

8EHQ-0303-15294

Applera
Corporation

850 Lincoln Centre Drive
Foster City, CA
94404-1128 U.S.A.



February 19, 2003

VIA FEDERAL EXPRESS

Document Processing Center 7407
Room G-99, East Tower
Attn: Section 8(e)
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
401 "M" Street, S.W.
Washington, DC 20460-0001



Contain NO CBI

2003 MAR 20 AM 11:19
APPLERA
OPERATIONS

Re: *Section 8(e) Submittal for CE Phosphite*

Dear Section 8(e) Coordinator:

In accordance with the requirements of Section 8(e) of the Toxic Substances Control Act (TSCA), 15 U.S.C. § 2607(e), Applera Corporation, Applied Biosystems Group is submitting information to the U.S. Environmental Protection Agency (EPA) concerning the health effects of a chemical substance that is manufactured and processed by Applied Biosystems and the Corporation's subsidiaries.

According to our analysis, it appears that the attached information may not pose a "substantial risk" to human health or the environment, and thus may not be reportable under Section 8(e). However, given the breadth of the Section 8(e) requirements as they apply to the attached information, Applied Biosystems is reporting this information with a desire that EPA have all information that it may need to evaluate the risks of chemical products.

We received the attached information on February 3, 2003. Accordingly, I am submitting this information within fifteen (15) working days as required by TSCA Section 8(e).

64699

1-850-470-0957
1-850-470-2000

Identity of Chemical Substance

Applied Biosystems is submitting information regarding the following chemical substance:

2-CYANOETHYL N,N,N',N'-TETRAISOPROPYLPHOSPHORDIAMIDITE
(CE PHOSPHITE)

CAS Number: 102691-36-1

Applied Biosystems manufactures this substance and uses it internally as a raw material for the manufacture of phosphoramidites. These phosphoramidites are sold and intended for use only in research and development (R&D) applications or FDA-regulated activities. Specifically, these are used by scientists in academic, governmental and industrial laboratories to synthesize oligonucleotides (e.g., synthetic DNA) for further R&D activities, or for diagnostic purposes. Although not sold by Applied Biosystems, other suppliers of biotechnology reagents do sell CE Phosphite.

Name and Address of Person and Company Reporting

This information is being submitted by me on behalf of Applied Biosystems:

Lauren E Kamer
Regulatory Analyst
Applied Biosystems
850 Lincoln Centre Drive
Foster City, CA 94404
Telephone: (650) 554-2860
Facsimile: (650) 638-6786

Summary of Adverse Effects and Source of Information

Attached at Tab 1 is a draft report, entitled "Draft Report #1, Local Lymph Node Assay, Study No. 0787XA66.013, CE Phosphite," prepared by Calvert Laboratories, Inc. (hereafter, the "CE Phosphite Study"). The CE Phosphite Study was sponsored by Applied Biosystems.

Based on the results of the CE Phosphite Study, CE Phosphite was found to have induced a hypersensitivity response in mice, at concentrations of 25% and 50%, as measured by the proliferation of lymphocytes in the draining lymph nodes. Consequently, CE Phosphite is considered to be a potential sensitizer.

Applied Biosystems has received no reportable allegations of adverse health effects related to CE Phosphite.

Attached at Tab 2 is a copy of the revised Applied Biosystems Material Safety Data Sheet (MSDS) for CE Phosphite.

Please contact me if you have any questions about this submittal.

Sincerely,

A handwritten signature in black ink, appearing to read "Lauren E. Kamer". The signature is fluid and cursive, written over a white background.

Lauren E. Kamer
Regulatory Analyst
Applied Biosystems

Attachments



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DRAFT REPORT #1

STUDY SPONSOR

Applied Biosystems
850 Lincoln Centre Drive
Foster City, CA 94404

STUDY TITLE

Local Lymph Node Assay

STUDY NUMBER

0787XA66.013

TEST ARTICLE

CE PHOSPHITE

ISSUE DATE OF FINAL REPORT

DRAFT

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COMPLIANCE STATEMENT

I, the undersigned, hereby declare that this report is a true and accurate record of the results obtained. Except as noted below, the work described in this report was performed in accordance with the following:

“Good Laboratory Practice Regulations” of the United States Food and Drug Administration (21 CFR Part 58) and subsequent revisions; and

“OECD Principles of Good Laboratory Practice” concerning Mutual Acceptance of Data in the Assessment of Chemicals dated 26 November 1997 [C (97) 186/Final].

- Exceptions:
1. The test article was not characterized for identity, purity or stability.
 2. The test article dosing solutions were not analyzed for concentration and stability.

Joan M. Chapdelaine, Ph.D.
Study Director
Calvert Laboratories, Inc.
(formerly Calvert Preclinical Services, Inc.)

Date

QUALITY ASSURANCE UNIT STATEMENT

Study Title: Local Lymph Node Assay

Study Director: Joan M. Chapdelaine, Ph.D.

The following inspections were made in accordance with appropriate Standard Operating Procedures.

