TRADE SECRET

Study Title

USP Biocompatibility Testing of H-22239 (Control Item)
and H-22240 (Test Item)

Contracting Laboratory

North American Science Associates, Inc.
2261 Tracy Road
Northwood, Ohio 43619-1397

for

E. I. du Pont de Nemours and Company
Haskell Laboratory for Toxicology and Industrial Medicine
Elkton Road, P.O. Box 50
Newark, Delaware 19714

Study Monitor

Susan A. MacKenzie, V.M.D., Ph.D., D.A.B.T.

Study Completed on

January 17, 1997

[redacted]

Laboratory Project ID

Haskell Laboratory Report No. HLO-1997-00026

GENERAL INFORMATION

Test Substance #1

Substance Tested: [redacted]

Synonyms/Other Codes: H-22239
H-22239 (Control Item)

NAmSA Lab. No.: 96T-16837-00

Physical Form: Blue fabric

Purity: [redacted]

Composition: [redacted]

Contaminants: [redacted]

CAS Registry No.: [redacted]

Test Substance #2

Substance Tested: [redacted]

Synonyms/Other Codes: H-22240
H-22240 (Test Item)

NAmSA Lab. No.: 96T-16836-00

Physical Form: Blue fabric

Purity: [redacted]

Composition: [redacted]

Contaminants: [redacted]

CAS Registry No.: [redacted]

Study Initiated-Completed: 12/02/96 - 01/17/97

DuPont Central Research and Development
Haskell Laboratory for Toxicology
and Industrial Medicine

January 17, 1997

[redacted]
DuPont Non-Woven
Old Hickory, Tennessee

USP Biocompatibility Testing of H-22239 (Control Item)
and H-22240 (Test Item)

Attached you will find reports from North American Science Associates, Inc.

The results for the H-22239 and H-22240 samples are:

		Test Results		
Test Substance	Cytotoxicity (Agarose Overlay)	USP Intracutaneous SC[a]	USP Intracutaneous CSO[b]	USP Muscle Implantation (7 day)
H-22239	Non-toxic	Pass	Pass	NS[c]
H-22240	Non-toxic	Pass	Pass	NS

a Sodium chloride extract
b Cotton Seed oil extract
c Not significant

Susan A. MacKenzie 1/17/97
Susan A. MacKenzie, V.M.D., Ph.D., D.A.B.T.
Research Toxicologist

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MG030-110

Lab No. 96T 16837 00
P.O. No. LGX-593521

ID No. H-22239

E. I. du Pont de Nemours & Co.
HASKELL LABORATORY
ELKTON RD. P.O. BOX 50
NEWARK, DE 19714-0050
ATTN: DR. SUSAN A. MACKENZIE

CYTOTOXICITY - AGAROSE OVERLAY, SOLID

Test Article: H-22239 (control item)

Test Article Description: Sheet - 1 cm^2 piece

Experimental Procedure: A monolayer of L-929 mouse fibroblast cells was grown to confluency and overlaid with Minimum Essential Medium supplemented with serum, antibiotics, neutral red, and 2% agarose. The test article, a 0.5 cm x 0.5 cm piece of 725871H as a positive control, and a 1.0 cm length piece of UP-1 as a negative control were placed on the solidified overlay surface. Following incubation for 24 hours, the culture was macroscopically examined for evidence of cell decolorization to determine the zone of cell lysis. Any decolorized zone present was examined microscopically to confirm cell lysis.

Score	Observations
Nontoxic (N)	Normal cell morphology in proximity to test sample.
Toxic (T)	Cellular death and degeneration associated with the area beneath the test sample and possibly extended beyond the perimeter of the test sample. Where a zone of lysis was observed, the distance from the edge of the sample to the edge of the zone was measured and reported in millimeters (mm).

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CYTOTOXICITY - AGAROSE OVERLAY, SOLID

Results:	Test/Control Articles	Score	Zone of Lysis (mm)
	Test Article	N	0
	Negative Control: UP-1	N	0
	NAmSA Positive Control: 725871H	T	7

Results and conclusions apply only to the test article tested. No further evaluation of these results is made by NAmSA. Any extrapolation of these data to other samples is the responsibility of the sponsor.

Conclusion: The above test article was nontoxic for L-929 mouse fibroblast cells under the above described test parameters.

Comments: Placed the bluest side of the test article down on agarose.

Record Storage: All raw data pertaining to this study and a copy of the final report are to be retained in designated NAmSA archive files.

