Institute Report No. 238

Dermal Sensitization Potential of Ball Powder® in Guinea Pigs

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and
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DIVISION OF TOXICOLOGY

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BALL POWDER® was tested for its potential to produce sensitazation via contact with the skin. Testing was performed on male quinea pigs using the Buehler Dermal Sensitization method. No evidence of dermal sensitization to BALL POWDER® was obtained in this study.

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ABSTRACT

BALL POWDER® was tested for its potential to produce sensitization via contact with the skin. Testing was performed on male guinea pigs. The Buehler Dermal Sensitization method was used. No evidence of dermal sensitization to BALL POWDER® was obtained in this study.

Key Words: Dermal Sensitization, Toxicology; ~~BALL POWDER®~~, Nitrocellulose; Buehler Test; Guinea Pigs; Ball powder propellants; ammunition; small arms ammunition; 5.56 mm ammunition

PREFACE

TYPE REPORT: Dermal Sensitization GLP Report

TESTING FACILITY: U.S. Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR: US Army Medical Research and Development Command
US Army Medical Bioengineering Research and Development Laboratory
Fort Detrick, MD 21701-5010
Project Officer: Gunda Reddy, PhD

WORK UNIT: 3E162720A835, Nitrocellulose-Nitroguanidine Projects
WU 180, TL09

GLP STUDY NO.: 84032

STUDY DIRECTOR: Don W. Korte Jr, PhD, MAJ, MS

PRINCIPAL INVESTIGATOR: Earl W. Morgan, DVM, MAJ, VC
Diplomate of American College of Veterinary Preventive Medicine
Diplomate, American Board of Toxicology

CO-PRINCIPAL INVESTIGATOR: John R.G. Ryabik, BS, SP4

REPORT AND DATA MANAGEMENT: A copy of the final report, study protocols, raw data, SOPs, and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: BALL POWDER® (Olin WC 844 for cartridge 5.56 mm, Ball, M193)

INCLUSIVE STUDY DATES: 10 Apr - 3 Jun 1985

OBJECTIVE: The objective of the study was to evaluate the dermal sensitization potential of BALL POWDER® in guinea pigs.

ACKNOWLEDGMENT

MAJ Larry D. Brown, DVM, SSG James D. Justus, BS, SP4 Steven K. Sano, BS, and Gerald F.S. Hiatt, PhD, provided research assistance; Yvonne C. Johnson, BS, provided statistical and research assistance; SP4 James F. Fisher, Scott L. Schwebe, Richard D. Spieler, and Charlotte L. Speckman provided animal care and facility management; Colleen S. Kamiyama, Brenda Goce and Dianna B. Johnson provided office management during performance of this study and preparation of the report.

SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS INVOLVED IN THE STUDY

We, the undersigned, declare that GLP Study 84032 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

DON W. KORTE JR., PhD / DATE
MAJ, MS
Study Director

LANCE O. LOLLINI, DVM / DATE
LTC, VC
Pathologist

EARL W. MORGAN, DVM / DATE
CPT, VC
Principal Investigator

CONRAD R. WHEELER, PhD / DATE
DAC
Analytical Chemist

JOHN R.G. RYABIK, BS / DATE
SP4, USA
Co-Principal Investigator

DEPARTMENT OF THE ARMY

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129-6800

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA

14 May 1987

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

1. I hereby cerify that in relation to LAIR GLP Study 84032 the following inspections were made:

23 April 1985 - 1st Induction Dose Applied

10 May 1985 - 72 Hour Score for 3rd Induction Dose

23 May 1985 - 48 Hour Score for Challenge Dose

2. The report and raw data for this study were audited on 15 January 1987.

for CAROLYN M. LEWIS
DAC
C, Quality Assurance Section

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Dermal Sensitization Potential of BALL POWDER® in Guinea Pigs -- Morgan et al

Nitroguanidine, a primary component of US Army triple base propellants, is now produced in a Government-owned contractor-operated ammunition plant. The US Army Biomedical Research and Development Laboratory (USABRDL), as part of its mission to evaluate the environmental and health hazards of propellants generated by US Army munitions manufacturing facilities, conducted a review of the nitroguanidine data base and identified significant gaps in the toxicity data (1). The Toxicology Division, LAIR, was tasked by USABRDL to develop a genetic and mammalian toxicity profile for nitroguanidine, related intermediates/by-produces of its manufacture, and its environmental degradation. A genetic and acute mammalian toxicity profile of BALL POWDER®, a fielded nitrocellulose-based propellant (cartridge 5.56 mm, Ball, M193) was also requested as a baseline against which future formulations will be compared.