Protocol Inspection:

Date of Inspection	Report to Study Director	Report to Management
01 Oct 2002	01 Oct 2002	01 Oct 2002

Protocol Amendments:

Amendment Inspection	Date of Inspection	Report to Study Director	Report to Management
1	24 Jan 2003	24 Jan 2003	24 Jan 2003

Data/Draft Report Inspection:

Inspected Phase	Date of Inspection	Report to Study Director	Report to Management
Raw data and draft report #1	24-27 Jan 2003	27 Jan 2003	27 Jan 2003

Procedures:

Inspected Phase	Date of Inspection	Report to Study Director	Report to Management
Test Article preparation	20 Oct 2002	20 Oct 2002	20 Oct 2002

This report has been reviewed by the Quality Assurance Unit, employing methods detailed in appropriate Standard Operating Procedures. The results constitute an accurate representation of recorded data. Any data supplied by the Sponsor were not audited by the Quality Assurance Unit at Calvert.

Senior Quality Assurance Auditor
Calvert Laboratories, Inc.
(formerly Calvert Preclinical Services, Inc.)

Date

SUMMARY

The test article, CE PHOSPHITE, was tested for its capacity to induce a hypersensitivity response in mice as measured by the proliferation of lymphocytes in the draining lymph nodes.

CBA/J female mice were treated on the dorsal surface of both ears once per day for 3 days with the vehicle (acetone/olive oil 4:1 (v/v)), the test article, CE PHOSPHITE, at either 10, 25 or 50%, or the positive control (0.1% DNCB). On Day 6, the mice were injected with 20 μ Ci of 3 H-thymidine. Five hours later, the mice were euthanized and the draining auricular lymph nodes were removed. The lymph node cells were precipitated with 5% trichloroacetic acid (TCA) and the pellets counted in a β -scintillation counter to determine incorporation of the 3 H-thymidine.

The positive control, 0.1% DNCB, resulted in a stimulation index greater than 3 (5.73) indicating a positive response. This response compared to the vehicle control was also statistically significant ($p \leq 0.05$).

The test article was also positive at concentrations of 25 and 50% with the response at 50% being statistically significant ($p < 0.01$). The stimulation indices were 3.30 and 9.08, respectively. CE PHOSPHITE was negative at a concentrations of 10%. The stimulation index at 10% was 1.49.

Mean body weights at Days 1 and 6 and mean changes in body weights for each treatment group were compared to the vehicle control group. No statistically significant differences were observed for any of the test article treated groups when compared to the vehicle control group. Therefore, the test article did not cause any overt toxicity.

Based on the data from this study, CE PHOSPHITE at concentrations of 25 and 50% induced a hypersensitivity response and therefore is considered to be a potential sensitizer.

The undersigned have reviewed the format and content of this report and have approved the report for final issuance.

Joan M. Chapdelaine, Ph.D.
Study Director
Calvert Laboratories, Inc.
(formerly Calvert Preclinical Services, Inc.)

Date

Scientific Management
Calvert Laboratories, Inc.
(formerly Calvert Preclinical Services, Inc.)

Date

GENERAL INFORMATION

Study Number: 0787XA66.013

Study Sponsor:

Applied Biosystems
850 Lincoln Centre Drive
Foster City, CA 94404
Sang-Tae Kim, Ph.D., *Study Monitor*

Testing Facility:

Calvert Preclinical Services, Inc.
(Calvert)
Scott Technology Park
100 Discovery Drive
Olyphant, PA 18447

Test Article: CE PHOSPHITE

Study Objective:

To determine if the test article will induce a hypersensitivity response in mice as measured by the proliferation of lymphocytes in the draining lymph nodes.

Responsible Personnel:

Study Director: Joan M. Chapdelaine, Ph.D.

Study Schedule:

Date Protocol Signed
by Study Director: 24 Sep 2002

Experimental Start Date: 20 Oct 2002

Experimental Completion
Date: 26 Oct 2002

TEST/CONTROL ARTICLE AND VEHICLE

Test Article:

Identification: CE PHOSPHITE

Supplier: Applied Biosystems

Lot Number: 0204061

Description: Light yellow liquid

Storage: Room temperature

Vehicle:

Identification: Acetone/Olive oil (4:1 v/v)

Identification	Supplier	Lot#
Acetone	Fisher	993009
Olive oil	Sigma	012K6042

Description: Pale yellow liquid

Storage: Room temperature

Preparation: 80 ml of acetone was mixed with 20 ml of olive oil on 26 Sep 2003.

Positive Control:

Identification: Dinitrochlorobenzene (DNCB)

Supplier: Sigma

Lot Number: 100K1323

Description: Pale yellow crystals

Storage: Room temperature

Test Article Formulation:

Preparation: CE PHOSPHITE was prepared at concentrations of 10, 25 and 50% in 4:1 acetone/olive oil. All solutions were prepared fresh daily on the days of dosing as follows:

Date	Solution %	ml of test article	ml of vehicle
20 Oct 2002	10%	0.2	1.8
	25%	0.5	1.5
	50%	1.0	1.0
21 Oct 2002	10%	0.2	1.8
	25%	0.5	1.5
	50%	1.0	1.0
22 Oct 2002	10%	0.2	1.8
	25%	0.5	1.5
	50%	1.0	1.0

The positive control, DNCB, was prepared at a concentration of 0.1% in the 4:1 acetone/olive oil vehicle. A 10% stock solution was formulated and a 1:100 dilution was made to achieve the 0.1% positive control dosing solution. The solution was mixed by vortexing and stored at room temperature.