Test Article Received: 12-5-96

Date Prepared: 12-11-96 Date Terminated: 12-12-96

Company Sanitized. Does not contain TSCA CBI

mm
bsj

Date Completed 12-17-96

Approved By Barry Phelps, BS

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Lab No. 96T 16837 00

TU013-800

P.O. No. LGX-593521

STUDY TITLE:

USP INTRACUTANEOUS TOXICITY STUDY IN THE RABBIT

(Extracts)

TEST ARTICLE:

H-22239 (control item)

IDENTIFICATION NO.:

H-22239

SPONSOR:

DR. SUSAN A. MACKENZIE
E. I. du Pont de Nemours & Co.
HASKELL LABORATORY
ELKTON RD. P.O. BOX 50
NEWARK, DE 19714-0050

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SUMMARY

The test article, H-22239 (control item), H-22239, was extracted in 0.9% sodium chloride USP solution and cottonseed oil, NF. These extracts were evaluated for intracutaneous toxicity in accordance with the guidelines of the current USP.

A 0.2 ml dose of the appropriate test article extract was injected by the intracutaneous route into five separate sites on the right side of the back of each rabbit. Similarly, the corresponding blank vehicle was injected on the left side of the back of each rabbit. Two rabbits were used for each pair of extracts. Observations for erythema and edema were conducted at 24, 48, and 72 hours after intracutaneous injection.

Under the conditions of this study, there was no evidence of significant irritation or toxicity from the extracts injected intracutaneously into rabbits. Each test article extract met the USP requirements.

Study and Supervisory Personnel:

Donald P. Barda, LAT
Michael A. Safron, AAS (HT)
Patricia Mitchell
Barbara M. Monroe
Darcy Irons
Cindy Harper
Tina M. Cox, BS, LAT

Approved by:

Steven J. Hermansky, PharmD, PhD, DABT
Manager, Toxicology

December 23, 1996
Date Completed

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/las
bs)

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INTRODUCTION

The test article identified below was extracted and the extracts were evaluated for biocompatibility in accordance with the guidelines of the current USP. The purpose of the study was to determine whether leachables extracted from the material would cause local dermal irritant or toxic effects following injection into rabbit skin. The test article was received on December 5, 1996. The animals were injected on December 18, 1996, and the observations were concluded on December 21, 1996.

MATERIALS

The sample provided by the sponsor was identified and handled as follows:

Test Article:	H-22239 (control item)
Identification No:	H-22239
Storage Conditions:	Room temperature
Vehicles:	0.9% sodium chloride USP solution (SC) cottonseed oil, NF (CSO)
Preparation:	A ratio of 120 cm^2:20 ml (surface area of test article to volume of vehicle) was used for each preparation. The test article was extracted in SC and CSO at 121°C for 1 hour. The extraction vehicles without test article were similarly prepared to serve as control blanks.

Condition of Extracts:	Test	Control
SC:	clear	clear
CSO:	clear	clear

Sample Disposition: Any remaining sample was discarded.

METHODS

Test System:

Species:	Rabbit (Oryctolagus cuniculus)
Breed:	New Zealand White
Source:	Myrtle's Rabbitry, Inc.
Sex:	No particular gender was prescribed for this test
Body Weight Range:	No particular body weight range was prescribed for this test
Age:	No particular age was prescribed for this test
Acclimation Period:	Minimum 2 days
Number of Animals:	Two per pair of extracts
Identification Method:	Ear tag or tattoo

Justification of Test System:

The intracutaneous injection test in rabbits is specified in the current USP and has been used historically to evaluate biomaterial extracts.

Animal Management:

Husbandry:	Conditions conformed to Standard Operating Procedures which are based on the "Guide for the Care and Use of Laboratory Animals."
Food:	PROLAB® High Fiber Rabbit Diet was provided daily.

Water: Freely available, municipal (Toledo, OH) water was delivered through an automatic watering system.

Contaminants: Reasonably expected contaminants in feed or water supplies did not have the potential to influence the outcome of this test.

Housing: Animals were individually housed in stainless steel cages identified by a card indicating the lab number, animal number, test code, sex, and date on test.

Environmental: The room temperature was monitored daily. The temperature range for the rabbit was 64-74°F.

The room humidity was monitored daily. The humidity range for the rabbit was 30-70%.

The light cycle was controlled using an automatic timer (12 hours light, 12 hours dark).

Facility: NAmSA is an AAALAC accredited facility.

Personnel: Associates involved were appropriately qualified and trained.

Selection: Only healthy, thin-skinned animals free of mechanical irritation or trauma that could interfere with the test were selected.