Objective of Study

The objective of this study was to evaluate the dermal sensitization potential of BALL POWDER® in guinea pigs.

MATERIALS

Test Substance

Chemical name: BALL POWDER® (Olin WC 844 for cartridge 5.56 mm, Ball, M193). BALL POWDER® is a registered trademark of Olin Winchester Corporation.

Code number: LAIR Code No. TA045

Chemical Composition:

	Percent
Nitroglycerin	10.235
Dinitrotoluene	0.685
Diphenylamine	1.105
Dibutylphthalate	5.255
Nitrocellulose	83.23
Total Volatiles	1.045
Moisture and Volatiles	0.895
Residual Solvent	0.49
Calcium Carbonate	0.09
Sodium Sulfate	0.12

Source: Badger Army Ammunition Plant
Baraboo, WI 53913

Other test substance information is presented in Appendix A.

Vehicle for Test Substance

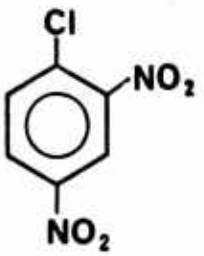
Sterile, isotonic saline (Travenol Laboratories, Deerfield, IL) was used as the vehicle for BALL POWDER®. The expiration date for this lot (7C950X0) was October 1985.

Positive Control

Chemical name: Dinitrochlorobenzene (DNCB)

Chemical Abstract Service Registry No.: 97-00-7

Structural formula:



Molecular formula: $C_6H_3N_2O_4Cl$

Vehicle for Positive Control

The vehicle for DNCB was a propylene glycol (3%) and isotonic saline (97%) mixture. Propylene glycol (lot number 36485) was obtained from Certified Laboratories, Inc. (Philadelphia, PA). Saline was the same as for the BALL POWDER® vehicle. Other positive control substance information is presented in Appendix A.

Animal Data

Forty-six male guinea pigs, Hartley strain, (Charles River, Kingston, NY) were used for this study. They were identified individually with ear tags numbered 85E00134 - 85E00179 inclusive. Two animals were selected randomly for quality control necropsy evaluation on receipt. Two of the animals were used for a pilot study to determine a non-irritating dose level. Animal weights on receipt (11 Apr 85) ranged from 189 to 256 g. Additional animal data are presented in Appendix B.

Husbandry

Guinea pigs were caged individually in stainless steel wire mesh cages in racks equipped with automatically flushing dump tanks. No bedding was used in any of the cages. The diet, fed ad libitum, consisted of Certified Purina Guinea Pig Chow Diet No. 5026 (Ralston Purina Company, Checkerboard Square, St Louis, MO); water was provided by continuous drip from a central line. The animal room temperature was maintained in a range from 21.7°C to 25.0°C and relative humidity in a range of 32 to 56 percent. The photoperiod was 12 hours of light per day.

METHODS

This study was conducted in accordance with LAIR SOP-OP-STX-82 "Buehler Dermal Sensitization Test" (2) and EPA guidelines (3).

Group Assignment/Acclimation

The guinea pigs were quarantined for 13 days before administration of the first induction dose. During the quarantine period, they were checked daily for signs of illness and weighed once a week. Ten animals were assigned to each of four groups by a stratified randomization technique based on their body weights.

Dosage Levels

BALL POWDER® (Olin WC 844) is a smooth, spheroidal pellet 0.5 - 1.5 mm in diameter. Since it is insoluble in water or physiologic solutions, BALL POWDER® was applied neat (without physical or chemical alterations). A pilot study, using BALL POWDER® moistened with isotonic saline, indicated that the neat compound was non-irritating under the conditions of this test.

Two sensitization control groups were included in the study. Dinitrochlorobenzene, a known potent sensitizing agent (4), was applied to one group, at a 0.1% concentration, as a positive control. Isotonic saline was applied to another group as a vehicle control. In addition, a negative control group received BALL POWDER® only on the day of challenge dosing.

Compound Preparation

The test compound was prepared by weighing 0.5 g BALL POWDER® and pouring it on a 2.5 cm^2 patch that had been

moistened with 0.5 ml of isotonic saline. The dinitrochlorobenzene dosing solution was prepared by first adding 30 mg DNCB to 1 ml of propylene glycol and heating until it dissolved (approximately 40°C). To this, 29 ml of 0.9% sodium chloride solution were added, to give a final concentration of 0.1% (w/v). This solution was heated to 65°C and vortexed before application to keep the DNCB in solution. DNCB solutions were prepared fresh for each application day.

