INTERNATIONAL COURT OF JUSTICE

CASE CONCERNING
AERIAL HERBICIDE SPRAYING

(ECUADOR v. COLOMBIA)

REJOINDER OF THE REPUBLIC OF COLOMBIA

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1 FEBRUARY 2012

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United States Environmental Protection Agency (EPA), Memorandum of 13 May 2003, Technical Review of the six acute toxicity studies on the spray mixture for Eradication of Illicit Crops in Colombia

(United States Embassy in Bogotá, 2011)

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION,

AND
TOXIC

PESTICIDES

SUBSTANCES

May 13, 2003

MEMORANDUM

DP Barcode: D289806
Case No: 296097
Submission: S634325
PC Codes: 103601 Glyphosate, isopropylamine salt

From: Byron T. Backus, Ph.D., Toxicologist
Technical Review Branch
Registration Division (7505C)

To: Jim Tompkins PM 25
Herbicide Branch
Registration Division (7505C)

ACTION REQUESTED: "Please review the acute six pack by the Department of State for the spray mixture being used by the Department of State for illicit drug crop control in Columbia."

BACKGROUND: This package contains the following 6 acute toxicity studies conducted on test material identified as Spray–Charlie: acute oral LD_{50} (rat; MRID 45929403), acute dermal LD_{50} (rat; MRID 45929402), acute inhalation LC_{50} (rat; MRID 45929404) primary eye irritation (rabbit; MRID 45929405); primary skin irritation (rabbit; MRID 45929406), and dermal sensitization (guinea pig; MRID 45929407). There is also a study titled "Purity Analysis for Glyphosate of Spray–Charlie (Active Ingredient)" in MRID 45929401. All studies were conducted at Springborn Laboratories, Inc. (SLI), Spencerville, OH.

The material received also includes a label for GLY-41 Herbicide (EPA Reg. No. 524-475) with a label declaration of 41.0% Glyphosate (as the isopropylamine salt) as sole

active ingredient, as well as a label (in Spanish) for COSMO-FLUX® 411F. Spray–Charlie (the end-use spray formulation) is prepared by mixing 44% (by volume) GLY-41 with 55% (by volume) water and 1% (by volume) of the surfactant Cosmo-Flux-411F.

COMMENTS AND RECOMMENDATIONS:

1. All 6 acute toxicity studies have been reviewed and classified as acceptable. The Data Evaluation Records (DERs) for each of these 6 studies are included in this memorandum.

2. The following is the acute toxicity profile for SPRAY–CHARLIE, based on the results of the acute toxicity studies:

Study Type	Tox. Cat.	Classification & MRID #
Oral LD_{50} (rat)	Tox. Cat. IV	Acceptable (MRID 45929403)
Dermal LD_{50} (rat)	Tox. Cat. IV	Acceptable (MRID 45929402)
Inhalation LC_{50}(rat)	Tox. Cat. IV	Acceptable (MRID 45929404)
Eye Irritation (rabbit)	Tox. Cat. III	Acceptable (MRID 45929405)
Dermal Irritation (rabbit)	Tox. Cat. IV	Acceptable (MRID 45929406)
Dermal Sensitization (guinea pig)	Non-Sensitizer	Acceptable (MRID 45929407)

3. Based on the acute toxicity profile above, the following would be the appropriate precautionary labeling for this product, as obtained from the Label Review System:

PRODUCT NAME: SPRAY - CHARLIE

PRECAUTIONARY STATEMENTS

SIGNAL WORD: CAUTION

Hazards to Humans and Domestic Animals:

Causes moderate eye irritation. Avoid contact with eyes or clothing. Wash thoroughly with soap and water after handling and before eating, drinking, chewing gum, or using tobacco. Wear: Long-sleeved shirt and long pants, Socks, and Shoes.

First Aid:

If in eyes:
-Hold eye open and rinse slowly and gently with water for 15-20 minutes.
-Remove contact lenses, if present, after the first 5 minutes, then continue rinsing.
-Call a poison control center or doctor for treatment advice.

Have the product container or label with you when calling a poison control center or doctor or going for treatment. You may also contact 1-800-xxx-xxxx for emergency medical treatment information.

4. The above labeling is consistent with that for GLY-41 Herbicide (EPA Reg. No. 524-475).

DATA REVIEW FOR ACUTE ORAL TOXICITY TESTING (870.1100, formerly §81-1)

Product Manager: 25 **Reviewer:** Byron T. Backus, Ph.D.
MRID No.: 45929403

CITATION: Bonnette, K.L. An Acute Oral Toxicity Study in Rats with Spray–Charlie. SLI Study No. 3596.16. Unpublished study prepared by Springborn Laboratories, Inc. (SLI), Spencerville, OH 45887. Study Completion Date: Feb. 20, 2003. MRID 45929403.

STUDY SPONSOR AND SUBMITTER: INL/A U.S. Dept. of State, Washington D.C. 20520

TEST MATERIAL: Sample pooled at SLI from five different mixes of Spray–Charlie. From SLI Study No. 3596.15 [Purity Analysis for Glyphosate of Spray–Charlie (Active Ingredient)] in MRID 45929401 the five separate mixes were prepared by adding together 0.439-0.44 by volume GLY-41 Herbicide; 0.01 by volume Cosmo Flux-411F; and 0.55-0.551 by volume Lake Water. The before use (pre-test?) mean for Glyphosate a.e. [acid equivalent] was 16.53% (S.D. 1.35%); and the after use mean percentage was 15.20% (S.D. 1.54%). Both values are above the expected 14.8%.

SPECIES: Rat, Hsd: Sprague Dawley® SD®
AGE(at dosing): "Young adult," males: approx. 9-10 weeks; females: approx. 8 weeks
WEIGHT (fasted): Males: 294-325 g; Females: 169-188 g
SOURCE: Harlan Sprague-Dawley, Inc., Indianapolis, IN

EXECUTIVE SUMMARY: ***In an acute oral toxicity study (MRID 45929403), 5 male & 5 female fasted (overnight; fasted body wts: males: 294-325 g; females: 169-188 g) young adult (males: ~9-10 wks; females: ~8 wks) Hsd: Sprague-Dawley® SD® rats (source: Harlan Sprague-Dawley, Indianapolis), were orally dosed with Spray-Charlie, containing at least 15.2% a.e. [acid equivalent] glyphosate. The test material (a liquid with a density of 1.08 g/mL) was administered undiluted at 5000 mg/kg.***

There was no mortality. Symptoms included soft stools (5M & 2F) and fecal stain (4M) on days 0-1. In addition, there was rough coat (3M), dark material around eyes and/or nose (4M) and congested breathing with rales (1F). Most symptoms were gone by day 6, although one male had transient dark material around the eyes on day 9 only. All rats had weight gains from day 0 to 7, and again from day 7 to 14.

There were no dose-related abnormalities observed at post-sacrifice necropsy.

Oral LD50 Males > 5000 mg/kg (0/5 died at this dose level)
Oral LD50 Females > 5000 mg/kg (0/5 died at this dose level)

Spray–Charlie, a liquid (density of 1.08 g/mL), with at least 15.2% a.e. glyphosate, is in toxicity category IV in terms of its oral LD50.

Study Classification: Acceptable

COMPLIANCE: Signed and dated GLP Compliance (p. 3), Quality Assurance (p. 4), and [No] Data Confidentiality (p. 2) statements are provided. There is no flagging statement.

Procedure (including deviations from 870.1100): The test article was an amber liquid, which was a pooled sample from five different mixes of Spray–Charlie.

Results:

Dose (mg/kg)	Dose (mL/kg)	Number of Deaths/Number Tested		
		Males	Females	Total
5000	4.63	0/5	0/5	0/10

Observations: Symptoms included soft stools (5M & 2F) and fecal stain (4M) on days 0-1. In addition, there was rough coat (3M), dark material around eyes and/or nose (4M) and congested breathing with rales (1F). Most symptoms were gone by day 6, although one male had transient dark material around the eyes on day 9 only. All rats had weight gains from day 0 to 7, and again from day 7 to 14.

Gross Necropsy: There were no dose-related abnormalities observed at post-sacrifice necropsy.

DATA REVIEW FOR ACUTE DERMAL TOXICITY TESTING (870.1200, formerly §81-2)

Product Manager: 25 **Reviewer:** Byron T. Backus, Ph.D.
MRID No.: 45929402

CITATION: Bonnette, K.L. An Acute Dermal Toxicity Study in Rats with Spray–Charlie. SLI Study No. 3596.17. Unpublished study prepared by Springborn Laboratories, Inc. (SLI), Spencerville, OH 45887. Study Completion Date: Feb. 20, 2003. MRID 45929402.

STUDY SPONSOR AND SUBMITTER: INL/A U.S. Dept. of State, Washington D.C. 20520

TEST MATERIAL: Sample pooled at SLI from five different mixes of Spray–Charlie. From SLI Study No. 3596.15 [Purity Analysis for Glyphosate of Spray–Charlie (Active Ingredient)] in MRID 45929401 the five separate mixes were prepared by adding together 0.439-0.44 by volume GLY-41 Herbicide; 0.01 by volume Cosmo Flux-411F; and 0.55-0.551 by volume Lake Water. The before use (pre-test?) mean for Glyphosate a.e. [acid equivalent] was 16.53% (S.D. 1.35%); and the after use mean percentage was 15.20% (S.D. 1.54%). Both values are above the expected 14.8%.

SPECIES: Rat, Hsd: Sprague Dawley® SD®
AGE(at exposure): "Young adult," approx. 9 weeks old
WEIGHT: Males: 265-290 g; Females: 189-207 g
SOURCE: Harlan Sprague-Dawley, Inc., Indianapolis, IN

EXECUTIVE SUMMARY: ***In an acute dermal toxicity study (MRID 45929402), 5M & 5F young adult (~9-week old; males: 265-290 g; females: 189-207 g) Sprague Dawley® SD® rats (source: Harlan Sprague-Dawley, Indianapolis, IN) were dermally exposed for 24 hrs (occluded exposure) to 5000 mg/kg of Spray–Charlie, containing at least 15.2% a.e. [acid equivalent] glyphosate. The test material (a liquid with a density of 1.08 g/mL) was administered undiluted.***

There was no mortality. Systemic symptoms included dark material around the eyes, nose and/or mouth (10/10 rats), few feces (2F) and soft stools (1M). These symptoms were gone by day 3. One male lost 1 g between day 7 and 14, and two females with weight gains in the period from day 0 to day 7 had moderate weight losses (31 g or 13.7% for #A6710 and 26 g or 12.5% for #A6715) between day 7 and 14. However, based on results from other acute dermal studies with glyphosate, as well as the findings from the oral toxicity study (MRID 45929403) on Spray–Charlie, it is concluded that these weight losses were not a result of exposure to the test material. There was dermal irritation (grade "1" erythema and/or edema) in some rats on day 1, still present in one on day 2, gone by day 3.

There were no significant gross findings at post-sacrifice necropsy.

Dermal LD50 Males > 5000 mg/kg (0/5 died at this dose level)
Dermal LD50 Females > 5000 mg/kg (0/5 died at this dose level)

Spray–Charlie, a liquid with a density of 1.08 g/mL, with at least 15.2% glyphosate a.e., is in toxicity category IV in terms of dermal toxicity, based on the LD50 (both sexes) > 5000 mg/kg.

Study Classification: Acceptable
COMPLIANCE: Signed and dated GLP Compliance (p. 3), Quality Assurance (p. 4), and [No] Data Confidentiality (p. 2) statements are provided. There is no flagging statement.

Procedure (including deviations from 870.1200): "On day -1, the fur was removed from the dorsal trunk area of the animals chosen for the limit test... The clipped area was approximately 10% of the animal's body surface area (BSA). The region included the scapula (shoulder) to the wing of the ilium (hipbone) and half way down the flank on each side of the animal... On the following day (day 0), the test article was administered dermally to approximately 10% of the body surface area (or as large an area as possible). The four corners of this area were delineated in the clipped area with an indelible marker. The test article was then spread evenly over the delineated test area and held in contact with the skin with an appropriately sized 4-ply porous gauze dressing backed with a plastic wrap which was placed over the gauze dressing (occlusive binding). Removal and ingestion of the test article was prevented by placing an elastic wrap over the trunk and test area. The elastic wrap was further secured with a tape harness on the cranial end of the trunk and then secured with adhesive tape around the trunk at the caudal end... Individual doses were calculated based on the animal's day 0 body weight. After an approximate 24-hour exposure period, the binding materials were removed... Residual test article was removed using gauze moistened with deionized water followed by dry gauze."

Results:

Dosage (mg/kg)	Number of Deaths/Number Tested		
	Males	Females	Combined
5000	0/5	0/5	0/10

Observations: Systemic symptoms included dark material around the eyes, nose and/or mouth (10/10 rats), few feces (2F) and soft stools (1M). These symptoms were gone by day 3. One male lost 1 g between day 7 and 14, and two females with weight gains in the period from day 0 to day 7 had moderate weight losses (31 g or 13.7% for #A6710 and 26 g or 12.5% for #A6715) between day 7 and 14. However, based on results from other acute dermal studies with glyphosate, as well as the findings from the oral toxicity study (MRID 45929403) on Spray–Charlie, it is concluded that these weight losses were not a result of exposure to the test material. There was dermal irritation (grade "1" erythema and/or edema) in some rats on day 1, still present in one on day 2, gone by day 3.

Gross Necropsy: There were no significant gross findings at post-sacrifice necropsy.

DATA REVIEW FOR ACUTE INHALATION TOXICITY TESTING (870.1300, formerly §81-3)

Product Manager: 25 **Reviewer:** Byron T. Backus, Ph.D.
MRID No.: 45929404

CITATION: Bonnette, K.L. An Acute Nose-Only Inhalation Toxicity Study in Rats with Spray–Charlie. SLI Study No. 3596.18. Unpublished study prepared by Springborn Laboratories, Inc. (SLI), Spencerville, OH 45887. Study Completion Date: March 14, 2003. MRID 45929404.

STUDY SPONSOR AND SUBMITTER: INL/A U.S. Dept. of State, Washington D.C. 20520

TEST MATERIAL: Sample pooled at SLI from five different mixes of Spray–Charlie. From SLI Study No. 3596.15 [Purity Analysis for Glyphosate of Spray–Charlie (Active Ingredient)] in MRID 45929401 the five separate mixes were prepared by adding together 0.439-0.44 by volume GLY-41 Herbicide; 0.01 by volume Cosmo Flux-411F; and 0.55-0.551 by volume Lake Water. The before use (pre-test?) mean for Glyphosate a.e. [acid equivalent] was 16.53% (S.D. 1.35%); and the after use mean percentage was 15.20% (S.D. 1.54%). Both values are above the expected 14.8%.

SPECIES: Rat, Hsd: Sprague Dawley® SD®
AGE(at exposure): "Young adult," approx. 9 weeks old
WEIGHT(at exposure): Males: 248-275 g; Females: 201-212 g
SOURCE: Harlan Sprague-Dawley, Inc., Indianapolis, IN

EXECUTIVE SUMMARY: ***In an acute inhalation toxicity study (MRID 45929404), a group of 5 male and 5 female young adult (~9 week old; males 248-275 g; females: 201-212 g) Hsd: Sprague Dawley® SD® rats (source: Harlan Sprague-Dawley, Indianapolis, IN) received 4-hr nose-only exposure to an aerosol with a mean time-weighted analytical concentration of 2.60 mg/L of Spray–Charlie, a liquid containing at least 15.2% a.e. [acid equivalent] glyphosate. A mean of 66% of the particles by weight had an effective cutoff diameter of $\leq$ 4 μm. The MMAD was 2.9 μm, and the GSD was 2.17.***

There was no mortality (0/5M & 0/5F died). No symptoms were observed during exposure. Symptoms after exposure included congested breathing and rales in all rats, with congested breathing persisting in 3M through day 14. Other symptoms included labored breathing (in some cases with gasping), no or few feces, dark material around mouth, and decreased food consumption. Two males and one female lost weight in the period from day 0 to day 7; but (except for one female which maintained weight) all gained weight in the period from day 0 to day 14, although overall body weight gains in two males (as well as this one female) appeared to be reduced.

At post-sacrifice necropsy there were no gross abnormalities.

Inhalation LC50 Males > 2.60 mg/L (0/5 died after 4-hr exposure to this concentration)
Inhalation LC50 Females > 2.60 mg/L (0/5 died after 4-hr exposure to this concentration)

The test material, Spray–Charlie, a liquid containing at least 15.2% a.e. glyphosate, is in toxicity category IV by the inhalation exposure route.

Study Classification: Acceptable

COMPLIANCE: Signed and dated GLP Compliance (p. 3), Quality Assurance (p. 4), and [No] Data Confidentiality (p. 2) statements are provided. There is no flagging statement.

Procedure (including deviations from 870.1300): "Prior to experimental initiation, preliminary aerosol generation trials were conducted. These trials were performed in order to determine the most efficient means of generating an aerosol of the appropriate concentration while utilizing equipment that would reduce the aerodynamic particle size... On day 0, the animals chosen for the limit test were weighed, placed in a nose-only exposure tube and allowed to acclimate to the exposure tube for at least 1 hour. Animals that appeared to have been acclimated to the exposure tube (i.e., minimal struggling and no inversion) were considered to be acceptable, removed from the exposure tube and returned to their cages until initiation of the aerosol exposure. Animals that did not...acclimate to the exposure tube were not acceptable...

"The acceptable animals were then placed in exposure tubes, the tubes inserted into the Multi-State 10L nose-only inhalation chamber and the test article aerosolized... The aerosol exposure consisted of a 3-minute T99 equilibration period, a 240-minute exposure period and a 3-minute de-equilibration period equal to the T99 equilibration period. After each aerosol exposure, animals were removed from the exposure tubes and residual test article was removed from the animal's exterior surfaces (where practical) by wiping the haircoat with a towel...

"The test aerosol was generated with a Pistol Spraying System and a Master Flex Pump... Conditioned high pressure external air was used in generating the test atmosphere..."

Results:

Mean Exposure Concentration mg/L (Analytically Determined)	Number of Deaths/Number Tested		
	Males	Females	Combined
2.60	0/5	0/5	0/10

The nominal concentration was 70.30 mg/L.

Clinical Observations: No symptoms were observed during exposure. Symptoms following exposure included congested breathing and rales in all rats, with congested breathing persisting in 3M through day 14. Other symptoms included labored breathing (in some cases with gasping), no or few feces, dark material around mouth, and decreased food consumption. Two males and one female lost weight in the period from day 0 to day 7; but (except for one female which only maintained weight) all gained weight in the period from day 0 to day 14, although overall body weight gains in two males (as well as this one female) appeared to be reduced.

Gross Necropsy: At post-sacrifice necropsy there were no gross abnormalities.

	Chamber Atmosphere	
Analytical Conc. (mg/L)	MMAD (μm)	GSD
2.60	2.9	2.17

Particle Size Distribution: A 7-stage Cascade Impactor was used to determine particle size distribution. A mean of 66% of the particles by mass were ≤ 4.0 μm.

Chamber Environment	

Internal Chamber Volume	10 L
Mean Air Flow Rate	24 LPM
Mean Chamber Temperature (range)	68.3-70.7° F
Mean Relative Humidity (range)	68.3-69.3%

DATA REVIEW FOR PRIMARY EYE IRRITATION TESTING (870.2400, formerly §81-4)

Product Manager: 25 **Reviewer:** Byron T. Backus, Ph.D.
MRID No.: 45929405

CITATION: Bonnette, K.L. A Primary Eye Irritation Study in Rabbits with Spray–Charlie. SLI Study No. 3596.19. Unpublished study prepared by Springborn Laboratories, Inc. (SLI), Spencerville, OH 45887. Study Completion Date: February 17, 2003. MRID 45929405.

STUDY SPONSOR AND SUBMITTER: INL/A U.S. Dept. of State, Washington D.C. 20520

TEST MATERIAL: Sample pooled at SLI from five different mixes of Spray–Charlie. From SLI Study No. 3596.15 [Purity Analysis for Glyphosate of Spray–Charlie (Active Ingredient)] in MRID 45929401 the five separate mixes were prepared by adding together 0.439-0.44 by volume GLY-41 Herbicide; 0.01 by volume Cosmo Flux-411F; and 0.55-0.551 by volume Lake Water. The before use (pre-test?) mean for Glyphosate a.e. [acid equivalent] was 16.53% (S.D. 1.35%); and the after use mean percentage was 15.20% (S.D. 1.54%). Both values are above the expected 14.8%. pH not reported.

SPECIES: Rabbit, albino, New Zealand White (males only)
AGE: "adult" (approximately 16 weeks)
WEIGHT: 3.172 - 3.607 kg
SOURCE: Myrtle's Rabbitry, Thompson Station, TN

EXECUTIVE SUMMARY: ***In a primary eye irritation study (MRID 45929405), 0.1 mL Spray–Charlie, a liquid (pH not reported) containing at least 15.2% a.e. [acid equivalent] glyphosate, was instilled into the conjunctival sac of one eye of each of three adult (16 week old) male (3.172-3.607 kg) New Zealand white rabbits (source: Myrtle's Rabbitry, Thompson Station, TN).***

No corneal opacity was observed. All 3 eyes were positive for iritis at 1 hr, but all were negative (scored zero) for iritis at 24 hrs and subsequently. All eyes were positive for conjunctival redness (score "2") and chemosis (score "2") at 24 hours, and all 3 eyes were positive for redness at 48 hrs. One eye was still positive for redness at 72 hrs. All eyes had cleared (all scores zero) by day 7.

As eye irritation was still present through 72 hours, but had cleared by day 7, the test material, Spray–Charlie, a liquid containing at least 15.2% a.e. glyphosate, is in toxicity category III for eye irritation potential.

Study Classification: Acceptable

COMPLIANCE: Signed and dated GLP Compliance (p. 3), Quality Assurance (p. 4), and [No] Data Confidentiality (p. 2) statements are provided. There is no flagging statement.

Procedure (including deviations from 870.2400): "A minimum of one hour after preliminary ocular examination, the test article was instilled...into the conjunctival sac of the right eye of each animal after gently pulling the lower lid away from the eye. Following instillation, the eyelids were gently held together for approximately one second in order to limit test article loss..."

Results:

Observations	Number scoring positive/total number				
	1 hr	24 hrs[b]	48 hrs	72 hrs	7 days
Corneal Opacity	0/3	0/3[b]	0/3	0/3	0/3
Iritis	3/3	0/3	0/3	0/3	0/3
Conjunctivae:					
Redness[a]	2/3	3/3	3/3	1/3	0/3
Chemosis[a]	3/3	3/3	1/3	0/3	0/1
Discharge[a]	1/3	1/3	0/3	0/3	0/1

[a]Score of 2 or more considered positive.
[b]Fluorescein examination at 24 hours; all eyes were negative.

No corneal opacity was observed. All 3 eyes were positive for iritis at 1 hr, but all were negative (scored zero) for iritis at 24 hrs and subsequently. All eyes were positive for conjunctival redness (score "2") and chemosis (score "2") at 24 hours, and all 3 eyes were positive for redness at 48 hrs. One eye was still positive for redness at 72 hrs. All eyes had cleared (all scores zero) by day 7.

DATA REVIEW FOR PRIMARY DERMAL IRRITATION TESTING (870.2500, formerly §81-5)

Product Manager: 21 **Reviewer:** Byron T. Backus, Ph.D.
MRID No.: 45929406

CITATION: Bonnette, K.L. A Primary Skin Irritation Study in Rabbits with Spray–Charlie. SLI Study No. 3596.20. Unpublished study prepared by Springborn Laboratories, Inc. (SLI), Spencerville, OH 45887. Study Completion Date: February 17, 2003. MRID 45929406.

STUDY SPONSOR AND SUBMITTER: INL/A U.S. Dept. of State, Washington D.C. 20520

TEST MATERIAL: Sample pooled at SLI from five different mixes of Spray–Charlie. From SLI Study No. 3596.15 [Purity Analysis for Glyphosate of Spray–Charlie (Active Ingredient)] in MRID 45929401 the five separate mixes were prepared by adding together 0.439-0.44 by volume GLY-41 Herbicide; 0.01 by volume Cosmo Flux-411F; and 0.55-0.551 by volume Lake Water. The before use (pre-test?) mean for Glyphosate a.e. [acid equivalent] was 16.53% (S.D. 1.35%); and the after use mean percentage was 15.20% (S.D. 1.54%). Both values are above the expected 14.8%. pH not reported.

SPECIES: Rabbit, albino, New Zealand White (1 male, 2 females)
AGE: "adult" (approximately 13 weeks)
WEIGHT: Male: 2.723 kg; Females: 2.494-2.814 kg [according to Table 1 p. 15 all 3 rabbits were female]
SOURCE: Myrtle's Rabbitry, Thompson Station, TN

EXECUTIVE SUMMARY: ***In a dermal irritation study (MRID 45929406), 0.5 mL undiluted Spray–Charlie, a liquid (pH not reported) containing at least 15.2% a.e. [acid equivalent] glyphosate was applied to a dermal site on each of 3 adult (13 weeks; male: 2.723 kg; females: 2.494 & 2.814 kg) New Zealand white rabbits, with 4-hr semioccluded exposure.***

All scores (1, 24, 48 & 72 hrs) for edema were zero. At 1 hour all 3 sites scored "1" for erythema; at 24 hrs and subsequently all scores for erythema were zero. The primary irritation index (mean of scores at 1, 24, 48 & 72 hrs) = 0.25. The primary irritation index (mean of scores at 1, 24, 48 & 72 hrs) = 0.25. At 1 hr 3/3 sites scored "1" for erythema; this was the only irritation seen in this study as all scores at 24 hrs and subsequently were zero.

The test material, Spray–Charlie, containing at least 15.2% a.e. glyphosate, is in toxicity category IV in terms of dermal irritation.

Study Classification: Acceptable

COMPLIANCE: Signed and dated GLP Compliance (p. 3), Quality Assurance (p. 4), and [No] Data Confidentiality (p. 2) statements are provided. There is no flagging statement.

Procedure (including deviations from 870.2500): "On day -1, the animals chosen for use...had the fur removed from the dorsal area of the trunk... On the following day (day 0), [0.5 mL of] the test article was applied to a small area of intact skin on each test animal (approximately 1 inch x 1 inch)... The test article was administered under the [1" x 1" square 4-ply] gauze patch. The gauze patch was held in contact with the skin...with a nonirritating tape. Removal and ingestion of the test article was prevented by placing an elastic wrap over the trunk and test area (semi-occlusive binding). The elastic wrap was the further secured with adhesive tape around the trunk at the cranial and caudal ends. After dosing, collars were placed on each animal and remained in place until removal on day 3. After a four-hour exposure period, the binding materials were removed from each animal... Residual test article was removed using gauze moistened with deionized water, followed by dry gauze."

Results: All scores (1, 24, 48 & 72 hrs) for edema were zero. At 1 hour all 3 sites scored "1" for erythema; at 24 hrs and subsequently all scores for erythema were zero. The primary irritation index (mean of scores at 1, 24, 48 & 72 hrs) = 0.25.

DATA REVIEW FOR DERMAL SENSITIZATION TESTING (870.2600, formerly §81-6)

Product Manager: 25 **Reviewer:** Byron T. Backus, Ph.D.
MRID No.: 45929407

CITATION: Bonnette, K.L. A Dermal Sensitization Study in Guinea Pigs with Spray–Charlie. SLI Study No. 3596.21. Unpublished study prepared by Springborn Laboratories, Inc. (SLI), Spencerville, OH 45887. Study Completion Date: March 14, 2003. MRID 45929407.

STUDY SPONSOR AND SUBMITTER: INL/A U.S. Dept. of State, Washington D.C. 20520

TEST MATERIAL: Sample pooled at SLI from five different mixes of Spray–Charlie. From SLI Study No. 3596.15 [Purity Analysis for Glyphosate of Spray–Charlie (Active Ingredient)] in MRID 45929401 the five separate mixes were prepared by adding together 0.439-0.44 by volume GLY-41 Herbicide; 0.01 by volume Cosmo Flux-411F; and 0.55-0.551 by volume Lake Water. The before use (pre-test?) mean for Glyphosate a.e. [acid equivalent] was 16.53% (S.D. 1.35%); and the after use mean percentage was 15.20% (S.D. 1.54%). Both values are above the expected 14.8%.

SPECIES: Guinea Pig, albino, Hartley-derived
AGE(at initiation of induction): Young adult (males: ~6-7 weeks; females: ~8-9 weeks)
WEIGHT(Day -1): Males: 394 - 464 g; Females: 366 - 420 g
SOURCE: Hilltop Lab Animals Inc., Scottdale, PA

EXECUTIVE SUMMARY: ***In a dermal sensitization study (MRID 45929407) using a Modified Buehler Design, 20 (10 male: 7 weeks; 394-464 g [day -1] & 10F: ~9 weeks; 366-420 g [day -1]) albino Hartley-derived guinea pigs received 3 6-hr occluded induction exposures, each to 0.3 mL of undiluted Spray–Charlie, a liquid containing at least 15.2% a.e. glyphosate, on study days 0, 7 & 14. Two weeks later the test (previously exposed) guinea pigs as well as a naive control group of 5M & 5F were similarly exposed at a previously unexposed test site. The concentration of test material in the induction and challenge exposures was based on results from a preliminary topical range-finding assay.***

Following challenge 0/20 previously exposed and 0/10 naive control guinea pigs scored zero at 24 hours; 2/20 previously exposed and 0/10 naive control guinea pigs scored ± (maximum response observed) at 48 hrs. These results indicate the test material is not a potential dermal sensitizer.

The report includes a positive control study utilizing alpha-Hexylcinnamaldehyde (HCA); this study was conducted from September 17, 2002 to October 17, 2002. Results were appropriate. The in-life study with Spray–Charlie began on December 31, 2002 and ended on January 30, 2003.

Study Classification: Acceptable. The results of this study indicate Spray–Charlie, a liquid containing at least 15.2 a.e. glyphosate, is not a potential dermal sensitizer.

COMPLIANCE: Signed and dated GLP Compliance (p. 3), Quality Assurance (p. 4), and [No] Data Confidentiality (p. 2) statements are provided. There is no flagging statement.

Procedure: The dosages used for induction and challenge were based on preliminary irritation studies. For induction: "On the day prior to each dose administration, the guinea pigs had the hair removed... A dose of 0.3 mL of the test article was placed on a 25 mm Hilltop chamber backed by adhesive tape (occlusive patch). The chambers were then applied to the clipped surface as quickly

as possible... The induction procedure was repeated on study day 7 and on study day 14 so that a total of three consecutive induction exposures were made to the test animals."

For challenge: "On the day prior to challenge dose administration, the test and challenge control animals were weighed and the hair was removed from the right side of the animals. On the day following...(day 28), chambers were applied... Approximately six hours after chamber application, the binding materials were removed. The test sites were wiped with gauze moistened in deionized water

Results: Following challenge 0/20 previously exposed and 0/10 naive control guinea pigs scored zero at 24 hours; 2/20 previously exposed and 0/10 naive control guinea pigs scored ± (maximum response observed) at 48 hrs. These results indicate the test material is not a potential dermal sensitizer.

The report includes a positive control study utilizing alpha-Hexylcinnamaldehyde (HCA); this study was conducted from September 17, 2002 to October 17, 2002. Results were appropriate. The in-life study with Spray–Charlie began on December 31, 2002 and ended on January 30, 2003.

ACUTE TOX ONE-LINERS

1. **DP BARCODE:** D289806
2. **PC CODES:** 103601 Glyphosate, isopropylamine salt
3. **CURRENT DATE:** May 12, 2003
4. **TEST MATERIAL:** Sample pooled at SLI from five different mixes of Spray–Charlie. From SLI Study No. 3596.15 [Purity Analysis for Glyphosate of Spray–Charlie (Active Ingredient)] in MRID 45929401 the five separate mixes were prepared by adding together 0.439-0.44 by volume GLY-41 Herbicide; 0.01 by volume Cosmo Flux-411F; and 0.55-0.551 by volume Lake Water. The before use (pre-test?) mean for Glyphosate a.e. [acid equivalent] was 16.53% (S.D. 1.35%); and the after use mean percentage was 15.20% (S.D. 1.54%). Both values are above the expected 14.8%.