Experimental Procedure:

Prior to treatment, each rabbit was clipped free of fur from the back and both sides of the spinal column to yield a sufficient injection area. Two rabbits were prepared per pair of extracts. A 0.2 ml dose of test article extract was injected intracutaneously into five separate sites on the right side of the back of each rabbit; 0.2 ml of the control blank was injected into five separate sites on the corresponding left side of the back. Injections were spaced approximately 2 cm apart. The animals were returned to their respective cages following the procedure.

Observations for erythema and edema were conducted at 24, 48, and 72 hours after injection. Reactions were scored on a 0 to 4 basis. Any reaction at the injection sites was also noted. The reactions were evaluated according to the following subjective rating scale:

ERYTHEMA (ER)		EDEMA (ED)	
0	No erythema	0	No edema
1	Very slight (barely perceptible)	1	Very slight (barely perceptible)
2	Well-defined (pink)	2	Slight (edges of area well-defined by definite swelling)
3	Moderate to severe (red)	3	Moderate (raised approximately 1 mm)
4	Severe (beet redness) to slight eschar formation (injuries in depth)	4	Severe (raised more than 1 mm, may extend beyond the area of exposure)

The cumulative average erythema and edema score for each test extract and corresponding control blank was calculated. For each extract, a difference in average scores (test minus control blank) of 1.0 or less was considered to be acceptable. A difference of 0.6 to 1.0 indicated a slight, but acceptable, reaction. A difference > 1.0 was considered to be unacceptable. Additionally, the average score for each test extract and blank was calculated for each interval. Any adverse reaction noted in the test extract was compared to the corresponding blank.

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RESULTS

Results of scores for individual rabbits appear in Table I. The findings are summarized below:

Extract	Average Test (-) Average Control = Difference = Assessment			
SC	0.0	0.0	0.0	Passed
CSO	1.0	1.0	0.0	Passed

The difference in average scores was considered acceptable. The difference between test and control average scores did not exceed 1.0 at any observation interval.

Results and conclusions apply only to the test article tested. No further evaluation of these results is made by NAmSA. Any extrapolation of these data to other samples is the responsibility of the sponsor.

CONCLUSION

Under the conditions of this study, there was no evidence of significant irritation or toxicity from the extracts injected intracutaneously into rabbits. Each test article extract met the USP requirements.

RECORD STORAGE

All raw data pertaining to this study and a copy of the final report are to be retained in designated NAmSA archive files.

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TABLE I

USP INTRACUTANEOUS TOXICITY OBSERVATIONS

RABBIT NUMBER	EXTRACT	TEST EXTRACT						CONTROL BLANK					
		Scoring Interval (Hours)						Scoring Interval (Hours)					
		24		48		72		24		48		72	
		ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED
99558	SC	0	0	0	0	0	0	0	0	0	0	0	0
99553	SC	0	0	0	0	0	0	0	0	0	0	0	0
99558	CSO	1	1	1	1	1	1	1	1	1	1	1	1
99553	CSO	1	1	1	1	1	1	1	1	1	1	1	1

ER = Erythema
ED = Edema

SC = 0.9% sodium chloride USP solution
CSO = cottonseed oil, NF

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FE014 807

HLO-1997-00026

Lab No. 96T 16837 00

P.O. No LGX 593521

STUDY TITLE:

USP MUSCLE IMPLANTATION STUDY IN THE RABBIT

(1 Week)

TEST ARTICLE:

H-22239 (control item)

IDENTIFICATION NO.:

H-22239

SPONSOR:

DR. SUSAN A. MACKENZIE
E. I. du Pont de Nemours & Co.
HASKELL LABORATORY
ELKTON RD. P.O. BOX 50
NEWARK, DE 19714-0050

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SUMMARY

The test article, H-22239 (control item), H-22239, was implanted in muscle tissue of the rabbit. The muscle tissue was evaluated for evidence of irritation or toxicity in accordance with the guidelines of the current USP.

Implant samples and USP negative control strips were loaded into needles and sterilized by steam. Rabbits were implanted and were then euthanatized 1 week later. Muscle tissues were excised and the implant sites were examined macroscopically.

Under the conditions of this study, the macroscopic reaction was not significant as compared to the USP negative control implant material. The implanted test article met the USP requirements.

Study and Supervisory Personnel:

Martha Oswanski, BS, LAT
Merle J. Heineke, BS, LAT
Jason A. Karsten
Tina M. Cox, BS, LAT

Approved by:

Steven J. Hermansky, PharmD, PhD, DABT
Manager, Toxicology

December 27, 1996
Date Completed

/mm
bsj

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INTRODUCTION

The test article identified below was evaluated for biocompatibility in accordance with the guidelines of the current USP. The purpose of the study was to evaluate the potential for a local irritant or toxic response to material implanted in direct contact with muscle tissue. The test article was received on December 5, 1996. The animals were implanted on December 17, 1996, and muscles were explanted on December 24, 1996.