Test Procedures

The closed patch dermal sensitization test procedures utilized in this study were developed by Buehler and Griffith (5-7). Test compounds were applied for six hours under a closed patch once a week for three weeks during the induction phase. The same application site was used for each induction dose. To distinguish between reactions from repeated insult and sensitization, duplicate patches of the challenge dose were applied, one on the old site and one on a new site. To distinguish between reactions from primary irritation and sensitization, negative control groups were added which received only the challenge dose.

During the induction phase, the experimental, saline control, and positive control groups were dosed with 0.5 g of the appropriate compound applied topically under a 2.5 cm^2 gauze patch. This procedure was performed for three consecutive weeks (23 Apr, 30 Apr, and 7 May 85). The day before each dosing a 8 cm^2 area on the left side of the animal was clipped with electric clippers (Oster® Model A5, size 40 blade, Sunbeam Corp, Milwaukee, WI) and then shaved with an electric razor (Norelco® Speed Razor Model HP1134® S, North American Phillips Corp, Stamford, CT). The patch was taped with Blenderm® hypo-allergenic surgical tape (3M Corp, St Paul, MN) to the same site each time and the animal was wrapped several times with Vetrap® (3M Corp, St Paul, MN). The patch was left in place for six hours. When the wrap and patch were removed, the area under the patch was marked with surgical felt tip marking pen for ease of scoring.

Animals were challenged two weeks (21 May 85) following the third induction dose. The experimental group and the positive control group received two 0.5 g doses, one applied to the old site on the left side and the other to a new site on the right side. Negative and vehicle control groups only received a single 0.5 g dose which was applied to the left side. The procedures for clipping, shaving, wrapping, and exposure period remained the same.

Since the positive control group exhibited only a weak positive response to the first challange dose, the animals were re-challenged on 29 May 85. The procedures were the same except the negative control group was not re-challenged.

In Buehler's procedure, skin reactions are scored 24 and 48 hours after the challenge dose only. In the present study, skin reactions were scored 24, 48, and 72 hours after each induction dose as well. Skin reactions were assigned scores according to Buehler's grading system: 0 (no reaction), 1 (slight erythema), 2 (moderate erythema) and 3 (marked erythema). The results are expressed both in terms of incidence (the number of animals showing responses of 1 or greater at either observation time) and severity (the sum of the test scores divided by the number of animals tested). Results from the left side are compared with right side and with the negative control group.

Some modifications of Buehler's procedures were made. Instead of placing animals in restraint during the 6-hour exposure period, the animals were wrapped several times with an elasticized tape to hold the patch in place. Consequently, the animals were able to move about freely in their cage during the exposure period. Buehler and Griffith (7) also recommended depilating the day before the challenge dose. For consistency with induction procedures, this step was replaced by clipping the animals as described previously.

Necropsy

All guinea pigs were submitted for a complete gross necropsy at the conclusion of the 14-day observation period.

Duration of Study

A historical listing of study events appears in Appendix C.

Deviations from Study Protocol

Only two guinea pigs and one "concentration," 100%, were studied in the pilot study. Since the test compound is a hard pellet and insoluble in the preferred vehicle, it was considered unlikely that the compound or one of its constituent chemicals would enter the skin and cause a reaction. Thus the pilot study was reduced to conserve animals.

The DNCB solution was maintained at approximately 65°C prior to dosing. This was necessary to keep the DNCB in solution, but did not result in thermal insult to the animal's skin, as the aliquot for dosing cooled quickly during pipetting and application to the patch. Sufficient sensitization was produced by DNCB with this method.

One guinea pig caught his foot in the caging and broke his leg. He was removed from the study and sacrificed. He was replaced with one of the unused "pilot" guinea pigs.

On 27 Mar 85, the building engineers turned off the exhaust fans and steam system for routine maintenance. During this outage the humidity fluctuated between 44 and 81 percent.

It is believed that these deviations from the protocol did not adversely affect study results.

Raw Data and Final Report Storage

A copy of the final report, study protocols, raw data, retired SOPs, and an aliquot of the test compound will be retained in the LAIR Archives.

RESULTS

Tables 1, 2, and 3 summarize the incidence of reactions 24, 48, and 72 hours after each dose. There was no reaction observed in response to BALL POWDER®, either at 24, 48, and 72 hours. This lack of response is reflected in Tables 4, 5, and 6 which report the severity of skin reactions at 24, 48, and 72 hours. Response severity for each group is calculated by summing the scores of responding animals and dividing by the total number of animals within that group. This produced a severity index of 0.0 for BALL POWDER®.

In contrast, dinitrochlorobenzene (DNCB) produced a positive response at all time points except the first induction. Between 70% and 100% of the DNCB-treated animals exhibited a response 24 hours following the second and/or third induction and challenge doses. These reactions persisted and yielded scorable effects in 20-100% of the animals at 48 hours after dosing and 10-100% of the animals at 72 hours after dosing.