Study/Species/Lab Study #/Date	MRID	Results	Tox. Cat.	Core Grade
Acute oral toxicity/rat/ Springborn Labs Inc. (SLI)/SLI Study No. 3596.16/FEB-20-2003	45929403	LD_{50}(M, F, combined) > 5000 mg/kg (0/5M & 0/5F died after dosage at this level). Only dose was 5000 mg/kg. Symptoms included soft stools and fecal stain on days 0-1. Also, there was rough coat, dark material around eyes and/or nose and congested breathing with rales (1F only). Most symptoms were gone by day 6, although one male had transient dark material around eyes on day 9 only. All gained weight from day 0-7 and from day 7-14. No dose-related abnormalities observed at post-sacrifice necropsy.	III	A
Acute dermal toxicity/rat/ Springborn Labs Inc. (SLI)/SLI Study No. 3596.17/FEB-20-2003	45929402	LD_{50}(M, F, combined) > 5000 mg/kg (0/5M & 0/5F died at this dose level). Symptoms: dark material around facial area, few feces and soft stools. One male lost 1 g day 7-14 and 2F which had gained weight days 0-7 had moderate wt losses (31 g or 13.7% for one and 26 g or 12.5% for the other) day 7-14. No significant findings at post-sacrifice necropsy.	IV	A
Acute inhalation toxicity/ rat/Springborn Labs Inc. (SLI)/SLI Study No. 3596.18/MAR-14-2003	45929404	Nose-only exposure. LC_{50}(M,F, combined) > 2.6 mg/L (0/5M & 0/5F died). No symptoms observed during exposure. Symptoms after included congested breathing and rales in all rats, with congested breathing persisting in 3M through day 14. Other symptoms: labored breathing (in some cases with gasping), no or few feces, dark material around mouth and decreased food consumption. 2M & 1F lost wt from day 0 to 7; but, except for 1F which maintained wt, all gained wt day 0 -14, though overall wt gains in 2M (as well as the 1F) were reduced. No abnormalities were observed at post-sacrifice necropsy. 66% of the particles by mass had an effective cut-off diameter of $\leq$4 µm. MMAD was 2.9 µm & GSD was 2.17.	IV	A
Primary eye irritation/ rabbit/Springborn Labs Inc. (SLI)/SLI Study No.	45929405	3 NZ white rabbit eyes exposed. 0.1 mL test material instilled. No corneal opacity observed. 3/3 eyes were positive for iridial irritation at 1 hr	III	A

3596.19/FEB-17-2003		but were subsequently clear. All 3 eyes were positive for conjunctival redness & chemosis at 24 hrs, and all 3 were positive for redness at 48 hrs. 1/3 eyes was still positive for redness at 72 hrs. All eyes had cleared (all scores zero) by day 7.		
Primary dermal irritation/ rabbit/Springborn Labs Inc. (SLI)/SLI Study No. 3596.20/FEB-17-2003	45929406	3 NZ white rabbits used. PII (av. of 1, 24, 48 & 72 hr scores) = 0.25; at 1 hr 3/3 sites scored "1" for erythema (max score for erythema) and "0" for edema. At 24 hrs & subsequently all scores were zero.	IV	A
Dermal sensitization/ guinea pig/Springborn Labs Inc. (SLI)/SLI Study No. 3596.21/MAY-30-2002	45929407	Modified Buehler test. 20 (10M & 10F) Hartley-derived albino guinea pigs received 1/week for 3 weeks induction exposures to 0.3 mL undiluted test material, with challenge 2 weeks after last induction treatment. At challenge 0/20 induced and 0/10 naive controls scored zero at 24 hrs; 2/20 induced scored ± at 48 hrs with all other scores zero. Results indicate a nonsensitizer. Positive control study used HCA, was within 6 months & was acceptable.	Non-Sensi-tizer	A

Core Grade Key: **A =Acceptable, S = Supplementary, U = Unacceptable, V = Self Validated**

Annex 56-A

Six Acute Toxicity Studies with Spray-Charlie, SLI Study N° 3596.16, 20 February 2003

(United States Embassy in Bogotá, 2011)

AN ACUTE ORAL TOXICITY STUDY IN RATS WITH SPRAY--CHARLIE

FINAL REPORT

OPPTS Guideline

870.1100

Author

Kimberly L. Bonnette, M.S., LATG

Study Completed on

February 20, 2003

Performing Laboratory

Springborn Laboratories (SLI),
a division of Charles River Company, Inc.
640 North Elizabeth Street
Spencerville, Ohio 45887

SLI Study No.

3596.16

Submitted to

INL/A
U.S. Department of State
2201 C St. NW SA-4
Washington, DC 20520

SLI Study No. 3596.16 (2)

1. STATEMENT OF *NO* DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B), or (C).

Company: _____

Company Agent: _____ Date _____

_____ _____

Title Signature

SLI Study No. 3596.16 (3)

FEB 14 2003

2. COMPLIANCE STATEMENT

This study was conducted in compliance with the Good Laboratory Practice Standards as described by the EPA (40 CFR Parts 160 and 792).

Kimberly L. Bonnette, M.S., LATG
Study Director/Author
Springborn Laboratories, Inc.

Date 2/20/03

Rogers Woolfolk
Senior Aviation Advisor
Sponsor/Submitter
INL/A
U.S. Department of State

Date 6 Feb 03

3. QUALITY ASSURANCE STATEMENT

This study was inspected by the Quality Assurance Unit and reports were submitted to management and the Study Director in accordance with SLI's Standard Operating Procedures as follows:

Phase	Date
Protocol Review	10/07/02
Necropsy	12/30/02
Data Audit	01/21/03
Draft Report Review	01/21/03
Final Report Review	02/20/03
Reports to Study Director and Management	01/21/03, 02/20/03

The final report has been reviewed to assure that it accurately describes the materials and methods, and the reported results accurately reflect the raw data.

Jennifer D. McGue

Date 2/20/03

Jennifer D. McGue
Quality Assurance Auditor

Anita M. Bosau

Date 2/20/03

Anita M. Bosau, RQAP-GLP
Senior Director, Compliance Assurance

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SLI Study No. 3596.16 (7)

6. SUMMARY

The single-dose oral toxicity of Spray--Charlie was evaluated in Sprague Dawley rats. A limit test was performed in which one group of five male and five female rats received a single oral administration of the test article at a dose of 5000 mg/kg body weight. Following dosing, the limit test rats were observed daily and weighed weekly. A gross necropsy examination was performed on all limit test animals at the time of scheduled euthanasia (day 14).

No mortality occurred during the limit test. Clinical abnormalities observed during the study included transient incidences of soft stools, fecal staining, rough coat, congested breathing, rales and dark material around the facial area. Body weight gain was noted for all animals during the test period. No significant gross internal findings were observed at necropsy on study day 14.

Under the conditions of this test, the acute oral LD50 of Spray--Charlie was estimated to be greater than 5000 mg/kg in the rat.

7. INTRODUCTION

This study was performed to assess the short-term toxicity of Spray--Charlie in Sprague Dawley rats when administered by gavage as a single oral dose. This study was intended to provide information on the potential health hazards of the test article with respect to oral exposure. Data from this study may serve as a basis for classification and/or labeling of the test article. This study was performed in accordance with the US EPA, Health Effects Test Guidelines, OPPTS 870.1100, Acute Oral Toxicity, August 1998. This study was performed at Springborn Laboratories (SLI), a division of Charles River Laboratories, Inc. 553 North Broadway, Spencerville, Ohio. The protocol was signed by the Study Director on October 9, 2002 (GLP initiation date). The in-life phase of the study was initiated with test article administration on December 16, 2002 (day 0) and concluded with necropsy on December 30, 2002.

8. MATERIALS AND METHODS

8.1. Test Article

The test article was received from the Sponsor and identified as follows:

Sponsor's ID	Assigned SLI ID	Physical Description	Receipt Date	Expiration Date
Spray--Charlie[a]	S02.003.3596	Amber liquid	12/09/02	None provided
Ingredients:[b]				
Herbicide: GLY-41 Lot No.: Manufactured 10/20/02				None provided
Surfactant: Cosmo Flux-411F Lot No.: Manufactured 11/29/02				None provided

[a]Sample pooled at SLI from five different mixes of Spray--Charlie (top/middle/bottom).
[b]Ingredients used in the five Spray--Charlie mixes that were prepared by the Sponsor.

The test article was stored at room temperature. The Sponsor was responsible for any necessary evaluations related to identity, strength, purity, composition, stability and method of synthesis of the test material according to 40 CFR 160.105 and 40 CFR 792.105. Springborn Laboratories, analyzed the test article for the glyphosate (a.e.) which is presented in SLI Study No. 3596.15.

8.2. Retention Sample

A 1 mL retention sample of each of the 5 test article mixtures (top/middle/bottom, maintained separately for a total of 15, 1 mL samples) was collected and maintained at SLI at room temperature. Also, a 10 mL retention sample of the pooled test article sample (from the 5 test article mixtures) was collected and maintained at SLI at room temperature. These samples serve as the retention samples for all studies conducted with this material.

8.3. Test Article Disposition

The remaining test article was returned to the Sponsor following completion of all studies with the test article.

8.4. Method of Test Article Preparation

The test articles were pooled and dispensed as received fresh on the day of dosing. The density of the test article was 1.08 g/mL. The test article preparation was stirred continuously during the dosing procedure.

8.5. Animals and Animal Husbandry

8.5.1. Description, Identification and Housing

Young adult, Hsd: Sprague Dawley® SD® rats were received from Harlan Sprague Dawley, Inc., Indianapolis, IN. Upon receipt, metal ear tags displaying unique identification numbers were used to individually identify the animals. Cage cards displaying at least the study number, animal number and sex were affixed to each cage. The animals were housed individually in suspended stainless steel cages. All housing and care were based on the standards recommended by the Guide for the Care and Use of Laboratory Animals [1].

8.5.2. Environment

The animal room temperature and relative humidity ranges were 71-74°F (22-23°C) and 33-53%, respectively. Environmental control equipment was monitored and adjusted as necessary to minimize fluctuations in the animal room environment. Light timers were set to maintain a 12-hour light/12-hour dark cycle and room ventilation was set to produce 10-15 air changes/hour. The animal room temperature and relative humidity were recorded a minimum of once daily.

8.5.3. Food

PMI Certified Rodent Chow #5002 (Purina Mills, Inc.) was provided ad libitum to the animals throughout the study (except during fasting). The lot number and expiration date of each batch of diet used during the study were recorded. The

feed was analyzed and certified by the supplier for nutritional components and environmental contaminants. Dietary limitations for various environmental contaminants, including heavy metals, pesticides, polychlorinated biphenyls and total aflatoxin are set by the manufacturer. Within these limits, contaminants which may have been present were not expected to compromise the purpose of this study. Results of the dietary analyses (Certificates of Analysis) are provided by the manufacturer for each lot of diet. These are maintained by SLI.

8.5.4. Water

Municipal tap water treated by reverse osmosis was available ad libitum throughout the study. The purified water was supplied by an automatic watering system. Monitoring of the drinking water for contaminants is conducted by SLI and the records are available for inspection. Within generally accepted limits, contaminants which may have been present were not expected to compromise the purpose of this study. The water meets the standards specified under the EPA National Drinking Water Regulations (40 CFR Part 141).

8.5.5. Acclimation

Upon receipt, the animals were removed randomly from the shipping cartons, examined by qualified personnel, identified with metal ear tags and then acclimated to the laboratory conditions for a minimum of five days. The animals were observed daily for overt physical or behavioral abnormalities, general health/moribundity and mortality.

8.5.6. Animal Selection

The animals chosen for study use were randomly selected from healthy stock animals using a computerized random numbers table to avoid potential bias. All animals received a detailed pretest observation prior to dosing. Only healthy animals were chosen for study use. Females were nulliparous and nonpregnant. The male animals were approximately 9-10 weeks of age and weighed 325-356 g prior to fasting. The female animals were approximately 8 weeks of age and weighed 190-208 g prior to fasting.

9. EXPERIMENTAL PROCEDURES

9.1. Dosing

On day -1, the animals chosen for the limit test were weighed and fasted overnight. On day 0, the test article was administered orally as a single dose using a ball tipped stainless steel gavage needle attached to a syringe at the following level:

Dose Level (mg/kg)	Concentration (%)	Dose Volume (mL/kg)	No. of Animals	
			Male	Female
5000	100[a]	4.63[b]	5	5

[a]Pooled test article.
[b]Adusted based on a density of 1.08 g/mL.

Individual doses were calculated based on the animal's fasted (day 0) body weight. Animals were returned to ad libitum feeding after dosing.

9.2. Clinical Observations

The animals were observed for clinical abnormalities a minimum of two times on study day 0 (post-dose) and daily thereafter (days 1-14). A general health/mortality check was performed twice daily (in the morning and in the afternoon).

9.3. Body Weights

Individual body weights were obtained for the animals prior to fasting (day -1), prior to dosing on day 0 and on days 7 and 14.

9.4. Gross Necropsy

All animals were euthanized by carbon dioxide inhalation at study termination (day 14) and were necropsied. Body cavities (cranial, thoracic, abdominal and pelvic) were opened and examined. No tissues were retained.

9.5. Protocol Deviations

No protocol deviations occurred during this study.

10. DATA ACQUISITION AND ELECTRONIC RECORDS

Electronic data were recorded on a Compaq Alpha Server DS10 utilizing the Toxicology Analysis System Customized, Acute Toxicology Module, Version 1.0.0 or higher. The SLI study number assigned to this study is 3596.16. The computer study number used to collect data for the study phases was 359616. The tables within the report display the applicable computer number.

11. ANALYSIS OF DATA

Data from the study were analyzed and an LD50 value estimated as follows:

< 50% Mortality: LD50 was estimated as greater than the administered dose.
= 50% Mortality: LD50 was estimated as equal to the administered dose.
> 50% Mortality: LD50 was estimated as less than the administered dose.

Body weight means and standard deviations were calculated separately for males and females.

12. MAINTENANCE OF RAW DATA AND RECORDS

All original raw data, the final report and electronic records were transferred to the SLI archives for a period of 10 years. The Sponsor will be contacted prior to final disposition of these items.

13. RESULTS

13.1. Mortality

Individual Data: Table 1

No mortality occurred during the limit test.

13.2. Clinical Observations

Individual Data: Table 1

Clinical abnormalities observed during the study included transient incidences of soft stools, fecal staining, rough coat, congested breathing, rales and dark material around the facial area.

13.3. Body Weight Data

Individual Data: Table 2

Body weight gain was noted for all animals during the test period.

13.4. Gross Necropsy

Individual Data: Table 3

No significant gross internal findings were observed at necropsy on study day 14.

Note: A hernia of the diaphragm was observed for 1/5 test males. However, this finding is congenital and common in this strain of rat and therefore, is not considered to be significant.

14. CONCLUSION

Under the conditions of this test, the acute oral LD50 of Spray--Charlie was estimated to be greater than 5000 mg/kg in the rat.

Kimberly L. Bonnette, M.S., LATG
Study Director

Date 2/20/03

15. REPORT REVIEW

Dawn D. Rodabaugh, B.S.
Toxicologist

Date 2/20/03

16. REFERENCE

1. Guide for the Care and Use of Laboratory Animals, DHHS Publication No. (NIH) 96-03, 1996.

INL/A, US DEPARTMENT OF STATE

TABLE 1

AN ACUTE ORAL TOXICITY STUDY IN RATS

INDIVIDUAL CLINICAL OBSERVATIONS
(POSITIVE FINDINGS)

MALES 5000 MG/KG

MALE#	OBSERVATIONS	DAY OF STUDY 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A6561	SCHEDULED EUTHANASIA															P
	SOFT STOOLS	P														
	FECAL STAIN	1	P													
A6626	SCHEDULED EUTHANASIA															P
	FECAL STAIN	1														
	ROUGH COAT		P	P												
	DARK MATERIAL AROUND NOSE		P													
A6640	SCHEDULED EUTHANASIA															P
	SOFT STOOLS	P														
	DARK MATERIAL AROUND EYE(S)				P											
	DARK MATERIAL AROUND NOSE				P		P									
A6638	SCHEDULED EUTHANASIA															P
	SOFT STOOLS	P														
	FECAL STAIN	1														
	ROUGH COAT		P													
	DARK MATERIAL AROUND NOSE		P													
A6646	SCHEDULED EUTHANASIA															P
	SOFT STOOLS	P														
	FECAL STAIN	1														
	ROUGH COAT		P													
	DARK MATERIAL AROUND EYE(S)										P					

GRADE CODE: 1=SLIGHT 2=MODERATE 3=SEVERE P=PRESENT L=LEFT R=RIGHT B=BILATERAL

STUDY NO.: 359616 PAGE 2
INL/A, US DEPARTMENT OF STATE

TABLE 1

AN ACUTE ORAL TOXICITY STUDY IN RATS

INDIVIDUAL CLINICAL OBSERVATIONS
(POSITIVE FINDINGS)

FEMALES 5000 MG/KG

FEMALE#	OBSERVATIONS	DAY OF STUDY 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A6711	SCHEDULED EUTHANASIA															P
A6712	SCHEDULED EUTHANASIA															P
A6713	SCHEDULED EUTHANASIA															P
	CONGESTED BREATHING	P	P	P												
	RALES		P													
A6714	SCHEDULED EUTHANASIA															P
	SOFT STOOLS	P														
A6718	SCHEDULED EUTHANASIA															P
	SOFT STOOLS	P														

GRADE CODE: 1=SLIGHT 2=MODERATE 3=SEVERE P=PRESENT L=LEFT R=RIGHT B=BILATERAL

STUDY NO.: 359616 PAGE 1

INL/A, US DEPARTMENT OF STATE

TABLE 2

AN ACUTE ORAL TOXICITY STUDY IN RATS

INDIVIDUAL BODY WEIGHTS (GRAMS)

MALES 5000 MG/KG

ANIMAL#	DAY OF STUDY -1	0	7	14 AT DEATH (DAY)
A6561	325	295	340	364
A6626	330	299	345	378
A6640	336	301	352	374
A6638	356	325	372	405
A6646	326	294	354	378
MEAN	335	303	353	380
S.D.	12.7	12.7	12.2	15.2
N	5	5	5	5

STUDY NO.: 359616 PAGE 2
INL/A, US DEPARTMENT OF STATE

TABLE 2

AN ACUTE ORAL TOXICITY STUDY IN RATS

INDIVIDUAL BODY WEIGHTS (GRAMS)

FEMALES 5000 MG/KG

	DAY OF STUDY			
ANIMAL#	-1	0	7	14 AT DEATH (DAY)
A6711	194	172	207	217
A6712	190	169	195	216
A6713	197	175	213	220
A6714	208	188	216	235
A6718	201	178	219	234
MEAN	198	176	210	224
S.D.	6.9	7.3	9.5	9.3
N	5	5	5	5

INL/A, US DEPARTMENT OF STATE

TABLE 3

AN ACUTE ORAL TOXICITY STUDY IN RATS

INDIVIDUAL GROSS NECROPSY OBSERVATIONS

MALES 5000 MG/KG

ANIMAL#	DAY OF DEATH	STUDY DAY	OBSERVATION	FATE
A6561	30-DEC-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A6626	30-DEC-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A6640	30-DEC-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A6638	30-DEC-02	14	DIAPHRAGM: HERNIA; PRESENT MUSCULOTENDINOUS PORTION, 0.5 X 0.4 CM, PORTION OF MEDIAL LIVER LOBE MISSHAPEN AND EXTENDS INTO THORACIC CAVITY	SCHEDULED EUTHANASIA
A6646	30-DEC-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA

TABLE 3

AN ACUTE ORAL TOXICITY STUDY IN RATS

INDIVIDUAL GROSS NECROPSY OBSERVATIONS

FEMALES 5000 MG/KG

ANIMAL#	DAY OF DEATH	STUDY DAY	OBSERVATION	FATE
A6711	30-DEC-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A6712	30-DEC-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A6713	30-DEC-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A6714	30-DEC-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A6718	30-DEC-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA

APPENDIX A

SLI Personnel Responsibilities

SLI Study No. 3596.16 (22)

SLI PERSONNEL RESPONSIBILITIES

Kimberly L. Bonnette, M.S., LATG	Study Director/Director, Acute Toxicology
Dawn D. Rodabaugh, B.S.	Alternate Contact/Toxicologist
Malcolm Blair, Ph.D.	Managing Director Emeritus
Joseph C. Siglin, Ph.D., DABT	General Manager
Jason W. Smedley, B.S.	Assistant Toxicologist
Pamela S. Smith, ALAT	Study Supervisor, Acute Toxicology
Christina L. Zehender, B.S.	Primary Technician/Acute Technician I
Delores P. Knippen	Supervisor, Pharmacy
Steven H. Magness, B.S., LATG	Senior Supervisor, Pathology
Anita M. Bosau, RQAP-GLP	Senior Director, Compliance Assurance
Cheryl Bellamy	Senior Supervisor, Report Writing
Deanna M. Talerico, RQAP-GLP	Senior Supervisor, Quality Assurance
J. Dale Thurman, D.V.M., M.S., DACVP	Senior Director, Pathology
Kathy M. Gasser	Archivist

AN ACUTE DERMAL TOXICITY STUDY IN RATS WITH SPRAY--CHARLIE

FINAL REPORT

OPPTS Guideline

870.1200

Author

Kimberly L. Bonnette, M.S., LATG

Study Completed on

February 20, 2003

Performing Laboratory

Springborn Laboratories (SLI),
a division of Charles River Company, Inc.
640 North Elizabeth Street
Spencerville, Ohio 45887

SLI Study No.

3596.17

Submitted to

INL/A
U.S. Department of State
2201 C St. NW SA-4
Washington, DC 20520

Page 1 of 29

(2)

SLI Study No. 3596.17

1. STATEMENT OF *NO* DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B), or (C).

Company: _____

Company Agent: _____ Date _____

_____ _____

Title Signature

SLI Study No. 3596.17 (3)

2. COMPLIANCE STATEMENT

This study was conducted in compliance with the Good Laboratory Practice Standards as described by the EPA (40 CFR Parts 160 and 792).

Kimberly L. Bonnette, M.S., LATG
Study Director/Author
Springborn Laboratories, Inc.

Date 2/20/03

Rogers Woolfolk
Senior Aviation Advisor
Sponsor/Submitter
INL/A
U.S. Department of State

Date 3 FEB 2003

SLI Study No. 3596.17

3. QUALITY ASSURANCE STATEMENT

This study was inspected by the Quality Assurance Unit and reports were submitted to management and the Study Director in accordance with SLI's Standard Operating Procedures as follows:

Phase	Date
Protocol Review	10/07/02
Dosing	12/19/02
Data Audit	01/23/03
Draft Report Review	01/23/03
Final Report Review	02/20/03
Reports to Study Director and Management	01/23/03, 02/20/03

The final report has been reviewed to assure that it accurately describes the materials and methods, and the reported results accurately reflect the raw data.

Jennifer D. McGue
Quality Assurance Auditor

Date 2/20/03

Anita M. Bosau, RQAP-GLP
Senior Director, Compliance Assurance

Date 2/20/03

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6. SUMMARY

The single-dose dermal toxicity of Spray--Charlie was evaluated in Sprague Dawley rats. A limit test was performed in which one group of five male and five female rats received a single dermal administration of the test article at a dose of 5000 mg/kg body weight. Following dosing, the limit test rats were observed daily and weighed weekly. A gross necropsy examination was performed on all animals at the time of scheduled euthanasia (day 14).

No mortality occurred during the limit test. Clinical abnormalities observed during the study included transient incidences of dark material around the facial area and decreased defecation. Dermal irritation was noted at the site of test article application. Body weight loss was noted in one male and two females during the study day 7 to 14 body weight interval. Body weight gain was noted for all other animals during the test period. No significant gross internal findings were observed at necropsy on study day 14.

Under the conditions of this test, the acute dermal LD50 of Spray--Charlie was estimated to be greater than 5000 mg/kg in the rat.

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7. INTRODUCTION

This study was performed to assess the short-term toxicity of Spray--Charlie in Sprague Dawley rats when administered by a single dermal dose. This study was intended to provide information on the potential health hazards of the test article with respect to dermal exposure. Data from this study may serve as a basis for classification and/or labeling of the test article. This study was performed in accordance with the US EPA, Health Effects Test Guidelines, OPPTS 870.1200, Acute Dermal Toxicity, August 1998. This study was performed at Springborn Laboratories (SLI), 553 North Broadway, Spencerville, Ohio. The protocol was signed by the Study Director on October 9, 2002 (GLP initiation date). The in-life phase of the study was initiated with test article administration on December 19, 2002 (day 0), and concluded with necropsy on January 2, 2003.

8. MATERIALS AND METHODS

8.1. Test Article

The test article was received from the Sponsor and identified as follows:

Sponsor's ID	Assigned SLI ID	Physical Description	Receipt Date	Expiration Date
Spray—Charlie[a]	S02.003.3596	Amber liquid	12/09/02	None provided
Ingredients:[b]				
Herbicide: GLY-41 Lot No.: Manufactured 10/20/02				None provided
Surfactant: Cosmo Flux-411F Lot No.: Manufactured 11/20/02				None provided

[a]Sample pooled at SLI from five different mixes of Spray--Charlie (top/middle/bottom).
[b]Ingredients used in the five Spray--Charlie mixes that were prepared by the Sponsor.

The test article was stored at room temperature. The Sponsor was responsible for any necessary evaluations related to identity, strength, purity, composition, stability and method of synthesis of the test material according to 40 CFR 160.105 and 40 CFR 792.105. Springborn Laboratories, Inc. analyzed the test article for the glyphosate (a.e.) which is presented in SLI Study No. 3596.15.

8.2. Retention Sample

An approximate 1 mL retention sample of each test article mixture sample (top/middle/bottom, maintained separately for a total of fifteen 1 mL samples) was taken and stored at SLI at room temperature. In addition, a 10 mL retention sample of the pooled test article samples (from the 5 test article mixtures) was collected and stored at SLI at room temperature. These samples serve as the retention samples for all studies conducted with this material.

8.3. Test Article Disposition

The test article was returned to the Sponsor following completion of all studies with the test article.

8.4. Method of Test Article Preparation

The test articles were pooled and administered as received from the Sponsor and dispensed fresh on the day of dosing. The test articles were stirred continuously during dosing. The density of the test article was determined to be 1.08 g/mL.

8.5. Animals and Animal Husbandry

8.5.1. Description, Identification and Housing

Adult, Hsd: Sprague Dawley® SD® rats were received from Harlan Sprague Dawley, Inc., Indianapolis, IN. Upon receipt, metal ear tags displaying unique identification numbers were used to individually identify the animals. Cage cards displaying at least the study number, animal number and sex were affixed to each cage. The animals were housed individually in suspended stainless steel cages. All housing and care were based on the standards recommended by the Guide for the Care and Use of Laboratory Animals [1].

8.5.2. Environment

The animal room temperature and relative humidity ranges were 71-74°F (22-23°C) and 40-53%, respectively. Environmental control equipment was monitored and adjusted as necessary to minimize fluctuations in the animal room environment. Light timers were set to maintain a 12-hour light/12-hour dark cycle and room ventilation was set to produce 10-15 air changes/hour. The animal room temperature and relative humidity were recorded a minimum of once daily.

8.5.3. Food

PMI Certified Rodent Chow #5002 (Purina Mills, Inc.) was provided ad libitum to the animals throughout the study. The lot number and expiration date of each

batch of diet used during the study were recorded. The feed was analyzed and certified by the supplier for nutritional components and environmental contaminants. Dietary limitations for various environmental contaminants, including heavy metals, pesticides, polychlorinated biphenyls and total aflatoxin are set by the manufacturer. Within these limits, contaminants which may have been present were not expected to compromise the purpose of this study. Results of the dietary analyses (Certificates of Analysis) were provided by the manufacturer for each lot of diet. These are maintained by SLI.

8.5.4. Water

Municipal tap water treated by reverse osmosis was available ad libitum throughout the study. The purified water was supplied by an automatic watering system. Monitoring of the drinking water for contaminants is conducted by SLI and the records are available for inspection. Within generally accepted limits, contaminants which may have been present were not expected to compromise the purpose of this study. The water meets the standards specified under the EPA National Drinking Water Regulations (40 CFR Part 141).

8.5.5. Acclimation

Upon receipt, the animals were removed randomly from the shipping cartons, examined by qualified personnel, identified with metal ear tags and then acclimated to the laboratory conditions for a minimum of five days. The animals were observed daily for overt physical or behavioral abnormalities, general health/moribundity and mortality.

8.5.6. Animal Selection

The animals chosen for study use were randomly selected from healthy stock animals using a computerized random numbers table to avoid potential bias. All animals received a detailed pretest observation prior to dosing. Only healthy animals were chosen for study use. Females were nulliparous and nonpregnant. The male animals were approximately 9 weeks of age and weighed 265-290 g prior to dosing. The female animals were approximately 9 weeks of age and weighed 189-207 g prior to dosing.

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9. EXPERIMENTAL PROCEDURES

9.1. Preliminary Procedures

On day -1, the fur was removed from the dorsal trunk area of the animals chosen for the limit test using an animal clipper. The clipped area was approximately 10% of the animal's body surface area (BSA). The region included the scapula (shoulder) to the wing of the ilium (hipbone) and half way down the flank on each side of the animal. Care was taken to avoid abrading the skin during the clipping procedure.

9.2. Dosing

On the following day (day 0), the test article was administered dermally to approximately 10% of the body surface area. The four corners of this area were delineated in the clipped area with an indelible marker. The test article was then spread evenly over the delineated test area and held in contact with the skin with an appropriately sized 4-ply porous gauze dressing backed with a plastic wrap which was placed over the gauze dressing (occlusive binding). Removal and ingestion of the test article was prevented by placing an elastic wrap over the trunk and test area. The elastic wrap was further secured with a tape harness on the cranial end of the trunk and then secured with adhesive tape around the trunk at the caudal end.

The test article was administered at the following level:

Dose Level (mg/kg)	Dose Volume (mL/kg)	Concentration (%)	No. of Animals	
			Male	Female
5000	4.63[a]	100[b]	5	5

[a]Adjusted based on a density of 1.08 g/mL.
[b]Pooled test article.

Individual doses were calculated based on the animal's day 0 body weight. After an approximate 24-hour exposure period, the binding materials were removed and the corners of the test site were re-delineated using a marker. Residual test article was removed using gauze moistened with deionized water followed by dry gauze.

9.3. Dermal Observations

The test animals were examined for erythema and edema following patch removal and the responses scored on study day 1 and daily thereafter

(days 2-14) according to the Macroscopic Dermal Grading System provided in Appendix A which is based on Draize [2]. The dermal test sites were reclipped as necessary to allow clear visualization of the skin.

9.4. Clinical Observations

The animals were observed for clinical abnormalities two times on study day 0 (postdose) and daily thereafter (days 1-14). A mortality check was performed twice daily, in the morning and afternoon.

9.5. Body Weights

Individual body weights were obtained for the animals prior to dosing on day 0 and on days 7 and 14.

9.6. Gross Necropsy

All animals were euthanized by carbon dioxide inhalation at study termination (day 14) and necropsied. Body cavities (cranial, thoracic, abdominal and pelvic) were opened and examined. No tissues were retained.

9.7. Protocol Deviations

On study day 1, edema was inadvertently not recorded for Animal No. A6709. This occurrence was considered to have had no adverse effect on the outcome of this study.

10. DATA ACQUISITION AND ELECTRONIC RECORDS

Electronic data were recorded on a Compaq Alpha Server DS10 utilizing the Toxicology Analysis System Customized, Acute Toxicology Module, Version 1.0.0 or higher. The SLI study number assigned to this study is 3596.17. The computer study number used to collect data for the study phases was 359617. The tables within the report will display the applicable computer number.

11. ANALYSIS OF DATA

Data from the study were analyzed and an LD50 value estimated as follows:

< 50% Mortality: LD50 was estimated as greater than the administered dose.
= 50% Mortality: LD50 was estimated as equal to the administered dose.
> 50% Mortality: LD50 was estimated as less than the administered dose.

Body weight means and standard deviations were calculated separately for males and females.

12. MAINTENANCE OF RAW DATA AND RECORDS

All original raw data, the final report and electronic records were transferred to the SLI archives for a period of 10 years. The Sponsor will be contacted prior to final disposition of these items.

13. RESULTS

13.1. Mortality

Individual Data: Table 1

No mortality occurred during the limit test.

13.2. Clinical/Dermal Observations

Individual Data: Table 1

Clinical abnormalities observed during the study included transient incidences of dark material around the facial area and decreased defecation. Dermal irritation was noted at the site of test article application.

13.3. Body Weight Data

Individual Data: Table 2

Body weight loss was noted in one male and two females during the study day 7 to 14 body weight interval. Body weight gain was noted for all other animals during the test period.

13.4. Gross Necropsy

Individual Data: Table 3

No significant gross internal findings were observed at necropsy on study day 14.

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14. CONCLUSION

Under the conditions of this test, the acute dermal LD50 of Spray--Charlie was estimated to be greater than 5000 mg/kg in the rat.

Kimberly L. Bonnette, M.S., LATG
Study Director

Date 2/20/03

15. REPORT REVIEW

Rusty E. Rush, M.S., LATG
Director, Neurotoxicity and Transgenics

Date 2-20-03

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SLI Study No. 3596.17

16. REFERENCES

1. Guide for the Care and Use of Laboratory Animals, DHHS Publication No. (NIH) 96-03, 1996.

2. Draize, J.H., Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics, The Association of Food and Drug Officials of the United States, 49-51, 1959.