MATERIALS

The sample provided by the sponsor was identified and handled as follows:

Test Article:	H-22239 (control item)
Identification No:	H-22239
Storage Conditions:	Room temperature
Control Article:	USP plastic 1 mm diameter negative control reference strips purchased by NAmSA from the offices of the US Pharmacopeial Convention and cut into 10 mm sections.
Preparation:	A minimum of six sections of the test article per rabbit, each approximately 1 mm x 10 mm, were loaded into 16 gauge needles. The test article was cut into 1 mm. x 20 mm lengths and then folded in half to obtain 1 mm x 10 mm sections. For each rabbit, a minimum of four USP strips were loaded into the same size needles as used for the test article. Test and control articles were sterilized by steam.
Sample Disposition:	Any remaining sample was discarded.

METHODS

Test System:

Species:	Rabbit (Oryctolagus cuniculus)
Breed:	New Zealand White
Source:	Myrtle's Rabbitry, Inc.
Sex:	No particular gender was prescribed for this test
Body Weight Range:	3.2 kg to 3.3 kg at implantation
Age:	No particular age was prescribed for this test
Acclimation Period:	Minimum 2 days
Number of Animals:	Two
Identification Method:	Ear tag or tattoo

Justification of Test System:

The rabbit is suggested as an appropriate animal model for evaluating polymer materials by the current USP testing guidelines. The muscle tissue has been used historically because the response to implanted material is easily graded and compared to a known negative control material.

Animal Management:

Husbandry:	Conditions conformed to Standard Operating Procedures which are based on the "Guide for the Care and Use of Laboratory Animals."
Food:	PROLAB® High Fiber Rabbit Diet was provided daily.

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TU014-807

Water: Freely available, municipal (Toledo, OH) water was delivered through an automatic watering system.

Contaminants: Reasonably expected contaminants in feed or water supplies did not have the potential to influence the outcome of this test.

Housing: Animals were individually housed in stainless steel suspended cages identified by a card indicating the lab number, animal number, test code, sex, and date implanted.

Environmental: The room temperature was monitored daily. The temperature range for the rabbit was 64-74°F.

The room humidity was monitored daily. The humidity range for the rabbit was 30-70%.

The light cycle was controlled using an automatic timer (12 hours light, 12 hours dark).

Facility: NAmSA is an AAALAC accredited facility.

Personnel: Associates involved were appropriately qualified and trained.

Selection: Healthy, previously unused animals were selected.

Experimental Procedure:

The fur was clipped from the area over the paravertebral muscles and the rabbits were sedated with acepromazine maleate (0.2 ml/kg dose). After the muscle area was wiped clean with 70% alcohol, each paravertebral muscle was infiltrated with 1% lidocaine to achieve local anesthesia on both sides of the vertebral column.

Needles containing test article were inserted into the muscle at a 45° angle. A stylet was placed in the needle and the needle was withdrawn, leaving the sample in the paravertebral muscle. Six test article sections were implanted in the right paravertebral muscle of each rabbit. In the opposite muscle, four USP negative control strips were similarly implanted.

Rabbits were returned to their respective cages following the procedure. Rabbits were observed daily for general health. Body weights were recorded prior to implantation and at termination.

At 1 week, the rabbits were weighed and euthanatized by an intravenous injection of a sodium pentobarbital based drug. The paravertebral muscles were dissected free and methodically cut to locate four of the test article sites and two of the USP negative control sites in each rabbit. Capsule formation or other evidence of irritation was scored using appropriate magnification and an auxiliary light source. Scores were assigned and recorded as follows:

0 No capsule, no adverse reaction (other than minimal hemorrhage)
1 Up to 0.5 mm capsule or reaction area
2 0.6 to 1.0 mm capsule or reaction area
3 1.1 to 2.0 mm capsule or reaction area
4 >2.0 mm capsule or reaction area

Average macroscopic scores for test implants were compared to average scores of the control sites. Calculations were rounded off to the nearest 0.1. A Reaction Index difference of 0.0 to 0.5 in scores (test minus control) was regarded as "not significant," 0.6 to 1.0 as a "trace," 1.1 to 2.0 as "slight," 2.1 to 3.0 as "moderate" and ≥3.1 as "marked." The requirements of the USP test were met if the difference between test and control score averages (macroscopic) was not greater than 1.0. The requirements of the test were not met if the difference between the test and control score for two (or more) implant sites exceeded 1 for any animal implanted.