Severity scores for these responses to DNCB ranged from 0.0 to 1.3 at the 24-hour scoring period (Table 4). The highest score, 1.3, was observed on the left (induction) side in response to the re-challenge dose. By 48 and 72

hours after dosing the reactions had subsided somewhat except for the re-challenge; consequently, the severity range decreased from 0.0 to 0.7 (Tables 5 and 6). However, severity scores for the re-challenge increased slightly from 0.9 to 1.5.

No responses whatsoever were observed in the negative control (challenge dose of BALL POWDER® only) or the vehicle control (normal saline-treated) groups. The individual 24, 48, and 72 hour scores for all animals are provided, by group, in Appendix D.

No lesions were found at necropsy which could be attributed to the test procedure or the test compound. The veterinary pathologist's report appears in Appendix E.

TABLE 1

Incidences of Skin Reactions
after 24 Hours
(n=10 in each test group)

Test Group	Induction			Challenge		Re-Challenge	
	First	Second	Third	Left	Right	Left	Right
BALL POWDER®	0	0	0	0	0	0	0
Negative Control*	---	---	---	0	0	---	---
Saline Vehicle	0	0	0	0	0	0	0
DNCB	0	7	10	8	8	10	10

*The Negative Control Group received only a challenge dose of the test compound.

TABLE 2

Incidences of Skin Reactions
after 48 Hours
(n=10 in each test group)

Test Group	Induction			Challenge		Re-Challenge	
	First	Second	Third	Left	Right	Left	Right
BALL POWDER®	0	0	0	0	0	0	0
Negative Control*	---	---	---	0	0	---	---
Saline Vehicle	0	0	0	0	0	0	0
DNCB	0	5	7	1	2	10	6

*The Negative Control Group received only a challenge dose of the test compound.

TABLE 3

Incidences of Skin Reactions
after 72 Hours
(n=10 in each test group)

Test Group	Induction			Challenge		Re-Challenge	
	First	Second	Third	Left	Right	Left	Right
BALL POWDER®	0	0	0	0	0	0	0
Negative Control*	---	---	---	0	0	---	---
Saline Vehicle	0	0	0	0	0	0	0
DNCB	0	1	3	1	1	10	6

*The Negative Control Group received only a challenge dose of the test compound.

TABLE 4

Severity of Skin Reactions
after 24 Hours

Test Group	Induction			Challenge		Re-Challenge	
	First	Second	Third	Left	Right	Left	Right
BALL POWDER®	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Negative Control*	---	---	---	0.0	0.0	---	---
Saline Vehicle	0.0	0.0	0.0	0.0	0.0	0.0	0.0
DNCB	0.0	0.7	1.2	0.8	0.9	1.3	1.0

*The Negative Control Group received only a challenge dose of the test compound.

TABLE 5

Incidences of Skin Reactions
after 24 Hours

Test Group	Induction First	Induction Second	Induction Third	Challenge Left	Challenge Right	Re-Challenge Left	Re-Challenge Right
BALL POWDER®	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Negative Control*	---	---	---	0.0	0.0	---	---
Saline Vehicle	0.0	0.0	0.0	0.0	0.0	0.0	0.0
DNCB	0.0	0.5	0.7	0.1	0.2	1.5	0.9

*The Negative Control Group received only a challenge dose of the test compound.

TABLE 6

Incidences of Skin Reactions
after 72 Hours

Test Group	Induction First	Induction Second	Induction Third	Challenge Left	Challenge Right	Re-Challenge Left	Re-Challenge Right
BALL POWDER®	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Negative Control*	---	---	---	0.0	0.0	---	---
Saline Vehicle	0.0	0.0	0.0	0.0	0.0	0.0	0.0
DNCB	0.0	0.1	0.3	0.1	0.1	1.5	1.0

*The Negative Control Group received only a challenge dose of the test compound.

DISCUSSION

Skin reactions arising from direct contact with chemicals are classified as either irritative or sensitizing reactions. Both reactions produce similar inflammatory responses; the primary difference being the basic mechanism responsible for the response.

Primary irritation is direct inflammation in response to injury to the skin produced by the eliciting chemical. Irritation is a locally mediated response ranging from mild, reversible inflammation to severe ulceration progressing to necrosis.

Sensitization is manifested as indirect inflammation mediated by components of the immune system in response to activation by the eliciting chemical (8). Dermal sensitization is usually a delayed hypersensitivity or cellular immunologic reaction. During the induction phase (3 weeks in the present study) there is proliferation of a clone of T lymphocytes specifically sensitized to the eliciting antigen. Upon subsequent exposure to the antigen these T lymphocytes release mediators, lymphokines, which initiate and amplify an inflammatory reaction at the site of contact (9).

