeding

Animals received ssniff SM R/M-Z+H "Autoclavable complete diet for rats and mice – breeding and maintenance" (Batch number: 767 3000 Expiry Date: March 2010) produced by ssniff Spezialdiäten GmbH (Ferdinand-Gabriel-Weg 16, D-59494 Soest, Germany), *ad libitum*. The contents of the standard diet are detailed in Appendix 2.

3.5.3. Water supply

Animals received tap water from the municipal supply from 500 ml bottle, *ad libitum*. Water quality control analysis was performed once every three months and microbiological assessment was performed monthly, by Veszprém County Institute of State Public Health and Medical Officer Service (ÁNTSZ, H-8201 Veszprém, József A.u.36., Hungary). Copies of the relevant Certificates of Analysis are retained in the Archive at LAB Research Ltd.

3.5.4. Bedding

Lignocel[®] Hygienic Animal Bedding produced by J. Rettenmaier & Söhne GmbH+Co.KG (D-73494 Rosenberger (Germany) Holzmühle 1) was available to animals during the study.

3.5.5. Identification

A unique number written on the tail with a permanent marker identified each animal. The animal number was assigned on the basis of LAB Research Ltd.'s master file. The cages were marked with identity cards with information including study code, cage number, and dose group, sex and individual animal number. The animals were randomised and allocated to the experimental groups. The randomisation was checked by computer software according to the actual body weights, verifying the homogeneity and variability between the groups.

3.6. ADMINISTRATION OF THE TEST ITEM

3.6.1. Dose Selection and Justification of Dose Selection

The Preliminary Irritation/Toxicity Test was performed in CBA/J mice using two doses (test item concentrations of 50 and 25 (w/v) %). This preliminary experiment was conducted in a similar experimental manner to the main study, but it was terminated on Day 6 with a body weight measurement (radioactive proliferation assay was not performed).

During the Preliminary Irritation/Toxicity Test no mortality was observed in any treatment groups. No significant body weight loss was observed in the treated groups. The observed clinical signs are summarized in Appendix 3.

The experimental groups and dose levels are summarized in Table 2.

Table 2: Experimental Groups and Treatments.

Groups	Test item concentration (% w/v)	No. of animals
Vehicle control (AOO)	-	4
AMGARD TBEP	50	4
AMGARD TBEP	25	4
AMGARD TBEP	10	4

3.6.2. Topical application

During the assay each mouse was topically dosed on the dorsal surface of each ear with 25 μ l of the appropriate formulation applied using a pipette. Each animal was dosed once a day for three consecutive days (Days 1, 2 and 3). There was no treatment on Days 4, 5 and 6.

3.7. PROLIFERATION ASSAY

3.7.1. Injection of Tritiated Thymidine ($^3\text{HTdR}$)

On Day 6, animals were taken to the radioactive suite and each mouse was intravenously injected via the tail vein with 250 μl of sterile PBS (phosphate buffered saline) containing approximately 20 μCi of $^3\text{HTdR}$ using a gauge 25G1" hypodermic needle with 1 ml sterile syringe. Once injected, the mice were left for 5 hours (\pm 30 minutes).

3.7.2. Removal and Preparation of Draining Auricular Lymph Nodes

Five hours (\pm 30 minutes) after intravenous injection the mice were euthanized by asphyxiation with ascending doses of carbon dioxide (deep anaesthesia was confirmed before making incision, death was confirmed before discarding carcasses). The draining auricular lymph nodes were excised by making a small incision on the skin between the jaw and sternum, pulling the skin gently back towards the ears and exposing the lymph nodes. The nodes were then removed using forceps. The carcasses were discarded after cervical dislocation or after cutting through major cervical blood vessels. Once removed, the nodes of mice from each test group was pooled and collected in separate Petri dishes containing a small amount (1-2 ml) of PBS to keep the nodes wet before processing.