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RESULTS

Clinical Observations: All animals appeared clinically normal throughout the duration of the study. Body weight data for individual rabbits were considered acceptable.

Macroscopic Observations: The findings for the macroscopic evaluation are shown below.

Rabbit Number	Weight (kg)		Site	Test	USP Negative Control
	Day 0	Day 7			
99472	3.2	3.3	1 2 3 4	0 0 0 0	0 0
99474	3.3	3.6	1 2 3 4	0 0 0 0	0 0
			Average:	0.0	0.0

Macroscopically, there was no difference between the reactions of the test and control materials. This resulted in a macroscopic reaction of "not significant" tissue contact irritation.

Results and conclusions apply only to the test article tested. No further evaluation of these results is made by NAmSA. Any extrapolation of these data to other samples is the responsibility of the sponsor.

CONCLUSION

Under the conditions of this study, the macroscopic reaction was not significant as compared to the USP negative control implant material. The implanted test article met the USP requirements.

RECORD STORAGE

All raw data pertaining to this study and a copy of the final report are to be retained in designated NAmSA archive files.

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Lab No. 96T 16836 00
P.O. No. LGX-593521

ID No. H-22240

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ATTN: DR. SUSAN A. MACKENZIE

CYTOTOXICITY - AGAROSE OVERLAY, SOLID

Test Article: H-22240 (test item)

Test Article Description: Sheet - 1 cm^2 piece

Experimental Procedure: A monolayer of L-929 mouse fibroblast cells was grown to confluency and overlaid with Minimum Essential Medium supplemented with serum, antibiotics, neutral red, and 2% agarose. The test article, a 0.5 cm x 0.5 cm piece of 725871H as a positive control, and a 1.0 cm length piece of UP-1 as a negative control were placed on the solidified overlay surface. Following incubation for 24 hours, the culture was macroscopically examined for evidence of cell decolorization to determine the zone of cell lysis. Any decolorized zone present was examined microscopically to confirm cell lysis.

Score	Observations
Nontoxic (N)	Normal cell morphology in proximity to test sample.
Toxic (T)	Cellular death and degeneration associated with the area beneath the test sample and possibly extended beyond the perimeter of the test sample. Where a zone of lysis was observed, the distance from the edge of the sample to the edge of the zone was measured and reported in millimeters (mm).

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Lab No. 96T 16836 00

CYTOTOXICITY - AGAROSE OVERLAY, SOLID

Results:	Test/Control Articles	Score	Zone of Lysis (mm)
	Test Article	N	0
	Negative Control: UP-1	N	0
	NAmSA Positive Control: 725871H	T	6

Results and conclusions apply only to the test article tested. No further evaluation of these results is made by NAmSA. Any extrapolation of these data to other samples is the responsibility of the sponsor.

Conclusion: The above test article was nontoxic for L-929 mouse fibroblast cells under the above described test parameters.

Comments: Placed the bluest side of the test article down on agarose.

Record Storage: All raw data pertaining to this study and a copy of the final report are to be retained in designated NAmSA archive files.

Test Article Received: 12-5-96

Date Prepared: 12-11-96 Date Terminated: 12-12-96

Company Sanitized. Does not contain TSCA CBI

mm
bsj

Date Completed 12-17-96

Approved By Barry Phelps, BS

Confidential

Lab No. 96T 16836 00

TU013-800

P.O. No. LGX-593521

STUDY TITLE:

USP INTRACUTANEOUS TOXICITY STUDY IN THE RABBIT

(Extracts)

TEST ARTICLE:

H-22240 (test item)

IDENTIFICATION NO.:

H-22240

SPONSOR:

DR. SUSAN A. MACKENZIE
E. I. du Pont de Nemours & Co.
HASKELL LABORATORY
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NEWARK, DE 19714-0050

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SUMMARY

The test article, H-22240 (test item), H-22240, was extracted in 0.9% sodium chloride USP solution and cottonseed oil, NF. These extracts were evaluated for intracutaneous toxicity in accordance with the guidelines of the current USP.

A 0.2 ml dose of the appropriate test article extract was injected by the intracutaneous route into five separate sites on the right side of the back of each rabbit. Similarly, the corresponding blank vehicle was injected on the left side of the back of each rabbit. Two rabbits were used for each pair of extracts. Observations for erythema and edema were conducted at 24, 48, and 72 hours after intracutaneous injection.

Under the conditions of this study, there was no evidence of significant irritation or toxicity from the extracts injected intracutaneously into rabbits. Each test article extract met the USP requirements.