Although both irritation and sensitization appear grossly similar in experimental animals, and may even be produced by the same agent, it is possible to distinguish between them. Irritation is an immediate response and can be produced upon first contact with the chemical, whereas sensitization requires at least one innocuous "conditioning" exposure before a reaction can be elicited.

Irritative responses usually require a relatively high concentration or dose of the offending chemical, while sensitization reactions may occur in response to minute quantities. Essentially all individuals in a population will express an irritative response to a reactive chemical, provided the dose is high enough, while only a fraction of the population normally becomes sensitized to the same chemical. A fully developed response can be produced by first contact with an irritant, but initial contact with a sensitizer produces no reaction (a conditioning exposure is necessary). Unless there is accumulation of damage, subsequent exposures to an irritant produce inflammation of essentially similar intensity/severity, while the reaction to a sensitizer increases over 2 to 4 exposures after the initial contact. An irritant produces inflammation of rapid onset with short duration while a sensitization reaction is somewhat delayed and prolonged. The

inflammatory response to an irritant may spread beyond the area of contact while sensitization reactions are usually circumscribed.

The features of irritation and sensitization were applied by Buehler and Griffith (5-7) to establish guidelines for differentiation between the two. In evaluating a dermal sensitization study they recommend comparing the results from a challenge dose in the experimental group with those for the negative control group:

Irritative Responses:

- occur in a large proportion of test animals.
- develop in response to the first or second exposure.
- usually fade within 24 to 48 hr, unless damage is severe.
- may be stronger at challenge to a previously unexposed area of skin (contralateral flank).

Sensitization Reactions:

- occur in only a few animals, unless the compound is a potent sensitizer.
- are absent after the initial (conditioning) exposure, but appear in response to subsequent exposures.
- develop slowly, the intensity/severity of inflammation being greater at 72 to 96 hr than at 24 to 48 hr.
- increase in intensity/severity from one exposure to the next (at sites previously exposed or unexposed).

Dermal irritancy is evaluated by the method of Draize et al (10) in which the chemical is applied once, at high concentration, and the resulting acute inflammatory response is graded. Evaluation of sensitization potential is accomplished by repeated application at lower non-irritating concentrations, over a few weeks. There is then a latent period, usually two weeks, to allow the immune system to elaborate and increase its specific reactivity to the chemical. A challenge dose is then given and the resulting inflammatory reaction is graded. Analysis of the

incidence, severity and timing of the reaction to the challenge dose gives an estimate of the sensitizing potential of the study compound.

BALL POWDER®

In the present study, BALL POWDER® was evaluated for its ability to elicit a delayed-hypersensitivity reaction via dermal contact. As tested by the Buehler and Griffith method (5-7), BALL POWDER® produced no response indicative of dermal sensitization. Therefore in this study BALL POWDER® showed no evidence of potential to elicit an immunologic response.

Because the guinea pig exhibits a somewhat lower sensitizing responsiveness than man, this result does not guarantee that BALL POWDER® will not sensitize humans. It does indicate that BALL POWDER® is unlikely to sensitize humans and its potential is low enough to permit testing in humans.

Any sensitization produced by BALL POWDER® would have been easily detected by this study. A hypersensitivity-type response was reliably elicted by DNCB in the present group of animals. This response to DNCB was characteristic of that observed previously within the Institute (11). Although DNCB is capable of producing primary irritation, the characteristics of responses observed in this study are indicative of a reaction due to sensitization. The concentration of DNCB used for induction and challenge is too low to produce primary irritation. Also, the response to DNCB was observed only after two or more exposures and the severity and persistency generally increased with the number of previous exposures.

CONCLUSION

BALL POWDER®, based on a zero percent sensitization rate in this study, exhibited no potential for inducing dermal sensitization.