*Identity, Strength, Purity,
Composition and Stability:*

A certificate of analysis was not provided by the Sponsor. This exception is noted on the Compliance page of this report.

*Analysis of Test
Article Formulation:*

Samples (1 ml) of the formulated test article and vehicle were obtained during the study on Days 1, 2, and 3. Samples were stored at 2-8°C. However, at the request of the Sponsor the samples were not shipped to the Sponsor. This protocol deviation had no impact on the quality, integrity or validity of the study. Samples were therefore not analyzed. This exception is noted on the compliance page of this report.

METHODS AND EXPERIMENTAL DESIGN

Test System:

<i>Species/strain:</i>	Mouse - CBA/J
<i>Supplier:</i>	Jackson Laboratories, Bar Harbor, ME 04609
<i>Number of Animals:</i>	25 females
<i>Age Range at Experimental Start:</i>	7-8 weeks. The age range deviated from the range stated in the protocol (8-12 weeks) due to the availability of the mice. However, this protocol deviation had no impact on the quality, integrity or validity of the study.
<i>Weight Range at Experimental Start:</i>	18-22 grams
<i>Justification:</i>	The mouse is the standard species used in the local lymph node assay (LLNA), which has been developed as an alternative to the Guinea Pig Maximization Test (GPMT). The LLNA is a refinement in terms of reducing or eliminating distress in the animals compared to the GPMT. The number used is the minimum number recommended. (NIH Publication No. 99-4494).

Acclimation/Quarantine:

Following arrival at Calvert, mice were assessed as to their general health by a member of the veterinary staff or other authorized personnel. Mice were acclimated/quarantined for 11 days prior to treatment initiation, during which each mouse was observed at least once daily for any abnormalities or for the development of infectious disease.

Animal Husbandry:

Animals were housed (grouped 5 per cage) in compliance with the National Research Council "Guide for the Care and Use of Laboratory Animals". Calvert is a USDA registered and a fully AAALAC accredited facility.

The animal room environment was controlled (targeted conditions: temperature 18 to 26°C, relative humidity 30 to 70%, 12 hours artificial light and 12 hours dark).

Temperatures and relative humidity were monitored daily. The humidity was below 30% and was as low as 26% for about 6 hours on 15 Oct 2002 and for about 24 hours on 24 Oct 2002. On 22 Oct 2002, the humidity was below 30% for about 12 hours and was as low as 28%. These deviations from the protocol were minimal and of slight duration and did not adversely affect the results of the study.

All animals had access to Certified Rodent Diet #7012C (Harlan Teklad) or equivalent *ad libitum*, unless otherwise specified. The lot number(s) and specifications of each lot used are archived at Calvert.

Water was provided to the animals *ad libitum*, unless otherwise specified. Periodic analyses of the water are performed and the results are archived at Calvert.

There were no known contaminants in the diet or water, which at the levels detected would be expected to interfere with the purpose, conduct or outcome of the study.

Allocation to Treatment Groups:

Only mice considered suitable for use were placed on the study. Prior to treatment initiation, all mice were weighed and assigned to treatment groups using a computer-generated randomization method based on body weight. Mice were given the following identification numbers and identified by tail mark:

Group Number	Number
1	1-5
2	6-10
3	11-15
4	16-20
5	21-25

Experimental Design:

The test article, positive or vehicle control were applied daily (25 µl/ear) on the dorsal surface of both ears for 3 days according to the following table:

Group	Treatment	Dose	No. of Female Mice
1	Acetone/Olive oil	-	5
2	CE PHOSPHITE	10%	5
3	CE PHOSPHITE	25%	5
4	CE PHOSPHITE	50%	5
5	DNCB	0.1%	5

Route of Administration: Dermal

Rationale for Route: This route is required for this model of hypersensitivity.

METHOD OF PERFORMANCE

Mice were weighed on Days 1 and 6. Mice were treated on the dorsal surface of both ears, once per day on Days 1, 2, and 3. Any irritation observed after test article application was recorded. On Day 6 the mice were injected i.v. with 20 μ Ci of 3 H-thymidine in 250 μ l of saline. Five hours later the mice were euthanized with CO₂ and the draining auricular lymph nodes removed. A single cell suspension was prepared from the lymph nodes of each mouse. Cells were washed twice with phosphate buffered saline (PBS) and precipitated with 5% trichloroacetic acid (TCA) overnight at 2-8°C. The pellets were recovered by centrifugation and resuspended in 1 ml of TCA and transferred to 10 ml of scintillation fluid. Incorporation of 3 H-thymidine was measured in a β -scintillation counter.

OBSERVATIONS

Morbidity/Mortality:

Animals were observed daily.

Clinical Observations:

Cage side observations were made daily.

METHOD OF ANALYSIS

The mean DPM for each group was determined. Increases in ³H-thymidine incorporation relative to vehicle-treated control were derived for each group and recorded as stimulation indices (SI). The criterion for a positive response is that one or more concentrations of a test article elicits a 3-fold or greater increase in isotope incorporation relative to the vehicle control. Mean body weights and mean differences in body weights between Days 1 and 6 were also determined.