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INL/A, U.S. DEPARTMENT OF STATE

TABLE 1

AN ACUTE DERMAL TOXICITY STUDY IN RATS

INDIVIDUAL CLINICAL OBSERVATIONS
(POSITIVE FINDINGS)

MALES 5000 MG/KG

MALE#	OBSERVATIONS	DAY OF STUDY 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A6684	SCHEDULED EUTHANASIA															P
	SOFT STOOLS			P												
	EDEMA GRADE 0		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	ERYTHEMA GRADE 0			P	P	P	P	P	P	P	P	P	P	P	P	P
	DARK MATERIAL AROUND EYE(S)	P	P													
	DARK MATERIAL AROUND NOSE	P														
	ERYTHEMA GRADE 1		P													
A6696	SCHEDULED EUTHANASIA															P
	EDEMA GRADE 0		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	ERYTHEMA GRADE 0		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	DARK MATERIAL AROUND EYE(S)	P	P													
	DARK MATERIAL AROUND NOSE	P	P	P												
A6698	SCHEDULED EUTHANASIA															P
	EDEMA GRADE 0		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	ERYTHEMA GRADE 0		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	DARK MATERIAL AROUND EYE(S)	P	P													
	DARK MATERIAL AROUND NOSE	P	P	P												
A6697	SCHEDULED EUTHANASIA															P
	EDEMA GRADE 0			P	P	P	P	P	P	P	P	P	P	P	P	P
	ERYTHEMA GRADE 0			P	P	P	P	P	P	P	P	P	P	P	P	P
	DARK MATERIAL AROUND EYE(S)	P	P													
	DARK MATERIAL AROUND NOSE	P	P													
	DARK MATERIAL AROUND MOUTH	P														
	ERYTHEMA GRADE 2		P													
	EDEMA GRADE 1		P													

GRADE CODE: 1=SLIGHT 2=MODERATE 3=SEVERE P=PRESENT L=LEFT R=RIGHT B=BILATERAL

TABLE 1

AN ACUTE DERMAL TOXICITY STUDY IN RATS

INDIVIDUAL CLINICAL OBSERVATIONS
(POSITIVE FINDINGS)

MALES 5000 MG/KG

MALE#	OBSERVATIONS	DAY OF STUDY 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A6703	SCHEDULED EUTHANASIA															P
	EDEMA GRADE 0		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	ERYTHEMA GRADE 0		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	DARK MATERIAL AROUND EYE(S)	P														
	DARK MATERIAL AROUND NOSE	P		P												

GRADE CODE: 1=SLIGHT 2=MODERATE 3=SEVERE P=PRESENT L=LEFT R=RIGHT B=BILATERAL

TABLE 1

AN ACUTE DERMAL TOXICITY STUDY IN RATS

INDIVIDUAL CLINICAL OBSERVATIONS
(POSITIVE FINDINGS)

FEMALES 5000 MG/KG

FEMALE#	OBSERVATIONS	DAY OF STUDY 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A6709	SCHEDULED EUTHANASIA															P
	EDEMA GRADE 0			P	P	P	P	P	P	P	P	P	P	P	P	P
	ERYTHEMA GRADE 0				P	P	P	P	P	P	P	P	P	P	P	P
	DARK MATERIAL AROUND EYE(S)	P	P													
	DARK MATERIAL AROUND NOSE	P														
	ERYTHEMA GRADE 1		P	P												
A6710	SCHEDULED EUTHANASIA															P
	EDEMA GRADE 0		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	ERYTHEMA GRADE 0		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	DARK MATERIAL AROUND EYE(S)		P													
	OCULAR DISCHARGE	P														
A6715	SCHEDULED EUTHANASIA															P
	FEW FECES			P												
	EDEMA GRADE 0		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	ERYTHEMA GRADE 0		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	DARK MATERIAL AROUND EYE(S)	P	P													
	DARK MATERIAL AROUND NOSE	P														
A6716	SCHEDULED EUTHANASIA															P
	FEW FECES			P												
	EDEMA GRADE 0		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	ERYTHEMA GRADE 0		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	DARK MATERIAL AROUND NOSE	P														
A6720	SCHEDULED EUTHANASIA															P
	EDEMA GRADE 0		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	ERYTHEMA GRADE 0		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	DARK MATERIAL AROUND EYE(S)		P	P												
	DARK MATERIAL AROUND NOSE	P														

GRADE CODE: 1=SLIGHT 2=MODERATE 3=SEVERE P=PRESENT L=LEFT R=RIGHT B=BILATERAL

TABLE 2

AN ACUTE DERMAL TOXICITY STUDY IN RATS

INDIVIDUAL BODY WEIGHTS (GRAMS)

MALES 5000 MG/KG

ANIMAL#	DAY OF STUDY 0	7	14 AT DEATH (DAY)
A6684	273	300	299
A6696	290	313	334
A6698	265	281	318
A6697	271	292	315
A6703	271	280	308
MEAN	274	293	315
S.D.	9.4	13.8	13.0
N	5	5	5

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TABLE 2

AN ACUTE DERMAL TOXICITY STUDY IN RATS

INDIVIDUAL BODY WEIGHTS (GRAMS)

FEMALES 5000 MG/KG

	DAY OF STUDY		
ANIMAL#	0	7	14 AT DEATH (DAY)
A6709	204	215	223
A6710	207	227	196
A6715	191	208	182
A6716	189	201	209
A6720	204	210	220
MEAN	199	212	206
S.D.	8.3	9.7	17.1
N	5	5	5

TABLE 3

AN ACUTE DERMAL TOXICITY STUDY IN RATS

INDIVIDUAL GROSS NECROPSY OBSERVATIONS

MALES 5000 MG/KG

ANIMAL#	DAY OF DEATH	STUDY DAY	OBSERVATION	FATE
A6684	2-JAN-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A6696	2-JAN-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A6698	2-JAN-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A6697	2-JAN-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A6703	2-JAN-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA

TABLE 3

AN ACUTE DERMAL TOXICITY STUDY IN RATS

INDIVIDUAL GROSS NECROPSY OBSERVATIONS

FEMALES 5000 MG/KG

ANIMAL#	DAY OF DEATH	STUDY DAY	OBSERVATION	FATE
A6709	2-JAN-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A6710	2-JAN-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A6715	2-JAN-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A6716	2-JAN-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A6720	2-JAN-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA

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APPENDIX A

Macroscopic Dermal Grading System

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MACROSCOPIC DERMAL GRADING SYSTEM

ERYTHEMA AND EDEMA OBSERVATIONS		
OBSERVATION	DEFINITION	CODE
Erythema – Grade 0	No erythema	0
Erythema – Grade 1	Very slight erythema (barely perceptible)	1
Erythema – Grade 2	Well-defined erythema	2
Erythema – Grade 3	Moderate to severe erythema	3
Erythema – Grade 4	Severe erythema (beet redness)	4
Maximized Grade 4	Notable dermal lesions (see below)	M – 4 (see below)
Edema – Grade 0	No edema	0
Edema – Grade 1	Very slight edema (barely perceptible)	1
Edema – Grade 2	Slight edema (edges of area well defined by definite raising)	2
Edema – Grade 3	Moderate edema (raised approximately 1 millimeter)	3
Edema – Grade 4	Severe edema (raised more than 1 millimeter and extends beyond the area of exposure)	4
NOTE: Each animal was assigned an erythema and edema score. The most severely affected area within the test site was graded. If eschar, blanching, ulceration and/or necrosis greater than grade 1 was observed, then the “Maximized Grade 4" was assigned to the test site in place of the erythema score and the type of notable dermal lesion(s) (e.g., eschar - grade 2, blanching - grade 3, ulceration - grade 4, etc.) was noted. The presence of any other dermal changes (e.g., desquamation, fissuring, eschar exfoliation, etc.) was also recorded.		

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MACROSCOPIC DERMAL GRADING SYSTEM

NOTABLE DERMAL LESIONS		
OBSERVATION	CODE	DEFINITION
Eschar – Grade 1	ES-1	Focal and/or pinpoint areas up to 10% of test site.
Eschar – Grade 2	ES-2	> 10% < 25% of test site.
Eschar – Grade 3	ES-3	> 25% < 50% of test site.
Eschar – Grade 4	ES-4	> 50% of test site.
Blanching – Grade 1	BLA-1	Focal and/or pinpoint areas up to 10% of test site.
Blanching – Grade 2	BLA-2	> 10% < 25% of test site.
Blanching – Grade 3	BLA-3	> 25% < 50% of test site.
Blanching – Grade 4	BLA-4	> 50% of test site.
Ulceration – Grade 1	U-1	Focal and/or pinpoint areas up to 10% of test site.
Ulceration – Grade 2	U-2	> 10% < 25% of test site.
Ulceration – Grade 3	U-3	> 25% < 50% of test site.
Ulceration – Grade 4	U-4	> 50% of test site.
Necrosis – Grade 1	NEC-1 *(color)*	Focal and/or pinpoint areas up to 10% of test site (Note color of necrosis).
Necrosis – Grade 2	NEC-2 *(color)*	> 10% < 25% of test site (Note color of necrosis).
Necrosis – Grade 3	NEC-3 *(color)*	> 25% < 50% of test site (Note color of necrosis).
Necrosis – Grade 4	NEC-4 *(color)*	> 50% of test site (Note color of necrosis).

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MACROSCOPIC DERMAL GRADING SYSTEM

ADDITIONAL DERMAL FINDINGS		
OBSERVATION	DEFINITION	CODE
Desquamation	Characterized by scaling or flaking of dermal tissue with or without denuded areas.	DES
Fissuring	Characterized by cracking of the skin with or without moist exudate. Fissuring should be checked prior to removing the animal from the cage and manipulating the test site.	FIS
Eschar Exfoliation	The process by which areas of eschar flake off the test site.	EXF
Test Site Staining	Skin located at test site appears to be discolored, possibly due to test article (note color of staining).	TSS *(color)*
Erythema Extends Beyond the Test Site	The erythema extends beyond the test site. Note: A study director should be contacted for erythema extending beyond the test site.	ERB
Superficial Lightening	Characterized by pale area(s) (almost a burn-like appearance) in the test site. However, erythema may still be observed through the pale area. Note: This observation may affect the overall erythema score of the test site. This observation may progress to other observations resulting in notable dermal lesions, but SL itself will not be considered a notable dermal lesion that will result in a dermal score to be maximized since it does not result in any in-depth injury. To be coded using an area designation (see below).	--
Superficial Lightening - Grade 1	Focal and/or pinpoint areas up to 10% of the test site	SL-1
Superficial Lightening - Grade 2	> 10% < 25% of test site	SL-2
Superficial Lightening - Grade 3	> 25% < 50% of test site	SL-3
Superficial Lightening - Grade 4	> 50% of test site	SL-4

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MACROSCOPIC DERMAL GRADING SYSTEM

ADDITIONAL FINDINGS		
OBSERVATION	DEFINITION	CODE
Dermal Irritation - Outside of the Test Site	Noticeable irritation outside of test site probably due to the binding tape material. This notation will only be made for reactions greater than what are normally observed from tape removal which do not interfere with the scoring of the test site.	IT

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APPENDIX B

SLI Personnel Responsibilities

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SLI PERSONNEL RESPONSIBILITIES

Kimberly L. Bonnette, M.S., LATG	Study Director/Director, Acute Toxicology
Dawn D. Rodabaugh, B.S.	Alternate Contact/Toxicologist
Malcolm Blair, Ph.D.	Managing Director Emeritus
Joseph C. Siglin, Ph.D., DABT	General Manager
Rusty E. Rush, M.S., LAT, DABT	Director, Neurotoxicity and Transgenics
Jason W. Smedley, B.S.	Assistant Toxicologist
Pamela S. Smith, ALAT	Study Supervisor, Acute Toxicology
Christina L. Zehender, B.S.	Primary Technician/Acute Technician I
Delores P. Knippen	Supervisor, Pharmacy
Steven H. Magness, B.S., LATG	Senior Supervisor, Pathology
Anita M. Bosau, RQAP-GLP	Senior Director, Compliance Assurance
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AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS WITH SPRAY--CHARLIE

FINAL REPORT

OPPTS Guidelines

870.1300

Author

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Study Completed on

March 14, 2003

Performing Laboratory

Springborn Laboratories (SLI),
a division of Charles River Laboratories, Inc.
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SLI Study No.

3596.18

Submitted to

INL/A
U.S. Department of State
2201 C St. NW SA-4
Washington, DC 20520

(2)

SLI Study No. 3596.18

1. STATEMENT OF *NO* DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B), or (C).

Company: _____

Company Agent: _____ Date_____

_____ _____

Title Signature

2. COMPLIANCE STATEMENT

This study was conducted in compliance with the Good Laboratory Practice Standards as described by the EPA (40 CFR Parts 160 and 792).

Kimberly L. Bonnette, M.S., LATG
Study Director/Author
Springborn Laboratories

Date 3/14/03

Rogers Woolfolk
Senior Aviation Advisor
Sponsor/Submitter
INL/A
U.S. Department of State

Date 11 MAR 03

SLI Study No. 3596.18

3. QUALITY ASSURANCE STATEMENT

This study was inspected by the Quality Assurance Unit and reports were submitted to management and the Study Director in accordance with SLI's Standard Operating Procedures as follows:

Phase	Date
Protocol Review	10/07/02
Animal Receipt	01/02/03
Clinical Observations	01/17/03
Analytical Chemistry Review	01/27/03
Analytical Chemistry Report Review	01/27/03
Data Audit	03/10/03
Draft Report Review	03/10/03
Final Report Review	03/14/03
Reports to Study Director and Management	01/02/03, 03/10/03, 03/14/03

The final report has been reviewed to assure that it accurately describes the materials and methods, and the reported results accurately reflect the raw data.

Rebecca A. Young

Rebecca A. Young
Quality Assurance Team Leader

Date 3/14/03

Anita M Bosau

Anita M. Bosau, RQAP-GLP
Senior Director, Compliance Assurance

Date 3/14/03

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6. SUMMARY

The four-hour nose-only inhalation toxicity of Spray--Charlie was evaluated in Sprague Dawley rats. A limit test was performed in which a group of five male and five female rats received a four-hour nose-only inhalation exposure to a time-weighted average aerosol concentration (analytically determined) of 2.60 mg/L. The mass median aerodynamic diameter and geometric standard deviation of the sampled particles were 2.9 μ $\pm$ 2.17. The percentage of particles $\leq$ 4.0 μ was determined to be 66%. Following the exposure, the limit test rats were observed daily and weighed weekly. A gross necropsy examination was performed on all limit test animals at the time of scheduled euthanasia (day 14).

No mortality occurred during the study. The most notable clinical abnormalities observed during the study included breathing abnormalities, no/decreased defecation, urine staining, rough haircoat, dark material around the facial area and decreased food consumption. Body weight loss was noted in two males and one female during the day 0 to 7 body weight interval. Body weight gain was noted for all other animals during the test period. At study termination, the animals had exceeded/maintained their initial body weight. No gross internal findings were observed at necropsy on study day 14.

Under the conditions of this test, the acute inhalation LC50 of Spray--Charlie was estimated to be greater than 2.60 mg/L in the rat (which was well above the EPA-required 2.00 mg/L).

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7. INTRODUCTION

This study was performed to assess the short-term toxicity of Spray--Charlie in Sprague Dawley rats when administered by a four-hour nose-only inhalation exposure. This study was intended to provide information on the potential health hazards of the test article with respect to inhalation exposure. Data from this study may serve as a basis for classification and/or labeling of the test article. This study was conducted in accordance with the US EPA, Health Effects Test Guidelines, OPPTS 870.1300, Acute Inhalation Toxicity, August, 1998. This study was performed at Springborn Laboratories (SLI), 553 North Broadway, Spencerville, Ohio. The protocol was signed by the Study Director on October 9, 2002 (GLP initiation date). The in-life phase of the study was initiated with test article administration on January 14, 2003 (day 0) and concluded with terminal euthanasia on January 28, 2003.

8. MATERIALS AND METHODS

8.1. Test Article

The test article was received from the Sponsor and identified as follows:

Sponsor's ID	Assigned SLI ID	Physical Description	Receipt Date	Expiration Date
Spray--Charlie[a]	S02.003.3596	Amber liquid	12/09/02	None provided
Ingredients:[b]				
Herbicide: GLY-41 Lot No.: Manufactured 10/20/02				None provided
Surfactant: Cosmo Flux-411F Lot No.: Manufactured 11/29/02				None provided

[a]Sample pooled at SLI from five different mixes of Spray--Charlie (top/middle/bottom).
[b]Ingredients used in the five Spray--Charlie mixes that were prepared by the Sponsor.

The test article was stored at room temperature. The Sponsor was responsible for any necessary evaluations related to identity, strength, purity, composition, stability and method of synthesis of the test material according to 40 CFR 160.105 and 40 CFR 792.105. Springborn Laboratories analyzed the test article for the glyphosate (a.e.) which is presented in SLI Study No. 3596.15.

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8.2. Retention Sample

An approximate 1 mL retention sample of each test article mixture sample (top/middle/bottom, maintained separately for a total of fifteen 1 mL samples) was taken and stored at SLI at room temperature. In addition, a 10 mL retention sample of the pooled test article samples (from the 5 test article mixtures) was collected and stored at SLI at room temperature. These samples serve as the retention samples for all studies conducted with this material.

8.3. Test Article Disposition

The test article was returned to the Sponsor following completion of all studies with the test article.

8.4. Method of Test Article Preparation

The test articles were pooled and administered as received from the Sponsor and dispensed fresh on the day of dosing. The pooled test article was stirred approximately 10 minutes prior to dispensation and stirred continuously during dosing.

8.5. Animals and Animal Husbandry

8.5.1. Description, Identification and Housing

Young adult, Hsd: Sprague Dawley® SD® rats were received from Harlan Sprague Dawley, Inc., Indianapolis, IN. Upon receipt, metal ear tags displaying unique identification numbers were used to individually identify the animals. Cage cards displaying at least the study number, animal number and sex were affixed to each cage. The animals were housed individually in suspended stainless steel cages. All housing and care were based on the standards recommended by the Guide for the Care and Use of Laboratory Animals [1].

8.5.2. Environment

The animal room temperature and relative humidity ranges were 64-68°F (18-20°C) and 37-55%, respectively. Environmental control equipment was monitored and adjusted as necessary to minimize fluctuations in the animal room environment. Light timers were set to maintain a 12-hour light/12-hour dark cycle and room ventilation was set to produce 10-15 air changes/hour. The animal room temperature and relative humidity were recorded a minimum of once daily.

8.5.3. Food

PMI Certified Rodent Chow #5002 (Purina Mills, Inc.) was provided ad libitum to the animals throughout the study (except during the time that the animals were

acclimated to the exposure tubes and maintained in the inhalation room for the exposure procedure). The lot number and expiration date of each batch of diet used during the study were recorded. The feed was analyzed and certified by the supplier for nutritional components and environmental contaminants. Dietary limitations for various environmental contaminants, including heavy metals, pesticides, polychlorinated biphenyls and total aflatoxin are set by the manufacturer. Within these limits, contaminants which may have been present were not expected to compromise the purpose of this study. Results of the dietary analyses (Certificates of Analysis) are provided by the manufacturer for each lot of diet. These are maintained by SLI.

8.5.4. Water

Municipal tap water treated by reverse osmosis was available ad libitum throughout the study (except during the time that the animals were acclimated to the exposure tubes and maintained in the inhalation room for the exposure procedure). The purified water was supplied by an automatic watering system. Monitoring of the drinking water for contaminants is conducted by SLI and the records are available for inspection. Within generally accepted limits, contaminants which may have been present were not expected to compromise the purpose of this study. The water meets the standards specified under the EPA National Drinking Water Regulations (40 CFR Part 141).

8.5.5. Acclimation

Upon receipt, the animals were removed randomly from the shipping cartons, examined by qualified personnel, identified with metal ear tags and then acclimated to the laboratory conditions for a minimum of five days. The animals were observed daily for overt physical or behavioral abnormalities, general health/moribundity and mortality.

8.5.6. Animal Selection

The animals chosen for study use were randomly selected from healthy stock animals using a computerized random numbers table to avoid potential bias. All animals received a detailed pretest observation prior to dosing. Only healthy animals were chosen for study use. Females were nulliparous and nonpregnant. The male animals were approximately 9 weeks of age and weighed 248-275 g on the day of exposure. The female animals were approximately 9 weeks of age and weighed 201-212 g on the day of exposure.

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9. EXPERIMENTAL PROCEDURES

9.1. Preliminary Procedures

9.1.1. Test Article Volatility Determination

The volatility of the test article relative to a distilled water standard was determined prior to experimental initiation. This procedure was performed in order to determine if the test article had sufficiently low volatility to allow for an accurate gravimetric determination of the aerosol concentration. A known quantity of the test article was placed on a preweighed filter disk and was allowed to evaporate for a total of ten minutes. The test article weight was determined each minute and the amount of evaporation of the test article was then determined. The results of this volatility trial indicated that the test article evaporation rate (0.82 mg/minute) was only slightly higher than the SLI determined distilled water evaporation rate (0.55 mg/minute); therefore was considered to not be volatile.

9.1.2. Preliminary Aerosol Generation Trials

Prior to experimental initiation, preliminary aerosol generation trials were conducted. These trials were performed in order to determine the most efficient means of generating an aerosol of the appropriate concentration while utilizing equipment that would reduce the aerodynamic particle size. Data obtained during the preliminary aerosol generation trials are presented in Appendix A.

9.2. Limit Test

9.2.1. Aerosol Generation Equipment

The test aerosol was generated with a Pistol Spraying System and a Master Flex Pump and Pump Heads 77200-60 and 7523-30. Conditioned high pressure external air was used in generating the test atmosphere. The aerosol was blown through a 5L Elutriator, the Multi-Stage 10L nose-only inhalation chamber and then vented from the chamber to an air treatment system which consisted of a prefilter, a HEPA filter, a charcoal bed and a water scrubbing tower (see Figure 1).

9.2.2. Dosing

On day 0, the animals chosen for the limit test were weighed, placed in a nose-only exposure tube and allowed to acclimate to the exposure tube for at least 1 hour. Animals that appeared to have been acclimated to the exposure tube (i.e., minimal struggling and no inversion) were considered to be acceptable,

removed from the exposure tube and returned to their cages until initiation of the aerosol exposure. Animals that did not appear to acclimate to the exposure tube were not acceptable, removed from the exposure tube and returned to their cages.

The acceptable animals were then placed in exposure tubes, the tubes inserted into the Multi-Stage 10L nose-only inhalation chamber and the test article aerosolized at the following level:

Exposure Level (mg/L)	No. of Animals	
	Male	Female
2.60	5	5

The aerosol exposure consisted of a 3-minute T99 equilibration period, a 240-minute exposure period and a 3-minute de-equilibration period equal to the T99 equilibration period. After each aerosol exposure, animals were removed from the exposure tubes and residual test article was removed from the animal's exterior surfaces (where practical) by wiping the haircoat with a towel. The animals were then returned to ad libitum feed and water. The following parameters were measured during the exposure.

9.2.2.1. Chamber Air Flow

Air flow readings were recorded at the initiation of the T99 equilibration period, at approximate 30-minute intervals during the aerosol exposure and at the conclusion of the de-equilibration period.

9.2.2.2. Aerosol Concentration

The aerosol concentration was measured at the beginning of the aerosol exposure (after equilibration), at approximate 30-minute intervals during the aerosol exposure and at the conclusion of the aerosol exposure (before de-equilibration). The concentration of the test article aerosol was collected in the inhalation chamber by gravimetric technique. A 5 L sample of the aerosol was drawn from the breathing zone of the chamber through a preweighed glass fiber filter. The change in weight of the filter (mg) was then determined and this value was divided by the volume of chamber atmosphere sampled (L) to yield the gravimetric concentration (mg/L). The average time-weighted gravimetric concentration of the test atmosphere was then calculated for the exposure. For the analytical concentration, the gravimetrically obtained samples were analyzed by Springborn Laboratories for the glyphosate component, a non-volatile component of the test article. These analyses were performed in order to determine the analytical (actual) concentrations of the aerosol in the chamber for each sampling period. The average time weighted analytical concentration of the

test atmosphere was then calculated for the exposure. Chemistry methods and results are detailed in the Analytical Chemistry Report (Appendix B).

9.2.2.3. Chamber Temperature and Humidity

The chamber temperature and humidity were measured electronically and recorded at approximate 30-minute intervals during the aerosol exposure using a Vaisala HMI 41 Thermometer.

9.2.2.4. Aerosol Aerodynamic Particle-Size Distribution

The aerosol aerodynamic particle-size distribution was determined three times during the aerosol exposure using the ITP 7 Stage Cascade Impactor. Each stage of the impactor was fitted with a preweighed glass fiber filter. Five liters per minute of the chamber air were drawn through the impactor and the change in weight of each filter was then determined and recorded. The mean particle-size distribution was subsequently determined using an Excel computer adaptation of the manual method. The Mass Median Aerodynamic Diameter, Geometric Standard Deviation and percentage of particles $\leq 4.0\,\mu$ were then determined. At least one hour passed between each aerosol particle-size analysis.

9.2.2.5. Chamber Oxygen

Chamber oxygen content was measured and recorded at approximate 30-minute intervals during the aerosol exposure using a GC-501 Oxygen Sensor.

9.2.3. Clinical Observations

The limit test animals were observed for clinical abnormalities during each aerosol exposure, two times on study day 0 (post-exposure) and daily thereafter (days 1-14). A general health/mortality check was performed twice daily (in the morning and in the afternoon).

9.2.4. Body Weights

Individual body weights were obtained for the limit test animals prior to dosing on day 0 and on days 7 and 14.

9.2.5. Gross Necropsy

All limit test animals were euthanized by carbon dioxide inhalation at study termination (day 14) and necropsied. Body cavities (cranial, thoracic, abdominal and pelvic) were opened and examined. No tissues were retained.

9.3. Protocol Deviations

The temperature of the animal room [64-68°F (18-20°C)] exceeded the preferred range [66-77°F (19-25°C)] during this study. This occurrence was considered to have had no adverse effect on the outcome of this study.

10. ANALYSIS OF DATA

Data from the limit tests were analyzed and an LC50 value estimated as follows:

< 50% Mortality: LC50 was estimated as greater than the administered dose.
= 50% Mortality: LC50 was estimated as equal to the administered dose.
> 50% Mortality: LC50 was estimated as less than the administered dose.

Body weight means and standard deviations were calculated separately for males and females. The aerodynamic particle-size distribution of the test article aerosol was plotted using an Excel computer adaptation of the three cycle logarithmic probability paper as per the ITP Cascade Impactor instruction manual. The Mass Median Aerodynamic Diameter, Geometric Standard Deviation and particles $\leq$ 4.0 µ were determined based on the plotted distribution.

11. MAINTENANCE OF RAW DATA AND RECORDS

All original raw data, the final report and electronic encoded records were transferred to the SLI archives for a period of 10 years. The Sponsor will be contacted prior to final disposition of these items.

12. RESULTS

12.1. Aerosol Generation and Chamber Environmental Data

12.1.1. Aerosol Generation Data

Individual Data: Table 1

The average time-weighted analytical concentration for the aerosol exposure was determined to be 2.60 mg/L. The mass median aerodynamic diameter and geometric standard deviation of the sampled particles were 2.9µ ± 2.17. The percentage of particles $\leq$ 4.0 µ was determined to be 66%.

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12.1.2. Chamber Environmental Data

Individual Data: Table 1

Chamber temperature and relative humidity for the aerosol exposure ranged from 68.3-70.7°F and 68.3-69.3%, respectively. Oxygen content was maintained at 20.9% throughout the exposure.

12.2. Limit Test Data

12.2.1. Mortality

Individual Data: Table 2

No mortality occurred during the study.

12.2.2. Clinical Observations

Individual Data: Table 2

No positive findings were noted at the time of observation during the 4-hour exposure period. The most notable clinical abnormalities observed during the study included breathing abnormalities, no/decreased defecation, urine staining, rough haircoat, dark material around the facial area and decreased food consumption.

12.2.3. Body Weight Data

Individual Data: Table 3

Body weight loss was noted in two males and one female during the day 0 to 7 body weight interval. Body weight gain was noted for all other animals during the test period. At study termination, the animals had exceeded/maintained their initial body weight.

12.2.4. Gross Necropsy

Individual Data: Table 4

No gross internal findings were observed at necropsy on study day 14.

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13. CONCLUSION

Under the conditions of this test, the acute inhalation LC50 of Spray--Charlie was estimated to be greater than 2.60 mg/L in the rat (which was well above the EPA-required 2.00 mg/L).

Kimberly L. Bonnette, M.S., LATG
Study Director

Date 3/14/03

14. REPORT REVIEW

Dawn D. Rodabaugh, B.S.
Toxicologist

Date 3/14/03

15. REFERENCE

1. Guide for the Care and Use of Laboratory Animals, DHHS Publication No. (NIH) 96-03, 1996.

TABLE 1

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INL/A, U.S. DEPARTMENT OF STATE SUMMARY OF AEROSOL GENERATION AND CHAMBER ENVIRONMENTAL DATA

	EXPOSURE LEVEL (MG/L)
	2.60
CHAMBER AND EXPOSURE DATA	
CHAMBER VOLUME (L):	10
ELUTRIATOR VOLUME (L):	5
MEAN AIR FLOW RATE (L/MIN):	24
MEAN AIR CHANGES PER HOUR:	95.24
T99 EQUILIBRATION PERIOD (MIN.):	3
EXPOSURE TIME (MIN):	240
DE-EQUILIBRATION PERIOD (MIN):	3
AEROSOL CONCENTRATIONS	
CALCULATED NOMINAL CONCENTRATION (MG/L):	70.30
TIME-WEIGHTED MEAN ANALYTICAL CONCENTRATION (MG/L):	2.60
AEROSOL PARTICLE-SIZE ANALYSIS	
MASS MEDIAN AERODYNAMIC DIAMETER (μ):	2.9
GEOMETRIC STANDARD DEVIATION:	±2.17
PERCENTAGE OF PARTICLES ≤ 4.0 μ (%):	66
CHAMBER ENVIRONMENTAL DATA	
TEMPERATURE RANGE (°F):	68.3-70.7
HUMIDITY RANGE (%):	68.3-69.3
OXYGEN CONTENT (%):	20.9

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TABLE 2
AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS
INDIVIDUAL CLINICAL OBSERVATIONS
(POSITIVE FINDINGS)

MALES 2.60 MG/L

		DAY OF STUDY														
MALE#	OBSERVATIONS	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A6829	SCHEDULED EUTHANASIA															P
	CONGESTED BREATHING	P	P	P	P	P			P	P						
	RALES		P	P												
	NO FECES		P													
	FEW FECES			P												
	DARK MATERIAL AROUND NOSE		P													
	DECREASED FOOD CONSUMPTION			P												
A6830	SCHEDULED EUTHANASIA															P
	CONGESTED BREATHING	P	P	P												
	RALES	P														
A6831	SCHEDULED EUTHANASIA															P
	CONGESTED BREATHING	P	P	P					P	P	P	P	P	P	P	P
	RALES	P	P	P							P	P	P			P
	FEW FECES		P	P												
	ROUGH COAT		P													
	DARK MATERIAL AROUND NOSE	P														
	DECREASED FOOD CONSUMPTION		P	P												
A6832	SCHEDULED EUTHANASIA															P
	CONGESTED BREATHING	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	RALES		P	P	P						P	P	P			
	LABORED BREATHING				P	P					P					
	GASPING				P											
	FEW FECES		P	P												
	ROUGH COAT		P	P	P	P										
	DARK MATERIAL AROUND EYE(S)	P														
	DARK MATERIAL AROUND NOSE	P														
	DECREASED FOOD CONSUMPTION		P	P												

GRADE CODE: 1=SLIGHT 2=MODERATE 3=SEVERE P=PRESENT L=LEFT R=RIGHT B=BILATERAL

TABLE 2
AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS
INDIVIDUAL CLINICAL OBSERVATIONS
(POSITIVE FINDINGS)

MALES 2.60 MG/L

		DAY OF STUDY														
MALE#	OBSERVATIONS	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A6833	SCHEDULED EUTHANASIA															P
	CONGESTED BREATHING	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	RALES	P	P	P	P	P	P	P	P	P	P	P	P			
	LABORED BREATHING		P								P					
	GASPING		P													
	NO FECES			P												
	FEW FECES		P		P											
	UNKEMPT APPEARANCE		P													
	FECAL STAIN		1													
	ROUGH COAT		P	P												
	DARK MATERIAL AROUND NOSE		P	P	P											
	DARK MATERIAL AROUND MOUTH			P	P											
	DECREASED FOOD CONSUMPTION		P	P												

GRADE CODE: 1=SLIGHT 2=MODERATE 3=SEVERE P=PRESENT L=LEFT R=RIGHT B=BILATERAL

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TABLE 2
AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS

INDIVIDUAL CLINICAL OBSERVATIONS
(POSITIVE FINDINGS)

FEMALES 2.60 MG/L

		DAY OF STUDY													
FEMALE#	OBSERVATIONS	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A6849	SCHEDULED EUTHANASIA															P
	CONGESTED BREATHING	P	P	P					P	P						
	RALES	P	P	P												
	FEW FECES		P													
	MUCOID STOOLS			P												
	URINE STAIN		1													
	ROUGH COAT		P													
	DECREASED FOOD CONSUMPTION		P													
A6850	SCHEDULED EUTHANASIA															P
	CONGESTED BREATHING	P	P	P												
	RALES	P	P	P												
	LABORED BREATHING		P													
	GASPING		P													
	NO FECES		P	P												
	ROUGH COAT		P													
	DARK MATERIAL AROUND NOSE	P														
	DARK MATERIAL AROUND MOUTH		P													
	DECREASED FOOD CONSUMPTION			P												
A6851	SCHEDULED EUTHANASIA															P
	CONGESTED BREATHING	P	P	P					P	P						
	RALES		P	P												
	FEW FECES			P												
	DARK MATERIAL AROUND EYE(S)	P														
	DARK MATERIAL AROUND NOSE	P														
	DECREASED FOOD CONSUMPTION			P												

GRADE CODE: 1=SLIGHT 2=MODERATE 3=SEVERE P=PRESENT L=LEFT R=RIGHT B=BILATERAL

TABLE 2
AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS
INDIVIDUAL CLINICAL OBSERVATIONS
(POSITIVE FINDINGS)

FEMALES 2.60 MG/L

		DAY OF STUDY													
FEMALE#	OBSERVATIONS	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A6853	SCHEDULED EUTHANASIA															P
	CONGESTED BREATHING	P	P	P	P											
	RALES	P	P													
	LABORED BREATHING			P												
	GASPING			P												
	NO FECES			P												
	FEW FECES		P													
	ROUGH COAT		P													
	COOL TO TOUCH			P												
	DARK MATERIAL AROUND EYE(S)	P														
	DARK MATERIAL AROUND NOSE	P		P												
	DARK MATERIAL AROUND MOUTH		P	P												
	DECREASED FOOD CONSUMPTION			P												
A6860	SCHEDULED EUTHANASIA															P
	CONGESTED BREATHING	P	P	P	P	P	P	P	P	P	P					
	RALES	P	P	P			P	P								
	LABORED BREATHING		P													
	GASPING		P													
	NO FECES			P												
	FEW FECES				P											
	URINE STAIN		1													
	ROUGH COAT		P													
	DARK MATERIAL AROUND EYE(S)	P														
	DARK MATERIAL AROUND NOSE	P	P	P												
	DARK MATERIAL AROUND MOUTH		P	P												
	DECREASED FOOD CONSUMPTION			P												

GRADE CODE: 1=SLIGHT 2=MODERATE 3=SEVERE P=PRESENT L=LEFT R=RIGHT B=BILATERAL

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TABLE 3

AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS

INDIVIDUAL BODY WEIGHTS (GRAMS)

MALES 2.60 MG/L

ANIMAL#	DAY OF STUDY 0	7	14	AT DEATH (DAY)
A6829	274	278	318	
A6830	259	269	304	
A6831	270	272	279	
A6832	248	243	257	
A6833	275	256	303	
MEAN	265	264	292	
S.D.	11.5	14.0	24.2	
N	5	5	5	

TABLE 3

AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS

INDIVIDUAL BODY WEIGHTS (GRAMS)

FEMALES 2.60 MG/L

	DAY OF STUDY			
ANIMAL#	0	7	14	AT DEATH (DAY)
A6849	211	227	248	
A6850	212	235	252	
A6851	201	216	227	
A6853	205	195	205	
A6860	208	219	246	
MEAN	207	218	236	
S.D.	4.5	15.0	19.6	
N	5	5	5	

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TABLE 4

AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS

INDIVIDUAL GROSS NECROPSY OBSERVATIONS

MALES 2.60 MG/L

ANIMAL#	DAY OF DEATH	STUDY DAY	OBSERVATION	FATE
A6829	28-JAN-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A6830	28-JAN-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A6831	28-JAN-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A6832	28-JAN-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A6833	28-JAN-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA

TABLE 4

AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS

INDIVIDUAL GROSS NECROPSY OBSERVATIONS

FEMALES 2.60 MG/L

ANIMAL#	DAY OF DEATH	STUDY DAY	OBSERVATION	FATE
A6849	28-JAN-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A6850	28-JAN-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A6851	28-JAN-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A6853	28-JAN-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A6860	28-JAN-03	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA

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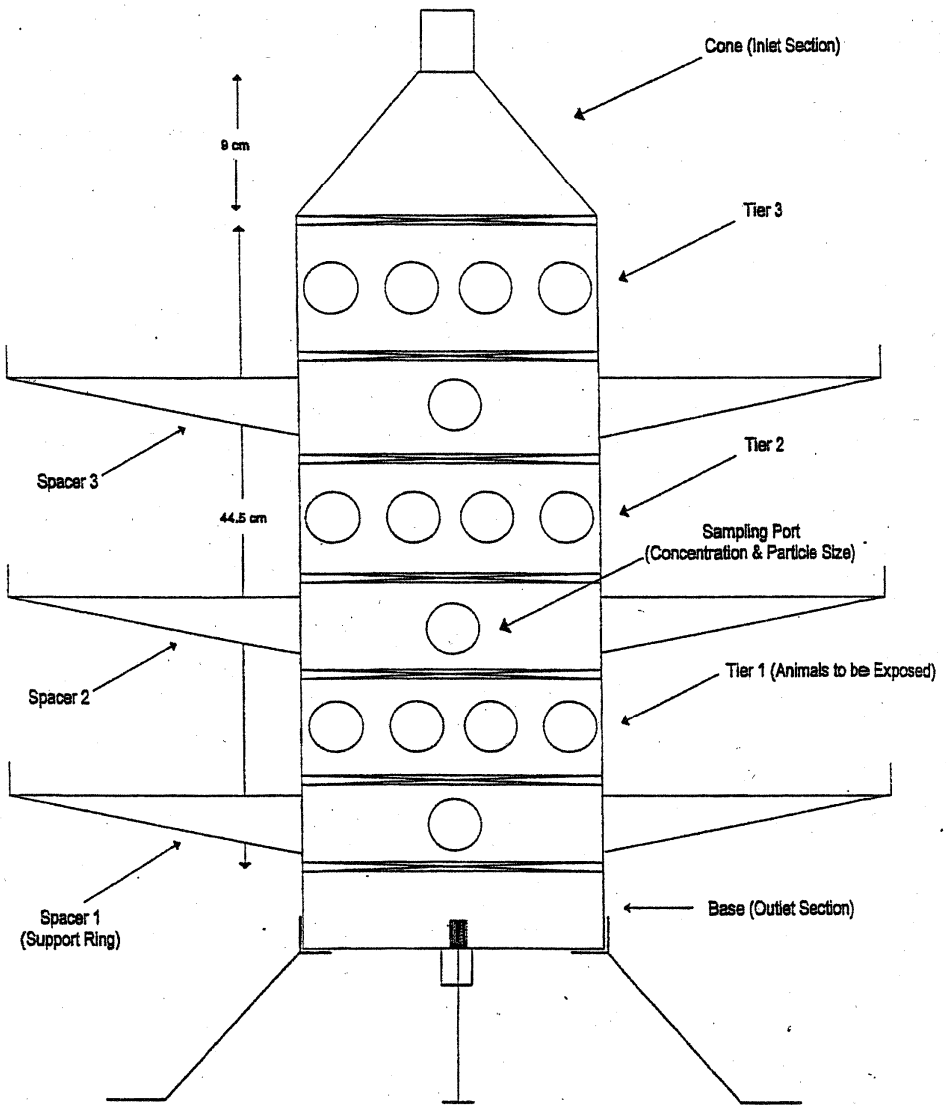

MULTI-STAGE 10 L NOSE-ONLY INHALATION CHAMBER

Figure 1

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APPENDIX A

Preliminary Aerosol Generation Trials

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1. PRELIMINARY AEROSOL GENERATION TRIALS

Prior to experimental initiation, preliminary aerosol generation trials were conducted. These trials were performed to determine the appropriate means of generating the aerosol exposure atmosphere of the test article at the targeted gravimetric/analytical concentration of (2.00 mg/L, initially) and the aerodynamic particle size (1-4 microns Mass Median Aerodynamic Diameter). The type of equipment used during each trial procedure is presented in the table that follows.