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APPENDICES

PROPELLANT DESCRIPTION SHEET

REPORTS CONTROL SYMBOL
EXEMPT – PARA 7-2e
AR 335-15

TO	FROM	DATE
	Badger Army Ammunition Plant Baraboo, Wisconsin 53913	10 August 1984

DA LOT NUMBER 50/50 blend of lots BAJ-47670 and BAJ-47671	COMPOSITION NUMBER WC 844 for Cartridge 5.56 mm, BALL, M193
MFG AT Badger Army Ammunition Plant	PACKED AMOUNT LB
CONTRACT NUMBER DAAA09-73-C-0004	SPECIFICATION NUMBER MIL-P-3984E w/Amendment 4 and Drawing No. C10542743 Rev. C

NITROCELLULOSE

ACCEPTED BLEND NUMBERS	NITROGEN CONTENT	KI STARCH(65.5°C)	STABILITY (134.5°C)
Nitrocellulose (NC) extracted from excessed Single Base Propellant.	MAX %	MIN	MIN
	MIN %	MIN	MIN
	AVG %	MIN	MIN
NC complied with MIL-N-244A			EXPLOSION HR

MANUFACTURE OF PROPELLANT

_____POUNDS SOLVENT PER POUND NC/DRY WEIGHT INGREDIENTS CONSISTING OF _____ POUNDS ALCOHOL AND _____ POUNDS _____ PER 100 POUNDS SOLVENT. PERCENTAGE REMIX TO WHOLE _____.

TEMPERATURE FROM	TEMPERATURE TO	PROCESS–SOLVENT RECOVERY AND DRYING	TIME DAYS	TIME HOURS

PROPELLANT COMPOSITION — TESTS OF FINISHED PROPELLANT — STABILITY AND PHYSICAL TESTS

CONSTITUENT	% FORMULA	% TOLERANCE	% MEASURED		FORMULA	ACTUAL
Nitroglycerin			10.235	HEAT TEST 120°	Min 60 min.	65 min.*
Dinitrotoluene			0.685	No Explosion (HRS)	Min 5	5+*
Diphenylamine			1.105	FORM OF PROPELLANT		
Dibutylphthalate			5.255	Dust&Foreign Mat.		0.02
Nitrocellulose			83.23	Graphite		0.075
Total Volatiles			1.045	Grav. Density		1.008
Moisture and Volatiles			0.895	Nitrogen		13.075
Residual Solvent			0.49			
Calcium Carbonate			0.09			
Sodium Sulfate			0.12			

CLOSED BOMB	LOT NUMBER	TEMP °F	RELATIVE BURNRATE	RELATIVE FORCE
TEST				
STANDARD			100.00%	100.00%

PROPELLANT DIMENSIONS (INCHES)	SPEC	DIE	FINISHED	MEAN VARIATION IN % OF MEAN DIMENSIONS SPEC	MEAN VARIATION IN % OF MEAN DIMENSIONS ACTUAL
LENGTH (L)					
DIAMETER (D)					
PERF DIA (d)					
				PACKED	
				SAMPLED	
WEB DIFFERENCE/STD DEV IN % OF WEB AVERAGE				TEST FINISHED	
				OFFERED	
L:D				DESCRIPTION SHEETS FORWARDED	
D:d					

REMARKS

TYPE OF PACKING CONTAINER

REMARKS
*Tested 29 February 1984.

SIGNATURE OF CONTRACTOR'S REPRESENTATIVE	SIGNATURE OF GOVERNMENT QUALITY ASSURANCE REPRESENTATIVE

ANIMAL DATA

Species: Cavia porcellus

Strain: Hartley

Source: Charles River Kingston, NY

Sex: Male

Date of birth: 23 March 1985

Method of randomization: Weight bias, stratified animal allocation

Animals in each group: 10 male animals

Condition of animals at start of study: Normal

Identification procedures: Ear tagging procedure, tag numbers 85E00134 to 85E00179 inclusive.

Pretest conditioning: Quarantine/acclimation 10 Apr - 22 Apr 1985

Justification: The laboratory guinea pig has proven to be a sensitive and reliable model for detection of delayed hypersensitivity from dermal contact.

HISTORICAL LISTING OF EVENTS

Date	Event
10 Apr 85	Forty-six animals arrived, were examined, placed in cages, and fed.
11 Apr 85	Animals were ear-tagged and weighed. Two animals were submitted for necropsy as quality controls.
10 Apr-3 Jun 85	Animals were checked daily.
1,16,22,29 Apr, 6,13,20,28	Animals were weighed.
May 85 15 Apr 85	Four pilot animals were randomly selected and two were shaved. Pilot doses were prepared.
16 Apr 85	Pilot animals were patch tested. One animal was sacrificed due to a broken leg.
17 Apr 85	Pilot animals were scored for 24-hour skin reaction.
18 Apr 85	Pilot animals were scored for 48-hour skin reaction. Pilot results evaluated, test concentration determined.
22 Apr 85	Animals were randomized into groups.