Evaluation of equality of means was made by a one way analysis of variance using the F distribution to assess statistical significance using Systat (version 9.01, SYSTAT, Inc.). If statistically significant differences between the means were found, a Dunnett's test was used to determine the degree of significance from control means.

ARCHIVING OF MATERIALS

Test article preparation, test article tracking, in-life data, protocol, protocol amendments (if applicable), draft report(s) that have been submitted to a regulatory agency, and the original final report generated as a result of this study will be archived at Calvert, 105 Edella Road, Suite 100, Clarks Summit, PA 18411. After 5 years, the Sponsor will be contacted to determine final disposition of all study materials.

RESULTS

The local lymph node assay was used to determine if the test article, CE PHOSPHITE, would induce a hypersensitivity response. The concentrations tested were 10, 25 and 50%. The results are shown in Table 1.

The positive control, 0.1% DNCB, resulted in a stimulation index greater than 3 (5.73) indicating a positive response. This response compared to the vehicle control was also statistically significant ($p \leq 0.05$).

The test article was also positive at concentrations of 25 and 50% with the response at 50% being statistically significant ($p < 0.01$). The stimulation indices were 3.30 and 9.08, respectively. CE PHOSPHITE was negative at a concentrations of 10%. The stimulation index at 10% was 1.49.

Mean body weights at Days 1 and 6 and mean changes in body weights for each treatment group were compared to the vehicle control group. No statistically significant differences were observed for any of the test article treated groups when compared to the vehicle control group. Therefore, the test article did not cause any overt toxicity.

CONCLUSION

Based on the data from this study, CE PHOSPHITE at concentrations of 25 and 50% induced a hypersensitivity response and therefore is considered to be a potential sensitizer.

REFERENCE

The Murine Local Lymph Node Assay: A Test Method for Assessing the Allergic Contact Dermatitis Potential of Chemicals/Compounds. NIH Publication No. 99-4494, Feb. 1999.

TABLE 1

Local Lymph Node Assay

Group	Treatment	Dose	DPM (mean ± sem)	SI (Test/control Ratio)	Results ¹
1	Acetone/Olive oil	-	715 ± 223	-	-
2	CE PHOSPHITE	10%	1063 ± 247	1.49	-
3	CE PHOSPHITE	25%	2363 ± 591	3.30	+
4	CE PHOSPHITE	50%	6492 ± 1203**	9.08	+
5	DNCB	0.1%	4099 ± 1497*	5.73	+

¹Test/control ratio of 3.0 or greater represents a positive result

*Statistically significant difference compared to the vehicle control group (P ≤ 0.05)

**Statistically significant difference compared to the vehicle control group (P < 0.01)

TABLE 2
Body Weights

Group	Treatment	Dose	Body Weights (g) (mean ± sem)		Change in Body Weight (g) (mean ± sem)
			Day 1	Day 6	
1	Acetone/Olive oil	-	20.0 ± 0.8	21.2 ± 1.0	1.2 ± 0.4
2	CE PHOSPHITE	10%	20.0 ± 0.6	21.4 ± 0.9	1.4 ± 0.2
3	CE PHOSPHITE	25%	20.2 ± 0.4	21.0 ± 0.4	0.8 ± 0.2
4	CE PHOSPHITE	50%	20.0 ± 0.7	21.0 ± 0.7	1.0 ± 0.3
5	DNCB	0.1%	19.6 ± 0.7	20.8 ± 0.5	1.2 ± 0.4

APPENDIX I—PROTOCOL/PROTOCOL AMENDMENT

STUDY TITLE

Local Lymph Node Assay

STUDY NUMBER

0787XA66.013

DRAFT



CALVERT PRECLINICAL
S E R V I C E S , I N C .

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In North Carolina:
Tel: 919.854.4453
Fax: 919.854.2860

LOCAL LYMPH NODE ASSAY

Sponsor:
Applied Biosystems
850 Lincoln Centre Drive
Foster City, CA 94404

Study No.: 0787XA66.013

Protocol-787
Local Lymph Node Assay

1. **INTRODUCTION**

- 1.1. **Study Title:** Local Lymph Node Assay
- 1.2. **Purpose:** To determine if the test article will induce a hypersensitivity response in mice as measured by the proliferation of lymphocytes in the draining lymph nodes.
- 1.3. **Regulatory Compliance:** This Study will be conducted in compliance with the Good Laboratory Practice Regulations 21 CFR Part 58 and in accordance with OECD Principles of Good Laboratory Practice as revised 26 November 1997 [C(97)186/final], and in accordance with the appropriate Standard Operating Procedures (SOP) of Calvert.
- 1.4. **Calvert Study No.:** 0787XA66.013
- 1.5. **Testing Facility:** Calvert Preclinical Services, Inc. (Calvert)
Scott Technology Park
100 Discovery Drive
Olyphant, PA 18447
- 1.6. **Sponsor:** Applied Biosystems
850 Lincoln Centre Drive
Foster City, CA 94404
- 1.7. **Proposed Experimental Start Date:** October 2002
- 1.8. **Proposed Experimental Completion Date:** October 2002