Study and Supervisory Personnel:

Donald P. Barda, LAT
Michael A. Safron, AAS (HT)
Patricia Mitchell
Barbara M. Monroe
Darcy Irons
Cindy Harper
Tina M. Cox, BS, LAT

Approved by:

Steven J. Hermansky, PharmD, PhD, DABT
Manager, Toxicology

December 23, 1996
Date Completed

Company Sanitized. Does not contain TSCA CBI

/las

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INTRODUCTION

The test article identified below was extracted and the extracts were evaluated for biocompatibility in accordance with the guidelines of the current USP. The purpose of the study was to determine whether leachables extracted from the material would cause local dermal irritant or toxic effects following injection into rabbit skin. The test article was received on December 5, 1996. The animals were injected on December 18, 1996, and the observations were concluded on December 21, 1996.

MATERIALS

The sample provided by the sponsor was identified and handled as follows:

Test Article:	H-22240 (test item)
Identification No:	H-22240
Storage Conditions:	Room temperature
Vehicles:	0.9% sodium chloride USP solution (SC) cottonseed oil, NF (CSO)
Preparation:	A ratio of 120 cm^2:20 ml (surface area of test article to volume of vehicle) was used for each preparation. The test article was extracted in SC and CSO at 121°C for 1 hour. The extraction vehicles without test article were similarly prepared to serve as control blanks.

Condition of Extracts:		Test	Control
	SC:	clear	clear
	CSO:	clear	clear

Sample Disposition: Any remaining sample was discarded.

METHODS

Test System:

Species:	Rabbit (Oryctolagus cuniculus)
Breed:	New Zealand White
Source:	Myrtle's Rabbitry, Inc.
Sex:	No particular gender was prescribed for this test
Body Weight Range:	No particular body weight range was prescribed for this test
Age:	No particular age was prescribed for this test
Acclimation Period:	Minimum 2 days
Number of Animals:	Two per pair of extracts
Identification Method:	Ear tag or tattoo

Justification of Test System:

The intracutaneous injection test in rabbits is specified in the current USP and has been used historically to evaluate biomaterial extracts.

Animal Management:

Husbandry:	Conditions conformed to Standard Operating Procedures which are based on the "Guide for the Care and Use of Laboratory Animals."
Food:	PROLAB® High Fiber Rabbit Diet was provided daily.

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Water: Freely available, municipal (Toledo, OH) water was delivered through an automatic watering system.

Contaminants: Reasonably expected contaminants in feed or water supplies did not have the potential to influence the outcome of this test.

Housing: Animals were individually housed in stainless steel suspended cages identified by a card indicating the lab number, animal number, test code, sex, and date dosed.

Environmental: The room temperature was monitored daily. The temperature range for the rabbit was 64-74°F.

The room humidity was monitored daily. The humidity range for the rabbit was 30-70%.

The light cycle was controlled using an automatic timer (12 hours light, 12 hours dark).

Facility: NAmSA is an AAALAC accredited facility.

Personnel: Associates involved were appropriately qualified and trained.

Selection: Only healthy, thin-skinned animals free of mechanical irritation or trauma that could interfere with the test were selected.

Experimental Procedure:

Prior to treatment, each rabbit was clipped free of fur from the back and both sides of the spinal column to yield a sufficient injection area. Two rabbits were prepared per pair of extracts. A 0.2 ml dose of test article extract was injected intracutaneously into five separate sites on the right side of the back of each rabbit; 0.2 ml of the control blank was injected into five separate sites on the corresponding left side of the back. Injections were spaced approximately 2 cm apart. The animals were returned to their respective cages following the procedure.

Observations for erythema and edema were conducted at 24, 48, and 72 hours after injection. Reactions were scored on a 0 to 4 basis. Any reaction at the injection sites was also noted. The reactions were evaluated according to the following subjective rating scale:

ERYTHEMA (ER)		EDEMA (ED)	
0	No erythema	0	No edema
1	Very slight (barely perceptible)	1	Very slight (barely perceptible)
2	Well-defined (pink)	2	Slight (edges of area well-defined by definite swelling)
3	Moderate to severe (red)	3	Moderate (raised approximately 1 mm)
4	Severe (beet redness) to slight eschar formation (injuries in depth)	4	Severe (raised more than 1 mm, may extend beyond the area of exposure)

The cumulative average erythema and edema score for each test extract and corresponding control blank was calculated. For each extract, a difference in average scores (test minus control blank) of 1.0 or less was considered to be acceptable. A difference of 0.6 to 1.0 indicated a slight, but acceptable, reaction. A difference >1.0 was considered to be unacceptable. Additionally, the average score for each test extract and blank was calculated for each interval. Any adverse reaction noted in the test extract was compared to the corresponding blank.