It was determined that since the gravimetric concentration was proportional to the analytical concentration it could be used as a “real time” estimate for the actual analytical concentration thus allowing for changes during the exposure. The results of the trials indicated that the equipment utilized during Trials # 1-7 produced an analytical concentration greater than 2.00 mg/L utilizing a pump speed of 1.2 mL/minute or greater. In addition, the aerodynamic particle size distribution was determined using the ITP 7 Stage Cascade Impactor during Trial # 2 and was acceptable ($3.0 \pm 1.78\ \mu$). Therefore, this equipment design was used for the study exposure.

Note: The ability to generate a target gravimetric concentration of $\geq$ 0.5 mg/L (Trials # 8-10) were also explored. These trials revealed that the gravimetric concentrations were also proportional to the analytical concentration at lower concentrations. The trials provide an indication of the settings necessary to achieve the target analytical concentration and that the gravimetric concentrations could be used as a “real time” estimate of the analytical concentration at lower concentrations in case additional levels would have been required.

TRIAL NO.	EQUIPMENT USED	INPUT AIR (PSI)	TEST ARTICLE CONCEN-TRATION (%)	MAXIMUM ATTAINABLE CONCENTRATIONS (MG/L)	
				GRAVIMETRIC	ANALYTICAL
1	One Multi-Stage 10L Nose-Only Chamber 5L Elutriator Master Flex Pump and Pump Heads 7523-30 and 77200-60 Spraying Systems, Pistol Air/Fluid Mixing Nozzle 5.0 mL/min pump speed 14 gauge tubing size	30	100	2.94	--
2	One Multi-Stage 10L Nose-Only Chamber 5L Elutriator ITP 7 Stage Cascade Impactor Master Flex Pump and Pump Heads 7523-30 and 77200-60 Spraying Systems, Pistol Air/Fluid Mixing Nozzle 4.0 mL/min pump speed 14 gauge tubing size	30	100	2.52	4.829
3	One Multi-Stage 10L Nose-Only Chamber 5L Elutriator Master Flex Pump and Pump Heads 7523-30 and 77200-60 Spraying Systems, Pistol Air/Fluid Mixing Nozzle 4.0 mL/min pump speed 14 gauge tubing size	30	100	2.54	4.688

Note: Targeting ≥ 4.50 mg/L analytical and ≥ 2.50 gravimetric concentration for Trials 1-3.

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STUDY NO.: 3596.18 TRIAL TABLE 1 PAGE 2
INL/A, U.S. DEPARTMENT OF STATE PRELIMINARY AEROSOL GENERATION TRIALS

TRIAL NO.	EQUIPMENT USED	INPUT AIR (PSI)	TEST ARTICLE CONCEN-TRATION (%)	MAXIMUM ATTAINABLE CONCENTRATIONS (MG/L)	
				GRAVIMETRIC	ANALYTICAL
4	One Multi-Stage 10L Nose-Only Chamber 5L Elutriator Master Flex Pump and Pump Heads 7523-30 and 77200-60 Spraying Systems, Pistol Air/Fluid Mixing Nozzle 2.0 mL/min pump speed 14 gauge tubing size	30	100	1.60	--
5	One Multi-Stage 10L Nose-Only Chamber 5L Elutriator Master Flex Pump and Pump Heads 7523-30 and 77200-60 Spraying Systems, Pistol Air/Fluid Mixing Nozzle 1.5 mL/min pump speed 14 gauge tubing size	30	100	1.36	--
6	One Multi-Stage 10L Nose-Only Chamber 5L Elutriator Master Flex Pump and Pump Heads 7523-30 and 77200-60 Spraying Systems, Pistol Air/Fluid Mixing Nozzle 1.8 mL/min pump speed 14 gauge tubing size	30	100	1.50	3.169

Note: Targeting ≥ 3.00 mg/L analytical and ≥ 1.50 mg/L gravimetric concentration for Trials 4-6.

TRIAL NO.	EQUIPMENT USED	INPUT AIR (PSI)	TEST ARTICLE CONCEN-TRATION (%)	MAXIMUM ATTAINABLE CONCENTRATIONS (MG/L)	
				GRAVIMETRIC	ANALYTICAL
7	One Multi-Stage 10L Nose-Only Chamber 5L Elutriator Master Flex Pump and Pump Heads 7523-30 and 77200-60 Spraying Systems, Pistol Air/Fluid Mixing Nozzle 1.8 mL/min pump speed 14 gauge tubing size	30	100	1.60	2.940
8	One Multi-Stage 10L Nose-Only Chamber 5L Elutriator Master Flex Pump and Pump Heads 7523-30 and 77200-60 Spraying Systems, Pistol Air/Fluid Mixing Nozzle 0.8 mL/min pump speed 14 gauge tubing size	30	100	0.86	--
9	One Multi-Stage 10L Nose-Only Chamber 5L Elutriator Master Flex Pump and Pump Heads 7523-30 and 77200-60 Spraying Systems, Pistol Air/Fluid Mixing Nozzle 0.5 mL/min pump speed 14 gauge tubing size	30	100	0.52	1.202

Note: Targeting ≥ 3.00 mg/L analytical and ≥ 1.50 gravimetric concentration for Trial 7.
Targeting ≥ 1.00 mg/L analytical and gravimetric concentration for Trials 8-9.

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STUDY NO.: 3596.18 TRIAL TABLE 1 PAGE 4
INL/A, U.S. DEPARTMENT OF STATE PRELIMINARY AEROSOL GENERATION TRIALS

TRIAL NO.	EQUIPMENT USED	INPUT AIR (PSI)	TEST ARTICLE CONCEN-TRATION (%)	MAXIMUM ATTAINABLE CONCENTRATIONS (MG/L)	
				GRAVIMETRIC	ANALYTICAL
10	One Multi-Stage 10L Nose-Only Chamber 5L Elutriator Master Flex Pump and Pump Heads 7523-30 and 77200-60 Spraying Systems, Pistol Air/Fluid Mixing Nozzle 0.5 mL/min pump speed 14 gauge tubing size	30	100	0.46	1.311
11	One Multi-Stage 10L Nose-Only Chamber 5L Elutriator Master Flex Pump and Pump Heads 7523-30 and 77200-60 Spraying Systems, Pistol Air/Fluid Mixing Nozzle 1.2 mL/min pump speed 14 gauge tubing size	30	100	1.30	--
12	One Multi-Stage 10L Nose-Only Chamber 5L Elutriator Master Flex Pump and Pump Heads 7523-30 and 77200-60 Spraying Systems, Pistol Air/Fluid Mixing Nozzle 1.0 mL/min pump speed 14 gauge tubing size	30	100	0.64	--

Note: Targeting ≥ 1.00 mg/L analytical and gravimetric concentration for Trials 10-12.

TRIAL NO.	EQUIPMENT USED	INPUT AIR (PSI)	TEST ARTICLE CONCEN-TRATION (%)	MAXIMUM ATTAINABLE CONCENTRATIONS (MG/L)	
				GRAVIMETRIC	ANALYTICAL
13	One Multi-Stage 10L Nose-Only Chamber 5L Elutriator Master Flex Pump and Pump Heads 7523-30 and 77200-60 Spraying Systems, Pistol Air/Fluid Mixing Nozzle 1.1 mL/min pump speed 14 gauge tubing size	30	100	0.72	--

Note: Targeting ≥ 1.00 mg/L gravimetric concentration for Trial 13.

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AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS
AERODYNAMIC PARTICLE SIZE DATA
TRIAL 2

Stage	Effective Cutoff Diameter	Filter Weights (mg) Pre-sample	Post-sample	Difference Weights	% of Total	Cumulative % <ECD
1	10.00	103.2	103.3	0.1	1.4	98.6
2	6.11	102.9	103.7	0.8	11.4	87.1
3	3.70	103.6	105.0	1.4	20.0	67.1
4	2.22	103.4	106.1	2.7	38.6	28.6
5	1.39	103.1	104.5	1.4	20.0	8.6
6	0.79	103.5	104.0	0.5	7.1	1.4
7	0.50	103.8	103.9	0.1	1.4	0.0
Filter	-	103.6	103.6	0.0	0.0	
		Total of Difference Weights:		7.0		

Mass Median Aerodynamic Diameter = 3.0 microns
Geometric Standard Deviation = 1.78
Percentage ≤ 4.0 microns = 70 %

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APPENDIX B

Analytical Chemistry Report

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1. SPRAY--CHARLIE ANALYSIS

The analytical method for the analysis of the glyphosate component of Spray--Charlie was validated prior to the analytical chamber concentration analyses performed at Springborn Laboratories, Inc. This method was utilized to determine the inhalation chamber concentration during the Acute Nose-Only Inhalation Toxicity Study.

1.1. Experimental System

1.1.1. HPLC System

Pump:	Waters 600E System Controller
Injector:	Waters WISP 717
Detector:	Waters 2487
Data System:	HP 3396B Integrator
Precolumn	Phenomenex, SecurityGuard, C18, 4.0 x 3.0 mm ID
Column:	Phenomenex, Spherex, C18, 5μ, 250 x 4.6 mm ID
Mobile Phase:	A: 0.05 M HCO_2NH_4, pH 3.6/5% Acetonitrile B: 100% HPLC Acetonitrile
Gradient:	100% A, hold for 6 minutes; linear change to 25% A/75% B over 1 minute; hold for 5 minutes; linear change to 100% A over 1 minute; hold at 100% A for 15 minutes
Injection Volume:	10 μL
Flow Rate:	1.0 mL/min
Detection:	500nm; 0.4000 AUFS

1.1.2. Apparatus

Balance:	Mettler AG 245, accuracy of 0.0001 gram
Glassware:	Assorted volumetric glassware
Filters:	Gelman, glass fiber, Whatman Puradisc 25PP, 0.45 μm; 0.2 μ Nylon-66 filter
Shaker:	Labline, Multi-Wrist Shaker
Oven:	Boekel, Model 107905
Pipet:	Mettler-Toledo 100-1000 μL, 500 – 5000 μL
pH Meter	Corning 320

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1.1.3. Solutions and Reagents

1.1.3.1. Reagents

Water, Fisher, HPLC Grade, Lot # 023349
Acetonitrile, J.T. Baker, HPLC Grade, Lot # M15811
NBD-Chloride, Aldrich, Lot # 10926TO
Hydrochloric Acid, A.C.S. Grade, Lot # 012161
Potassium Tetraborate Tetrahydrate, Aldrich, Lot # 15325DI
Ammonium Formate, Fisher, Certified Grade, Lot # 990125
Formic Acid, Fisher, Laboratory Grade, Lot # 003630
Methanol, Fisher, HPLC Grade, Lot # 023883

1.1.3.2. Solutions

0.37M Borate Solution: Prepared by dissolving approximately 11.44 g of potassium tetraborate tetrahydrate in 100 mL of HPLC grade water. The resulting solution was mixed thoroughly and was stable for 6 months post-preparation at room temperature.

1.2 N HCl: Prepared by diluting 10 mL of HCl in 90 mL of HPLC grade water. The resulting solution was mixed thoroughly and was stable for 6 months post-preparation at room temperature.

25 mM NBD-Cl: Prepared by dissolving approximately 2.5 g of NBD-Cl in 500 mL of HPLC grade methanol. The resulting solution was mixed thoroughly and was stable for 6 months post-preparation at room temperature.

Mobile Phase A: Prepared by dissolving approximately 1.57 g of ammonium formate in 950 mL of HPLC grade water. The pH of the resulting solution was adjusted to approximately 3.6 with formic acid. Then, 50 mL of HPLC grade acetonitrile was added. The resulting solution was mixed thoroughly, filtered through a 0.2 μm Nylon-66 filter, and degassed by helium sparging prior to use. Different volumes were also prepared using the same ratio of components.

Mobile Phase B: 100% HPLC grade acetonitrile used as received.

Diluent: 100% HPLC grade water used as received.

Stock Standard Solution (Trial Work): Prepared by dissolving 116.8 mg of Spray--Charlie in a 25 mL flask with diluent.

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Standard Solutions (Trial Work): Prepared by serially diluting the stock standard solution with diluent. The final concentrations of the solutions were in the range of approximately 0.47 to 3.3 mg/mL. These solutions were then filtered through Whatman Puradisc 25PP 0.45 μm filters and diluted with HPLC water at a ratio of 1:10 prior to the derivatization.

Stock Standard Solution (Exposure #1): Prepared by dissolving 100.2 mg of Spray--Charlie in a 25 mL flask with diluent.

Standard Solutions (Exposure #1): Prepared by serially diluting the stock standard solution with diluent. The final concentrations of the solutions were in the range of approximately 0.4 to 1.6 mg/mL. These solutions were then filtered through Whatman Puradisc 25PP 0.45 μm filters and diluted with HPLC water at a ratio of 1:10 prior to the derivatization.

Chamber Concentration Solutions: Prepared by placing the weighed glass fiber filter used for gravimetric concentration determination in a capped container with 10 mL of diluent. The solutions were then agitated mechanically for 15 minutes and filtered through Whatman Puradisc 25PP 0.45 μm filters. The sample solutions were then diluted at a ratio of 1:10 with HPLC water prior to derivatization.

Precolumn Derivatization: In order to analyze the glyphosate component, a precolumn derivatization was performed by adding 1.2 mL of the appropriate control, standard, or sample solution to a labeled scintillation vial. Both 0.8 mL of the borate solution and 2.4 mL of the NBD-Cl solution were added to each vial. The vials were then capped and shaken by hand prior to being heated in an oven at 80° C for 30 minutes. After removal from the oven, the vials were allowed to cool for 10 minutes followed by the addition of 0.9 mL of the HCl solution. After the vials were again shaken by hand, they were allowed to stand for 10 minutes in order for incipient precipitation to occur. These solutions were then transferred to injection vials.

1.2. Analytical Procedures

1.2.1. Standard Curve Analysis

The peak areas of the glyphosate component of each standard were determined, measured, and plotted as a function of concentration to generate a standard curve. The actual values used for the calculations are shown in Chemistry Tables 1 and 2.

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1.2.2. Sample Analysis

The peak areas of the glyphosate component of each sample were measured and the concentration was determined by linear fit to the standard curve. The actual values used for the calculations are shown in Chemistry Tables 1 and 2.

1.3. Results and Conclusions

1.3.1. Analytical Chamber Concentration

The actual sample results of the trial work are shown in Chemistry Table 1. The individual sample results of the analytical chamber analysis are shown in Chemistry Table 2.

M. Gardner Clemons Date: 3/14/2003

M. Gardner Clemons, B.A.
Manager of Analytical Chemistry
and Pharmacy

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Chemistry Table 1

Standard Curve and Sample Analysis Values for Trial Work

Sample No.	Theoretical Conc. (mg/L)	Peak Area	Analytical Chamber Conc. (mg/L)
Std 1	0.9344	31125	NA
Std 2	2.804	97258	NA
Std 3	4.672	170507	NA
Std 4	6.540	249444	NA
Trial # 2	NA	179632	4.829
Trial # 3	NA	174130	4.688
Trial # 6	NA	114911	3.169
Trial # 6	NA	105992	2.940
Trial # 9	NA	38278	1.202
Trial # 10	NA	42531	1.311

NA – Not Applicable
Correlation coefficient = 0.9992

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Chemistry Table 2

Standard Curve and Sample Analysis Values for Exposure #1

Sample No.	Theoretical Conc. (mg/L)	Peak Area	Analytical Chamber Conc. (mg/L)
Std 1	0.8016	25636	NA
Std 2	1.603	51542	NA
Std 3	2.404	70695	NA
Std 4	3.206	98772	NA
# 1	NA	81029	2.654
# 2	NA	62864	2.044
# 3	NA	85271	2.797
# 4	NA	87625	2.876
# 5	NA	79437	2.601
# 6	NA	80738	2.645
# 7	NA	80393	2.633
# 8	NA	77142	2.524
# 9	NA	82645	2.709

NA – Not Applicable
Correlation coefficient = 0.998

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APPENDIX C

Individual Aerosol Generation and
Chamber Environmental Data

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2.60 mg/L Exposure Level

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AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS
CHAMBER ENVIRONMENTAL DATA
EXPOSURE: 2.60 MG/L

TIME (MIN.)	TEMPERATURE (°F)	RELATIVE HUMIDITY (%)	OXYGEN CONTENT (%)
0	69.4	69.3	20.9
30	68.3	68.7	20.9
60	69.3	68.8	20.9
90	69.7	68.4	20.9
120	69.8	68.6	20.9
150	70.3	68.3	20.9
180	70.2	68.5	20.9
210	70.6	69.0	20.9
240	70.7	68.9	20.9

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AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS
TIME WEIGHTED ANALYTICAL CONCENTRATION
ANALYTICAL EXPOSURE: 2.60 MG/L

Sample No.	Sample Time (min.)	Aerosol Concentration (mg/L)	Mean Concentration Per Interval (mg/L)	Interval Length (min.)	Time Weighted Concentration Per Interval
1	0	2.65			
			2.35	30.00	70.35
2	30	2.04			
			2.42	30.00	72.60
3	60	2.80			
			2.84	30.00	85.20
4	90	2.88			
			2.74	30.00	82.20
5	120	2.60			
			2.63	30.00	78.75
6	150	2.65			
			2.64	30.00	79.20
7	180	2.63			
			2.58	30.00	77.25
8	210	2.52			
			2.62	30.00	78.45
9	240	2.71			
TOTAL				240.00	624.00
TIME WEIGHTED MEAN ANALYTICAL CONCENTRATION (MG/L)					2.60

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AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS
AERODYNAMIC PARTICLE SIZE DATA
SAMPLE NO. A
ANALYTICAL EXPOSURE: 2.60 MG/L

Stage	Effective Cutoff Diameter	Filter Weights (mg) Pre-sample	Post-sample	Difference Weights	% of Total	Cumulative % <ECD
1	10.00	102.0	102.2	0.2	8.0	92.0
2	6.11	102.2	102.4	0.2	8.0	84.0
3	3.70	102.1	102.5	0.4	16.0	68.0
4	2.22	102.7	103.7	1.0	40.0	28.0
5	1.39	103.5	103.9	0.4	16.0	12.0
6	0.79	103.7	103.9	0.2	8.0	4.0
7	0.50	103.3	103.4	0.1	4.0	0.0
Filter	-	102.7	102.7	0.0	0.0	
		Total of Difference Weights:		2.5		

Mass Median Aerodynamic Diameter = 3.1 microns
Geometric Standard Deviation = 2.10
Percentage ≤ 4.0 microns = 63 %

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AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS
AERODYNAMIC PARTICLE SIZE DATA
SAMPLE NO.: B
ANALYTICAL EXPOSURE: 2.60 MG/ML

Stage	Effective Cutoff Diameter	Filter Weights (mg) Pre-sample	Post-sample	Difference Weights	% of Total	Cumulative % <ECD
1	10.00	101.7	102.2	0.5	10.4	89.6
2	6.11	103.7	104.0	0.3	6.2	83.3
3	3.70	101.9	102.8	0.9	18.7	64.6
4	2.22	103.0	104.4	1.4	29.2	35.4
5	1.39	102.3	103.1	0.8	16.7	18.8
6	0.79	102.0	102.2	0.2	4.2	14.6
7	0.50	102.1	102.7	0.6	12.5	2.1
Filter	-	102.3	102.4	0.1	2.1	
		Total of Difference Weights:		4.8		

Mass Median Aerodynamic Diameter = 2.8 microns
Geometric Standard Deviation = 2.47
Percentage ≤ 4.0 microns = 65 %

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SLI Study No. 3596.18

AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS
AERODYNAMIC PARTICLE SIZE DATA
SAMPLE NO.: C
ANALYTICAL EXPOSURE: 2.60 MG/L

Stage	Effective Cutoff Diameter	Filter Weights (mg) Pre-sample	Filter Weights (mg) Post-sample	Difference Weights	% of Total	Cumulative % <ECD
1	10.00	102.7	102.9	0.2	4.4	95.6
2	6.11	103.4	103.7	0.3	6.7	88.9
3	3.70	103.2	103.9	0.7	15.6	73.3
4	2.22	102.8	104.3	1.5	33.3	40.0
5	1.39	102.7	103.8	1.1	24.4	15.6
6	0.79	102.9	103.5	0.6	13.3	2.2
7	0.50	103.0	103.1	0.1	2.2	0.0
Filter	-	103.6	103.6	0.0	0.0	
		Total of Difference Weights:		4.5		

Mass Median Aerodynamic Diameter = 2.8 microns
Geometric Standard Deviation = 1.95
Percentage ≤ 4.0 microns = 71 %

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AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS
AERODYNAMIC PARTICLE SIZE DATA

ANALYTICAL EXPOSURE: 2.60 MG/L

Stage	Effective Cutoff Diameter	Cumulative % less than indicated size			
		Sample A	Sample B	Sample C	
1	10.00	92.0	89.6	95.6	
2	6.11	84.0	83.3	88.9	
3	3.70	68.0	64.6	73.3	
4	2.22	28.0	35.4	40.0	
5	1.39	12.0	18.8	15.6	
6	0.79	4.0	14.6	2.2	
7	0.50	0.0	2.1	0.0	
					Mean
Mass Median Aerodynamic Diameter		3.1	2.8	2.8	2.9
Geometric Standard Deviation		2.10	2.47	1.95	2.17
Percentage ≤ 4.0 microns		63	65	71	66

SLI Study No. 3596.18

APPENDIX D

SLI Personnel Responsibilities

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SLI Study No. 3596.18

SLI PERSONNEL RESPONSIBILITIES

Kimberly L. Bonnette, M.S., LATG	Study Director/Director, Acute Toxicology
Dawn D. Rodabaugh, B.S.	Alternate Contact/Toxicologist
Malcolm Blair, Ph.D.	Managing Director Emeritus
Rusty E. Rush, M.S., LAT, DABT	Director, Neurotoxicity and Transgenics
Joseph C. Siglin, Ph.D., DABT	General Manager
Jason W. Smedley, B.S.	Assistant Toxicologist
Pamela S. Smith, ALAT	Study Supervisor, Acute Toxicology
Kevin V. Weitzel, A.S.	Primary Technician/Inhalation Team Leader
Delores P. Knippen	Supervisor, Pharmacy
Steven H. Magness, B.S., LATG	Senior Supervisor, Pathology
Anita M. Bosau, RQAP-GLP	Senior Director, Compliance Assurance
Cheryl A. Bellamy	Senior Supervisor, Report Writing
Deanna M. Talerico, RQAP-GLP	Senior Supervisor, Quality Assurance
J. Dale Thurman, D.V.M., M.S., DACVP	Senior Director, Pathology
Kathy M. Gasser	Archivist

A DERMAL SENSITIZATION STUDY IN GUINEA PIGS WITH SPRAY--CHARLIE •MODIFIED BUEHLER DESIGN•

FINAL REPORT

OPPTS Guidelines

870.2600

Author

Kimberly L. Bonnette, M.S., LATG

Study Completed on

March 14, 2003

Performing Laboratory

Springborn Laboratories (SLI),
a division of Charles River Laboratories, Inc.
640 North Elizabeth Street
Spencerville, Ohio 45887

SLI Study No.

3596.21

Submitted to

INL/A
U.S. Department of State
2201 C St. NW SA-4
Washington, DC 20520

SLI Study No. 3596.21 (2)

1. STATEMENT OF *NO* DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B), or (C).

Company: _____

Company Agent: _____ Date: _____

_____ _____

Title Signature

2. COMPLIANCE STATEMENT

This study was conducted in compliance with the Good Laboratory Practice Standards as described by the EPA (40 CFR Parts 160 and 792) with the following exception:

The dose preparations used during the range-finding study were not analyzed to confirm test article concentration, stability or homogeneity.

Kimberly L. Bonnette, M.S., LATG
Study Director/Author
Springborn Laboratories

Date 3/14/03

Rogers Woolfolk
Senior Aviation Advisor
Sponsor/Submitter
INL/A
U.S. Department of State

Date 21 Feb 03

SLI Study No. 3596.21 (4)

3. QUALITY ASSURANCE STATEMENT

This study was inspected by the Quality Assurance Unit and reports were submitted to management and the Study Director in accordance with SLI's Standard Operating Procedures as follows:

Phase	Date
Protocol Review	10/07/02
Body Weight	12/30/02
Data Audit	02/18/03
Draft Report Review	02/18/03
Final Report Review	03/14/03
Reports to Study Director and Management	02/18/03, 03/14/03

The final report has been reviewed to assure that it accurately describes the materials and methods, and the reported results accurately reflect the raw data.

Rebecca A. Young Date 3/14/03
Rebecca A. Young
Quality Assurance Team Leader

Anita M Bosau Date 3/14/03
Anita M. Bosau, RQAP-GLP
Senior Director, Compliance Assurance

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6. SUMMARY

The dermal sensitization potential of Spray--Charlie was evaluated in Hartley-derived albino guinea pigs. Ten male and ten female guinea pigs were topically treated with 100% Spray--Charlie, once per week, for three consecutive weeks. Following a two-week rest period, a challenge was performed whereby the 20 test and 10 previously untreated (naive) challenge control guinea pigs were topically treated with 100% Spray--Charlie. Challenge responses in the test animals were compared with those of the challenge control animals.

6.1. Spray--Charlie

Following challenge with 100% Spray--Charlie, dermal reactions in the test and challenge control animals were limited to scores of 0 to ±. Group mean dermal scores were noted to be similar in the test animals as compared with the challenge control animals.

6.2. HCA

Using α-Hexylcinnamaldehyde (HCA) as a positive control, Springborn Laboratories, Inc., Spencerville, Ohio, has completed a study during the past six months which provided historical control data for contact sensitization to this agent utilizing the test system described herein (Modified Buehler Design). Following induction at 5% w/v HCA in ethanol and challenge at levels of 2.5% and 1% w/v HCA in acetone, a contact sensitization response was observed, thereby demonstrating the susceptibility of the test system to this sensitizing agent.

6.3. Conclusion

Based on the results of this study, Spray--Charlie is not considered to be a contact sensitizer in guinea pigs. The results of the HCA historical control study demonstrated that a valid test was performed and indicated that the test design would detect potential contact sensitizers.

7. INTRODUCTION

This study was performed to assess the dermal sensitization potential (delayed contact hypersensitivity) of Spray--Charlie in Hartley-derived albino guinea pigs when administered by multiple topical applications. This study was intended to provide information on the potential health hazards of the test article with respect to dermal exposure. Data from this study may serve as a basis for classification and/or labeling of the test article. This study was performed in accordance with the US EPA, Health Effects Test Guidelines, OPPTS 870.2600, Skin Sensitization, August 1998. This study was performed at Springborn Laboratories, 553 North Broadway, Spencerville, Ohio. The protocol was signed by the Study Director on October 9, 2002 (GLP initiation date). The in-life phase of the main sensitization study was initiated with test article administration on December 31, 2002 (day 0) and concluded with final scoring on January 30, 2003.

Prior to initiation of the main sensitization study, a topical range-finding study was conducted in guinea pigs to aid in the selection of dosage levels. The in-life phase of the range-finding study was initiated with test article administration on December 17, 2002 and concluded on December 19, 2002. The experimental methods and results of the range-finding study are included in Appendix A.

8. MATERIALS AND METHODS

8.1. Test Article

The test article was received from the Sponsor and identified as follows:

Sponsor's ID	Assigned SLI ID	Physical Description	Receipt Date	Expiration Date
Spray--Charlie[a]	S02.003.3596	Amber liquid	12/09/02	None provided
Ingredients:[b]				
Herbicide: GLY-41 Lot No.: Manufactured 10/20/02				None provided
Surfactant: Cosmo Flux-411F Lot No.: Manufactured 11/29/02				None provided

[a]Sample pooled at SLI from five different mixes of Spray--Charlie (top/middle/bottom).
[b]Ingredients used in the five Spray--Charlie mixes that were prepared by the Sponsor.

The test article was stored at room temperature. The Sponsor was responsible for any necessary evaluations related to identity, strength, purity, composition, stability and method of synthesis of the test material according to 40 CFR 160.105 and 40 CFR 792.105. Springborn Laboratories analyzed the test article for the glyphosate (a.e.) which is presented in SLI Study No. 3596.15.

8.2. Retention Sample

An approximate 1 mL retention sample of each test article mixture sample (top/middle/bottom, maintained separately for a total of fifteen 1 mL samples) was taken and stored at SLI at room temperature. In addition, a 10 mL retention sample of the pooled test article samples (from the 5 test article mixtures) was collected and stored at SLI at room temperature. These samples serve as the retention samples for all studies conducted with this material.

8.3. Test Article Disposition

The remaining test article was returned to the Sponsor following completion of all studies with the test article.

8.4. Method of Test Article Preparation

The test article was utilized at 100% (Induction and Challenge). The test article was dispensed fresh on each day of dosing

8.5. Animals and Animal Husbandry

8.5.1. Description, Identification and Housing

Young adult, Hartley-derived albino guinea pigs were received from Hilltop Lab Animals, Inc., Scottdale, PA. Upon receipt, plastic ear tags displaying unique identification numbers were used to individually identify the animals. Cage cards displaying at least the study number, animal number and sex were affixed to each cage. The animals were housed individually in suspended stainless steel cages. All housing and care were based on the standards recommended by the Guide for the Care and Use of Laboratory Animals [1].