APPENDIX C

Date	Event
22,29 Apr, 6 May 85	Test animals, except negative control group, were clipped and shaved. Doses were prepared.
23,30 Apr, 7 May 87	Test animals, except negative control group, were given induction dose.
24 Apr, 1,8 May 86	Test animals, except negative control group, were scored for 24-hour skin reaction.
25 Apr, 2,9 May 86	Test animals, except negative control group, were scored for 48-hour skin reaction.
26 Apr, 3,10 May 86	Test animals, except negative control group, were scored for 72-hour skin reaction.
20 May 85	Test animals were clipped and shaved. Doses were prepared.
21 May 85	Test animals were given challenge dose.
22 May 85	Test animals were scored for 24-hour skin reaction.
23 May 85	Test animals were scored for 48-hour skin reaction.

Date	Event
28 May 85	Test animals, except negative control group, were clipped and shaved. Doses were prepared.
29 May 85	Test animals, except negative control group, were given induction dose.
30 May 85	Test animals, except negative control group, were scored for 24-hour skin reaction.
1 May 85	Test animals, except negative control group, were scored for 48-hour skin reaction.
2 May 85	Test animals, except negative control group, were scored for 72-hour skin reaction.
3 May 85	Animals were observed and delivered to Necropsy Suite for sacrifice and gross necropsy.

Individual Dermal Scores

Page

APPENDIX D-1

INDIVIDUAL DERMAL SCORES

BALL POWDER® STUDY GROUP

ANIMAL NUMBER	INDUCTION 1			INDUCTION 2			INDUCTION 3			CHALLENGE (LEFT/RIGHT)					
	24H	48H	72H	24H	48H	72H	24H	48H	72H	24H	48H	72H	24H	48H	72H
85E0135	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
85E0140	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
85E0144	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
85E0156	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
85E0167	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
85E0169	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
85E0170	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
85E0171	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
85E0176	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
85E0178	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
AVERAGE	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

INDIVIDUAL DERMAL SCORES

BALL POWDER® STUDY GROUP

RE-CHALLENGE DOSING						
ANIMAL NUMBER	LEFT FLANK			RIGHT FLANK		
	24H	48H	72H	24H	48H	72H
85E0135	0.0	0.0	0.0	0.0	0.0	0.0
85E0140	0.0	0.0	0.0	0.0	0.0	0.0
85E0144	0.0	0.0	0.0	0.0	0.0	0.0
85E0156	0.0	0.0	0.0	0.0	0.0	0.0
85E0167	0.0	0.0	0.0	0.0	0.0	0.0
85E0169	0.0	0.0	0.0	0.0	0.0	0.0
85E0170	0.0	0.0	0.0	0.0	0.0	0.0
85E0171	0.0	0.0	0.0	0.0	0.0	0.0
85E0176	0.0	0.0	0.0	0.0	0.0	0.0
85E0178	0.0	0.0	0.0	0.0	0.0	0.0
AVERAGE	0.0	0.0	0.0	0.0	0.0	0.0

INDIVIDUAL DERMAL SCORES

DNCB POSITIVE CONTROL GROUP

ANIMAL NUMBER	INDUCTION 1			INDUCTION 2			INDUCTION 3			CHALLENGE (LEFT/RIGHT)					
	24H	48H	72H	24H	48H	72H	24H	48H	72H	24H	48H	72H	24H	48H	72H
85E0134	0.0	0.0	0.0	1.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
85E0137	0.0	0.0	0.0	1.0	1.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
85E0138	0.0	0.0	0.0	1.0	1.0	1.0	2.0	1.0	1.0	1.0	0.0	0.0	1.0	0.0	0.0
85E0139	0.0	0.0	0.0	1.0	1.0	0.0	1.0	1.0	0.0	1.0	0.0	0.0	1.0	0.0	0.0
85E0157	0.0	0.0	0.0	0.0	0.0	0.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
85E0160	0.0	0.0	0.0	1.0	1.0	0.0	1.0	1.0	0.0	1.0	0.0	0.0	2.0	0.0	0.0
85E0162	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	1.0	0.0	0.0	1.0	0.0	0.0
85E0166	0.0	0.0	0.0	1.0	0.0	0.0	2.0	1.0	1.0	2.0	1.0	1.0	1.0	0.0	0.0
85E0175	0.0	0.0	0.0	0.0	0.0	0.0	1.0	1.0	0.0	1.0	0.0	0.0	1.0	0.0	0.0
85E0179	0.0	0.0	0.0	1.0	1.0	0.0	1.0	1.0	0.0	1.0	0.0	0.0	1.0	1.0	1.0
AVERAGE	0.0	0.0	0.0	0.7	0.5	0.1	1.2	0.7	0.3	0.8	0.1	0.1	0.9	0.2	0.1