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RESULTS

Results of scores for individual rabbits appear in Table I. The findings are summarized below:

Extract	Average Test (-) Average Control = Difference = Assessment			
SC	0.0	0.0	0.0	Passed
CSO	1.0	1.0	0.0	Passed

The difference in average scores was considered acceptable. The difference between test and control average scores did not exceed 1.0 at any observation interval.

Results and conclusions apply only to the test article tested. No further evaluation of these results is made by NAmSA. Any extrapolation of these data to other samples is the responsibility of the sponsor.

CONCLUSION

Under the conditions of this study, there was no evidence of significant irritation or toxicity from the extracts injected intracutaneously into rabbits. Each test article extract met the USP requirements.

RECORD STORAGE

All raw data pertaining to this study and a copy of the final report are to be retained in designated NAmSA archive files.

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TABLE I

USP INTRACUTANEOUS TOXICITY OBSERVATIONS

RABBIT NUMBER	EXTRACT	TEST EXTRACT						CONTROL BLANK					
		Scoring Interval (Hours)						Scoring Interval (Hours)					
		24		48		72		24		48		72	
		ER	ED	ER	ED	ER	ED	ER	ED	ER	ED	ER	ED
99555	SC	0	0	0	0	0	0	0	0	0	0	0	0
99557	SC	0	0	0	0	0	0	0	0	0	0	0	0
99555	CSO	1	1	1	1	1	1	1	1	1	1	1	1
99557	CSO	1	1	1	1	1	1	1	1	1	1	1	1

ER = Erythema
ED = Edema

SC = 0.9% sodium chloride USP solution
CSO = cottonseed oil, NF

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Confidential

Lab No. 96T 16836 00

TU014-807

P.O. No. LGX-593521

STUDY TITLE:

USP MUSCLE IMPLANTATION STUDY IN THE RABBIT

(1 Week)

TEST ARTICLE:

H-22240 (test item)

IDENTIFICATION NO.:

H-22240

SPONSOR:

DR. SUSAN A. MACKENZIE
E. I. du Pont de Nemours & Co.
HASKELL LABORATORY
ELKTON RD. P.O. BOX 50
NEWARK, DE 19714-0050

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SUMMARY

The test article, H-22240 (test item), H-22240, was implanted in muscle tissue of the rabbit. The muscle tissue was evaluated for evidence of irritation or toxicity in accordance with the guidelines of the current USP.

Implant samples and USP negative control strips were loaded into needles and sterilized by steam. Rabbits were implanted and were then euthanatized 1 week later. Muscle tissues were excised and the implant sites were examined macroscopically.

Under the conditions of this study, the macroscopic reaction was not significant as compared to the USP negative control implant material. The implanted test article met the USP requirements.

Study and Supervisory Personnel:

Martha Oswanski, BS, LAT
Merle J. Heineke, BS, LAT
Jason A. Karsten
Tina M. Cox, BS, LAT

Approved by:

Steven J. Hermansky, PharmD, PhD, DABT
Manager, Toxicology

December 27, 1996
Date Completed

/mm
bsj

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INTRODUCTION

The test article identified below was evaluated for biocompatibility in accordance with the guidelines of the current USP. The purpose of the study was to evaluate the potential for a local irritant or toxic response to material implanted in direct contact with muscle tissue. The test article was received on December 5, 1996. The animals were implanted on December 17, 1996, and muscles were explanted on December 24, 1996.

MATERIALS

The sample provided by the sponsor was identified and handled as follows:

Test Article:	H-22240 (test item)
Identification No:	H-22240
Storage Conditions:	Room temperature
Control Article:	USP plastic 1 mm diameter negative control refcrence strips purchased by NAmSA from the offices of the US Pharmacopeial Convention and cut into 10 mm sections.
Preparation:	A minimum of six sections of the test article per rabbit, each approximately 1 mm x 10 mm, were loaded into 16 gauge needles. The test article was cut into 1 mm x 20 mm lengths and then folded in half to obtain 1 mm x 10 mm sections. For each rabbit, a minimum of four USP strips were loaded into the same size needles as used for the test article. Test and control articles were sterilized by steam.
Sample Disposition:	Any remaining sample was discarded.

METHODS

Test System:

Species:	Rabbit (Oryctolagus cuniculus)
Breed:	New Zealand White
Source:	Myrtle's Rabbitry, Inc.
Sex:	No particular gender was prescribed for this test
Body Weight Range:	2.5 kg to 2.6 kg at implantation
Age:	No particular age was prescribed for this test
Acclimation Period:	Minimum 2 days
Number of Animals:	Two
Identification Method:	Ear tag or tattoo

Justification of Test System:

The rabbit is suggested as an appropriate animal model for evaluating polymer materials by the current USP testing guidelines. The muscle tissue has been used historically because the response to implanted material is easily graded and compared to a known negative control material.