3.7.3. Preparation of Single Cell Suspension of Lymph Node Cells

A single cell suspension (SCS) of pooled lymph node cells (LNCs) was prepared and collected in disposable tubes by gentle mechanical disaggregating of the lymph nodes through a cell strainer using the plunger of a disposable syringe. The cell strainer was washed with PBS (up to 10 ml). Pooled LNCs were pelleted with a relative centrifugal force (RCF) of 190 x g (approximately) for 10 minutes at 4 $^{\circ}\text{C}$. After centrifugation supernatants were discarded. Pellets were gently resuspended and 10 ml of PBS was added to the tubes. The washing step was repeated twice.

This procedure was repeated for each group of pooled lymph nodes.

3.7.4. Determination of Incorporated $^3\text{HTdR}$

After the final wash, supernatant were removed leaving a small volume (<0.5 ml) of supernatant above each pellet. Each pellet was gently agitated before suspending the LNCs in 3 ml of 5% TCA (trichloroacetic acid) for precipitation of macromolecules. After incubation with 5% TCA at 2-8 °C overnight (approximately 18 hours) precipitate was recovered by centrifugation at 190 x g for 10 minutes, supernatants were removed and pellets were suspended in 1 ml of 5% TCA and dispersed using ultrasonic water bath. Each precipitate was transferred to a suitable sized scintillation vial with 10 ml of scintillation liquid and thoroughly mixed. The vials were loaded to a β -scintillation counter and $^3\text{HTdR}$ incorporation was measured for up to 10 minutes per sample.

The β -counter expresses the $^3\text{HTdR}$ incorporation as the number of radioactive disintegrations per minute (DPM). Similarly, background $^3\text{HTdR}$ levels were also measured in two 1 ml aliquots of 5% TCA.

3.8. OBSERVATIONS

3.8.1. Clinical Observations

During the study (Day 1 to Day 6) each animal was observed daily for any clinical signs, including local irritation and systemic toxicity. Clinical observations were performed twice a day (before and after treatments) on Days 1, 2 and 3 and once daily on Days 4, 5 and 6. Individual records were maintained.

3.8.2. Measurement of Body Weight

Individual body weights were recorded on Day 1 (beginning of the test) and on Day 6 (prior to $^3\text{HTdR}$ injection) with a precision of ± 0.1 g.

3.9. EVALUATION OF THE RESULTS

DPM was measured for each pooled group of nodes. The measured DPM values were corrected with the background DPM value ("DPM"). The average of the two measured DPM values of 5 (w/v) % TCA solutions was used as the background DPM value. The results were expressed as "DPN" (DPM divided by the number of lymph nodes) following the industry standard for data presentation. Stimulation index (SI = DPN value of a treated group divided by the DPN value of the negative control group) for each treatment group was also calculated.

A stimulation index of 3 or greater is an indication of a positive result.

3.9.1. Interpretation of Results

The test item is regarded as a sensitizer if both of the following criteria are fulfilled:

- That exposure to at least one concentration of the test item resulted in an incorporation of $^3\text{HTdR}$ at least 3-fold or greater than recorded in control mice, as indicated by the stimulation index.
- The data are compatible with a conventional dose response, although allowance must be made (especially at high topical concentrations) for either local toxicity or immunological suppression.

4. USE OF RADIOACTIVE MATERIALS

Use of radioactive materials was recorded in the appropriate register. Regular decontamination of the working area with a verification of decontamination was carried out. Radioactive waste materials were processed according to normal laboratory standards.

5. PERMISSION OF THE IACUC

The conduct of the study was permitted by Institutional Animal Care and Use Committee (IACUC) of LAB Research Ltd.
Date of IACUC approval: 24 November 2009

6. ARCHIVES

The study documents and samples:

- study plan and amendment,
- all raw data,
- sample of the test item,
- study report and any amendments,
- correspondence

will be stored in the archives of LAB Research Ltd., 8200 Veszprém-Szabadságpuszta, Hungary according to the Hungarian GLP regulation and to test facility SOPs.