8.5.2. Environment

The animal room temperature and relative humidity ranges were 58-72°F (14-22°C) and 19-71%, respectively. Environmental control equipment was monitored and adjusted as necessary to minimize fluctuations in the animal room environment. Light timers were set to maintain a 12-hour light/12-hour dark cycle and room ventilation was set to produce 10-15 air changes/hour. The room temperature and relative humidity were recorded a minimum of once daily.

8.5.3. Food

PMI Certified Guinea Pig Chow #5026 (Purina Mills, Inc.) was provided ad libitum to the animals throughout the study. The lot number and expiration date of each batch of diet used during the study were recorded. The feed was analyzed and certified by the supplier for nutritional components and environmental contaminants. Dietary limitations for various environmental contaminants, including heavy metals, pesticides, polychlorinated biphenyls and total aflatoxin are set by the manufacturer. Within these limits, contaminants which may have been present were not expected to compromise the purpose of this study. Results of the dietary analyses (Certificates of Analysis) are provided by the manufacturer for each lot of diet. These are maintained by SLI.

8.5.4. Water

Municipal tap water treated by reverse osmosis was available ad libitum throughout the study. The purified water was supplied by an automatic watering system. Monitoring of the drinking water for contaminants is conducted by SLI and the records are available for inspection. Within generally accepted limits, contaminants which may have been present were not expected to compromise the purpose of this study. The water meets the standards specified under the EPA National Drinking Water Regulations (40 CFR Part 141).

8.5.5. Acclimation

Upon receipt, the animals were removed randomly from the shipping cartons, examined by qualified personnel, identified with plastic ear tags and then acclimated to the laboratory conditions for a minimum of five days. The animals were observed daily for overt physical or behavioral abnormalities, general health/moribundity and mortality.

8.5.6. Animal Selection

The animals chosen for study use were arbitrarily selected from healthy stock animals to avoid potential bias. All animals received a detailed pretest observation prior to dosing. Only healthy animals were chosen for study use. Females were nulliparous and nonpregnant. The male animals were approximately 7 weeks of age and weighed 394-464 g on the day prior to Induction 1 dosing. The female animals were approximately 9 weeks of age and weighed 366-420 g on the day prior to Induction 1 dosing.

SLI Study No. 3596.21 (11)

9. EXPERIMENTAL PROCEDURES

9.1. Study Design

This study consisted of a topical range-finding group, a test group and a challenge control group [2]. A rechallenge control group was maintained on this study; however, the rechallenge procedure was not required since the challenge results were definitive.

9.2. Sensitization Study

9.2.1. Preliminary Procedures

On the day prior to each dose administration, the guinea pigs had the hair removed with a small animal clipper. Care was taken to avoid abrading the skin.

9.2.2. Dosing

A dose of 0.3 mL of the test article was placed on a 25 mm Hilltop chamber backed by adhesive tape (occlusive patch). The chambers were then applied to the clipped surface as quickly as possible.

Following chamber application, the trunk of the animal was wrapped with elastic wrap which was secured with adhesive tape to prevent removal of the chamber and the animal was returned to its cage.

9.2.2.1. Induction

On the day prior to the first induction dose administration (day -1), all test and control animals were weighed and the hair was removed from the left side of the test animals. On the day following clipping (day 0), chambers were applied as follows:

Group	Material	Induction No.	Concentration[a] (%)	Test Site No.	No. of Animals Male	No. of Animals Female
Test	Spray--Charlie	1	100	1	10	10
		2	100	1		
		3	100	1		

[a]Pooled test article.

The induction procedure was repeated on study day 7 and on study day 14 so that a total of three consecutive induction exposures were made to the test animals.

9.2.2.2. Challenge

On the day prior to challenge dose administration, the test and challenge control animals were weighed and the hair was removed from the right side of the animals. On the day following clipping (day 28), chambers were applied as follows:

Group	Material	Concentration[a] (%)	Test Site No.	No. of Animals Male	No. of Animals Female
Test	Spray--Charlie	100	2	10	10
Challenge Control	Spray--Charlie	100	2	5	5

[a]Pooled test article.

9.2.3. Test Article Removal

Approximately six hours after chamber application, the binding materials were removed. The test sites were wiped with gauze moistened in deionized water, followed by dry gauze, to remove test article residue. The animals were then returned to their cages.

9.2.4. Dermal Observations

The test sites were graded for irritation at approximately 24 and 48 hours following chamber application (induction) or chamber removal (challenge) using the Dermal Grading System presented in Appendix B.

9.2.5. Clinical Observations

Any unusual observations and mortality were recorded. The animals were observed for general health/mortality twice daily, once in the morning and once in the afternoon.

9.2.6. Body Weights

Individual body weights were obtained for all sensitization study animals on the day prior to the first induction (day -1) and for the appropriate test and challenge control animals on the day prior to challenge dosing.

9.2.7. Scheduled Euthanasia

All sensitization study animals were euthanized by carbon dioxide inhalation following each animal's final scoring interval. Gross necropsy examinations were not required for these animals.

9.3. Protocol Deviations

The animal room temperature and relative humidity ranges [58-72°F (14-22°C) and 19-71%, respectively] exceeded the preferred ranges [63-73°F (17-23°C) and 30-70%, respectively] during this study. These occurrences were considered to have had no adverse effect on the outcome of this study.

10. ANALYSIS OF DATA

The sensitization potential of the test article was based on the dermal responses observed on the test and control animals at challenge. Generally, dermal scores of ≥1 in the test animals with scores of 0 to ± noted in the controls are considered indicative of sensitization. Dermal scores of 1 in both the test and control animals are generally considered equivocal unless a higher dermal response (≥ grade 2) is noted in the test animals. Group mean dermal scores were calculated for challenge.

11. MAINTENANCE OF RAW DATA AND RECORDS

All original raw data, the final report and electronic records were transferred to the SLI archives for a period of 10 years. The Sponsor will be contacted prior to final disposition of these items.

12. RESULTS

12.1. Topical Range-Finding Study

Individual Topical Range-Finding Data: Appendix A

The results of the range-finding study indicated that a test article concentration of 100% was considered appropriate for induction and challenge since it was the highest possible concentration which was nonirritating.

12.2. Sensitization Study

Individual Data: Tables 1-2

Following challenge with 100% Spray--Charlie, dermal reactions in the test and challenge control animals were limited to scores of 0 to ±. Group mean dermal scores were noted to be similar in the test animals as compared with the challenge control animals.

12.3. Body Weights

Individual Body Weight Data: Appendix C

The sensitization study animals gained weight during the test period and generally appeared in good health.

12.4. Historical Control

HCA Historical Control Data: Appendix D

Using α-Hexylcinnamaldehyde (HCA) as a positive control, Springborn Laboratories, Inc., Spencerville, Ohio, has completed a study during the past six months which provided historical control data for contact sensitization to this agent utilizing the test system described herein (Modified Buehler Design). Following induction at 5% w/v HCA in ethanol and challenge at levels of 2.5% and 1% w/v HCA in acetone, a contact sensitization response was observed, thereby demonstrating the susceptibility of the test system to this sensitizing agent.

13. CONCLUSION

Based on the results of this study, Spray--Charlie is not considered to be a contact sensitizer in guinea pigs. The results of the HCA historical control study demonstrated that a valid test was performed and indicated that the test design would detect potential contact sensitizers.

Kimberly L. Bonnette, M.S., LATG
Study Director

Date 3/14/03

14. REPORT REVIEW

Dawn D. Rodabaugh, B.S.
Toxicologist

Date 3/14/03

15. REFERENCES

1. Guide for the Care and Use of Laboratory Animals, DHHS Publication No. (NIH) 96-03, 1996.

2. E. V. Buehler, Delayed Contact Hypersensitivity in the Guinea Pig, Arch. Dermat., 91:171-177, 1965.

STUDY NO.: 3596.21
INL/A, U.S. DEPARTMENT OF STATE

TABLE 1
A DERMAL SENSITIZATION STUDY IN GUINEA PIGS
INDIVIDUAL INDUCTION DATA
(SPRAY--CHARLIE)

Group	Animal No./ Sex	Induction 1 Dermal Scores 100%[a] 24 Hr	Induction 1 Dermal Scores 100%[a] 48 Hr	Induction 2 Dermal Scores 100%[a] 24 Hr	Induction 2 Dermal Scores 100%[a] 48 Hr	Induction 3 Dermal Scores 100%[a] 24 Hr	Induction 3 Dermal Scores 100%[a] 48 Hr
Test	G1598/M	0	0	0	0	0	0
	G1599/M	0	0	0	0	0	0
	G1600/M	0	0	0	0	0	0
	G1601/M	0^{IT}	0	0^{IT}	0	0	0
	G1602/M	0	0	0^{IT}	0	0	0
	G1603/M	0	0	±	0	0	0
	G1604/M	0^{IT}	0	0^{IT}	0	0	0
	G1605/M	0	0	0	0	0	0
	G1606/M	0	0	0	0	0	0
	G1607/M	0^{IT}	0	0	0	0	0
	G1623/F	0^{IT}	0	0	0	0	0
	G1624/F	$\pm^{IT}$	0	0	0	±	0
	G1625/F	0	0	0	0	0	0
	G1626/F	0	0	0	0	0	0
	G1627/F	0	0	0	0	0	±
	G1628/F	0	0	0	0	0	0
	G1629/F	0	0	0	0	0	0
	G1630/F	0	0	0	0	0	0
	G1631/F	0	0	0	0	0	0
	G1632/F	0	0	0	0	0	0

[a]Pooled test article.
Note: See Appendix B for definition of codes.

STUDY NO.: 3596.21
INL/A, U.S. DEPARTMENT OF STATE

TABLE 2
A DERMAL SENSITIZATION STUDY IN GUINEA PIGS
INDIVIDUAL CHALLENGE DATA
(SPRAY--CHARLIE)

Group	Animal No./ Sex	Dermal Scores 100%[a] 24 Hr	48 Hr
Test	G1598/M	0	0
	G1599/M	0	0
	G1600/M	0	0
	G1601/M	0	0
	G1602/M	0	0
	G1603/M	0	0
	G1604/M	0	0
	G1605/M	0	0
	G1606/M	0^{IT}	0^{IT}
	G1607/M	0	0
	G1623/F	0	0
	G1624/F	0	±
	G1625/F	0	0
	G1626/F	0	0
	G1627/F	0	0
	G1628/F	0	0
	G1629/F	0	0
	G1630/F	0	0
	G1631/F	0	±
	G1632/F	0	0
	Mean	0.0	0.1

[a]Pooled test article.
Notes: For the purpose of calculation, ± = 0.5. See Appendix B for definition of codes.

STUDY NO.: 3596.21
INL/A, U.S. DEPARTMENT OF STATE

TABLE 2
A DERMAL SENSITIZATION STUDY IN GUINEA PIGS
INDIVIDUAL CHALLENGE DATA
(SPRAY--CHARLIE)

Group	Animal No./ Sex	Dermal Scores 100%[a] 24 Hr	48 Hr
Challenge Control	G1608/M	0	0
	G1609/M	0[IT]	0
	G1610/M	0[IT]	0
	G1611/M	0[IT]	0
	G1612/M	0[IT]	0
	G1633/F	0	0
	G1634/F	0[IT]	0
	G1635/F	0	0
	G1636/F	0	0
	G1637/F	0[IT]	0
	Mean	0.0	0.0

[a]Pooled test article.
Note: See Appendix B for definition of codes.

APPENDIX A

Topical Range-Finding Study

SLI Study No. 3596.21 (20)

1. TOPICAL RANGE-FINDING STUDY

This appendix provides the experimental procedures and results of a topical range-finding study in guinea pigs with Spray--Charlie. The procedures for animal husbandry were similar to those described for the main sensitization study animals. The male animals were approximately 8 weeks of age and weighed 420-473 g; the female animals were approximately 9 weeks of age and weighed 385-420 g on the day prior to dosing.

1.1. Method of Test Article Preparation

The test article was utilized at 100% and at 75%, 50% and 25% w/v in deionized water for the range-finding study. The test article was prepared and dispensed fresh on the day of dosing. The dosing preparations were stirred continuously during dosing.

1.2. Dosing

On the day prior to dose administration, four topical range-finding guinea pigs were weighed and the hair removed from the right and left side of the animals with a small animal clipper. Care was taken to avoid abrading the skin during clipping procedures.

On the following day, four concentrations of the test article were prepared and each concentration was applied to the clipped area of each topical range-finding animal as indicated below:

Group	Material	Concentration (%)	Test Site No.	Amount Applied	Patch Design[a]
Topical Range-Finding	Spray--Charlie	100[b]	1	0.3 mL	25 mm Hilltop Chamber
		75[c]	2	0.3 mL	25 mm Hilltop Chamber
		50[c]	3	0.3 mL	25 mm Hilltop Chamber
		25[c]	4	0.3 mL	25 mm Hilltop Chamber

[a]Occlusive patch.
[b]Pooled test article.
[c]The vehicle used was deionized water.

The chambers were applied to the clipped surface as quickly as possible. The trunk of the animal was wrapped with elastic wrap which was secured with adhesive tape to prevent removal of the chambers and the animal was returned to its cage.

Approximately six hours after chamber application, the binding materials were removed. The test sites were then wiped with gauze moistened in deionized water, followed by dry gauze, to remove test article residue and the animals returned to their cages.

1.3. Dermal Observations

The test sites of the topical range-finding animals were graded for irritation at approximately 24 and 48 hours following chamber application using the Dermal Grading System in Appendix B.

1.4. Clinical Observations

Any unusual observations and mortality were recorded. The topical range-finding animals were observed for general health/mortality twice daily, once in the morning and once in the afternoon.

1.5. Body Weights

Individual body weights were obtained for the topical range-finding animals on the day prior to dosing.

1.6. Scheduled Euthanasia

Following the 48-hour scoring interval, all topical range-finding animals were euthanized by carbon dioxide inhalation. Gross necropsy examinations were not required for these animals.

1.7. Results

The results of the range-finding study indicated that a test article concentration of 100% was considered appropriate for induction and challenge since it was the highest possible concentration which was nonirritating.

A DERMAL SENSITIZATION STUDY IN GUINEA PIGS
TOPICAL RANGE-FINDING DATA
(SPRAY--CHARLIE)

Group	Animal No./Sex Body Weight (g)	Range-Finding Dermal Scores							
		100%[a]		75%[a,b]		50%[a,b]		25%[a,b]	
		24 Hr	48 Hr	24 Hr	48 Hr	24 Hr	48 Hr	24 Hr	48 Hr
Range-Finding	G1471/M 473	0	0	0	0	0	0	0	0
	G1472/M 420	0	0	0	0	0	0	0	0
	G1539/F 420	0	0	0	0	0	0	0	0
	G1540/F 385	0	0	0	0	0[IT]	0	0	0

[a]Pooled test article.
[b]The vehicle used was deionized water.
Note: See Appendix B for definition of codes.

SLI Study No. 3596.21 (23)

APPENDIX B

Dermal Grading System

SLI Study No. 3596.21 (24)

DERMAL GRADING SYSTEM

ERYTHEMA AND EDEMA OBSERVATIONS		
OBSERVATION	DEFINITION	CODE
Erythema – Grade 0	No reaction	0
Erythema – Grade ±	Slight patchy erythema	±
Erythema – Grade 1	Slight, but confluent or moderate patchy erythema	1
Erythema – Grade 2	Moderate, confluent erythema	2
Erythema – Grade 3	Severe erythema with or without edema	3
Maximized Grade 3	Notable dermal lesions	M – 3 (see below)
Edema – Grade 1	Very slight edema (barely perceptible)	ED-1
Edema – Grade 2	Slight edema (edges of area well defined by definite raising)	ED-2
Edema – Grade 3	Moderate edema (raised approximately 1 millimeter)	ED-3
Edema – Grade 4	Severe edema (raised more than 1 millimeter and extends beyond the area of exposure)	ED-4
An erythema code was assigned to each test site. An edema code was assigned only if edema was present at the test site. If notable dermal lesion(s) (> grade 1) were present, then the "Maximized Grade 3" was assigned to the test site in place of the erythema score and the type of the notable dermal lesion(s) was noted (e.g., M-3^{ES-2}).		

SLI Study No. 3596.21 (25)

DERMAL GRADING SYSTEM

NOTABLE DERMAL LESIONS		
OBSERVATION	CODE	DEFINITION
Eschar – Grade 1	ES-1	Focal and/or pinpoint areas up to 10% of test site.
Eschar – Grade 2	ES-2	> 10% < 25% of test site.
Eschar – Grade 3	ES-3	> 25% < 50% of test site.
Eschar – Grade 4	ES-4	> 50% of test site.
Blanching – Grade 1	BLA-1	Focal and/or pinpoint areas up to 10% of test site.
Blanching – Grade 2	BLA-2	> 10% < 25% of test site.
Blanching – Grade 3	BLA-3	> 25% < 50% of test site.
Blanching – Grade 4	BLA-4	> 50% of test site.
Ulceration – Grade 1	U-1	Focal and/or pinpoint areas up to 10% of test site.
Ulceration – Grade 2	U-2	> 10% < 25% of test site.
Ulceration – Grade 3	U-3	> 25% < 50% of test site.
Ulceration – Grade 4	U-4	> 50% of test site.
Necrosis – Grade 1	NEC-1 *(color)*	Focal and/or pinpoint areas up to 10% of test site (note color of necrosis).
Necrosis – Grade 2	NEC-2 *(color)*	> 10% < 25% of test site (Note color of necrosis).
Necrosis – Grade 3	NEC-3 *(color)*	> 25% < 50% of test site (Note color of necrosis).
Necrosis – Grade 4	NEC-4 *(color)*	> 50% of test site (Note color of necrosis).

SLI Study No. 3596.21 (26)

DERMAL GRADING SYSTEM

ADDITIONAL DERMAL FINDINGS		
OBSERVATION	DEFINITION	CODE
Desquamation	Characterized by scaling or flaking of dermal tissue or without denuded areas.	DES
Fissuring	Characterized by cracking of the skin with or without moist exudate. Fissuring should be checked prior to removing the animal from the cage and manipulating the test site.	FIS
Eschar Exfoliation	The process by which areas of eschar flake off the test site.	EXF
Test Site Staining	Skin located at test site appears to be discolored, possibly due to test article (note color of staining).	TSS (*color*)
Erythema Extends Beyond the Test Site	The erythema extends beyond the test site. Note: A study director should be contacted for erythema extending beyond the test site.	ERB
Superficial Lightening	Characterized by pale area(s) (almost a burn-like appearance) in the test site. However, erythema may still be observed through the pale area. Note: This observation may affect the overall erythema score of the test site. This observation may progress to other observations resulting in notable dermal lesions, but SL itself will not be considered a notable dermal lesion that will result in a dermal score to be maximized since it does not result in any in-depth injury. To be coded using an area designation (see below).	--
Superficial Lightening - Grade 1	Focal and/or pinpoint areas up to 10% of the test site	SL-1
Superficial Lightening - Grade 2	> 10% < 25% of test site	SL-2
Superficial Lightening - Grade 3	> 25% < 50% of test site	SL-3
Superficial Lightening - Grade 4	> 50% of test site	SL-4
Dermal Irritation - Outside of the Test Site	Noticeable irritation outside of test site probably due to the binding tape material. This notation will only be made for reactions greater than what are normally observed from tape removal which do not interfere with the scoring of the test site.	IT

SLI Study No. 3596.21 (27)

APPENDIX C

Individual Body Weight Data

		Body Weight (g)	
Group	Animal No./Sex	Day -1	Day 27
Test	G1598/M	412	590
	G1599/M	462	656
	G1600/M	405	611
	G1601/M	409	597
	G1602/M	423	586
	G1603/M	443	627
	G1604/M	464	644
	G1605/M	453	670
	G1606/M	407	575
	G1607/M	394	534
	G1623/F	385	443
	G1624/F	394	536
	G1625/F	387	502
	G1626/F	395	516
	G1627/F	366	508
	G1628/F	376	554
	G1629/F	377	562
	G1630/F	390	573
	G1631/F	369	491
	G1632/F	420	511

		Body Weight (g)	
Group	Animal No./Sex	Day -1	Day 27
Challenge Control	G1608/M	398	588
	G1609/M	428	628
	G1610/M	410	641
	G1611/M	437	611
	G1612/M	413	643
	G1633/F	392	550
	G1634/F	382	525
	G1635/F	378	497
	G1636/F	404	552
	G1637/F	374	466
Rechallenge Control[a]	G1613/M	414	--
	G1614/M	419	--
	G1615/M	402	--
	G1616/M	438	--
	G1617/M	405	--
	G1638/F	390	--
	G1639/F	409	--
	G1640/F	396	--
	G1641/F	407	--
	G1642/F	393	--

[a]A rechallenge control group was maintained on the study; however, the rechallenge procedure was not required since the challenge results were definitive.

APPENDIX D

HCA Historical Control Data

SLI Study No. 3596.21 (31)

SPRINGBORN LABORATORIES, INC.
MODIFIED BUEHLER HISTORICAL CONTROL DATA
USING α-HEXYLCINNAMALDEHYDE
(SLI Study No. 999.176)

1. OBJECTIVE

This study was performed to assess the dermal sensitization potential of α-Hexylcinnamaldehyde (HCA) when administered by multiple topical applications. This study may be used to provide information on the ability of the test system to detect potential contact sensitizers and to update the historical positive control of the testing facility. The protocol was signed by the Study Director on September 6, 2002 (GLP initiation date). The in-life phase of the study was initiated with test article administration on September 17, 2002, and concluded with final scoring on October 17, 2002.

2. TEST ARTICLE

The test article was received from the manufacturer, TCI America, and identified as follows:

Supplier's ID	Assigned SLI ID	Physical Description	Receipt Date	SLI Assigned Expiration Dates
HCA Lot No.: GJ01	S02.004.N	Pale yellow liquid	02/11/02	02/11/04

The bulk compound was stored desiccated, protected from light, at room temperature. The manufacturer provided a Certificate of Analysis for the test article which is presented as Attachment 1 of this Appendix.

The HCA was mixed with ethanol or acetone to produce the appropriate concentrations for dose administration. For the sensitization study, the test article concentrations utilized were 5% w/v in ethanol (induction) and 1% and 2.5% w/v in acetone (challenge).

SLI Study No. 3596.21 (32)

3. EXPERIMENTAL PROCEDURES [1]

Young adult Hartley-derived albino guinea pigs were received on September 12, 2002, from Hilltop Lab Animals, Inc., Scottdale, PA. The guinea pigs were uniquely identified by ear tag, individually housed in suspended stainless steel cages and received Purina Certified Guinea Pig Chow #5026 and water purified by reverse osmosis ad libitum. The animals were acclimated for a minimum of 5 days prior to experimental initiation. The male guinea pigs were approximately 6 weeks of age and weighed 380-437 g; the female guinea pigs were approximately 8 weeks of age and weighed 320-391 g on the day prior to Induction I dosing.

On the day prior to the first induction dose administration (day -1), the hair was removed from the left side of the twenty test animals. On the following day, 0.3 mL of 5% w/v HCA in ethanol was placed on a Hilltop chamber and applied to the clipped area of each animal's back. The trunk of each animal was wrapped with elastic wrap which was secured with adhesive tape to prevent removal of the chamber. Approximately six hours after chamber application, the binding materials were removed. The test sites were wiped with gauze moistened with deionized water, followed by dry gauze, to remove test article residue. The test sites were graded for irritation at approximately 24 and 48 hours following chamber application using the Dermal Grading System. The induction procedure was repeated on study day 8 and on study day 15 so that a total of three induction exposures were made to the animals.

On the day prior to challenge dose administration, the hair was removed from the right side of the twenty test and ten challenge control animals. On the following day (day 28), 0.3 mL of 1% and 2.5% w/v HCA in acetone was placed on a 25 mm Hilltop chamber and applied to the clipped area of each animal's back. Wrapping, unwrapping and rinsing procedures were the same as those utilized for the induction phase. The test sites were graded for irritation at approximately 24 and 48 hours following chamber removal.

Any unusual observations and/or mortality were recorded. Body weights were recorded for the test, challenge control and rechallenge control animals on the day prior to first induction (day -1) and for the test and challenge control animals on the day prior to challenge dosing. All sensitization study animals were euthanized by carbon dioxide inhalation following each animal's final scoring interval. Gross necropsy examinations were not required for these animals.

Note: The animal room temperature range [64-74°F (18-23°C)] exceeded the preferred range [63-73°F (17-23°C)] during this study. This occurrence was considered to have had no adverse effect on the outcome of this study.

4. RESULTS

Individual Data: Tables 1-2

Following challenge with 2.5% w/v HCA in acetone, dermal scores of 1 were noted in 5/20 test animals at the 24-hour scoring interval and 4/20 test animals at the 48-hour scoring interval. Dermal reactions in the remaining test and challenge control animals were limited to scores of 0 to ±. Group mean dermal scores were noted to be higher in the test animals as compared with the challenge control animals.

Following challenge with 1% w/v HCA in acetone, dermal scores of 1 were noted in 1/20 test animals at the 24-hour scoring interval. Dermal reactions in the remaining test and challenge control animals were limited to scores of 0 to ±. Group mean dermal scores were noted to be higher in the test animals as compared with the challenge control animals.

5. CONCLUSION

The results of this α-Hexylcinnamaldehyde positive control study demonstrated that a valid test was performed and indicated that the test design would detect potential contact sensitizers. Based on the results of this study, α-Hexylcinnamaldehyde is considered to be a contact sensitizer in guinea pigs.

6. REFERENCE

1. E.V. Buehler, Occlusive Patch Method for Skin Sensitization in Guinea Pigs: The Buehler Method, Fd. Chem. Toxic., Vol. 32, No. 2, pp. 97-101, 1994.

SLI HISTORICAL CONTROL
STUDY NO.: 999.176

TABLE 1
A DERMAL SENSITIZATION STUDY IN GUINEA PIGS
INDIVIDUAL INDUCTION DATA
(α-HEXYLCINNAMALDEHYDE)

Group	Animal No./ Sex	Induction 1 Dermal Scores 5%[a] 24 Hr	Induction 1 Dermal Scores 5%[a] 48 Hr	Induction 2 Dermal Scores 5%[a] 24 Hr	Induction 2 Dermal Scores 5%[a] 48 Hr	Induction 3 Dermal Scores 5%[a] 24 Hr	Induction 3 Dermal Scores 5%[a] 48 Hr
Test	G0168/M	±	±	M-3$^{BLA-2, ED-2, ES-2}$	M-3$^{ES-2, BLA-2, ED-1}$	1$^{BLA-1, ED-1}$	±$^{BLA-1}$
	G0169/M	±	±	±	±	1$^{BLA-1, ED-2}$	±$^{BLA-1}$
	G0170/M	1$^{BLA-1, ED-1b}$	±$^{BLA-1b}$	±$^{BLA-1}$	±$^{BLA-1}$	±	0
	G0171/M	±$^{BLA-1b}$	±$^{BLA-1b}$	1^{ED-1}	±	1^{ED-1}	±
	G0172/M	1$^{BLA-1, ED-1b}$	±$^{BLA-1, ED-1b}$	1$^{ED-1, ES-1}$	M-3$^{ES-2, ED-1}$	1$^{BLA-1, ED-2}$	1$^{BLA-1, ED-1}$
	G0173/M	±	±	M-3$^{BLA-1, NEC-2(BN),ED-1}$	M-3 $^{ES-2, NEC-1 (BN), ED-2}$	2$^{BLA-1, ED-2}$	1$^{BLA-1, ED-2}$
	G0174/M	±^{IT}	0	1$^{BLA-1, ED-1}$	1$^{BLA-1, ED-1}$	1^{ED-1}	±
	G0175/M	±	±	M-3$^{BLA-2, NEC-1(BN), ED-1}$	M-3$^{BLA-2, ES-1, NEC-1 (BN), ED-1}$	2^{ED-2}	1
	G0176/M	±	±	M-3$^{BLA-1, ES-3, ED-1}$	M-3$^{ES-3, NEC-1(BN), ED-1}$	M-3$^{BLA-2, NEC-1(BN), ED-2}$	M-3$^{BLA-2, NEC-(BN), ED-2}$
	G0177/M	±	±	1$^{BLA-1, ED-1}$	1$^{BLA-1, ED-1}$	2$^{BLA-1, SL-3, ED-1}$	±$^{ED-1}$
	G0137/F	±$^{BLA-1, ED-1b}$	±$^{BLA-1, ED-1b}$	M-3$^{BLA-1, NEC-2(BN), ED-2}$	M-3$^{ES-2, NEC-1(BN), ED-2}$	2$^{BLA-1, ED-2, SL-4}$	2$^{BLA-1, ED-1}$
	G0143/F	±$^{BLA-1b}$	±$^{BLA-1,b}$	M-3$^{BLA-1, NEC-2(BN), ED-2}$	M-3$^{BLA-1, NEC-2(BN), ED-2}$	2$^{SL-4, ED-1}$	1^{ED-1}
	G0140/F	±^{IT}	±	M-3$^{BLA-2, ED-2}$	M-3$^{BLA-2, NEC-1(BN), ED-1}$	2^{ED-2}	±
	G0146/F	±^{IT}	±	1$^{ED-1, IT}$	1 $^{ED-1}$	1^{ED-1}	1^{ED-1}
	G0147/F	±^{IT}	0	1$^{BLA-1, ED-2}$	1$^{BLA-1, ED-1}$	2^{ED-1}	1^{ED-1}
	G0154/F	1$^{BLA-1, ED-1,IT}$	±$^{BLA-1b}$	M-3$^{BLA-1, ED-1, ES-2}$	M-3$^{BLA-1,ES-2, ED-1}$	1^{ED-1}	1^{ED-1}
	G0161/F	±	±	M-3$^{BLA-2, ED-2}$	M-3$^{BLA-1, NEC-2(BN), ED-1}$	1^{ED-1}	1
	G0157/F	0	0	M-3$^{BLA-2, ED-2}$	M-3$^{BLA-2, ED-1}$	1^{ED-1}	±
	G0159/F	±	±	M-3$^{BLA-2, NEC-2(BN), ED-1}$	M-3$^{BLA-1, ES-1, NEC-2(BN), ED-1}$	2$^{SL-4, ED-1}$	1^{ED-1}
	G0220/F	±	±	1$^{BLA-1, ED-1}$	1$^{BLA-1, ED-1}$	2^{ED-1}	1^{ED-1}

[a]The vehicle was ethanol.
[b]The score of BLA-1 was associated with the rim of the Hilltop chambers.
Note: See Appendix B for definition of codes.

SLI HISTORICAL CONTROL
STUDY NO.: 999.176

TABLE 2
A DERMAL SENSITIZATION STUDY IN GUINEA PIGS
INDIVIDUAL CHALLENGE DATA
(α-HEXYLCINNAMALDEHYDE)

Group	Animal No./ Sex	Dermal Scores 2.5%[a] 24 Hr	2.5%[a] 48 Hr	1%[a] 24 Hr	1%[a] 48 Hr
Test	G0168/M	0	0	0	0
	G0169/M	0	0	0	0
	G0170/M	0	±	±	±
	G0171/M	0	0	0	0
	G0172/M	1	1	±	±
	G0173/M	1	1	1	±
	G0174/M	0	0	0	0
	G0175/M	1	±	±	±
	G0176/M	±	±	0	0
	G0177/M	±	0	±	0
	G0137/F	1	1	±	±
	G0143/F	±[IT]	±	±	±
	G0140/F	±	±	±	±
	G0146/F	0	0	±[IT]	0
	G0147/F	±	0	0	0
	G0154/F	±	±	±[IT]	±
	G0161/F	±	±	±	±
	G0157/F	0[IT]	0	0[IT]	0
	G0159/F	1	1	±	±
	G0220/F	±	±	±	0
	Mean	0.5	0.4	0.4	0.3

[a]The vehicle was acetone.
Notes: For the purpose of calculation, ± = 0.5. See Appendix B for definition of codes.

TABLE 2
A DERMAL SENSITIZATION STUDY IN GUINEA PIGS
INDIVIDUAL CHALLENGE DATA
(α-HEXYLCINNAMALDEHYDE)

SLI HISTORICAL CONTROL
STUDY NO.: 999.176

Group	Animal No./ Sex	Dermal Scores			
		2.5%[a]		1%[a]	
		24 Hr	48 Hr	24 Hr	48 Hr
Challenge	G0178/M	0	0	0[IT]	0
Control	G0179/M	0	0	0	0
	G0180/M	0	0	0	0
	G0181/M	0	0	0	0
	G0182/M	0	0	0[IT]	0
	G0221/F	0	0	0	0
	G0222/F	0	0	0	0
	G0223/F	0[IT]	0	0	0
	G0224/F	0	0	0	0
	G0225/F	0[IT]	0	0	0
	Mean	0.0	0.0	0.0	0.0

[a]The vehicle was acetone.
Note: See Appendix B for definition of codes.