Animal Management:

Husbandry:	Conditions conformed to Standard Operating Procedures which are based on the "Guide for the Care and Use of Laboratory Animals."
Food:	PROLAB® High Fiber Rabbit Diet was provided daily.

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Water:	Freely available, municipal (Toledo, OH) water was delivered through an automatic watering system.
Contaminants:	Reasonably expected contaminants in feed or water supplies did not have the potential to influence the outcome of this test.
Housing:	Animals were individually housed in stainless steel suspended cages identified by a card indicating the lab number, animal number, test code, sex, and date implanted.
Environmental:	The room temperature was monitored daily. The temperature range for the rabbit was 64-74°F. The room humidity was monitored daily. The humidity range for the rabbit was 30-70%. The light cycle was controlled using an automatic timer (12 hours light, 12 hours dark).
Facility:	NAmSA is an AAALAC accredited facility.
Personnel:	Associates involved were appropriately qualified and trained.
Selection:	Healthy animals were selected. To reduce the number of animals used for testing, and to comply with the directives of the NAmSA IACUC, rabbits on this study may have been used previously in an unrelated test model. Any previously evaluated test or control articles did not cause a response in the animals. Complete history of animal usage is traceable in laboratory records. Animals used for previous evaluations are identified in the report.

Experimental Procedure:

The fur was clipped from the area over the paravertebral muscles and the rabbits were sedated with acepromazine maleate (0.2 ml/kg dose). After the muscle area was wiped clean with 70% alcohol, each paravertebral muscle was infiltrated with 1% lidocaine to achieve local anesthesia on both sides of the vertebral column.

Needles containing test article were inserted into the muscle at a 45° angle. A stylet was placed in the needle and the needle was withdrawn, leaving the sample in the paravertebral muscle. Six test article sections were implanted in the right paravertebral muscle of each rabbit. In the opposite muscle, four USP negative control strips were similarly implanted.

Rabbits were returned to their respective cages following the procedure. Rabbits were observed daily for general health. Body weights were recorded prior to implantation and at termination.

At 1 week, the rabbits were weighed and euthanatized by an intravenous injection of a sodium pentobarbital based drug. The paravertebral muscles were dissected free and methodically cut to locate four of the test article sites and two of the USP negative control sites in each rabbit. Capsule formation or other evidence of irritation was scored using appropriate magnification and an auxiliary light source. Scores were assigned and recorded as follows:

0 No capsule, no adverse reaction (other than minimal hemorrhage)
1 Up to 0.5 mm capsule or reaction area
2 0.6 to 1.0 mm capsule or reaction area
3 1.1 to 2.0 mm capsule or reaction area
4 >2.0 mm capsule or reaction area

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Average macroscopic scores for test implants were compared to average scores of the control sites. Calculations were rounded off to the nearest 0.1. A Reaction Index difference of 0.0 to 0.5 in scores (test minus control) was regarded as "not significant," 0.6 to 1.0 as a "trace," 1.1 to 2.0 as "slight," 2.1 to 3.0 as "moderate" and ≥3.1 as "marked." The requirements of the USP test were met if the difference between test and control score averages (macroscopic) was not greater than 1.0. The requirements of the test were not met if the difference between the test and control score for two (or more) implant sites exceeded 1 for any animal implanted.

RESULTS

Clinical Observations: All animals appeared clinically normal throughout the duration of the study. Body weight data for individual rabbits were considered acceptable.

Macroscopic Observations: The findings for the macroscopic evaluation are shown below.

Rabbit Number	Weight (kg)		Site	Test	USP Negative Control
	Day 0	Day 7			
98944*	2.6	2.8	1 2 3 4	0 0 0 0	0 0
99122*	2.5	2.8	1 2 3 4	0 0 0 0	0 0
			Average:	0.0	0.0

*Previous use history traceable in laboratory records.

Macroscopically, there was no difference between the reactions of the test and control materials. This resulted in a macroscopic reaction of "not significant" tissue contact irritation.

Results and conclusions apply only to the test article tested. No further evaluation of these results is made by NAmSA. Any extrapolation of these data to other samples is the responsibility of the sponsor.

CONCLUSION

Under the conditions of this study, the macroscopic reaction was not significant as compared to the USP negative control implant material. The implanted test article met the USP requirements.

RECORD STORAGE

All raw data pertaining to this study and a copy of the final report are to be retained in designated NAmSA archive files.

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