After the retention time agreed with the Sponsor has elapsed, all the archived materials listed above will be offered to the Sponsor or retained for a further period if agreed by a contract. Otherwise the materials will be discarded.

7. DEVIATIONS FROM THE STUDY PLAN

There was no deviation from the Study Plan.

8. RESULTS AND DISCUSSION

8.1. CLINICAL OBSERVATION

No mortality or signs of systemic toxicity were observed during the study. No cutaneous reactions were observed at the site of the treatment in any treatment groups.

8.2. BODY WEIGHT MEASUREMENT

No treatment related effects were observed on animal body weights. Individual and mean body weights are given in Table 3.

Table 3: Individual Body Weights for all Animals with Group Means

Animal Number	Identity Number	Test Group Name	Initial Body Weight (g)	Terminal Body Weight (g)
4134	1	Negative control (vehicle): AOO	21.1	22.5
4146	2		21.7	23.0
4153	3		21.4	22.4
4148	4		20.8	22.1
Mean			21.3	22.5
4139	5	AMGARD TBEP 50 % in AOO	21.6	22.6
4178	6		21.5	22.3
4163	7		21.0	22.0
4141	8		20.8	21.1
Mean			21.2	22.0
4150	9	AMGARD TBEP 25 % in AOO	21.5	21.5
4182	10		21.4	22.3
4188	11		21.2	21.9
4144	12		20.7	21.9
Mean			21.2	21.9
4168	13	AMGARD TBEP 10 % in AOO	21.4	21.2
4173	14		21.7	22.8
4171	15		20.9	21.1
4184	16		20.7	21.2
Mean			21.2	21.6

8.3. PROLIFERATION ASSAY

The results of the proliferation assay are summarized in Table 4 and Figure 1. Appearance of the lymph nodes was normal in the negative control group and in the 10 (w/v) % test item treated group. Larger than normal lymph nodes was observed in the 50 and 25 (w/v) % test item treated group.

Table 4: DPM, DPN and Stimulation Index Values for all Groups

Test Group Name	Measured DPM/group	DPM	No. of Node	DPN	Stimulation Index Values
Background (5 (w/v) % TCA)	33		-		
Negative control AOO	892	859	8	107.4	1.0
AMGARD TBEP 50 % in AOO	4791	4758	8	594.8	5.5
AMGARD TBEP 25 % in AOO	2670	2637	8	329.6	3.1
AMGARD TBEP 10 % in AOO	1436	1403	8	175.4	1.6

8.4. INTERPRETATION OF OBSERVATIONS

The test item was clear colourless liquid with characteristic odour, which was dissolved in the selected vehicle (AOO).

Since there were no confounding effects of irritation or systemic toxicity at the applied concentrations, the proliferation values obtained are considered to reflect the real potential of the test item to cause lymphoproliferation in the Local Lymph Node Assay.

A significant lymphoproliferative response ($SI \geq 3$) was noted for AMGARD TBEP at concentration of 50 and 25 % (w/v). The stimulation index values were 5.5, 3.1 and 1.6 at concentrations of 50 %, 25 % and 10 %, respectively. The stimulation index values were compatible with a biological dose-related response (Figure 1).