SLI Study No. 3596.21 (37)

ATTACHMENT 1

Certificate of Analysis
(Provided by the Manufacturer)

SLI Study No. 3596.21

(38)

TCI AMERICA

CERTIFICATE OF ANALYSIS

H0685
Lot# GJ01
CAS# 101-86-0

ALPHA-N-HEXYLCINNAMALDEHYDE

Appearance:	Yellow clear liquid
SG(20/20):	0.958
n(20/D):	1.550
Assay(GC):	93.6%

9211N. Harborgate St. Portland, OR 97203 Phone: (503)283-1681 (800)423-8616 Fax: (503)283-1987

APPENDIX E

SLI Personnel Responsibilities

SLI Study No. 3596.21 (40)

SLI PERSONNEL RESPONSIBILITIES

Kimberly L. Bonnette, M.S., LATG	Study Director/Director, Acute Toxicology
Dawn D. Rodabaugh, B.S.	Alternate Contact/Toxicologist
Malcolm Blair, Ph.D.	Managing Director Emeritus
Joseph C. Siglin, Ph.D., DABT	General Manager
Jason W. Smedley, B.S.	Associate Toxicologist
Pamela S. Smith, ALAT	Study Supervisor, Acute Toxicology
Lyndsay K. Simindinger, A.S.	Primary Technician/Acute Technician II
Delores P. Knippen	Supervisor, Pharmacy
Steven H. Magness, B.S., LATG	Senior Supervisor, Pathology
Anita M. Bosau, RQAP-GLP	Senior Director, Compliance Assurance
Cheryl Bellamy	Senior Supervisor, Report Writing
Deanna M. Talerico, RQAP-GLP	Senior Supervisor, Quality Assurance
J. Dale Thurman, D.V.M., M.S., DACVP	Senior Director, Pathology
Kathy M. Gasser	Archivist

A PRIMARY EYE IRRITATION STUDY IN RABBITS WITH SPRAY--CHARLIE

FINAL REPORT

OPPTS Guideline

870.2400

Author

Kimberly L. Bonnette, M.S., LATG

Study Completed on

February 17, 2003

Performing Laboratory

Springborn Laboratories (SLI),
a division of Charles River Laboratories, Inc.
640 North Elizabeth Street
Spencerville, Ohio 45887

SLI Study No.

3596.19

Submitted to

INL/A
U.S. Department of State
2201 C St. NW SA-4
Washington, DC 20520

SLI Study No. 3596.19 (2)

1. STATEMENT OF *NO* DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B), or (C).

Company: _____

Company Agent: _____ Date: _____

_____ _____

Title Signature

2. COMPLIANCE STATEMENT

This study was conducted in compliance with the Good Laboratory Practice Standards as described by the EPA (40 CFR Parts 160 and 792).

Kimberly L. Bonnette, M.S., LATG
Study Director/Author
Springborn Laboratories, Inc.

Date 2/17/03

Rogers Woolfolk
Senior Aviation Advisor
Sponsor/Submitter
INL/A
U.S. Department of State

Date 6 Feb 03

SLI Study No. 3596.19 (4)

3. QUALITY ASSURANCE STATEMENT

This study was inspected by the Quality Assurance Unit and reports were submitted to management and the Study Director in accordance with SLI's Standard Operating Procedures as follows:

Phase	Date
Protocol Review	10/07/02
Animal Receipt	11/11/02
Ocular Observations	12/23/02
Data Audit	01/22/03
Draft Report Review	01/22/03
Final Report Review	02/17/03
Reports to Study Director and Management	11/11/02, 01/22/03, 02/17/03

The final report has been reviewed to assure that it accurately describes the materials and methods, and the reported results accurately reflect the raw data.

Jennifer D. McGue Date 2/17/03
Jennifer D. McGue
Quality Assurance Auditor

Rebecca A. Young Date 2/17/03
for Anita M. Bosau, RQAP-GLP
Senior Director, Compliance Assurance

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SLI Study No. 3596.19 (7)

6. SUMMARY

The potential irritant and/or corrosive effects of Spray--Charlie were evaluated on the eyes of New Zealand White rabbits. Each of three rabbits received a 0.1 mL dose of the test article in the conjunctival sac of the right eye. The contralateral eye of each animal remained untreated and served as a control. Test and control eyes were examined for signs of irritation for up to seven days following dosing.

Exposure to the test article produced iritis in 3/3 test eyes at the 1-hour scoring interval which resolved completely in all test eyes by the 24-hour scoring interval. Conjunctivitis (redness, swelling and discharge) was noted in 3/3 test eyes at the 1-hour scoring interval. The conjunctival irritation resolved completely in all test eyes by study day 7. An additional ocular finding of slight dulling of normal luster of the cornea was noted in 1/3 test eyes.

Based on the Kay and Calandra, Spray--Charlie is considered to be a moderate irritant to the ocular tissue of the rabbit.

SLI Study No. 3596.19 (8)

7. INTRODUCTION

This study was performed to assess the irritant and/or corrosive effects of Spray--Charlie in New Zealand White rabbits when administered by a single ocular dose. This study was intended to provide information on the potential health hazards of the test article with respect to ocular exposure. Data from this study may serve as a basis for classification and/or labeling of the test article. This study was conducted in accordance with the US EPA, Health Effects Test Guidelines, OPPTS 870.2400, Acute Eye Irritation, August 1998. This study was performed at Springborn Laboratories (SLI), a division of Charles River Laboratories, Inc., 553 North Broadway, Spencerville, Ohio. The protocol was signed by the Study Director on October 9, 2002 (GLP initiation date). The in-life phase of the study was initiated with test article administration on December 23, 2002 (day 0), and concluded with final scoring on December 30, 2002.

8. MATERIALS AND METHODS

8.1. Test Article

The test article was received from the Sponsor and identified as follows:

Sponsor's ID	Assigned SLI ID	Physical Description	Receipt Date	Expiration Date
Spray--Charlie[a]	S02.003.3596	Amber liquid	12/09/02	None provided
Ingredients:[b]				
Herbicide: GLY-41 Lot No.: Manufactured 10/20/02				None provided
Surfactant: Cosmo Flux-411F Lot No.: Manufactured 11/29/02				None provided

[a]Sample pooled at SLI from five different mixes of Spray--Charlie (top/middle/bottom).
[b]Ingredients used in the five Spray--Charlie mixes that were prepared by the Sponsor.

The test article was stored at room temperature. The Sponsor was responsible for any necessary evaluations related to the identity, strength, purity, composition, stability and method of synthesis of the test material according to 40 CFR 160.105 and 40 CFR 792.105. Springborn Laboratories, Inc. analyzed the test article for the glyphosate (a.e.) which is presented in SLI Study No. 3596.15.

SLI Study No. 3596.19 (9)

8.2. Retention Sample

An approximate 1 mL retention sample of each test article mixture sample (top/middle/bottom, maintained separately for a total of fifteen 1 mL samples) was taken and stored at SLI at room temperature. In addition, a 10 mL retention sample of the pooled test article samples (from the 5 test article mixtures) was collected and stored at SLI at room temperature. These samples serve as the retention samples for all studies conducted with this material.

8.3. Test Article Disposition

The remaining test article was returned to the Sponsor at the completion of all studies with the test article.

8.4. Method of Test Article Preparation

The test articles were pooled and administered as received from the Sponsor and dispensed fresh on the day of dosing. The test articles were stirred continuously during dosing.

8.5. Animals and Animal Husbandry

8.5.1. Description, Identification and Housing

Adult, New Zealand White rabbits were received from Myrtle's Rabbitry, Thompson Station, TN. Upon receipt, plastic ear tags displaying unique identification numbers were used to individually identify the animals. Cage cards displaying at least the study number, animal number and sex were affixed to each cage. The animals were housed individually in suspended stainless steel cages. All housing and care were based on the standards recommended by the Guide for the Care and Use of Laboratory Animals [1].

8.5.2. Environment

The animal room temperature and relative humidity ranges were 71-75°F (22-24°C) and 42-50%, respectively. Environmental control equipment was monitored and adjusted as necessary to minimize fluctuations in the animal room environment. Light timers were set to maintain a 12-hour light/12-hour dark cycle and room ventilation was set to produce 10-15 air changes/hour. The animal room temperature and relative humidity were recorded a minimum of once daily.

8.5.3. Food

PMI Certified Rabbit Chow #5322 (Purina Mills, Inc.) was provided ad libitum to the animals throughout the study. The lot number and expiration date of each

batch of diet used during the study were recorded. The feed was analyzed and certified by the supplier for nutritional components and environmental contaminants. Dietary limitations for various environmental contaminants, including heavy metals, pesticides, polychlorinated biphenyls and total aflatoxin are set by the manufacturer. Within these limits, contaminants which may have been present were not expected to compromise the purpose of this study. Results of the dietary analyses (Certificates of Analysis) are provided by the manufacturer for each lot of diet. These are maintained by SLI.

8.5.4. Water

Municipal tap water treated by reverse osmosis was available ad libitum throughout the study. The purified water was supplied by an automatic watering system. Monitoring of the drinking water for contaminants is conducted by SLI and the records are available for inspection. Within generally accepted limits, contaminants which may have been present were not expected to compromise the purpose of this study. The water meets the standards specified under the EPA National Drinking Water Regulations (40 CFR Part 141).

8.5.5. Acclimation

Upon receipt, the animals were removed randomly from the shipping cartons, examined by qualified personnel, identified with plastic ear tags and then acclimated to the laboratory conditions for a minimum of five days. The animals were observed daily for overt physical or behavioral abnormalities, general health/moribundity and mortality.

8.5.6. Animal Selection

The animals chosen for study use were arbitrarily selected from healthy stock animals to avoid potential bias. All animals received a detailed pretest observation prior to dosing. Only healthy animals were chosen for study use. The male animals were approximately 16 weeks of age and weighed 3.2-3.6 kg prior to dosing.

9. EXPERIMENTAL PROCEDURES

9.1. Preliminary Examination

On day 0 prior to dosing, both eyes of each animal provisionally selected for test use were examined macroscopically for ocular irritation with the aid of an auxiliary light source. In addition, the corneal surface was examined using fluorescein sodium dye. One drop of a fluorescein/physiological saline mixture was gently dropped onto the superior sclera of each eye. Following an

approximate 15 second exposure, the eyes were thoroughly rinsed with physiological saline. The corneal surface was then examined for dye retention under a long-wave UV light source. Animals exhibiting ocular irritation, preexisting corneal injury or fluorescein dye retention were not used on study. All animals found to be acceptable for test use were returned to their cages until dosing.

9.2. Dosing

A minimum of one hour after preliminary ocular examination, the test article was instilled as follows:

	Concentration		No. of Animals
Group	(%)	Amount Instilled	Male
No Rinse	100[a]	0.1 mL	3

[a]Pooled test article.

The test article was instilled into the conjunctival sac of the right eye of each animal after gently pulling the lower lid away from the eye. Following instillation, the eyelids were gently held together for approximately one second in order to limit test article loss and the animal was returned to its cage. The contralateral eye remained untreated to serve as a control.

9.3. Ocular Observations

The eyes were macroscopically examined with the aid of an auxiliary light source for signs of irritation at 1, 24, 48 and 72 hours and up to 7 days after dosing according to the Ocular Grading System presented in Appendix A which is based on Draize [2]. Following macroscopic observations at the 24-hour scoring interval, the fluorescein examination procedure was repeated on all test and control eyes and any residual test article was gently rinsed from the eye at this time (if possible) using physiological saline. If any fluorescein findings were noted at 24 hours, a fluorescein exam was conducted on the affected eyes at each subsequent interval until a negative response was obtained and/or until all corneal opacity had cleared, or as directed by the Study Director.

9.4. Clinical Observations

Any unusual observations and/or mortality were recorded. General health/mortality checks were performed twice daily (in the morning and in the afternoon).

9.5. Body Weights

Individual body weights were obtained for each animal prior to dosing on day 0.

9.6. Scheduled Euthanasia

Each animal was euthanized by an intravenous injection of sodium pentobarbital following its final observation interval. Gross necropsy examinations were not required for these animals.

9.7. Protocol Deviations

On two occasions, the temperature of the animal room [71-75°F (22-24°C), respectively] exceeded the preferred range [63-73°F (17-23°C), respectively] during this study. These occurrences are considered to have had no adverse effect on the outcome of this study.

10. ANALYSIS OF DATA

For each group, the ocular irritation score for each parameter (i.e., corneal opacity x area, iritis and conjunctival redness + swelling + discharge) was multiplied by the appropriate factor (i.e., corneal injury x 5, iritis x 5, conjunctivitis x 2) and the totals added for each animal/interval. The group mean irritation score was then calculated for each scoring interval based on the number of animals initially dosed in each group. The calculated group mean ocular irritation scores for each interval were used to classify the test article according to the Ocular Evaluation Criteria [3] presented in Appendix B.

11. MAINTENANCE OF RAW DATA AND RECORDS

All original raw data, the final report and magnetically encoded records were transferred to the SLI archives for a period of 10 years. The Sponsor will be contacted prior to final disposition of these items.

12. RESULTS

12.1. Ocular Observations

Individual Data: Table 1

Exposure to the test article produced iritis in 3/3 test eyes at the 1-hour scoring interval which resolved completely in all test eyes by the 24-hour scoring interval. Conjunctivitis (redness, swelling and discharge) was noted in 3/3 test eyes at the 1-hour scoring interval. The conjunctival irritation resolved completely in all test eyes by study day 7. An additional ocular finding of slight dulling of normal luster of the cornea was noted in 1/3 test eyes.

No corneal opacity, iritis or conjunctivitis was observed in the control eyes.

13. CONCLUSION

Based on the Kay and Calandra, Spray--Charlie is considered to be a moderate irritant to the ocular tissue of the rabbit.

Kimberly L. Bonnette, M.S., LATG
Study Director

Date 2/17/03

14. REPORT REVIEW

Dawn D. Rodabaugh, B.S.
Toxicologist

Date 2/17/03

15. REFERENCES

1. Guide for the Care and Use of Laboratory Animals, DHHS Publication No. (NIH) 96-03, 1996.

2. Draize, J.H., Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics, The Association of Food and Drug Officials of the United States, 49-51, 1959.

3. Kay, J.H. and Calandra, J.C., “Interpretation of Eye Irritation Tests”, Journal of the Society of Cosmetic Chemists, 13, 281-289, 1962.

TABLE 1

STUDY NO.: 3596.19 — A PRIMARY EYE IRRITATION STUDY IN RABBITS — PAGE 1

INL/A, US DEPARTMENT OF STATE — INDIVIDUAL OCULAR IRRITATION SCORES

(NO RINSE GROUP)

Animal No./Sex Body Weight (kg)	Scoring Interval	Cornea			Iris		Conjunctivae				Total	Test Eye*		Control Eye*	
		O	A	OxAx5	I	Ix5	R	S	D	(R+S+D)2		Fluorescein Examination	Secondary Ocular Findings	Fluorescein Examination	Secondary Ocular Findings
R3365/M	1 Hour	0	0	0	1	5	2	2	1	10	15				
3.172	24 Hours	0	0	0	0	0	2	2	2	12	12	[-]		[-]	
	48 Hours	0	0	0	0	0	2	1	0	6	6				
	72 Hours	0	0	0	0	0	1	1	0	4	4				
	7 Days	0	0	0	0	0	0	0	0	0	0				
R3366/M	1 Hour	0	0	0	1	5	2	2	1	10	15		SDL		
3.246	24 Hours	0	0	0	0	0	2	2	0	8	8	[-]		[-]	
	48 Hours	0	0	0	0	0	2	1	0	6	6				
	72 Hours	0	0	0	0	0	2	1	0	6	6				
	7 Days	0	0	0	0	0	0	0	0	0	0				
R3380/M	1 Hour	0	0	0	1	5	1	2	2	10	15				
3.607	24 Hours	0	0	0	0	0	2	2	1	10	10	[-]		[-]	
	48 Hours	0	0	0	0	0	2	1	0	6	6				
	72 Hours	0	0	0	0	0	1	1	0	4	4				
	7 Days	0	0	0	0	0	0	0	0	0	0				

*See Appendix A for definition of codes.

Mean Ocular Scores

1 Hour	-	15.00
24 Hours	-	10.00
48 Hours	-	6.00
72 Hours	-	4.67
7 Days	-	0.00

Moderate Irritant

APPENDIX A

Ocular Grading System

SLI Study No. 3596.19 (17)

OCULAR GRADING SYSTEM

(O) CORNEAL OPACITY—DEGREE OF DENSITY (AREA MOST DENSE TAKEN FOR READING)	
OBSERVATION	CODE
No ulceration or opacity	0
Scattered or diffuse areas of opacity (other than slight dulling of normal luster), details of iris clearly visible	1*
Easily discernible translucent area, details of iris slightly obscured	2*
Nacreous (opalescent) area, no details of iris visible, size of pupil barely discernible	3*
Opaque cornea, iris not discernible through opacity	4*

(A) AREA OF CORNEA INVOLVED (TOTAL AREA EXHIBITING ANY OPACITY, REGARDLESS OF DEGREE)	
OBSERVATION	CODE
No ulceration or opacity	0
One quarter (or less) but not zero	1
Greater than one quarter, but less than half	2
Greater than half, but less than three quarters	3
Greater than three quarters, up to whole area	4

Cornea Score = O x A x 5 Total Maximum = 80

(I) IRITIS	
OBSERVATION	CODE
Normal	0
Markedly deepened rugae (folds above normal), congestion, swelling, moderate circumcorneal hyperemia or injection, any or all of these or combination of any thereof, iris is still reacting to light (sluggish reaction is positive)	1*
No reaction to light, hemorrhage, gross destruction (any or all of these)	2*

Iris Score = I x 5 Total Maximum = 10

*Starred figures indicate positive effect.

SLI Study No. 3596.19 (18)

OCULAR GRADING SYSTEM

(R) CONJUNCTIVAL REDNESS (REFERS TO PALPEBRAL AND BULBAR CONJUNCTIVAE EXCLUDING CORNEA AND IRIS)	
OBSERVATION	CODE
Blood vessels normal	0
Some blood vessels definitely hyperemic (injected) above normal (slight erythema)	1
Diffuse, crimson color, individual vessels not easily discernible (moderate erythema)	2*
Diffuse beefy red (marked erythema)	3*

(S) CONJUNCTIVAL SWELLING (LIDS AND/OR NICTITATING MEMBRANE)	
OBSERVATION	CODE
No swelling	0
Any swelling above normal (includes nictitating membrane, slightly swollen)	1
Obvious swelling with partial eversion of lids	2*
Swelling with lids about half closed	3*
Swelling with lids more than half closed	4*

(D) CONJUNCTIVAL DISCHARGE	
OBSERVATION	CODE
No discharge	0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)	1
Discharge with moistening of the lids and hairs just adjacent to lids	2
Discharge with moistening of the lids and hairs and considerable area around the eye	3

Conjunctival Score = (R + S + D) x 2 Total Maximum = 20

*Starred figures indicate positive effect.

SLI Study No. 3596.19 (19)

OCULAR GRADING SYSTEM

CORNEAL NEOVASCULARIZATION		
OBSERVATION	CODE	DEFINITION
Neovascularization – Very Slight	VAS-1	Total area of vascularized corneal tissue is < 10% of corneal surface
Neovascularization – Mild	VAS-2	Total area of vascularized corneal tissue is > 10% but < 25% of corneal surface
Neovascularization – Moderate	VAS-3	Total area of vascularized corneal tissue is > 25% but < 50% of corneal surface
Neovascularization – Severe	VAS-4	Total area of vascularized corneal tissue is > 50% of corneal surface

SECONDARY OCULAR FINDINGS		
OBSERVATION	CODE	DEFINITION
Sloughing of the corneal epithelium	SCE	Corneal epithelial tissue is observed to be peeling off the corneal surface.
Corneal bulging	CB	The entire corneal surface appears to be protruding outward further than normal.
Slight dulling of normal luster of the cornea	SDL	The normal shiny surface of the cornea has a slightly dulled appearance.
Raised area on the corneal surface	RAC	A defined area on the corneal surface that is raised above the rest of the cornea. This area is generally associated with neovascularization and has an off-white to yellow color.
Corneal edema	CE	The cornea has a swollen appearance.
Test article present in eye	TAE	Apparent residual test article is observed on the eye or in the conjunctival sac/inner canthus.
Observation confirmed by slit lamp	OCS	A slit lamp examination was performed to confirm the initial observation.
Corneal mineralization	CM	Small white or off-white crystals that are observed in the corneal tissue.

SLI Study No. 3596.19 (20)

OCULAR GRADING SYSTEM

FLUORESCEIN EXAMINATION OF CORNEA	
OBSERVATION	CODE
Fluorescein Dye Retention Fluorescein dye retention associated with the area of corneal opacity Fluorescein dye retention is not associated with any other finding	 FAO FNF
Negative Results No fluorescein retention is observed	 (-)
Secondary Ocular Findings Superficial mechanical abrasion to the cornea observed during the fluorescein examination period Fine stippling on the cornea observed during the fluorescein examination procedure	 MI ST

POST-DOSE CLINICAL OBSERVATIONS	
OBSERVATION	CODE
Animal vocalized following dosing	VOC
Animal excessively pawed test eye following dosing	PAW
Animal exhibited excessive hyperactivity following dosing	HYP
Animal exhibited excessive head tilt following dosing	HT
Animal exhibited excessive squinting of test eye following dosing	SQ

APPENDIX B

Ocular Evaluation Criteria

SLI Study No. 3596.19 (22)

OCULAR EVALUATION CRITERIA

Maximum Mean Score (Days 0-3)	Maximum Mean Score	Persistence of Individual Scores	Descriptive Rating and Class	
0.00 – 0.49	24 hours = 0		Non-Irritating	1
	24 hours > 0		Practically Non-irritating	2
0.50 – 2.49	24 hours = 0		Non-Irritating	1
	24 hours > 0		Practically Non-irritating	2
2.50 – 14.99	48 hours = 0		Slight Irritant	3
	48 hours > 0		Mild Irritant	4
15.00 – 24.99	72 hours = 0		Mild Irritant	4
	72 hours > 0		Moderate Irritant	5
25.00 – 49.99	7 day ≤ 20	> half of day 7 scores ≤ 10	Moderate Irritant	5
		> half of day 7 scores > 10, but no score > 20	Moderate Irritant	5
		> half of day 7 scores > 10, and any score > 20	Severe Irritant	6
	7 day > 20		Severe Irritant	6
50.00 – 79.99	7 day ≤ 40	> half of day 7 scores ≤ 30	Severe Irritant	6
		> half of day 7 scores > 30, but no score > 60	Severe Irritant	6
		> half of day 7 scores > 30, and any score > 60	Very Severe Irritant	7
	7 day > 40		Very Severe Irritant	7
80.00 – 99.99	7 day ≤ 80	> half of day 7 scores ≤ 60	Very Severe Irritant	7
		> half of day 7 scores > 60, but no score > 100	Very Severe Irritant	7
		> half of day 7 scores > 60, and any score > 100	Extremely Severe Irritant	8
	7 day > 80		Extremely Severe Irritant	8
100.00 – 110.00	7 day ≤ 80		Very Severe Irritant	7
	7 day > 80		Extremely Severe Irritant	8

APPENDIX C

SLI Personnel Responsibilities

SLI Study No. 3596.19 (24)

SLI PERSONNEL RESPONSIBILITIES

Kimberly L. Bonnette, M.S., LATG	Study Director/Director, Acute Toxicology
Dawn D. Rodabaugh, B.S.	Alternate Contact/Toxicologist
Malcolm Blair, Ph.D.	Managing Director Emeritus
Joseph C. Siglin, Ph.D., DABT	General Manager
Jason W. Smedely, B.S.	Assistant Toxicologist
Pamela S. Smith, ALAT	Study Supervisor, Acute Toxicology
Lyndsay K. Simindinger, A.S.	Primary Technician/Acute Technician II
Delores P. Knippen	Supervisor, Pharmacy
Steven H. Magness, B.S., LATG	Senior Supervisor, Pathology
Anita M. Bosau, RQAP-GLP	Senior Director, Compliance Assurance
Cheryl Bellamy	Senior Supervisor, Report Writing
Deanna M. Talerico, RQAP-GLP	Senior Supervisor, Quality Assurance
J. Dale Thurman, D.V.M., M.S., DACVP	Senior Director, Pathology
Kathy M. Gasser	Archivist

A PRIMARY SKIN IRRITATION STUDY IN RABBITS WITH SPRAY--CHARLIE

FINAL REPORT

OPPTS Guideline

870.2500

Author

Kimberly L. Bonnette, M.S., LATG

Study Completed on

February 17, 2003

Performing Laboratory

Springborn Laboratories (SLI),
a division of Charles River Laboratories, Inc.
640 North Elizabeth Street
Spencerville, Ohio 45887

SLI Study No.

3596.20

Submitted to

INL/A
U.S. Department of State
2201 C St. NW SA-4
Washington, DC 20520

SLI Study No. 3596.20 (2)

1. STATEMENT OF *NO* DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B), or (C).

Company: _____

Company Agent: _____ Date _____

_____ _____

Title Signature

2. COMPLIANCE STATEMENT

This study was conducted in compliance with the Good Laboratory Practice Standards as described by the EPA (40 CFR Parts 160 and 792).

Kimberly L. Bonnette, M.S., LATG
Study Director/Author
Springborn Laboratories, Inc.

Date 2/17/03

Rogers Woolfolk
Senior Aviation Advisor
Sponsor/Submitter
INL/A
U.S. Department of State

Date 7 Feb 03

3. QUALITY ASSURANCE STATEMENT

This study was inspected by the Quality Assurance Unit and reports were submitted to management and the Study Director in accordance with SLI's Standard Operating Procedures as follows:

Phase	Date
Protocol Review	10/07/02
Animal Receipt	12/02/02
Dose Preparation	12/20/02
Data Audit	01/21/03
Draft Report Review	01/21/03
Final Report Review	02/17/03
Reports to Study Director and Management	12/02/02, 01/21/03, 02/17/03

The final report has been reviewed to assure that it accurately describes the materials and methods, and the reported results accurately reflect the raw data.

Rebecca A. Young
Rebecca A. Young
Quality Assurance Team Leader

Date 2/17/03

for Jennifer D. McGee
Anita M. Bosau, RQAP-GLP
Senior Director, Compliance Assurance

Date 2/17/03

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6. SUMMARY

The potential irritant and/or corrosive effects of Spray--Charlie were evaluated on the skin of New Zealand White rabbits. Each of three rabbits received a 0.5 mL dose of the test article as a single dermal application. The dose was held in contact with the skin under a semi-occlusive binder for an exposure period of four hours. Following the exposure period, the binder was removed and the remaining test article was wiped from the skin using gauze moistened with deionized water followed by dry gauze. Test sites were subsequently examined and scored for dermal irritation for up to 72 hours following patch application.

Exposure to the test article produced very slight erythema on 3/3 test sites at the 1-hour scoring interval. The dermal irritation resolved completely on all test sites by the 24-hour scoring interval.

Under the conditions of the test, Spray--Charlie is considered to be a slight irritant to the skin of the rabbit. The calculated Primary Irritation Index for the test article was 0.25.

SLI Study No. 3596.20 (8)

7. INTRODUCTION

This study was performed to assess the potential irritant and/or corrosive effects of Spray--Charlie in New Zealand White rabbits when administered by a single dermal dose. This study was intended to provide information on the potential health hazards of the test article with respect to dermal exposure. Data from this study may serve as a basis for classification and/or labeling of the test article. This study was conducted in accordance with the US EPA, Health Effects Test Guidelines, OPPTS 870.2500, Acute Dermal Irritation, August 1998. This study was performed at Springborn Laboratories (SLI), a division of Charles River Laboratories, Inc., 553 North Broadway, Spencerville, Ohio. The protocol was signed by the Study Director on October 9, 2002 (GLP initiation date). The in-life phase of the study was initiated with test article administration on December 20, 2003 (day 0) and concluded with final scoring on December 23, 2002.

8. MATERIALS AND METHODS

8.1. Test Article

The test article was received from the Sponsor and identified as follows:

Sponsor's ID	Assigned SLI ID	Physical Description	Receipt Date	Expiration Date
Spray--Charlie[a]	S02.003.3596	Amber liquid	12/09/02	None provided
Ingredients:[b]				
Herbicide: GLY-41 Lot No.: Manufactured 10/20/02				None provided
Surfactant: Cosmo Flux-411F Lot No.: Manufactured 11/29/02				None provided

[a]Sample pooled at SLI from five different mixes of Spray--Charlie (top/middle/bottom).
[b]Ingredients used in the five Spray--Charlie mixes that were prepared by the Sponsor.

The test article was stored at room temperature. The Sponsor was responsible for any necessary evaluations related to identity, strength, purity, composition, stability and method of synthesis of the test material according to 40 CFR 160.105 and 40 CFR 792.105. Springborn Laboratories, Inc. analyzed the test article for the glyphosate (a.e.) which is presented in SLI Study No. 3596.15.

8.2. Retention Sample

An approximate 1 mL retention sample of each test article mixture sample (top/middle/bottom, maintained separately for a total of fifteen 1 mL samples) was taken and stored at SLI at room temperature. In addition, a 10 mL retention sample of the pooled test article sample (from the 5 test article mixtures) was collected and stored at SLI at room temperature. These samples serve as the retention samples for all studies conducted with this material.

8.3. Test Article Disposition

The remaining test article was returned to the Sponsor following completion of all studies with the test article.

8.4. Method of Test Article Preparation

The test articles were pooled and administered as received from the Sponsor. The test article was dispensed fresh on the day of dosing and stirred continuously during dosing.

8.5. Animals and Animal Husbandry

8.5.1. Description, Identification and Housing

Adult, New Zealand White rabbits were received from Myrtle's Rabbitry, Thompson Station, TN. Upon receipt, plastic ear tags displaying unique identification numbers were used to individually identify the animals. Cage cards displaying at least the study number, animal number and sex were affixed to each cage. The animals were housed individually in suspended stainless steel cages. All housing and care were based on the standards recommended by the Guide for the Care and Use of Laboratory Animals [1].

8.5.2. Environment

The animal room temperature and relative humidity ranges were 71-72°F (22°C) and 46-55%, respectively. Environmental control equipment was monitored and adjusted as necessary to minimize fluctuations in the animal room environment. Light timers were set to maintain a 12-hour light/12-hour dark cycle and room ventilation was set to produce 10-15 air changes/hour. The animal room temperature and relative humidity were recorded a minimum of once daily.

8.5.3. Food

PMI Certified Rabbit Chow #5322 (Purina Mills, Inc.) was provided ad libitum to the animals throughout the study. The lot number and expiration date of each batch of diet used during the study were recorded. The feed was analyzed and

certified by the supplier for nutritional components and environmental contaminants. Dietary limitations for various environmental contaminants, including heavy metals, pesticides, polychlorinated biphenyls and total aflatoxin are set by the manufacturer. Within these limits, contaminants which may have been present were not expected to compromise the purpose of this study. Results of the dietary analyses (Certificates of Analysis) were provided by the manufacturer for each lot of diet. These are maintained by SLI.

8.5.4. Water

Municipal tap water treated by reverse osmosis was available ad libitum throughout the study. The purified water was supplied by an automatic watering system. Monitoring of the drinking water for contaminants is conducted by SLI and the records are available for inspection. Within generally accepted limits, contaminants which may have been present were not expected to compromise the purpose of this study. The water meets the standards specified under the EPA National Drinking Water Regulations (40 CFR Part 141).

8.5.5. Acclimation

Upon receipt, the animals were removed randomly from the shipping cartons, examined by qualified personnel, identified with plastic ear tags and then acclimated to the laboratory conditions for a minimum of five days. The animals were observed daily for overt physical or behavioral abnormalities, general health/moribundity and mortality.

8.5.6. Animal Selection

The animals chosen for study use were arbitrarily selected from healthy stock animals to avoid potential bias. All animals received a detailed pretest observation prior to dosing. Only healthy animals were chosen for study use. Females were nulliparous and nonpregnant. The male animal was approximately 13 weeks of age and weighed 2.7 kg prior to dosing. The female animals were approximately 13 weeks of age and weighed 2.5-2.8 kg prior to dosing.

9. EXPERIMENTAL PROCEDURES

9.1. Preliminary Procedures

On day -1, the animals chosen for use on the primary skin irritation study had the fur removed from the dorsal area of the trunk using an animal clipper. Care was taken to avoid abrading the skin during the clipping procedure.

9.2. Dosing

On the following day (day 0), the test article was applied to a small area of intact skin on each test animal (approximately 1 inch x 1 inch) as indicated below:

Concentration (%)	Amount Applied	Patch Design	No. of Animals	
			Male	Female
100[a]	0.5 mL	~1" x 1" square 4-ply gauze patch	1	2

[a]Pooled test article.

The test article was administered under the gauze patch. The gauze patch was held in contact with the skin at the cut edges with a nonirritating tape. Removal and ingestion of the test article was prevented by placing an elastic wrap over the trunk and test area (semi-occlusive binding). The elastic wrap was then further secured with adhesive tape around the trunk at the cranial and caudal ends. After dosing, collars were placed on each animal and remained in place until removal on day 3. After a four-hour exposure period, the binding materials were removed from each animal and the corners of the test site delineated using a marker. Residual test article was removed using gauze moistened with deionized water, followed by dry gauze.

9.3. Dermal Observations

Animals were examined for signs of erythema and edema and the responses scored at 1 hour after patch removal and 24, 48 and 72 hours after patch application according to the Macroscopic Dermal Grading System presented in Appendix A which is based on Draize [2].

9.4. Clinical Observations

Any unusual observations and/or mortality were recorded. General health/mortality checks were performed twice daily (in the morning and in the afternoon).

9.5. Body Weights

Individual body weights were obtained for each animal prior to dosing on day 0.

9.6. Scheduled Euthanasia

Each animal was euthanized by an intravenous injection of sodium pentobarbital following its final scoring interval. Gross necropsy examinations were not required for these animals.

9.7. Protocol Deviations

No protocol deviations occurred during this study.

10. ANALYSIS OF DATA

The 1-, 24-, 48- and 72-hour erythema and edema scores for all animals were added and the total divided by the number of test sites x 4. The calculated Primary Irritation Index (P.I.I.) was classified according to the Dermal Evaluation Criteria [3] presented in Appendix B.

11. MAINTENANCE OF RAW DATA AND RECORDS

All original raw data, the final report and electronic encoded records were transferred to the SLI archives for a period of 10 years. The Sponsor will be contacted prior to final disposition of these items.

12. RESULTS

12.1. Dermal Observations

Individual Data: Table 1

Exposure to the test article produced very slight erythema on 3/3 test sites at the 1-hour scoring interval. The dermal irritation resolved completely on all test sites by the 24-hour scoring interval.

13. CONCLUSION

Under the conditions of the test, Spray--Charlie is considered to be a slight irritant to the skin of the rabbit. The calculated Primary Irritation Index for the test article was 0.25.