2. **TEST/CONTROL ARTICLE AND VEHICLE INFORMATION**

2.1. **Test Article:**

- 2.1.1. **Identification:** CE PHOSPHITE
- 2.1.2. **Lot Number:** Will be documented in the raw data
- 2.1.3. **Description:** White powder
- 2.1.4. **Storage:** Desiccated at room temperature
- 2.1.5. **Test Article Disposition:** Unused test article will be returned to the Sponsor or designee at the termination of this study or, if necessary, retained for use on related future studies. The Sponsor will be notified in advance of shipping and a transmittal letter will accompany the shipment. The material will be packed in a suitable container to maintain the conditions specified by the Sponsor during transit plus an adequate margin of safety to account for any possible transit delays.
- 2.1.6. **Analysis for Identity, Purity and Stability:** Good Laboratory Practice (GLP) regulations require that all test articles be appropriately characterized. Compliance necessitates documentation that characterization has been done. The Sponsor will, at a minimum, provide the Study Director with a certificate of analysis of the characterization and whether it was performed under GLP. If characterization was not performed under GLP, this fact will be reflected in the compliance statement. This information should be supplied prior to study initiation, but must be supplied before finalization of the report.

2.2. **Vehicle:**

- 2.2.1. **Identification:** Acetone/olive oil (4:1 v/v)
- 2.2.2. **Lot Number:** Will be documented in the raw data
- 2.2.3. **Description:** Clear, colorless liquid
- 2.2.4. **Storage:** Room temperature

2.3. **Control Article:**

2.3.1. **Identification:** Dinitrochlorobenzene (DNCEB)

2.3.2. **Lot Number:** Will be documented in the raw data

2.3.3. **Description:** Pale yellow crystals

2.3.4. **Storage:** Room temperature

2.4. **Test Article Formulation:**

2.4.1. **Preparation:** On the day of dosing the test article will be prepared at the appropriate concentration in the vehicle in which it is found to be soluble or as specified by the Sponsor.

2.4.2. **Storage:** Test article will be prepared fresh daily.

2.4.3. **Formulated Test Article Analysis:** Samples (1 ml) of the formulated test article will be obtained during the study on Days 1, 2, and 3 and shipped to the Sponsor. Good Laboratory Practice (GLP) regulations require that all formulated test articles be appropriately verified for concentration, homogeneity, and stability. Compliance necessitates documentation that verification has been done. The Sponsor will provide the Study Director with the results of the analyses and whether it was performed under GLP. If verification was not performed under GLP, this fact will be reflected in the compliance statement. This information must be supplied before finalization of the report.

3. **METHODS AND EXPERIMENTAL DESIGN**

3.1. **Test System:**

Species	: Mouse
Strain and/or Substrain	: CBA/Ca or CBA/J
Source	: Jackson Laboratories or Harlan Spague Dawley, Inc.
Sex	: Female

Age Range at Experimental Start	:	8-12 weeks
Weight Range at Experimental Start	:	18-25 g
Total Number of Animals	:	25
Justification	:	The mouse is the standard species used in the local lymph node assay (LLNA), which has been developed as an alternative to the Guinea Pig Maximization Test (GPMT). The LLNA is a refinement in terms of reducing or eliminating distress in the animals compared to the GPMT. The number used is the minimum number recommended. (NIH Publication No. 99-4494).
Identification	:	Tail marked with an indelible marker

3.2. **Acclimation/Quarantine:** Following arrival at Calvert, animals will be assessed as to their general health by a member of the veterinary staff or other authorized personnel. A minimum of at least 5 days will be allowed between animal receipt and the start of dosing in order to acclimate animals to the laboratory environment and to observe them for the development of infectious disease. Any animals considered unacceptable for use in this study will be replaced with animals of similar age and weight from the same vendor.

3.3. **Animal Husbandry:** Animals will be housed (grouped 5 per cage) in compliance with the National Research Council "Guide for the Care and Use of Laboratory Animals". Calvert is a USDA Registered and fully Accredited AAALAC Facility.

The animal room environment will be controlled (targeted conditions:

Temperature 18 to 26°C, relative humidity 30 to 70%, 12 hours artificial light and 12 hours dark). Temperatures and relative humidity will be monitored daily.

All animals will have access to Certified Rodent Diet #7012C (Harlan Teklad) or equivalent *ad libitum*, unless otherwise specified. The lot number(s) and specifications of each lot used will be archived at Calvert.

Water will be provided to the animals *ad libitum*, unless otherwise specified. Periodic analyses of the water are performed and the results are archived at Calvert.

There are no known contaminants in the diet or water which at the levels detected would be expected to interfere with the purpose, conduct or outcome of the study.

- 3.4. **Experimental Design:** Animals will be assigned to study groups using a computer randomization program/selection process. Animals will be assigned a permanent identification number at the time of randomization and then marked on the tail with an indelible marker. Animals will be assigned to the following groups:

Group	Treatment	Dose	Number of Animals
1	Vehicle	-	5
2	Test Article	10%	5
3	Test Article	25%	5
4	Test Article	50%	5
5	DNCB	0.1%	5

- 3.5. **Administration of the Test/Control Article:** The test/control article dosing solutions, will be applied once a day for 3 consecutive days to the dorsal surfaces of both ears of each mouse. A volume of 25 µl/ear will be used.
- 3.6. **Rationale for Route of Administration:** The dermal route was selected as this is the route required for this model of hypersensitivity.
- 3.7. **Selection of Dosage Levels:** The recommended doses for this assay are selected from the concentration series 100%, 50%, 25%, 10%, 5%, 2.5%, 1%,

0.5% etc. Three consecutive concentrations should be chosen, the top one being the highest level that can be achieved while avoiding systemic toxicity and excessive local irritation.