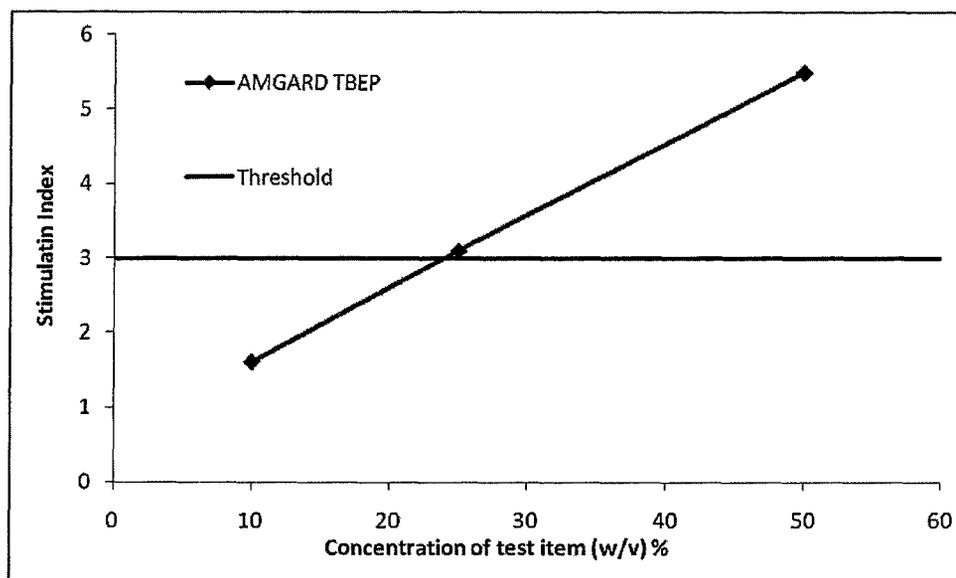


Figure 1. Test Item Stimulation Index Values

8.5. RELIABILITY OF THE TEST

The result of the latest reliability check (performed within an interval of no longer than six months, Study code: 09/188-037E) was used to demonstrate the appropriate performance of the assay in accordance with the OECD guideline 429[1]. The positive control substance α -Hexylcinnamaldehyde (HCA) was examined at a concentration of 25 % in the relevant vehicle. A significant lymphoproliferative response ($SI \geq 3$) was noted for HCA with stimulation index value of 4.9.

Results of this reliability check and the historical control data for the positive and negative control substances are given in Appendix 5 and Appendix 6, respectively.

9. REFERENCES

1. OECD Guidelines for Testing of Chemicals No. 429. Skin Sensitisation: Local Lymph Node Assay. Adopted: 24th April 2002.
2. Hungarian GLP Regulations: 9/2001. (III.30.) EüM-FVM joint decree of the Minister of Health and the Minister of Agriculture and Regional Development, which corresponds to the OECD GLP, ENV/MC/CHEM (98) 17.

10. CONCLUSION

In conclusion, under the conditions of the present assay AMGARD TBEP (Batch No.: JK20AHT021), tested in a suitable vehicle, was shown to have a sensitization potential (sensitizer) in the Local Lymph Node Assay..

APPENDICES

APPENDIX 1
STUDY SCHEDULE

	Relative Day	Absolute Day
PRE-EXPERIMENTAL PERIOD		
Animal receipt:	Day (-26)	13 November 2009
Veterinary control and acclimatisation:	from Day (-26) to Day 1	from 13 November 2009 to 09 December 2009
Animal identification:	Day 1	09 December 2009
Randomisation:	Day 1	09 December 2009
EXPERIMENTAL PERIOD		
Treatment days:	Day 1 Day 2 Day 3	09 December 2009 10 December 2009 11 December 2009
Body weight measurement:	Day 1 (beginning of the test) and Day 6 (prior to ³ HTdR injection)	09 December 2009 14 December 2009
Clinical observation:	daily from Day 1 to Day 6	09 December 2009 14 December 2009
Injection of ³ HTdR:	Day 6	14 December 2009
Preparation of LNC:	Day 6	14 December 2009
Sample measurement:	Day 7	15 December 2009
Date of Draft Report:		07 January 2010

APPENDIX 2

CONTENTS OF THE DIET

SSNIFF® SM R/M-Z+H COMPLETE DIET FOR RATS AND MICE

Batch No.: 767 3000 Best before: 03/2010

Crude Nutrients

Crude protein	19.00 %
Crude fat	3.50 %
Crude fiber	3.60 %
Crude ash	6.50 %
Calcium	1.00 %
Phosphorus	0.70 %
Sodium	0.20 %
Magnesium	0.22 %

Feed Additives

Vitamin A	25000 IU (per kg)
Vitamin D3	1000 IU (per kg)
Vitamin E	125 mg (per kg)
Copper,copper-(II)-sulfate pentahydrate	5 mg (per kg)
Lysine	1.10 %
Methionine	0.56 %

These data are standard and guaranteed values provided by the supplier.