INDIVIDUAL DERMAL SCORES

DNCB POSITIVE CONTROL GROUP

RE-CHALLENGE DOSING						
ANIMAL NUMBER	LEFT FLANK			RIGHT FLANK		
	24H	48H	72H	24H	48H	72H
85E0134	1.0	1.0	1.0	1.0	0.0	0.0
85E0137	2.0	2.0	1.0	1.0	1.0	1.0
85E0138	2.0	2.0	2.0	1.0	2.0	2.0
85E0139	1.0	1.0	2.0	1.0	1.0	1.0
85E0157	1.0	2.0	2.0	1.0	2.0	1.0
85E0160	1.0	2.0	2.0	1.0	2.0	2.0
85E0162	1.0	1.0	1.0	1.0	0.0	0.0
85E0166	2.0	1.0	2.0	1.0	1.0	2.0
85E0175	1.0	1.0	1.0	1.0	0.0	0.0
85E0179	1.0	2.0	1.0	1.0	0.0	0.0
AVERAGE	1.3	1.5	1.5	1.0	0.9	1.0

INDIVIDUAL DERMAL SCORES

NORMAL SALINE VEHICLE CONTROL GROUP

ANIMAL NUMBER	INDUCTION 1			INDUCTION 2			INDUCTION 3			CHALLENGE (LEFT/RIGHT)					
	24H	48H	72H	24H	48H	72H	24H	48H	72H	24H	48H	72H	24H	48H	72H
85E0136	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
85E0143	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
85E0145	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
85E0147	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
85E0151	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
85E0158	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
85E0159	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
85E0161	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
85E0164	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
85E0168	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
AVERAGE	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

NORMAL SALINE VEHICLE CONTROL GROUP

INDIVIDUAL DERMAL SCORES

RE-CHALLENGE DOSING						
ANIMAL NUMBER	LEFT FLANK			RIGHT FLANK		
	24H	48H	72H	24H	48H	72H
85E0136	0.0	0.0	0.0	0.0	0.0	0.0
85E0143	0.0	0.0	0.0	0.0	0.0	0.0
85E0145	0.0	0.0	0.0	0.0	0.0	0.0
85E0147	0.0	0.0	0.0	0.0	0.0	0.0
85E0151	0.0	0.0	0.0	0.0	0.0	0.0
85E0158	0.0	0.0	0.0	0.0	0.0	0.0
85E0159	0.0	0.0	0.0	0.0	0.0	0.0
85E0161	0.0	0.0	0.0	0.0	0.0	0.0
85E0164	0.0	0.0	0.0	0.0	0.0	0.0
85E0168	0.0	0.0	0.0	0.0	0.0	0.0
AVERAGE	0.0	0.0	0.0	0.0	0.0	0.0

INDIVIDUAL DERMAL SCORES

BALL POWDER® NEGATIVE CONTROL GROUP

ANIMAL NUMBER	CHALLENGE DOSING					
	LEFT FLANK			RIGHT FLANK		
	24H	48H	72H	24H	48H	72H
85E0141	0.0	0.0	0.0	0.0	0.0	0.0
85E0148	0.0	0.0	0.0	0.0	0.0	0.0
85E0149	0.0	0.0	0.0	0.0	0.0	0.0
85E0150	0.0	0.0	0.0	0.0	0.0	0.0
85E0153	0.0	0.0	0.0	0.0	0.0	0.0
85E0154	0.0	0.0	0.0	0.0	0.0	0.0
85E0155	0.0	0.0	0.0	0.0	0.0	0.0
85E0163	0.0	0.0	0.0	0.0	0.0	0.0
85E0172	0.0	0.0	0.0	0.0	0.0	0.0
85E0173	0.0	0.0	0.0	0.0	0.0	0.0
AVERAGE	0.0	0.0	0.0	0.0	0.0	0.0

LAIR Gross Pathology Report
GLP Study 84032

Study: GLP #84032, Toxicology Services Group

Test: Buehler Dermal Sensitization

Investigator: CPT Morgan

Test Substance: BALL POWDER®

History: Study conducted in accordance with SOP-OP-STX-82. Number of animals: 40. Sex: male Species: Guinea pig, Hartley Albino.

Findings:

Group	Animals/Group	Lesions
Test compound	10	0
Positive control (DNCB)	10	0
Saline	10	0
Negative control	10	1*

*Animal number 85E00149 had a discrete focal yellow (2 x 12 mm) area on the liver. Microscopically there were a few discrete (5 mm or less in diameter) subcapsular foci of coagulative necrosis bordered by a prominent layer of collapsed stroma/fibrosis and a marked infiltrate of macrophages.

Comments: The liver lesion was considered incidental and in no way related to the procedure or test compound. This type of lesion is frequently seen in guinea pigs.

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Pathology Services Group

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10 June 1985

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