3.8. **Method of Performance:** Mice will be weighed on Days 1 and 6. Mice will be treated on the dorsal surface of both ears, once per day on Days 1, 2, and 3. Any irritation observed after test article application will be recorded. On Day 6 the mice will be injected i.v. with 20 μ C of 3 H-thymidine in 250 μ l. Five hours later the mice will be euthanized with CO₂ and the draining auricular lymph nodes removed. A single cell suspension will be prepared from the lymph nodes of each mouse. Cells will be washed twice with phosphate buffered saline (PBS) and precipitated with 5% trichloroacetic acid (TCA) overnight at 2-8°C. The pellets will be recovered by centrifugation and resuspended in 1 ml of TCA and transferred to 10 ml of scintillation fluid. Incorporation of 3 H-thymidine will be measured in a β -scintillation counter.

3.9. **Method of Euthanasia:** CO₂ asphyxiation

4. **OBSERVATIONS**

4.1. **Morbidity/Mortality:** Animals will be observed daily. Any animal found dead or judged to be in a moribund condition will be necropsied. Animals will be euthanized for humane reasons based on the criteria outlined in Calvert's SOP VET-14, "Criteria for Determination of Moribund Animals and for Sacrifice for Humane Reasons".

4.2. **Clinical Observations:** Cage side observations will be made daily.

5. **METHOD OF ANALYSIS**

5.1. **Parameters:** The mean DPM for each group will be determined. Increases in 3 H-thymidine incorporation relative to vehicle-treated control will be derived for each group and recorded as stimulation indices (SI). The criterion for a positive response is that one or more concentrations of a test article elicits a 3-fold or greater increase in isotope incorporation relative to the vehicle control.

5.2. **Statistical Method(s):** Using SYSTAT (version 9.01) evaluation of equality of means will be made by a one way analysis of variance using the F distribution to assess statistical significance. If statistically significant differences between

the means are found, a Dunnett's test will be used to determine the degree of significance from control means.

6. **ARCHIVING OF MATERIALS:** Test article preparation, test article tracking, in-life data, protocol, protocol amendments (if applicable), draft report(s) that have been submitted to a regulatory agency, and the original final report generated as a result of this study will be archived at Calvert Archiving Services, 105 Edella Road, Suite 100, Clarks Summit, PA 18411. After 5 years, the Sponsor will be contacted to determine final disposition of all study materials.

7. **ANIMAL WELFARE PROVISIONS:** This study will be conducted in accordance with the current guidelines for animal welfare. No alternative test systems exist which have been adequately validated to permit replacement of the use of live animals in this study. Every effort has been made to obtain the maximum amount of information while reducing to a minimum the number of animals required for this study. The assessment of pain and distress in study animals and the use or non-use of pain alleviating medications will be in accordance with Standard Operating Procedure VET-19, Criteria for Assessing Pain and Distress in Laboratory Animals. The study will be terminated in part or whole for humane reasons if unnecessary pain occurs. This study is not unnecessary or duplicative.

This protocol has been reviewed by the Institutional Animal Care and Use Committee (IACUC) and complies with acceptable standard animal welfare and humane care.

8. **CONFIDENTIALITY STATEMENT:** The information contained herein is for the personal use of the intended recipient(s).

9. **REFERENCE:** The Murine Local Lymph Node Assay: A Test Method for Assessing the Allergic Contact Dermatitis Potential of Chemicals/Compounds. NIH Publication No. 99-4494, Feb. 1999.

Study No.: 0787XA66.013

Local Lymph Node Assay

PROTOCOL APPROVAL:

Joan M. Chapdelaine

Study Director
Joan M. Chapdelaine, Ph.D.
Calvert Preclinical Services, Inc.

24 Sep 2002

Date

[Signature]

Scientific Management
Calvert Preclinical Services, Inc.

20 Sep 2002

Date

Brian Clark

IACUC
Calvert Preclinical Services, Inc.

12 Sep 2002

Date

Sang-tae Kim

Sponsor's Approval Signature

19 Sep 2002

Date

Sang-tae Kim

(Typewritten or Printed Name)



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PROTOCOL AMENDMENT

STUDY NUMBER: 0787XA66.013

STUDY TITLE: Local Lymph Node Assay

AMENDMENT NUMBER: 1

EFFECTIVE DATE: 21st Oct 2002
© JMC
29 Jan 2003

ORIGINAL PROTOCOL STATEMENT: 2.1 Test Article:
2.1.3.: Description: White powder

AMENDED PROTOCOL STATEMENT: 2.1 Test Article:
2.1.3.: Description: Clear, light yellow liquid

REASON FOR CHANGE: Test article description was incorrect in the original protocol
© JMC
29 Jan 2003

SIGNATURES:



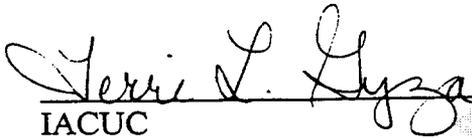
Joan M. Chapdelaine, Ph.D.
Study Director
Calvert Laboratories Inc.
(Formerly Calvert Preclinical Services, Inc.)