APPENDIX 3

RESULT OF THE PRELIMINARY
IRRITATION/TOXICITY TEST**Table 5:** Individual Body Weights for all Animals with Group Means (Preliminary Irritation/Toxicity Test)

Animal Number	Identity Number	Test Group Name	Initial Body Weight (g)	Terminal Body Weight (g)*
4127	1	50 %	22.0	21.8
4110	2	50 %	20.0	19.9
		Mean:	21.0	20.9
4125	3	25 %	23.4	24.2
4112	4	25 %	22.2	22.1
		Mean:	22.8	23.2

*: Terminal body weights were measured on Day 6.

Table 6: Summarized Clinical Observations (Preliminary Irritation/Toxicity Test)

Period	Group	Animal No.	Identity No.	Clinical observations
DAY 1	AMGARD TBEP, 50 % in AOO	1	4127	Before treatment: symptom-free After treatment: symptom-free
	AMGARD TBEP, 50 % in AOO	2	4110	Before treatment: symptom-free After treatment: symptom-free
	AMGARD TBEP, 25 % in AOO	3	4125	Before treatment: symptom-free After treatment: symptom-free
	AMGARD TBEP, 25 % in AOO	4	4112	Before treatment: symptom-free After treatment: symptom-free
DAY 2	AMGARD TBEP, 50 % in AOO	1	4127	Before treatment: symptom-free After treatment: symptom-free
	AMGARD TBEP, 50 % in AOO	2	4110	Before treatment: symptom-free After treatment: symptom-free
	AMGARD TBEP, 25 % in AOO	3	4125	Before treatment: symptom-free After treatment: symptom-free
	AMGARD TBEP, 25 % in AOO	4	4112	Before treatment: symptom-free After treatment: symptom-free

Table 6 (Continued)

DAY 3	AMGARD TBEP, 50 % in AOO	1	4127	Before treatment: symptom-free After treatment: symptom-free
	AMGARD TBEP, 50 % in AOO	2	4110	Before treatment: symptom-free After treatment: symptom-free
	AMGARD TBEP, 25 % in AOO	3	4125	Before treatment: symptom-free After treatment: symptom-free
	AMGARD TBEP, 25 % in AOO	4	4112	Before treatment: symptom-free After treatment: symptom-free
DAY 4	AMGARD TBEP, 50 % in AOO	1	4127	symptom-free
	AMGARD TBEP, 50 % in AOO	2	4110	symptom-free
	AMGARD TBEP, 25 % in AOO	3	4125	symptom-free
	AMGARD TBEP, 25 % in AOO	4	4112	symptom-free
DAY 5	AMGARD TBEP, 50 % in AOO	1	4127	symptom-free
	AMGARD TBEP, 50 % in AOO	2	4110	symptom-free
	AMGARD TBEP, 25 % in AOO	3	4125	symptom-free
	AMGARD TBEP, 25 % in AOO	4	4112	symptom-free
DAY 6	AMGARD TBEP, 50 % in AOO	1	4127	symptom-free
	AMGARD TBEP, 50 % in AOO	2	4110	symptom-free
	AMGARD TBEP, 25 % in AOO	3	4125	symptom-free
	AMGARD TBEP, 25 % in AOO	4	4112	symptom-free