Kimberly L. Bonnette, M.S., LATG
Study Director

Date 2/17/03

SLI Study No. 3596.20 (13)

14. REPORT REVIEW

Dawn D. Rodabaugh, B.S.
Toxicologist

Date 2/17/03

15. REFERENCES

1. Guide for the Care and Use of Laboratory Animals, DHHS Publication No. (NIH) 96-03, 1996.

2. Draize, J.H., Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics, The Association of Food and Drug Officials of the United States, 49-51, 1959.

3. Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals-Addendum 3 on Data Reporting, US EPA, 1988.

TABLE 1

STUDY NO. 3596.20 — A PRIMARY SKIN IRRITATION STUDY IN RABBITS — PAGE 1

INL/A, US DEPARTMENT OF STATE — INDIVIDUAL DERMAL IRRITATION SCORES

(SPRAY--CHARLIE)

Animal No./Sex Body Weight (kg)	Scoring Interval	Erythema	Edema	Comments
R3471/F	1 Hour	1	0	IT
2.814	24 Hours	0	0	
	48 Hours	0	0	
	72 Hours	0	0	
R3472/F	1 Hour	1	0	IT
2.494	24 Hours	0	0	IT
	48 Hours	0	0	IT
	72 Hours	0	0	
R3474/F	1 Hour	1	0	
2.723	24 Hours	0	0	
	48 Hours	0	0	
	72 Hours	0	0	

Note: See Appendix A for definition of codes.

Primary Irritation Index

0.25 = Slight Irritant

APPENDIX A

Macroscopic Dermal Grading System

SLI Study No. 3596.20 (17)

MACROSCOPIC DERMAL GRADING SYSTEM

ERYTHEMA AND EDEMA OBSERVATIONS		
OBSERVATION	DEFINITION	CODE
Erythema – Grade 0	No erythema	0
Erythema – Grade 1	Very slight erythema (barely perceptible)	1
Erythema – Grade 2	Well-defined erythema	2
Erythema – Grade 3	Moderate to severe erythema	3
Erythema – Grade 4	Severe erythema (beet redness)	4
Maximized Grade 4	Notable dermal lesions (see below)	M – 4 (see below)
Edema – Grade 0	No edema	0
Edema – Grade 1	Very slight edema (barely perceptible)	1
Edema – Grade 2	Slight edema (edges of area well defined by definite raising)	2
Edema – Grade 3	Moderate edema (raised approximately 1 millimeter)	3
Edema – Grade 4	Severe edema (raised more than 1 millimeter and extends beyond the area of exposure)	4
NOTE: Each animal was assigned an erythema and edema score. The most severely affected area within the test site was graded. If eschar, blanching, ulceration and/or necrosis greater than grade 1 was observed, then the “Maximized Grade 4" was assigned to the test site in place of the erythema score and the type of notable dermal lesion(s) (e.g., eschar - grade 2, blanching - grade 3, ulceration - grade 4, etc.) was noted. The presence of any other dermal changes (e.g., desquamation, fissuring, eschar exfoliation, etc.) was also recorded.		

SLI Study No. 3596.20 (18)

MACROSCOPIC DERMAL GRADING SYSTEM

NOTABLE DERMAL LESIONS		
OBSERVATION	CODE	DEFINITION
Eschar – Grade 1	ES-1	Focal and/or pinpoint areas up to 10% of test site.
Eschar – Grade 2	ES-2	> 10% < 25% of test site.
Eschar – Grade 3	ES-3	> 25% < 50% of test site.
Eschar – Grade 4	ES-4	> 50% of test site.
Blanching – Grade 1	BLA-1	Focal and/or pinpoint areas up to 10% of test site.
Blanching – Grade 2	BLA-2	> 10% < 25% of test site.
Blanching – Grade 3	BLA-3	> 25% < 50% of test site.
Blanching – Grade 4	BLA-4	> 50% of test site.
Ulceration – Grade 1	U-1	Focal and/or pinpoint areas up to 10% of test site.
Ulceration – Grade 2	U-2	> 10% < 25% of test site.
Ulceration – Grade 3	U-3	> 25% < 50% of test site.
Ulceration – Grade 4	U-4	> 50% of test site.
Necrosis – Grade 1	NEC-1 *(color)*	Focal and/or pinpoint areas up to 10% of test site (note color of necrosis).
Necrosis – Grade 2	NEC-2 *(color)*	> 10% < 25% of test site (note color of necrosis).
Necrosis – Grade 3	NEC-3 *(color)*	> 25% < 50% of test site (note color of necrosis).
Necrosis – Grade 4	NEC-4 *(color)*	> 50% of test site (note color of necrosis).

SLI Study No. 3596.20 (19)

MACROSCOPIC DERMAL GRADING SYSTEM

ADDITIONAL DERMAL FINDINGS		
OBSERVATION	DEFINITION	CODE
Desquamation	Characterized by scaling or flaking of dermal tissue or without denuded areas.	DES
Fissuring	Characterized by cracking of the skin with or without moist exudate. Fissuring should be checked prior to removing the animal from the cage and manipulating the test site.	FIS
Eschar Exfoliation	The process by which areas of eschar flake off the test site.	EXF
Test Site Staining	Skin located at test site appears to be discolored, possibly due to test article (note color of staining).	TSS (*color*)
Erythema Extends Beyond the Test Site	The erythema extends beyond the test site. Note: A study director should be contacted for erythema extending beyond the test site.	ERB
Superficial Lightening	Characterized by pale area(s) (almost a burn-like appearance) in the test site. However, erythema may still be observed through the pale area. Note: This observation may affect the overall erythema score of the test site. This observation may progress to other observations resulting in notable dermal lesions, but SL itself will not be considered a notable dermal lesion that will result in a dermal score to be maximized since it does not result in any in-depth injury. To be coded using an area designation (see below).	--
Superficial Lightening - Grade 1	Focal and/or pinpoint areas up to 10% of the test site	SL-1
Superficial Lightening - Grade 2	> 10% < 25% of test site	SL-2
Superficial Lightening - Grade 3	> 25% < 50% of test site	SL-3
Superficial Lightening - Grade 4	> 50% of test site	SL-4
Dermal Irritation - Outside of the Test Site	Noticeable irritation outside of test site probably due to the binding tape material. This notation will only be made for reactions greater than what are normally observed from tape removal which does not interfere with the scoring of the test site.	IT

SLI Study No. 3596.20 (20)

APPENDIX B

Dermal Evaluation Criteria

SLI Study No. 3596.20 (21)

DERMAL EVALUATION CRITERIA	
Primary Irritation Index (P.I.I.)	Irritation Rating
0.00	Nonirritant
0.01 - 1.99	Slight Irritant
2.00 - 5.00	Moderate Irritant
5.01 - 8.00	Severe Irritant

APPENDIX C

SLI Personnel Responsibilities

SLI Study No. 3596.20 (23)

SLI PERSONNEL RESPONSIBILITIES

Kimberly L. Bonnette, M.S., LATG	Study Director/Director, Acute Toxicology
Dawn D. Rodabaugh, B.S.	Alternate Contact/Toxicologist
Malcolm Blair, Ph.D.	Managing Director Emeritus
Joseph C. Siglin, Ph.D., DABT	General Manager
Jason W. Smedley, B.S.	Assistant Toxicologist
Pamela S. Smith, ALAT	Primary Technician/Study Supervisor, Acute Toxicology
Delores P. Knippen	Supervisor, Pharmacy
Steven H. Magness, B.S., LATG	Senior Supervisor, Pathology
Anita M. Bosau, RQAP-GLP	Senior Director, Compliance Assurance
Cheryl Bellamy	Senior Supervisor, Report Writing
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J. Dale Thurman, D.V.M., M.S., DACVP	Senior Director, Pathology
Kathy M. Gasser	Archivist

Report Amendment No. 1

PURITY ANALYSIS FOR GLYPHOSATE OF SPRAY--CHARLIE (ACTIVE INGREDIENT)

FINAL REPORT

Author

Kimberly L. Bonnette, M.S., LATG

Study Completed on

March 21, 2003

Amended Study Completed on

March 27, 2003

Performing Laboratory

Springborn Laboratories (SLI),
a division of Charles River Laboratories, Inc.
640 North Elizabeth Street
Spencerville, Ohio 45887

SLI Study No.

3596.15

Submitted to

INL/A
U.S. Department of State
2201 C St. NW SA-4
Washington, DC 20520

3. QUALITY ASSURANCE STATEMENT

This study was inspected by the Quality Assurance Unit and reports were submitted to management and the Study Director in accordance with SLI's Standard Operating Procedures as follows:

Phase	Date
Protocol Review	10/09/02
Mobile Phase and Standard Preparations	12/12/02
Data Audit	03/17/03
Draft Report Review	03/17/03
Protocol Amendment Review	03/20/03
Final Report Review	03/21/03
Amended Final Report Review	03/27/03
Reports to Study Director and Management	03/17/03, 03/21/03, 03/27/03

The final report has been reviewed to assure that it accurately describes the materials and methods, and the reported results accurately reflect the raw data.

Rebecca A Young Date 3/27/03
Rebecca A. Young
Quality Assurance Team Leader

for Deanna M Talerico Date 3/27/03
Anita M. Bosau, RQAP-GLP
Senior Director, Compliance Assurance

Page No.	Revision	Reason for Change
4	Mobile Phase and Standard Preparations date should 12/12/02 instead of 12/12/03.	To correct a typographical error.
17	Replace the entire table	Incorrect table.

K L Bonnette Date: 3/27/03
Kimberly L. Bonnette, M.S., LATG
Study Director

SLI Study No. 3596.15 (17) Report Amendment No. 1

Chemistry Table 2
Sample Analysis Value and % Error Based on Theoretical Value Before Use Purity Analysis

Test Mix No.	Sample Type	% Glyphosate (a.e.)	Average % Glyphosate (a.e.) by Sample Type	Average % Glyphosate (a.e.) by Test Mix	Overall Average % Glycosate (a.e.)	% Error	Average % Error by Sample Type	Average % Error by Test Mix
1	Top	18.70			16.53	26.4		
1	Top*	16.80	17.75			13.5	19.9	
1	Middle	16.66				12.6		
1	Middle*	15.41	16.04			4.1	8.3	
1	Bottom	16.98				14.7		
1	Bottom*	16.98	16.98	16.92		14.7	14.7	14.3
2	Top	16.48				11.4		
2	Top*	15.79	16.14			6.7	9.0	
2	Middle	16.36				10.5		
2	Middle*	14.69	15.53			0.7	5.6	
2	Bottom	17.33				17.1		
2	Bottom*	17.26	17.30	16.32		16.6	16.9	10.5
3	Top	17.66				19.3		
3	Top*	16.49	17.08			11.4	15.4	
3	Middle	16.98				14.7		
3	Middle*	18.45	17.72			24.7	19.7	
3	Bottom	18.88				27.6		
3	Bottom*	19.24	19.06	17.95		30.0	28.8	21.3
4	Top	13.98				5.5		
4	Top*	13.52	13.75			8.6	7.1	
4	Middle	15.75				6.4		
4	Middle*	15.21	15.48			2.8	4.6	
4	Bottom	15.79				6.7		
4	Bottom*	17.81	16.80	15.34		20.3	13.5	8.4
5	Top	15.72				6.2		
5	Top*	15.77	15.75			6.6	6.4	
5	Middle	16.31				10.2		
5	Middle*	16.13	16.22			9.0	9.6	
5	Bottom	15.46				4.5		
5	Bottom*	17.40	16.43	16.13		17.6	11.0	9.0

* = Duplicate

Report Amendment No. 1

AN ACUTE DERMAL TOXICITY STUDY IN RATS WITH SPRAY--CHARLIE

AMENDED FINAL REPORT

OPPTS Guideline

870.1200

Author

Kimberly L. Bonnette, M.S., LATG

Original Study Completion Date

February 20, 2003

Amended Study Completion Date

March 17, 2003

Performing Laboratory

Springborn Laboratories (SLI),
a division of Charles River Company, Inc.
640 North Elizabeth Street
Spencerville, Ohio 45887

SLI Study No.

3596.17

Submitted to

INL/A
U.S. Department of State
2201 C St. NW SA-4
Washington, DC 20520

4. QUALITY ASSURANCE STATEMENT

This study was inspected by the Quality Assurance Unit and reports were submitted to management and the Study Director in accordance with SLI's Standard Operating Procedures as follows:

Phase	Date
Protocol Review	10/07/02
Dosing	12/19/02
Data Audit	01/23/03
Draft Report Review	01/23/03
Final Report Review	02/20/03
Amended Report Review	03/17/03
Reports to Study Director and Management	01/23/03, 02/20/03, 03/17/03

The final report has been reviewed to assure that it accurately describes the materials and methods, and the reported results accurately reflect the raw data.

Jennifer D. McGue Date 3/17/03
Jennifer D. McGue
Quality Assurance Auditor

Anita M. Bosau Date 3/17/03
Anita M. Bosau, RQAP-GLP
Senior Director, Compliance Assurance

Page No.	Revision	Reason For Change
8	8.1. Test Article. In the table, change the lot number for Surfactant: Cosmo Flux-411F from "Lot No.: Manufactured 11/20/02" to "Lot No.: Manufactured 11/29/02".	Correct a typographical error

K. L. Bonnette Date 3/17/03
Kimberly L. Bonnette, M.S., LATG
Director, Acute Toxicology

7. INTRODUCTION

This study was performed to assess the short-term toxicity of Spray--Charlie in Sprague Dawley rats when administered by a single dermal dose. This study was intended to provide information on the potential health hazards of the test article with respect to dermal exposure. Data from this study may serve as a basis for classification and/or labeling of the test article. This study was performed in accordance with the US EPA, Health Effects Test Guidelines, OPPTS 870.1200, Acute Dermal Toxicity, August 1998. This study was performed at Springborn Laboratories (SLI), 553 North Broadway, Spencerville, Ohio. The protocol was signed by the Study Director on October 9, 2002 (GLP initiation date). The in-life phase of the study was initiated with test article administration on December 19, 2002 (day 0), and concluded with necropsy on January 2, 2003.

8. MATERIALS AND METHODS

8.1. Test Article

The test article was received from the Sponsor and identified as follows:

Sponsor's ID	Assigned SLI ID	Physical Description	Receipt Date	Expiration Date
Spray—Charlie[a]	S02.003.3596	Amber liquid	12/09/02	None provided
Ingredients:[b]				
Herbicide: GLY-41 Lot No.: Manufactured 10/20/02				None provided
Surfactant: Cosmo Flux-411F Lot No.: Manufactured 11/29/02				None provided

[a]Sample pooled at SLI from five different mixes of Spray--Charlie (top/middle/bottom).
[b]Ingredients used in the five Spray--Charlie mixes that were prepared by the Sponsor.

The test article was stored at room temperature. The Sponsor was responsible for any necessary evaluations related to identity, strength, purity, composition, stability and method of synthesis of the test material according to 40 CFR 160.105 and 40 CFR 792.105. Springborn Laboratories, analyzed the test article for the glyphosate (a.e.) which is presented in SLI Study No. 3596.15.

PURITY ANALYSIS FOR GLYPHOSATE OF SPRAY--CHARLIE (ACTIVE INGREDIENT)

PROTOCOL AMENDMENT NO. 1

1) PART TO BE CHANGED/REVISED (EFFECTIVE OCTOBER 21, 2002):

4.3.2. SLI Identification Number

CHANGE/REVISION:

Replace this section with the following:

R02.002.3596

REASON FOR CHANGE/REVISION:

To correct the SLI Identification Number for the reference standard.

2) PART TO BE CHANGED/REVISED (EFFECTIVE OCTOBER 21, 2002):

4.3.3. Lot Number

CHANGE/REVISION:

Replace this section with the following:

42K3650

REASON FOR CHANGE/REVISION:

To correct the lot number for the reference standard.

PURITY ANALYSIS FOR GLYPHOSATE OF SPRAY--CHARLIE (ACTIVE INGREDIENT)

PROTOCOL AMENDMENT NO. 1

Kimberly L. Bonnette, M.S., LATG
Study Director (SLI)

Date: 3/21/03

Rebecca A. Young
Quality Assurance Unit (SLI)

Date: 3/21/03

Rogers Woolfolk
Sponsor's Representative

Date: 21 MARCH 03

Annex 56-B

Six Acute Toxicity Studies with Spray-Alpha, SLI Study N° 3596.3, 3 September 2002

(United States Embassy in Bogotá, 2011)

AN ACUTE DERMAL TOXICITY STUDY IN RATS WITH SPRAY--ALPHA

FINAL REPORT

OPPTS Guideline

870.1200

Author

Kimberly L. Bonnette, M.S., LATG

Study Completed on

September 3, 2002

Performing Laboratory

Springborn Laboratories, Inc. (SLI)
Ohio Research Center
640 North Elizabeth Street
Spencerville, Ohio 45887

SLI Study No.

3596.3

Submitted to

INL/A
U.S. Department of State
2201 C St. NW SA-4
Washington, DC 20520

Page 1 of 30

SLI Study No. 3596.3 (2)

1. STATEMENT OF *NO* DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B), or (C).

Company: _____

Company Agent: Date _____ Date _____

_____ _____

Title Signature

SLI Study No. 3596.3 (3)

2. COMPLIANCE STATEMENT

This study was conducted in compliance with the Good Laboratory Practice Standards as described by the EPA (40 CFR Parts 160 and 792).

Kimberly L. Bonnette, M.S., LATG
Study Director/Author
Springborn Laboratories, Inc.

Date 9/3/02

Rogers Woolfolk
Senior Aviation Advisor
Sponsor/Submitter
INL/A
U.S. Department of State

Date 28 Aug 02

3. QUALITY ASSURANCE STATEMENT

This study was inspected by the Quality Assurance Unit and reports were submitted to management and the Study Director in accordance with SLI's Standard Operating Procedures as follows:

Phase	Date
Protocol Review	03/31/02
Dermal Observations	06/26/02
Data Audit	08/22/02
Draft Report Review	08/22/02
Protocol Amendment Review	08/28/02
Final Report Review	09/03/02
Reports to Study Director and Management	08/22/02, 09/03/02

The final report has been reviewed to assure that it accurately describes the materials and methods, and the reported results accurately reflect the raw data.

Jennifer D. McGue Date 9/3/02
Jennifer D. McGue
Quality Assurance Auditor

Deanna M Talarico Date 9/3/02
for Anita M. Bosau, RQAP-GLP
Senior Director, Compliance Assurance

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SLI Study No. 3596.3 (7)

6. SUMMARY

The single-dose dermal toxicity of Spray--Alpha was evaluated in Sprague Dawley rats. A limit test was performed in which one group of five male and five female rats received a single dermal administration of the test article at a dose of 5000 mg/kg body weight. Following dosing, the limit test rats were observed daily and weighed weekly. A gross necropsy examination was performed on all animals at the time of scheduled euthanasia (day 14).

No mortality occurred during the limit test. Clinical abnormalities observed during the study included dark material around the facial area and red ocular discharge. Minor/transient dermal irritation was noted at the site of test article application. Body weight loss was noted in two males and two females during the study day 0 to 7 body weight interval which is routinely observed in this study type due to experimental manipulation. Body weight gain was noted for all other animals during the test period. All animals exceeded their initial body weight by study termination (day 14). No significant gross internal findings were observed at necropsy on study day 14.

Under the conditions of this test, the acute dermal LD50 of Spray--Alpha was estimated to be greater than 5000 mg/kg in the rat.

7. INTRODUCTION

This study was performed to assess the short-term toxicity of Spray--Alpha in Sprague Dawley rats when administered by a single dermal dose. This study was intended to provide information on the potential health hazards of the test article with respect to dermal exposure. Data from this study may serve as a basis for classification and/or labeling of the test article. This study was performed in accordance with the US EPA, Health Effects Test Guidelines, OPPTS 870.1200, Acute Dermal Toxicity, August 1998. This study was performed at Springborn Laboratories, Inc., 553 North Broadway, Spencerville, Ohio. The protocol was signed by the Study Director on April 30, 2002 (GLP initiation date). The in-life phase of the study was initiated with test article administration on June 25, 2002 (day 0), and concluded with necropsy on July 9, 2002.

8. MATERIALS AND METHODS

8.1. Test Article

The test article was received from the Sponsor and identified as follows:

Sponsor's ID	Assigned SLI ID	Physical Description	Receipt Date	Expiration Date
Spray—Alpha[a]	S02.001.3596	Light amber liquid	05/13/02	None provided
Ingredients[b]				
Herbicide: Fuete-SL Lot No.: 02-01-02				None Provided
Surfactant: Cosmo Flux-411F Lot No.: 244301				10/2003

[a]Sample pooled at SLI from five different mixes of Spray--Alpha (top/middle/bottom).
[b]Ingredients used in the five Spray--Alpha mixes that were prepared by the Sponsor.

The test article was stored at room temperature. The Sponsor was responsible for any necessary evaluations related to identity, strength, purity, composition, stability and method of synthesis of the test material according to 40 CFR 160.105 and 40 CFR 792.105. Springborn Laboratories, Inc., analyzed the test article for the glyphosate (a.e.) which is presented in SLI Study No. 3596.1.

8.2. Retention Sample

An approximate 1 mL retention sample of each test article mixture (top/middle/bottom, maintained separately for a total of fifteen 1 mL samples) was taken and stored at SLI at room temperature. In addition, a 10 mL retention sample of the pooled test article samples (from the 5 test article mixtures) was collected and stored at SLI at room temperature. These samples serve as the retention samples for all studies conducted with this material.

8.3. Test Article Disposition

The test article was returned to the Sponsor following completion of all studies with the test article.

8.4. Method of Test Article Preparation

The test article was administered as received from the Sponsor and dispensed fresh on the day of dosing. The density of the test article was determined to be 1.08 g/mL.

8.5. Animals and Animal Husbandry

8.5.1. Description, Identification and Housing

Adult, Hsd: Sprague Dawley® SD® rats were received from Harlan Sprague Dawley, Inc., Indianapolis, IN. Upon receipt, metal ear tags displaying unique identification numbers were used to individually identify the animals. Cage cards displaying at least the study number, animal number and sex were affixed to each cage. The animals were housed individually in suspended stainless steel cages. All housing and care were based on the standards recommended by the Guide for the Care and Use of Laboratory Animals [1].

8.5.2. Environment

The animal room temperature and relative humidity ranges were 70-75°F (21-24°C) and 37-57%, respectively. Environmental control equipment was monitored and adjusted as necessary to minimize fluctuations in the animal room environment. Light timers were set to maintain a 12-hour light/12-hour dark cycle and room ventilation was set to produce 10-15 air changes/hour. The animal room temperature and relative humidity were recorded a minimum of once daily.

8.5.3. Food

PMI Certified Rodent Chow #5002 (Purina Mills, Inc.) was provided ad libitum to the animals throughout the study. The lot number and expiration date of each batch of diet used during the study were recorded. The feed was analyzed and

certified by the supplier for nutritional components and environmental contaminants. Dietary limitations for various environmental contaminants, including heavy metals, pesticides, polychlorinated biphenyls and total aflatoxin are set by the manufacturer. Within these limits, contaminants which may have been present were not expected to compromise the purpose of this study. Results of the dietary analyses (Certificates of Analysis) were provided by the manufacturer for each lot of diet. These are maintained by SLI.

8.5.4. Water

Municipal tap water treated by reverse osmosis was available ad libitum throughout the study. The purified water was supplied by an automatic watering system. Monitoring of the drinking water for contaminants is conducted by SLI and the records are available for inspection. Within generally accepted limits, contaminants which may have been present were not expected to compromise the purpose of this study. The water meets the standards specified under the EPA National Drinking Water Regulations (40 CFR Part 141).

8.5.5. Acclimation

Upon receipt, the animals were removed randomly from the shipping cartons, examined by qualified personnel, identified with metal ear tags and then acclimated to the laboratory conditions for a minimum of five days. The animals were observed daily for overt physical or behavioral abnormalities, general health/moribundity and mortality.

8.5.6. Animal Selection

The animals chosen for study use were randomly selected from healthy stock animals using a computerized (Alpha DS-10 AcuTox) random numbers table to avoid potential bias. All animals received a detailed pretest observation prior to dosing. Only healthy animals were chosen for study use. Females were nulliparous and nonpregnant. The male animals were approximately 11 weeks of age and weighed 335-374 g prior to dosing. The female animals were approximately 11 weeks of age and weighed 226-249 g prior to dosing.

9. EXPERIMENTAL PROCEDURES

9.1. Preliminary Procedures

On day -1, the fur was removed from the dorsal trunk area of the animals chosen for the limit test using an animal clipper. The clipped area was approximately 10% of the animal's body surface area (BSA). The region included the scapula (shoulder) to the wing of the ilium (hipbone) and half way down the flank on each

side of the animal. Care was taken to avoid abrading the skin during the clipping procedure.

9.2. Dosing

On the following day (day 0), the test article was administered dermally to approximately 10% of the body surface area. The four corners of this area were delineated in the clipped area with an indelible marker. The test article was then spread evenly over the delineated test area and held in contact with the skin with an appropriately sized 4-ply porous gauze dressing backed with a plastic wrap which was placed over the gauze dressing (occlusive binding). Removal and ingestion of the test article was prevented by placing an elastic wrap over the trunk and test area. The elastic wrap was further secured with a tape harness on the cranial end of the trunk and then secured with adhesive tape around the trunk at the caudal end.

The test article was administered at the following level:

Dose Level (mg/kg)	Dose Volume (mL/kg)	Concentration (%)	No. of Animals	
			Male	Female
5000	4.63[a]	100[b]	5	5

[a]Adjusted based on a density of 1.08 g/mL.
[b]Pooled test article.

Individual doses were calculated based on the animal's day 0 body weight. After an approximate 24-hour exposure period, the binding materials were removed. Residual test article was removed using gauze moistened with deionized water followed by dry gauze.

9.3. Dermal Observations

The test animals were examined for erythema and edema following patch removal and the responses scored on study day 1 and daily thereafter (days 2-14) according to the Macroscopic Dermal Grading System provided in Appendix A which is based on Draize [2]. The dermal test sites were reclipped as necessary to allow clear visualization of the skin.

9.4. Clinical Observations

The animals were observed for clinical abnormalities a minimum of two times on study day 0 (postdose) and daily thereafter (days 1-14). A mortality check was performed twice daily, in the morning and afternoon.

9.5. Body Weights

Individual body weights were obtained for the animals prior to dosing on day 0 and on days 7 and 14.

9.6. Gross Necropsy

All animals were euthanized by carbon dioxide inhalation at study termination (day 14) and necropsied. Body cavities (cranial, thoracic, abdominal and pelvic) were opened and examined. No tissues were retained.

9.7. Protocol Deviations

No protocol deviations occurred during this study.

10. ANALYSIS OF DATA

Data from the study were analyzed and an LD50 value estimated as follows:

< 50% Mortality: LD50 was estimated as greater than the administered dose.
= 50% Mortality: LD50 was estimated as equal to the administered dose.
> 50% Mortality: LD50 was estimated as less than the administered dose.

Body weight means and standard deviations were calculated separately for males and females.

11. MAINTENANCE OF RAW DATA AND RECORDS

All original raw data, the final report and magnetically encoded records were transferred to the SLI archives for a period of 10 years. The Sponsor will be contacted prior to final disposition of these items.

SLI Study No. 3596.3 (13)

12. RESULTS

12.1. Mortality

Individual Data: Table 1

No mortality occurred during the limit test.

12.2. Clinical/Dermal Observations

Individual Data: Table 1

Clinical abnormalities observed during the study included dark material around the facial area and red ocular discharge. Minor/transient dermal irritation was noted at the site of test article application.

12.3. Body Weight Data

Individual Data: Table 2

Body weight loss was noted in two males and two females during the study day 0 to 7 body weight interval which is routinely observed in this study type due to experimental manipulation. Body weight gain was noted for all other animals during the test period. All animals exceeded their initial body weight by study termination (day 14).

12.4. Gross Necropsy

Individual Data: Table 3

No significant gross internal findings were observed at necropsy on study day 14. Blood clots observed in one animal at necropsy were thought to have been caused by a possible accidental injury prior to euthanasia.

13. CONCLUSION

Under the conditions of this test, the acute dermal LD50 of Spray--Alpha was estimated to be greater than 5000 mg/kg in the rat.

Kimberly L. Bonnette, M.S., LATG
Study Director

Date 9/3/02

14. REPORT REVIEW

Dawn D. Rodabaugh, B.S.
Associate Toxicologist

Date 9/3/02

15. REFERENCES

1. Guide for the Care and Use of Laboratory Animals, DHHS Publication No. (NIH) 96-03, 1996.

2. Draize, J.H., Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics, The Association of Food and Drug Officials of the United States, 49-51, 1959.

STUDY NO.: 3596.3 PAGE 1

INL/A, U.S. DEPARTMENT OF STATE

TABLE 1

AN ACUTE DERMAL TOXICITY STUDY IN RATS

INDIVIDUAL CLINICAL OBSERVATIONS
(POSITIVE FINDINGS)

MALES 5000 MG/KG

MALE#	OBSERVATIONS	DAY OF STUDY 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A5297	SCHEDULED EUTHANASIA															P
	EDEMA GRADE 0		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	ERYTHEMA GRADE 0				P	P	P	P	P	P	P	P	P	P	P	P
	DARK MATERIAL AROUND EYE(S)	P	P													
	DARK MATERIAL AROUND NOSE	P		P					P							
	DARK MATERIAL AROUND MOUTH	P														
	ERYTHEMA GRADE 1		P	P												
A5302	SCHEDULED EUTHANASIA															P
	EDEMA GRADE 0		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	ERYTHEMA GRADE 0		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	DARK MATERIAL AROUND EYE(S)	P	P													
	OCULAR DISCHARGE - RED	P														
	DARK MATERIAL AROUND NOSE	P	P	P	P											
	DARK MATERIAL AROUND MOUTH	P														
A5304	SCHEDULED EUTHANASIA															P
	EDEMA GRADE 0		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	ERYTHEMA GRADE 0		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	DARK MATERIAL AROUND EYE(S)	P	P	P												
	OCULAR DISCHARGE - RED	P														
	DARK MATERIAL AROUND NOSE	P	P													
	DARK MATERIAL AROUND MOUTH	P														
	INCISOR(S) BROKEN		P	P		P	P									
A5305	SCHEDULED EUTHANASIA															P
	EDEMA GRADE 0		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	ERYTHEMA GRADE 0				P	P	P	P	P	P	P	P	P	P	P	P
	DARK MATERIAL AROUND EYE(S)	P	P													
	DARK MATERIAL AROUND NOSE	P	P	P												
	ERYTHEMA GRADE 1		P	P												

GRADE CODE: 1=SLIGHT 2=MODERATE 3=SEVERE P=PRESENT L=LEFT R=RIGHT B=BILATERAL

TABLE 1

AN ACUTE DERMAL TOXICITY STUDY IN RATS

INDIVIDUAL CLINICAL OBSERVATIONS
(POSITIVE FINDINGS)

MALES 5000 MG/KG

		DAY OF STUDY													
MALE#	OBSERVATIONS	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A5306	SCHEDULED EUTHANASIA															P
	EDEMA GRADE 0		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	ERYTHEMA GRADE 0		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	DARK MATERIAL AROUND EYE(S)	P	P													
	OCULAR DISCHARGE - RED	P														
	DARK MATERIAL AROUND NOSE	P	P	P												
	DARK MATERIAL AROUND MOUTH	P														

GRADE CODE: 1=SLIGHT 2=MODERATE 3=SEVERE P=PRESENT L=LEFT R=RIGHT B=BILATERAL

TABLE 1

AN ACUTE DERMAL TOXICITY STUDY IN RATS

INDIVIDUAL CLINICAL OBSERVATIONS
(POSITIVE FINDINGS)

FEMALES 5000 MG/KG

FEMALE#	OBSERVATIONS	DAY OF STUDY 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A5339	SCHEDULED EUTHANASIA															P
	EDEMA GRADE 0		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	ERYTHEMA GRADE 0		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	DARK MATERIAL AROUND EYE(S)	P	P													
	OCULAR DISCHARGE - RED	P														
	DARK MATERIAL AROUND NOSE	P														
	DARK MATERIAL AROUND MOUTH	P														
A5340	SCHEDULED EUTHANASIA															P
	EDEMA GRADE 0		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	ERYTHEMA GRADE 0		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	DARK MATERIAL AROUND EYE(S)	P														
	DARK MATERIAL AROUND NOSE	P														
A5341	SCHEDULED EUTHANASIA															P
	EDEMA GRADE 0		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	ERYTHEMA GRADE 0		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	DARK MATERIAL AROUND EYE(S)	P	P													
	DARK MATERIAL AROUND NOSE	P	P	P												
A5342	SCHEDULED EUTHANASIA															P
	EDEMA GRADE 0		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	ERYTHEMA GRADE 0			P	P	P	P	P	P	P	P	P	P	P	P	P
	DARK MATERIAL AROUND EYE(S)	P	P													
	DARK MATERIAL AROUND NOSE	P														
	ERYTHEMA GRADE 1		P													
A5343	SCHEDULED EUTHANASIA															P
	URINE STAIN		1													
	EDEMA GRADE 0		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	ERYTHEMA GRADE 0		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	DARK MATERIAL AROUND EYE(S)	P	P													
	OCULAR DISCHARGE - RED	P														

GRADE CODE: 1=SLIGHT 2=MODERATE 3=SEVERE P=PRESENT L=LEFT R=RIGHT B=BILATERAL

TABLE 1

AN ACUTE DERMAL TOXICITY STUDY IN RATS

INDIVIDUAL CLINICAL OBSERVATIONS
(POSITIVE FINDINGS)

FEMALES 5000 MG/KG

FEMALE#	OBSERVATIONS	DAY OF STUDY 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A5343	(CONTINUED)															
	DARK MATERIAL AROUND NOSE	P														
	DARK MATERIAL AROUND MOUTH	P														

GRADE CODE: 1=SLIGHT 2=MODERATE 3=SEVERE P=PRESENT L=LEFT R=RIGHT B=BILATERAL

STUDY NO.: 3596.3 PAGE 1

INL/A, U.S. DEPARTMENT OF STATE

TABLE 2

AN ACUTE DERMAL TOXICITY STUDY IN RATS

INDIVIDUAL BODY WEIGHTS (GRAMS)

MALES 5000 MG/KG

	DAY OF STUDY			
ANIMAL#	0	7	14	AT DEATH (DAY)
A5297	339	331	346	
A5302	374	388	410	
A5304	342	342	349	
A5305	360	367	392	
A5306	335	331	347	
MEAN	350	352	369	
S.D.	16.5	25.0	30.1	
N	5	5	5	

TABLE 2

AN ACUTE DERMAL TOXICITY STUDY IN RATS

INDIVIDUAL BODY WEIGHTS (GRAMS)

FEMALES 5000 MG/KG

	DAY OF STUDY			
ANIMAL#	0	7	14	AT DEATH (DAY)
A5339	249	236	259	
A5340	226	218	232	
A5341	244	247	258	
A5342	238	232	248	
A5343	230	233	241	
MEAN	237	233	248	
S.D.	9.5	10.4	11.5	
N	5	5	5	

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INL/A, U.S. DEPARTMENT OF STATE

TABLE 3

AN ACUTE DERMAL TOXICITY STUDY IN RATS

INDIVIDUAL GROSS NECROPSY OBSERVATIONS

MALES 5000 MG/KG

ANIMAL#	DAY OF DEATH	STUDY DAY	OBSERVATION	FATE
A5297	9-JUL-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A5302	9-JUL-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A5304	9-JUL-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A5305	9-JUL-02	14	CAVITY, ABDOMINAL: CONTENT ABNORMAL; PRESENT BLOOD AND BLOOD CLOTS DISPERSED THROUGHOUT ABDOMINAL VISCERA	SCHEDULED EUTHANASIA
A5306	9-JUL-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA

TABLE 3

AN ACUTE DERMAL TOXICITY STUDY IN RATS

INDIVIDUAL GROSS NECROPSY OBSERVATIONS

FEMALES 5000 MG/KG

ANIMAL#	DAY OF DEATH	STUDY DAY	OBSERVATION	FATE
A5339	9-JUL-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A5340	9-JUL-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A5341	9-JUL-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A5342	9-JUL-02	14	HAIRCOAT: DARK MATERIAL; PRESENT AROUND NOSE, RED	SCHEDULED EUTHANASIA
A5343	9-JUL-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA

SLI Study No. 3596.3 (24)

APPENDIX A

Macroscopic Dermal Grading System

SLI Study No. 3596.3 (25)

MACROSCOPIC DERMAL GRADING SYSTEM

ERYTHEMA AND EDEMA OBSERVATIONS		
OBSERVATION	DEFINITION	CODE
Erythema – Grade 0	No erythema	0
Erythema – Grade 1	Very slight erythema (barely perceptible)	1
Erythema – Grade 2	Well-defined erythema	2
Erythema – Grade 3	Moderate to severe erythema	3
Erythema – Grade 4	Severe erythema (beet redness)	4
Maximized Grade 4	Notable dermal lesions (see below)	M – 4 (see below)
Edema – Grade 0	No edema	0
Edema – Grade 1	Very slight edema (barely perceptible)	1
Edema – Grade 2	Slight edema (edges of area well defined by definite raising)	2
Edema – Grade 3	Moderate edema (raised approximately 1 millimeter)	3
Edema – Grade 4	Severe edema (raised more than 1 millimeter and extends beyond the area of exposure)	4

NOTE: Each animal was assigned an erythema and edema score. The most severely affected area within the test site was graded. If eschar, blanching, ulceration and/or necrosis greater than grade 1 was observed, then the “Maximized Grade 4" was assigned to the test site in place of the erythema score and the type of notable dermal lesion(s) (e.g., eschar - grade 2, blanching - grade 3, ulceration - grade 4, etc.) was noted. The presence of any other dermal changes (e.g., desquamation, fissuring, eschar exfoliation, etc.) was also recorded.