17 Jan 2003
Date



Scientific Management
Calvert Laboratories Inc.
(Formerly Calvert Preclinical Services, Inc.)

20 Jan 2003
Date



IACUC
Calvert Laboratories Inc.
(Formerly Calvert Preclinical Services, Inc.)

20 January 2003
Date

Sang-Tae Kim, Ph.D.
Applied Biosystems

Date

MATERIAL SAFETY DATA SHEET

SECTION 1 CHEMICAL PRODUCT AND COMPANY IDENTIFICATION

APPLIED BIOSYSTEMS
850 LINCOLN CENTRE DRIVE
FOSTER CITY, CA 94404
(650) 570-6667 (USA)
01925-825650 (UK)

24 HOUR EMERGENCY RESPONSE NUMBER:
1-800-424-9300 (NORTH AMERICA)
1-703-527-3887 (INTERNATIONAL)

SUBSTANCE: 2-CYANOETHYL N,N,N',N'-TETRAISOPROPYLPHOSPHORDIAMIDITE

TRADE NAMES/SYNONYMS:
MSDS P/N 902903; P/N 360154; CE PHOSPHITE; ABI33143

PRODUCT USE: process chemical

CREATION DATE: Oct 06 1993
REVISION DATE: Feb 19 2003

SECTION 2 COMPOSITION, INFORMATION ON INGREDIENTS

COMPONENT: 2-CYANOETHYL N,N,N',N'-TETRAISOPROPYLPHOSPHORDIAMIDITE
CAS NUMBER: 102691-36-1
PERCENTAGE: 100

SECTION 3 HAZARDS IDENTIFICATION

NFPA RATINGS (SCALE 0-4): HEALTH=2 FIRE=2 REACTIVITY=3

EMERGENCY OVERVIEW:

COLOR: colorless to pale yellow

PHYSICAL FORM: liquid

MAJOR HEALTH HAZARDS: respiratory tract irritation, skin irritation, eye irritation,
allergic reactions

PHYSICAL HAZARDS: May explode when heated. Combustible liquid and vapor. May
decompose on contact with water or moist air.

POTENTIAL HEALTH EFFECTS:

INHALATION:

SHORT TERM EXPOSURE: irritation (possibly severe), allergic reactions, loss
of voice, difficulty breathing

LONG TERM EXPOSURE: no information is available

SKIN CONTACT:

SHORT TERM EXPOSURE: irritation, allergic reactions

LONG TERM EXPOSURE: irritation

EYE CONTACT:

SHORT TERM EXPOSURE: irritation (possibly severe)

LONG TERM EXPOSURE: irritation

INGESTION:

SHORT TERM EXPOSURE: irritation, nausea, vomiting, stomach pain

LONG TERM EXPOSURE: no information is available

SECTION 4	FIRST AID MEASURES
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INHALATION: If adverse effects occur, remove to uncontaminated area. Give artificial respiration if not breathing. Get immediate medical attention.

SKIN CONTACT: Wash skin with soap and water for at least 15 minutes while removing contaminated clothing and shoes. Get medical attention, if needed. Thoroughly clean and dry contaminated clothing and shoes before reuse.

EYE CONTACT: Flush eyes with plenty of water for at least 15 minutes. Then get immediate medical attention.

INGESTION: If a large amount is swallowed, get medical attention.

SECTION 5	FIRE FIGHTING MEASURES
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FIRE AND EXPLOSION HAZARDS: Moderate fire hazard. Vapor/air mixtures are explosive above flash point.

EXTINGUISHING MEDIA: carbon dioxide, regular dry chemical, regular foam, water

FIRE FIGHTING: Move container from fire area if it can be done without risk. Cool containers with water spray until well after the fire is out. Flood with fine water spray. Do not scatter spilled material with high-pressure water streams. Avoid inhalation of material or combustion by-products. Stay upwind and keep out of low areas.

FLASH POINT: 142 F (61 C)

FLAMMABILITY CLASS (OSHA): IIIA

SECTION 6 ACCIDENTAL RELEASE MEASURES**OCCUPATIONAL RELEASE:**

Avoid heat, flames, sparks and other sources of ignition. Stop leak if possible without personal risk. Reduce vapors with water spray. Small spills: Absorb with sand or other non-combustible material. Collect spilled material in appropriate container for disposal.

SECTION 7 HANDLING AND STORAGE

STORAGE: Store and handle in accordance with all current regulations and standards. Store in a tightly closed container. Store below 10 C. Store under an inert atmosphere. Keep dry. Subject to storage regulations: U.S. OSHA 29 CFR 1910.106. Grounding and bonding required. Keep separated from incompatible substances.

SECTION 8 EXPOSURE CONTROLS, PERSONAL PROTECTION**EXPOSURE LIMITS:****2-CYANOETHYL N,N,N',N'-TETRAISOPROPYLPHOSPHORDIAMIDITE:**

No occupational exposure limits established.

VENTILATION: Ventilation equipment should be explosion-resistant if explosive concentrations of material are present. Provide local exhaust ventilation system. Ensure compliance with applicable exposure limits.

EYE PROTECTION: Wear splash resistant safety goggles with a faceshield. Provide an emergency eye wash fountain and quick drench shower in the immediate work area.