APPENDIX 4

SUMMARIZED CLINICAL OBSERVATIONS

Table 7: Summarized Clinical Observations

Group	Animal No.	Identity No.	CLINICAL OBSERVATIONS					
			DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6
Neg. control (AOO)	1	4134	B. T.: symptom-free A. T.: symptom-free	B. T.: symptom-free A. T.: symptom-free	B. T.: symptom-free A. T.: symptom-free	Symptom-free	Symptom-free	Symptom-free
	2	4146	B. T.: symptom-free A. T.: symptom-free	B. T.: symptom-free A. T.: symptom-free	B. T.: symptom-free A. T.: symptom-free	Symptom-free	Symptom-free	Symptom-free
	3	4153	B. T.: symptom-free A. T.: symptom-free	B. T.: symptom-free A. T.: symptom-free	B. T.: symptom-free A. T.: symptom-free	Symptom-free	Symptom-free	Symptom-free
	4	4148	B. T.: symptom-free A. T.: symptom-free	B. T.: symptom-free A. T.: symptom-free	B. T.: symptom-free A. T.: symptom-free	Symptom-free	Symptom-free	Symptom-free
AMGARD TBEP	5	4139	B. T.: symptom-free A. T.: symptom-free	B. T.: symptom-free A. T.: symptom-free	B. T.: symptom-free A. T.: symptom-free	Symptom-free	Symptom-free	Symptom-free
	6	4178	B. T.: symptom-free A. T.: symptom-free	B. T.: symptom-free A. T.: symptom-free	B. T.: symptom-free A. T.: symptom-free	Symptom-free	Symptom-free	Symptom-free
50 (w/v) %	7	4163	B. T.: symptom-free A. T.: symptom-free	B. T.: symptom-free A. T.: symptom-free	B. T.: symptom-free A. T.: symptom-free	Symptom-free	Symptom-free	Symptom-free
	8	4141	B. T.: symptom-free A. T.: symptom-free	B. T.: symptom-free A. T.: symptom-free	B. T.: symptom-free A. T.: symptom-free	Symptom-free	Symptom-free	Symptom-free

STUDY CODE: 09/276-037E

Table 7 (continued)

Group	Animal No.	Identity No.	CLINICAL OBSERVATIONS					
			DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6
AMGARD TBEP 25 (w/v) %	9	4150	B. T.: symptom-free A. T.: symptom-free	B. T.: symptom-free A. T.: symptom-free	B. T.: symptom-free A. T.: symptom-free	Symptom-free	Symptom-free	Symptom-free
	10	4182	B. T.: symptom-free A. T.: symptom-free	B. T.: symptom-free A. T.: symptom-free	B. T.: symptom-free A. T.: symptom-free	Symptom-free	Symptom-free	Symptom-free
	11	4188	B. T.: symptom-free A. T.: symptom-free	B. T.: symptom-free A. T.: symptom-free	B. T.: symptom-free A. T.: symptom-free	Symptom-free	Symptom-free	Symptom-free
	12	4144	B. T.: symptom-free A. T.: symptom-free	B. T.: symptom-free A. T.: symptom-free	B. T.: symptom-free A. T.: symptom-free	Symptom-free	Symptom-free	Symptom-free
AMGARD TBEP 10 (w/v) %	13	4168	B. T.: symptom-free A. T.: symptom-free	B. T.: symptom-free A. T.: symptom-free	B. T.: symptom-free A. T.: symptom-free	Symptom-free	Symptom-free	Symptom-free
	14	4173	B. T.: symptom-free A. T.: symptom-free	B. T.: symptom-free A. T.: symptom-free	B. T.: symptom-free A. T.: symptom-free	Symptom-free	Symptom-free	Symptom-free
	15	4171	B. T.: symptom-free A. T.: symptom-free	B. T.: symptom-free A. T.: symptom-free	B. T.: symptom-free A. T.: symptom-free	Symptom-free	Symptom-free	Symptom-free
	16	4184	B. T.: symptom-free A. T.: symptom-free	B. T.: symptom-free A. T.: symptom-free	B. T.: symptom-free A. T.: symptom-free	Symptom-free	Symptom-free	Symptom-free

Note: B.T.: Before treatment; A.T.: After treatment;

APPENDIX 5

RESULTS OF THE LATEST RELIABILITY CHECK
(Study Code: 09/188-037E)

Table 8: DPM, DPN and Stimulation Index Values for all Groups of Reliability Check

Test Group Name	Measured DPM/group	DPM	No. of Node	DPN	Stimulation Index Values
Background (5 (w/v) % TCA)	31	-	-	-	-
Solvent Control: AOO	725.0	694.0	8	86.8	1.0
25% HCA in AOO	3421.0	3390.0	8	423.8	4.9
Solvent Control: DMF	929.0	898.0	8	112.3	1.0
25% HCA in DMF	10795.0	10764.0	8	1345.5	12.0
Solvent Control: DMSO	1102.0	1071.0	8	133.9	1.0
25% HCA in DMSO	8656.0	8625.0	8	1078.1	8.1
Solvent Control: 70% EtOH	485.0	454.0	8	56.8	1.0
25% HCA in 70% EtOH	17461.0	17430.0	8	2178.8	38.4
Solvent Control: HOO	981.0	950.0	8	118.8	1.0
25% HCA in HOO	4993.0	4962.0	8	620.3	5.2
Solvent Control: MEK	698.0	667.0	8	83.4	1.0
25% HCA in MEK	3332.0	3301.0	8	412.6	4.9
Background (5 (w/v) % TCA)	36	-	-	-	-
Solvent Control: PG	1905.0	1869.0	8	233.6	1.0
25% HCA in PG	9822.0	9786.0	8	1223.3	5.2
Solvent Control: 1% Pluronic	1192.0	1156.0	8	144.5	1.0
25% HCA in 1% Pluronic	5201.0	5165.0	8	645.6	4.5

APPENDIX 6

HISTORICAL CONTROL DATA

Table 9: Historical Control Data of the Positive Control Substance

	Solvents					
	Acetone- Olive oil (AOO)			1% Pluronic PE9200 in water (1%Plu)		
	DPN values		SI value	DPN values		SI value
	Control	HCA 25%	HCA 25%	Control	HCA 25%	HCA 25%
<i>Average</i>	237.7	1950.2	9.7	160.9	1347.4	9.3
<i>Range: min</i>	46.9	423.8	3.2	75.0	645.6	3.4
<i>max</i>	586.9	3300.5	28.5	469.6	2157.5	20.1
<i>Number of cases</i>	38	25	25	23	13	13

	Solvents								
	N,N-Dimethylformamide (DMF)			Dimethyl sulfoxide (DMSO)			n-Hexane:Olive oil (HOO)		
	DPN values		SI value	DPN values		SI value	DPN values		SI value
	Control	HCA 25%	HCA 25%	Control	HCA 25%	HCA 25%	Control	HCA 25%	HCA 25%
<i>Average</i>	186.8	2380.6	12.7	278.6	1933.8	8.1	124.4	1059.9	8.9
<i>Range: min</i>	39.0	1045.1	7.1	133.3	1052.8	4.2	81.1	490.0	5.0
<i>max</i>	423.1	4438.9	20.8	553.3	5291.3	24.1	165.9	1296.4	14.0
<i>Number of cases</i>	47	17	20	14	11	11	9.0	9.0	9.0

	Solvents							
	Propylene glycol (PG)			Absolute ethanol: Distilled water 70:30 mixture (EtOH)				
	DPN values		SI value	DPN values			SI values	
	Control	HCA 25%	HCA 25%	Control	HCA 10%	HCA 25%	HCA 10%	HCA 25%
<i>Average</i>	169.9	1569.9	7.5	137.2	1264.2	4041.9	17.3	32.4
<i>Range: min</i>	93.8	583.8	5.2	56.8	1214.8	2178.8	17.1	25.7
<i>max</i>	288.8	3231.3	11.2	357.6	1313.5	9207.1	17.4	38.4
<i>Number of cases</i>	10	5	5	5	2	4	2	4

HCA = alpha-Hexylcinnamaldehyde

SI (Stimulation Index) = DPN of a treated group divided by DPN of the appropriate control group.

DPN (Disintegrations Per Node) = DPM (Disintegrations Per Minute) divided by the number of lymph nodes.