CLOTHING: Wear appropriate chemical resistant clothing.

GLOVES: Wear appropriate chemical resistant gloves.

PROTECTIVE MATERIAL TYPES: neoprene, rubber

RESPIRATOR: Under conditions of frequent use or heavy exposure, respiratory protection may be needed. Respiratory protection is ranked in order from minimum to maximum. Consider warning properties before use.
Any chemical cartridge respirator with organic vapor cartridge(s).
Any chemical cartridge respirator with a full facepiece and organic vapor cartridge(s).
Any air-purifying respirator with a full facepiece and an organic vapor canister.

For Unknown Concentrations or Immediately Dangerous to Life or Health -
Any supplied-air respirator with full facepiece and operated in a
pressure-demand or other positive-pressure mode in combination with a
separate escape supply.
Any self-contained breathing apparatus with a full facepiece.

SECTION 9	PHYSICAL AND CHEMICAL PROPERTIES
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PHYSICAL STATE: liquid
APPEARANCE: cloudy
COLOR: colorless to pale yellow
ODOR: Not available
MOLECULAR WEIGHT: 301.41
MOLECULAR FORMULA: (((C-H3)2-C-H)2-N)2-P-O-C-H2-C-H2-C-N
BOILING POINT: 212 F (100 C) @ 0.5 mmHg
FREEZING POINT: Not available
VAPOR PRESSURE: Not available
VAPOR DENSITY: Not available
SPECIFIC GRAVITY (water=1): 0.949
WATER SOLUBILITY: immiscible
PH: Not available
VOLATILITY: Not available
ODOR THRESHOLD: Not available
EVAPORATION RATE: Not available
COEFFICIENT OF WATER/OIL DISTRIBUTION: Not available

SECTION 10	STABILITY AND REACTIVITY
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REACTIVITY: May explode when heated. May decompose on contact with water or moist air.

CONDITIONS TO AVOID: Avoid heat, flames, sparks and other sources of ignition.
Containers may rupture or explode if exposed to heat. Keep out of water supplies and sewers.

INCOMPATIBILITIES: bases, oxidizing materials

HAZARDOUS DECOMPOSITION:

Thermal decomposition products: oxides of carbon, oxides of nitrogen, oxides of phosphorus, phosphine

POLYMERIZATION: Will not polymerize.

SECTION 11 TOXICOLOGICAL INFORMATION

2-CYANOETHYL N,N,N',N'-TETRAISOPROPYLPHOSPHORDIAMIDITE:

LOCAL EFFECTS:

Irritant: inhalation, skin, eye

ADDITIONAL DATA: The material induced a hypersensitivity response in mice as measured by the proliferation of lymphocytes in the draining lymph nodes (Local Lymph Node Assay).

Additional toxicological data is available on the component(s) of this product. Please call 650 554-2860 or contact hazcom@appliedbiosystems.com for more information.

SECTION 12 ECOLOGICAL INFORMATION

Not available

SECTION 13 DISPOSAL CONSIDERATIONS

Dispose in accordance with all applicable regulations. Subject to disposal regulations: U.S. EPA 40 CFR 262. Hazardous Waste Number(s): D003.

SECTION 14 TRANSPORT INFORMATION

U.S. DOT 49 CFR 172.101:

PROPER SHIPPING NAME: Combustible liquid, n.o.s. (2-CYANOETHYL N,N,N',N'-TETRAISOPROPYLPHOSPHORDIAMIDITE)

ID NUMBER: NA1993

HAZARD CLASS OR DIVISION: Combustible liquid

PACKING GROUP: III

CANADIAN TRANSPORTATION OF DANGEROUS GOODS: No classification assigned.

LAND TRANSPORT ADR: No classification assigned.

LAND TRANSPORT RID: No classification assigned.

AIR TRANSPORT IATA: No classification assigned.

AIR TRANSPORT ICAO: No classification assigned.

MARITIME TRANSPORT IMDG: No classification assigned.

U.S. REGULATIONS:

SARA TITLE III SECTION 302 EXTREMELY HAZARDOUS SUBSTANCES (40 CFR 355.30):
Not regulated.

SARA TITLE III SECTION 304 EXTREMELY HAZARDOUS SUBSTANCES (40 CFR 355.40):
Not regulated.

SARA TITLE III SARA SECTIONS 311/312 HAZARDOUS CATEGORIES (40 CFR 370.21):
ACUTE: Yes
CHRONIC: No
FIRE: Yes
REACTIVE: Yes
SUDDEN RELEASE: No

SARA TITLE III SECTION 313 (40 CFR 372.65): Not regulated.

OSHA PROCESS SAFETY (29CFR1910.119): Not regulated.

STATE REGULATIONS:

California Proposition 65: Not regulated.

CANADIAN REGULATIONS:

WHMIS CLASSIFICATION: This product has been classified in accordance with the hazard criteria of the CPR and the MSDS contains all of the information required by the CPR.

NATIONAL INVENTORY STATUS:

U.S. INVENTORY (TSCA): Not listed on inventory. This product is sold for Research and Development Use Only.

TSCA 12(b) EXPORT NOTIFICATION: Not listed.

SECTION 15	REGULATORY INFORMATION
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