In case of individual approach, SI values were calculated from the mean DPN values of the group.

APPENDIX 7

COPY OF CERTIFICATE OF ANALYSIS

CERTIFICATE of ANALYSIS			
		RHODIA UK LTD P O Box 89 Trinity Street Oldbury West Midlands B69 4LN Telephone: +44 (0) 121 541 3333 Facsimile: +44 (0) 121 541 3235	
Sample Name	AMGARD TBEP	Date of Manufacture	October 2009
Batch Number:	JK20A11T021	SDS Reference	29/05/09 Version 4
		Certificate of Analysis No:	ITC091105
Results of Analysis			
Test	Result		
Appearance	Clear colourless liquid		
TBEP assay (GC)	97.4% area		
Impurities - DBEP	1.5% area		
- Butoxyethanol	0.6% arca		
Acidity (mgKOH/g)	0.09		
Water content	0.02%w/w		
Specific Gravity @25oC	1.018g/ml		
Refractive index	1.436		
Mass spectrum	Consistent with reference spectrum		
NMR spectra (³¹ P, ¹ H, ¹³ C)	Consistent with reference spectra		
IR Spectrum	Consistent with reference spectrum		
UV -Vis spectrum	Consistent with reference spectrum		
Analyst:..... <i>H F Hau</i>	Date:..... 16/11/09		
H F Hau	Senior Analyst		
Rhodia UK Limited Place of Registration: England Registered Number: 36833 Registered Office: Oak House Needs Crescent Watford Herts WD24 4QQ			

APPENDIX 8

COPY OF GLP CERTIFICATE



ORSZÁGOS GYÓGYSZERÉSZETI INTÉZET
National Institute of Pharmacy

H-1051 Budapest, Zrínyi u. 3.

Mail: 1372 P.O. Box 450.

Phone: +36 1 8869-300

Fax: +36 1 8869-460

E-mail: ogyi@ogyi.hu



Budapest, 20th December 2008

No: 38623/48/2007

Our ref.: Szilvia Karsai

Subject: GLP Certificate

**GOOD LABORATORY PRACTICE (GLP)
CERTIFICATE**

Based on the Inspection report and the discussion of follow up activities it is hereby certified that the test facility

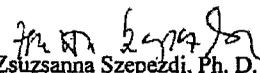
LAB Research Ltd.

H-8201 Veszprém, Szabadságpuszta, Hungary

is able to carry out Physical-chemical testing, Toxicity studies, Mutagenicity studies, Environmental toxicity studies on aquatic and terrestrial organisms, Studies on behaviour in water, soil and air; bioaccumulation, Bioanalytical, Analytical and clinical chemistry testing compliance with the Principles of GLP (Good Laboratory Practice).

Date of the inspection: 13-22 October 2008.

This GLP Certificate is valid for 2 years.


Zsuzsanna Szepezdi, Ph. D.
Director-General

From: Origin ID: ZRPA (809) 860-4474
Kathleen Porzl
Rhodia Inc
8 Cedar Brook Drive
Cranbury, NJ 08512



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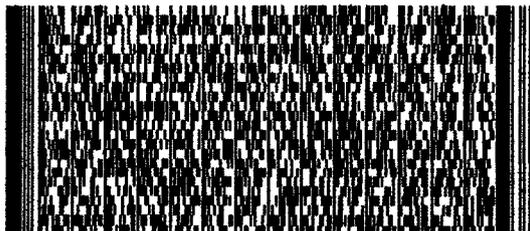
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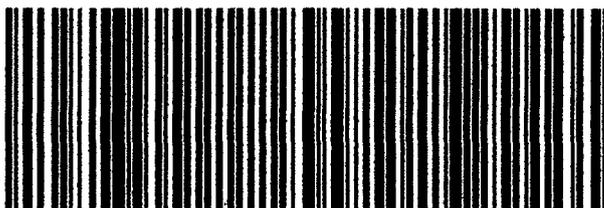


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