SLI Study No. 3596.3 (26)

MACROSCOPIC DERMAL GRADING SYSTEM

NOTABLE DERMAL LESIONS		
OBSERVATION	CODE	DEFINITION
Eschar – Grade 1	ES-1	Focal and/or pinpoint areas up to 10% of test site.
Eschar – Grade 2	ES-2	> 10% < 25% of test site.
Eschar – Grade 3	ES-3	> 25% < 50% of test site.
Eschar – Grade 4	ES-4	> 50% of test site.
Blanching – Grade 1	BLA-1	Focal and/or pinpoint areas up to 10% of test site.
Blanching – Grade 2	BLA-2	> 10% < 25% of test site.
Blanching – Grade 3	BLA-3	> 25% < 50% of test site.
Blanching – Grade 4	BLA-4	> 50% of test site.
Ulceration – Grade 1	U-1	Focal and/or pinpoint areas up to 10% of test site.
Ulceration – Grade 2	U-2	> 10% < 25% of test site.
Ulceration – Grade 3	U-3	> 25% < 50% of test site.
Ulceration – Grade 4	U-4	> 50% of test site.
Necrosis – Grade 1	NEC-1 *(color)*	Focal and/or pinpoint areas up to 10% of test site (Note color of necrosis).
Necrosis – Grade 2	NEC-2 *(color)*	> 10% < 25% of test site (Note color of necrosis).
Necrosis – Grade 3	NEC-3 *(color)*	> 25% < 50% of test site (Note color of necrosis).
Necrosis – Grade 4	NEC-4 *(color)*	> 50% of test site (Note color of necrosis).

SLI Study No. 3596.3 (27)

MACROSCOPIC DERMAL GRADING SYSTEM

ADDITIONAL DERMAL FINDINGS		
OBSERVATION	DEFINITION	CODE
Desquamation	Characterized by scaling or flaking of dermal tissue with or without denuded areas.	DES
Fissuring	Characterized by cracking of the skin with or without moist exudate. Fissuring should be checked prior to removing the animal from the cage and manipulating the test site.	FIS
Eschar Exfoliation	The process by which areas of eschar flake off the test site.	EXF
Test Site Staining	Skin located at test site appears to be discolored, possibly due to test article (note color of staining).	TSS *(color)*
Erythema Extends Beyond the Test Site	The erythema extends beyond the test site. Note: A study director should be contacted for erythema extending beyond the test site.	ERB
Superficial Lightening	Characterized by pale area(s) (almost a burn-like appearance) in the test site. However, erythema may still be observed through the pale area. Note: This observation may affect the overall erythema score of the test site. This observation may progress to other observations resulting in notable dermal lesions, but SL itself will not be considered a notable dermal lesion that will result in a dermal score to be maximized since it does not result in any in-depth injury. To be coded using an area designation (see below).	--
Superficial Lightening - Grade 1	Focal and/or pinpoint areas up to 10% of the test site	SL-1
Superficial Lightening - Grade 2	> 10% < 25% of test site	SL-2
Superficial Lightening - Grade 3	> 25% < 50% of test site	SL-3
Superficial Lightening - Grade 4	> 50% of test site	SL-4

SLI Study No. 3596.3 (28)

MACROSCOPIC DERMAL GRADING SYSTEM

ADDITIONAL FINDINGS		
OBSERVATION	DEFINITION	CODE
Dermal Irritation - Outside of the Test Site	Noticeable irritation outside of test site probably due to the binding tape material. This notation will only be made for reactions greater than what are normally observed from tape removal which do not interfere with the scoring of the test site.	IT

APPENDIX B

SLI Personnel Responsibilities

SLI Study No. 3596.3 (30)

SLI PERSONNEL RESPONSIBILITIES

Kimberly L. Bonnette, M.S., LATG	Study Director/Director, Acute Toxicology
Dawn D. Rodabaugh, B.S.	Alternate Contact/Associate Toxicologist
Robert C. Springborn, Ph.D.	Chairman, President and CEO
Malcolm Blair, Ph.D.	Senior Vice President, Managing Director Emeritus
Joseph C. Siglin, Ph.D., DABT	Vice President, Managing Director
Jason W. Smedley, B.S.	Assistant Toxicologist
Pamela S. Smith, ALAT	Supervisor of Acute Toxicology
Kathy A. Pugh, ALAT	Primary Technician/Team Leader
Delores P. Knippen	Supervisor of Pharmacy
Steven H. Magness, B.S., LATG	Senior Supervisor of Gross and Fetal Pathology
Anita M. Bosau, RQAP-GLP	Senior Director, Compliance Assurance
Deanna M. Talerico, RQAP-GLP	Senior Supervisor of Quality Assurance
J. Dale Thurman, D.V.M., M.S., DACVP	Senior Director, Pathology
Kathy M. Gasser	Supervisor of Archives

AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS WITH SPRAY--ALPHA

FINAL REPORT

OPPTS Guidelines

870.1300

Author

Kimberly L. Bonnette, M.S., LAGT

Study Completed on

September 3, 2002

Performing Laboratory

Springborn Laboratories, Inc. (SLI)
Ohio Research Center
640 North Elizabeth Street
Spencerville, Ohio 45887

SLI Study No.

3596.4

Submitted to

INL/A
U.S. Department of State
2201 C St. NW SA-4
Washington, DC 20520

Page 1 of 48

AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS WITH SPRAY--ALPHA

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(2)

SLI Study No. 3596.4

1. STATEMENT OF *NO* DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B), or (C).

Company: _____

Company Agent: _____ Date_____

_____ _____

Title Signature

2. COMPLIANCE STATEMENT

This study was conducted in compliance with the Good Laboratory Practice Standards as described by the EPA (40 CFR Parts 160 and 792).

Kimberly L. Bonnette, M.S., LAGT
Study Director/Author
Springborn Laboratories, Inc.

Date 9/3/02

Rogers Woolfolk
Senior Aviation Advisor
Sponsor/Submitter
INL/A
U.S. Department of State

Date 29 Aug 02

SLI Study No. 3596.4

3. QUALITY ASSURANCE STATEMENT

This study was inspected by the Quality Assurance Unit and reports were submitted to management and the Study Director in accordance with SLI's Standard Operating Procedures as follows:

Phase	Date
Protocol Review	03/31/02
Dose Preparation	06/06/02
Data Audit	08/27/02
Draft Report Review	08/27/02
Analytical Chemistry Report Review	08/27/02
Protocol Amendment Review	08/28/02
Final Report Review	09/03/02
Reports to Study Director and Management	08/27/02, 09/03/02

The final report has been reviewed to assure that it accurately describes the materials and methods, and the reported results accurately reflect the raw data.

Rebecca A. Young

Rebecca A. Young
Quality Assurance Team Leader

Date 9/3/02

for Deanna M Talenco

Anita M. Bosau, RQAP-GLP
Senior Director, Compliance Assurance

Date 9/3/02

(5)

SLI Study No. 3596.4

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6. SUMMARY

The four-hour nose-only inhalation toxicity of Spray--Alpha was evaluated in Sprague Dawley rats. A limit test was performed in which a group of five male and five female rats received a four-hour nose-only inhalation exposure to a time-weighted average aerosol concentration (analytically determined) of 3.27 mg/L. Following the exposure, the limit test rats were observed daily and weighed weekly. A gross necropsy examination was performed on all limit test animals at the time of scheduled euthanasia (day 14).

No mortality occurred during this study. The most notable clinical abnormalities observed during the study included decreased/no defecation, soft stools, feces small in size, rough coat, breathing abnormalities, decreased food consumption and dark material around the facial area. Body weight loss was noted for one male and one female during the study day 0-7 body weight interval. Body weight gain was noted for all other animals during the test period. All animals exceeded their initial body weight by study termination (day 14). No significant gross internal findings were observed at necropsy on study day 14.

Under the conditions of this test, the acute inhalation LC50 of Spray--Alpha was estimated to be greater than 3.27 mg/L in the rat.

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7. INTRODUCTION

This study was performed to assess the short-term toxicity of Spray--Alpha in Sprague Dawley rats when administered by a four-hour nose-only inhalation exposure. This study was intended to provide information on the potential health hazards of the test article with respect to inhalation exposure. Data from this study may serve as a basis for classification and/or labeling of the test article. This study was conducted in accordance with the US EPA, Health Effects Test Guidelines, OPPTS 870.1300, Acute Inhalation Toxicity, August 1998. This study was performed at Springborn Laboratories, Inc., 553 North Broadway, Spencerville, Ohio. The protocol was signed by the Study Director on April 30, 2002 (GLP initiation date). The in-life phase of the study was initiated with test article administration on June 6, 2002 (day 0) and concluded with terminal euthanasia on June 20, 2002.

8. MATERIALS AND METHODS

8.1. Test Article

The test article was received from the Sponsor and identified as follows:

Sponsor's ID	Assigned SLI ID	Physical Description	Receipt Date	Expiration Date
Spray--Alpha[a]	S02.001.3596	Light amber liquid	05/13/02	None provided
Ingredients[b]				
Herbicide: Fuete-SL Lot No.: 02-01-02				None Provided
Surfactant: Cosmo Flux-411F Lot No.: 244301				10/2003

[a]Sample pooled at SLI from five different mixes of Spray--Alpha (top/middle/bottom).
[b]Ingredients used in the five Spray--Alpha mixes that were prepared by the Sponsor.

The test article was stored at room temperature. The Sponsor was responsible for any necessary evaluations related to identity, strength, purity, composition, stability and method of synthesis of the test material according to 40 CFR 160.105 and 40 CFR 792.105. Springborn Laboratories, Inc., analyzed the test article for the glyphosate (a.e.) which is presented in SLI Study No. 3596.1.

8.2. Retention Sample

An approximate 1 mL retention sample of each test article mixture (top/middle/bottom, maintained separately for a total of fifteen 1 mL samples) was taken and stored at SLI at room temperature. In addition, a 10 mL retention sample of the pooled test article samples (from the 5 test article mixtures) was collected and stored at SLI at room temperature. These samples serve as the retention samples for all studies conducted with this material.

8.3. Test Article Disposition

The remaining test article was returned to the Sponsor following completion of all studies with the test article.

8.4. Method of Test Article Preparation

The test article was utilized as received from the Sponsor and dispensed fresh on the day of dosing. The test article was stirred continuously during exposure.

8.5. Animals and Animal Husbandry

8.5.1. Description, Identification and Housing

Young adult, Hsd: Sprague Dawley® SD® rats were received from Harlan Sprague Dawley, Inc., Indianapolis, IN. Upon receipt, metal ear tags displaying unique identification numbers were used to individually identify the animals. Cage cards displaying at least the study number, animal number and sex were affixed to each cage. The animals were housed individually in suspended stainless steel cages. All housing and care were based on the standards recommended by the Guide for the Care and Use of Laboratory Animals [1].

8.5.2. Environment

The animal room temperature and relative humidity ranges were 71-74°F (22-23°C) and 35-61%, respectively. Environmental control equipment was monitored and adjusted as necessary to minimize fluctuations in the animal room environment. Light timers were set to maintain a 12-hour light/12-hour dark cycle and room ventilation was set to produce 10-15 air changes/hour. The animal room temperature and relative humidity were recorded a minimum of once daily.

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8.5.3. Food

PMI Certified Rodent Chow #5002 (Purina Mills, Inc.) was provided ad libitum to the animals throughout the study (except during the time that the animals were acclimated to the exposure tubes and maintained in the inhalation room for the exposure procedure). The lot number and expiration date of each batch of diet used during the study were recorded. The feed was analyzed and certified by the supplier for nutritional components and environmental contaminants. Dietary limitations for various environmental contaminants, including heavy metals, pesticides, polychlorinated biphenyls and total aflatoxin are set by the manufacturer. Within these limits, contaminants which may have been present were not expected to compromise the purpose of this study. Results of the dietary analyses (Certificates of Analysis) are provided by the manufacturer for each lot of diet. These are maintained by SLI.

8.5.4. Water

Municipal tap water treated by reverse osmosis was available ad libitum throughout the study (except during the time that the animals were acclimated to the exposure tubes and maintained in the inhalation room for the exposure procedure). The purified water was supplied by an automatic watering system. Monitoring of the drinking water for contaminants is conducted by SLI and the records are available for inspection. Within generally accepted limits, contaminants which may have been present were not expected to compromise the purpose of this study. The water meets the standards specified under the EPA National Drinking Water Regulations (40 CFR Part 141).

8.5.5. Acclimation

Upon receipt, the animals were removed randomly from the shipping cartons, examined by qualified personnel, identified with metal ear tags and then acclimated to the laboratory conditions for a minimum of five days. The animals were observed daily for overt physical or behavioral abnormalities, general health/moribundity and mortality.

8.5.6. Animal Selection

The animals chosen for study use were randomly selected from healthy stock animals using a computerized (Alpha DS-10 AcuTox) random numbers table to avoid potential bias. All animals received a detailed pretest observation prior to dosing. Only healthy animals were chosen for study use. Females were nulliparous and nonpregnant. The male animals were approximately 10 weeks of age and weighed 248-293 g on the day of exposure. The female animals were approximately 10 weeks of age and weighed 170-190 g on the day of exposure.

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9. EXPERIMENTAL PROCEDURES

9.1. Preliminary Procedures

9.1.1. Test Article Volatility Determination

The volatility of the test article relative to a distilled water standard was determined prior to experimental initiation. This procedure was performed in order to determine if the test article had sufficiently low volatility to allow for an accurate gravimetric determination of the aerosol concentration. A known quantity of the test article was placed on a preweighed filter disk and was allowed to evaporate for a total of ten minutes. The test article weight was determined each minute and the amount of evaporation of the test article was then determined. The results of this volatility trial indicated that the test article evaporation rate (0.45 mg/minute) was comparable to the SLI determined distilled water evaporation rate (0.55 mg/minute); therefore, was considered to not be volatile.

9.1.2. Preliminary Aerosol Generation Trials

Prior to experimental initiation, preliminary aerosol generation trials were conducted. These trials were performed in order to determine the most efficient means of generating an aerosol of the appropriate concentration while utilizing equipment that would reduce the aerodynamic particle size. Data obtained during the preliminary aerosol generation trials are presented in Appendix A.

9.2. Limit Test

9.2.1. Aerosol Generation Equipment

The test aerosol was generated with a Pistol Spraying System. Conditioned high pressure external air was used in generating the test atmosphere. The aerosol was blown through the 5L Elutriator, the nose-only inhalation chamber and then vented from the chamber to an air treatment system which consisted of a prefilter, a HEPA filter, a charcoal bed and a water scrubbing tower (see Figure 1).

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9.2.2. Dosing

On day 0, the animals chosen for the limit test were weighed, placed in a nose-only exposure tube and allowed to acclimate to the exposure tube for at least 1 hour. Animals that appeared to have been acclimated to the exposure tube (i.e., minimal struggling and no inversion) were considered to be acceptable and removed from the exposure tube and returned to their cages until initiation of the aerosol exposure. Animals that did not appear to acclimate to the exposure tube were not acceptable and were removed from the exposure tube and returned to their cages.

The acceptable animals were then placed in exposure tubes and the tubes inserted into the Multistage 10L nose-only inhalation chamber and the test article aerosolized at the following level:

Exposure Level (mg/L)	No. of Animals	
	Male	Female
3.27	5	5

The aerosol exposure consisted of a 3-minute T99 equilibration period, a 240-minute exposure period and a 3-minute de-equilibration period equal to the T99 equilibration period. After each aerosol exposure, animals were removed from the exposure tubes and residual test article was removed from the animal's exterior surfaces (where practical) by wiping the haircoat with a towel. The animals were then returned to ad libitum feed and water. The following parameters were measured during the exposure.

9.2.2.1. Chamber Air Flow

Air flow readings were recorded at the initiation of the T99 equilibration period, at approximate 30-minute intervals during the aerosol exposure and at the conclusion of the de-equilibration period.

9.2.2.2. Aerosol Concentration

The aerosol concentration was measured at the beginning of the aerosol exposure (after equilibration), at approximate 30-minute intervals during the aerosol exposure and at the conclusion of the aerosol exposure (before de-equilibration). The concentration of the test article aerosol was collected in the inhalation chamber by gravimetric technique. A 5 L sample of the aerosol was drawn from the breathing zone of the chamber through a preweighed glass fiber filter. The change in weight of the filter (mg) was then determined and this value was divided by the volume of chamber atmosphere sampled (L) to yield the gravimetric concentration (mg/L). The average time-weighted gravimetric

concentration of the test atmosphere was then calculated for the exposure. For the analytical concentration, the gravimetrically obtained samples were analyzed by Springborn Laboratories, Inc. for the glyphosate component, a non-volatile component of the test article. These analyses were performed in order to determine the analytical (actual) concentrations of the aerosol in the chamber for each sampling period. The average time weighted analytical concentration of the test atmosphere was then calculated for the exposure. Chemistry methods and results are detailed in the Analytical Chemistry Report (Appendix B).

9.2.2.3. Chamber Temperature and Humidity

The chamber temperature and humidity were measured electronically and recorded at approximate 30-minute intervals during the aerosol exposure.

9.2.2.4. Aerosol Aerodynamic Particle-Size Distribution

The aerosol aerodynamic particle-size distribution was determined three times during the aerosol exposure using the ITP 7 Stage Cascade Impactor. Each stage of the impactor was fitted with a preweighed glass fiber filter. Five liters per minute of the chamber air were drawn through the impactor and the change in weight of each filter was then determined and recorded. The mean particle-size distribution was subsequently plotted using an Excel computer adaptation of the manual method. The Mass Median Aerodynamic Diameter, Geometric Standard Deviation and percentage of particles $\leq$ 4.0 μ were then determined. At least one hour passed between each aerosol particle-size analysis.

9.2.2.5. Chamber Oxygen

Chamber oxygen content was measured and recorded at approximate 30-minute intervals during the aerosol exposure.

9.2.3. Clinical Observations

The limit test animals were observed for clinical abnormalities during each aerosol exposure, two times on study day 0 (post-exposure) and daily thereafter (days 1-14). A general health/mortality check was performed twice daily (in the morning and in the afternoon).

9.2.4. Body Weights

Individual body weights were obtained for the limit test animals prior to dosing on day 0 and on days 7 and 14.

9.2.5. Gross Necropsy

All limit test animals were euthanized by carbon dioxide inhalation at study termination (day 14) and necropsied. Body cavities (cranial, thoracic, abdominal and pelvic) were opened and examined. No tissues were retained.

9.3. Protocol Deviations

No protocol deviations occurred during this study.

10. ANALYSIS OF DATA

Data from the limit tests were analyzed and an LC50 value estimated as follows:

< 50% Mortality: LC50 was estimated as greater than the administered dose.
= 50% Mortality: LC50 was estimated as equal to the administered dose.
> 50% Mortality: LC50 was estimated as less than the administered dose.

Body weight means and standard deviations were calculated separately for males and females. The aerodynamic particle-size distribution of the test article aerosol was plotted using an Excel computer adaptation of the three cycle logarithmic probability paper as per the ITP Cascade Impactor instruction manual. The Mass Median Aerodynamic Diameter, Geometric Standard Deviation and particles $\leq$ 4.0 µ was determined based on the plotted distribution.

11. MAINTENANCE OF RAW DATA AND RECORDS

All original raw data, the final report and magnetically encoded records were transferred to the SLI archives for a period of 10 years. The Sponsor will be contacted prior to final disposition of these items.

12. RESULTS

12.1. Aerosol Generation and Chamber Environmental Data

12.1.1. Aerosol Generation Data

Individual Data: Table 1

The average time-weighted analytical concentration for the aerosol exposure was determined to be 3.27 mg/L. The mass median aerodynamic diameter and geometric standard deviation of the sampled particles were 2.6 µ ± 1.8. The percentage of particles $\leq$ 4.0 µ was determined to be 77%.

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12.1.2. Chamber Environmental Data

Individual Data: Table 1

Chamber temperature and relative humidity for the aerosol exposure ranged from 72.6-73.7°F and 65.7-69.3%, respectively. Oxygen content was maintained at 20.9% throughout the exposure.

12.2. Limit Test Data

12.2.1. Mortality

Individual Data: Table 2

No mortality occurred during this study.

12.2.2. Clinical Observations

Individual Data: Table 2

The most notable clinical abnormalities observed during the study included decreased/no defecation, soft stools, feces small in size, decreased food consumption and rough coat. Clinical abnormalities also observed during the study included transient incidences of breathing abnormalities and dark material around the facial area, which were findings consistent with dosing an inhalation study. No positive findings were noted at the time of observation during the 4-hour exposure period.

In addition, the dose level actually conducted was significantly higher (3.27 mg/kg) than the required dose level (2.0 mg/L) and did not result in any mortality.

12.2.3. Body Weight Data

Individual Data: Table 3

Body weight loss was noted for one male and one female during the study day 0-7 body weight interval. Body weight gain was noted for all other animals during the test period. All animals exceeded their initial body weight by study termination (day 14).

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12.2.4. Gross Necropsy

Individual Data: Table 4

No significant gross internal findings were observed at necropsy on study day 14. One animal was observed to have a thin area of the diaphragm which was not considered to be test article-related.

13. CONCLUSION

Under the conditions of this test, the acute inhalation LC50 of Spray–Alpha was estimated to be greater than 3.27 mg/L in the rat.

Kimberly L. Bonnette, M.S., LATG
Study Director

Date 9/3/02

14. REPORT REVIEW

Dawn D. Rodabaugh, B.S.
Associate Toxicologist

Date 9/3/02

15. REFERENCE

1. Guide for the Care and Use of Laboratory Animals, DHHS Publication No. (NIH) 96-03, 1996.

TABLE 1

SLI STUDY NO.: 3596.4 AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS

CLIENT: INL/A,US DEPARTMENT OF STATE SUMMARY OF AEROSOL GENERATION AND CHAMBER ENVIRONMENTAL DATA

	EXPOSURE LEVEL (MG/L)
	3.27
CHAMBER AND EXPOSURE DATA	
CHAMBER VOLUME (L):	10
ELUTRIATOR VOLUME (L):	5
MEAN AIR FLOW RATE (L/MIN):	24
MEAN AIR CHANGES PER HOUR:	95.24
T99 EQUILIBRATION PERIOD (MIN.):	3
EXPOSURE TIME (MIN):	240
DE-EQUILIBRATION PERIOD (MIN):	3
AEROSOL CONCENTRATIONS	
CALCULATED NOMINAL CONCENTRATION (MG/L):	113.68
TIME-WEIGHTED MEAN ANALYTICAL CONCENTRATION (MG/L):	3.27
AEROSOL PARTICLE-SIZE ANALYSIS	
MASS MEDIAN AERODYNAMIC DIAMETER (μ):	2.6
GEOMETRIC STANDARD DEVIATION:	± 1.8
PERCENTAGE OF PARTICLES ≤ 4.0 μ (%):	77
CHAMBER ENVIRONMENTAL DATA	
TEMPERATURE RANGE (°F):	72.6-73.7
HUMIDITY RANGE (%):	65.7-69.3
OXYGEN CONTENT (%):	20.9

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INL/A, US DEPARTMENT OF STATE

TABLE 2

AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS

INDIVIDUAL CLINICAL OBSERVATIONS
(POSITIVE FINDINGS)

MALES 3.27 MG/L

MALE#	OBSERVATIONS	DAY OF STUDY 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A5241	SCHEDULED EUTHANASIA															P
	CONGESTED BREATHING			P							P	P				
	FEW FECES		P													
	SOFT STOOLS											P				
	DARK MATERIAL AROUND NOSE		P													
	DECREASED FOOD CONSUMPTION		P													
A5253	SCHEDULED EUTHANASIA															P
	RALES										P	P				
	CONGESTED BREATHING		P	P	P	P	P	P	P	P	P	P	P	P		
	LABORED BREATHING		P	P				P	P	P						
	FEW FECES		P		P						P	P				
	NO FECES			P												
	DARK MATERIAL AROUND NOSE		P													
	DARK MATERIAL AROUND MOUTH		P													
	NASAL DISCHARGE CLEAR		P													
	DECREASED FOOD CONSUMPTION		P	P	P											
A5252	SCHEDULED EUTHANASIA															P
	CONGESTED BREATHING						P									
	FEW FECES		P													
	DECREASED FOOD CONSUMPTION		P													
A5254	SCHEDULED EUTHANASIA															P
	RALES							P	P	P						
	CONGESTED BREATHING		P	P	P	P		P	P	P	P	P				
	LABORED BREATHING							P								
	FEW FECES		P	P												
	SOFT STOOLS			P												

GRADE CODE: 1=SLIGHT 2=MODERATE 3=SEVERE P=PRESENT L=LEFT R=RIGHT B=BILATERAL

TABLE 2

AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS

INDIVIDUAL CLINICAL OBSERVATIONS
(POSITIVE FINDINGS)

MALES 3.27 MG/L

		DAY OF STUDY														
MALE#	OBSERVATIONS	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A5254	(CONTINUED)															
	ROUGH COAT		P													
	DARK MATERIAL AROUND NOSE		P													
	DECREASED FOOD CONSUMPTION		P													
A5257	SCHEDULED EUTHANASIA															P
	CONGESTED BREATHING		P			P	P	P			P	P				
	LABORED BREATHING		P					P								
	FEW FECES		P													

GRADE CODE: 1=SLIGHT 2=MODERATE 3=SEVERE P=PRESENT L=LEFT R=RIGHT B=BILATERAL

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TABLE 2

AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS

INDIVIDUAL CLINICAL OBSERVATIONS
(POSITIVE FINDINGS)

FEMALES 3.27 MG/L

		DAY OF STUDY													
FEMALE#	OBSERVATIONS	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A5279	SCHEDULED EUTHANASIA															P
	RALES							P	P	P						
	CONGESTED BREATHING			P	P	P	P	P	P	P	P	P				
	LABORED BREATHING		P													
	FEW FECES		P	P	P											
	FECES SMALL IN SIZE			P												
	ROUGH COAT		P													
	DARK MATERIAL AROUND NOSE		P													
	DARK MATERIAL AROUND MOUTH		P													
	DECREASED FOOD CONSUMPTION		P		P											
A5284	SCHEDULED EUTHANASIA															P
	CONGESTED BREATHING		P	P	P	P	P	P	P	P	P	P	P	P		
	LABORED BREATHING		P													
	GASPING		P													
	FEW FECES		P	P	P											
	FECES SMALL IN SIZE			P												
	ROUGH COAT		P	P												
	DARK MATERIAL AROUND EYE(S)		P													
	DARK MATERIAL AROUND NOSE		P													
	DARK MATERIAL AROUND MOUTH		P													
	DECREASED FOOD CONSUMPTION		P	P												
A5283	SCHEDULED EUTHANASIA															P
	CONGESTED BREATHING				P	P										
	FEW FECES		P	P												
	FECES SMALL IN SIZE				P											
	DECREASED FOOD CONSUMPTION			P												

GRADE CODE: 1=SLIGHT 2=MODERATE 3=SEVERE P=PRESENT L=LEFT R=RIGHT B=BILATERAL

TABLE 2

AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS

INDIVIDUAL CLINICAL OBSERVATIONS
(POSITIVE FINDINGS)

FEMALES 3.27 MG/L

		DAY OF STUDY													
FEMALE#	OBSERVATIONS	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A5281	SCHEDULED EUTHANASIA															P
	RALES		P													
	CONGESTED BREATHING		P	P												
	LABORED BREATHING		P	P												
	FEW FECES		P	P	P											
	URINE STAIN			1												
	DARK MATERIAL AROUND EYE(S)			P												
	DARK MATERIAL AROUND NOSE			P												
	DARK MATERIAL AROUND MOUTH			P												
	DECREASED FOOD CONSUMPTION		P	P	P											
A5282	SCHEDULED EUTHANASIA															P
	RALES		P													
	CONGESTED BREATHING		P					P								
	LABORED BREATHING		P													
	GASPING		P													
	FEW FECES		P	P	P											
	DARK MATERIAL AROUND EYE(S)			P												
	DECREASED FOOD CONSUMPTION		P	P	P											

GRADE CODE: 1=SLIGHT 2=MODERATE 3=SEVERE P=PRESENT L=LEFT R=RIGHT B=BILATERAL

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TABLE 3

AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS

INDIVIDUAL BODY WEIGHTS (GRAMS)

MALES 3.27 MG/L

	DAY OF STUDY			
ANIMAL#	0	7	14	AT DEATH (DAY)
A5241	293	304	339	
A5253	286	264	292	
A5252	248	253	277	
A5254	265	269	306	
A5257	257	268	294	
MEAN	270	272	302	
S.D.	19.1	19.2	23.3	
N	5	5	5	

TABLE 3

AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS

INDIVIDUAL BODY WEIGHTS (GRAMS)

FEMALES 3.27 MG/L

ANIMAL#	DAY OF STUDY 0	7	14	AT DEATH (DAY)
A5279	171	176	193	
A5284	190	194	212	
A5283	172	180	195	
A5281	188	185	206	
A5282	170	180	191	
MEAN	178	183	199	
S.D.	9.9	6.9	9.1	
N	5	5	5	

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TABLE 4

AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS

INDIVIDUAL GROSS NECROPSY OBSERVATIONS

MALES 3.27 MG/L

ANIMAL#	DAY OF DEATH	STUDY DAY	OBSERVATION	FATE
A5241	20-JUN-02	14	DIAPHRAGM: THIN AREA(S); PRESENT TENDINOUS PORTION, ONE, 0.7 X 0.5 CM DIAMETER, PORTION OF MEDIAL LIVER LOBE MISSHAPEN AND EXTENDS INTO THIN AREA	SCHEDULED EUTHANASIA
A5253	20-JUN-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A5252	20-JUN-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A5254	20-JUN-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A5257	20-JUN-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA

TABLE 4

AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS

INDIVIDUAL GROSS NECROPSY OBSERVATIONS

FEMALES 3.27 MG/L

ANIMAL#	DAY OF DEATH	STUDY DAY	OBSERVATION	FATE
A5279	20-JUN-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A5284	20-JUN-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A5283	20-JUN-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A5281	20-JUN-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A5282	20-JUN-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA

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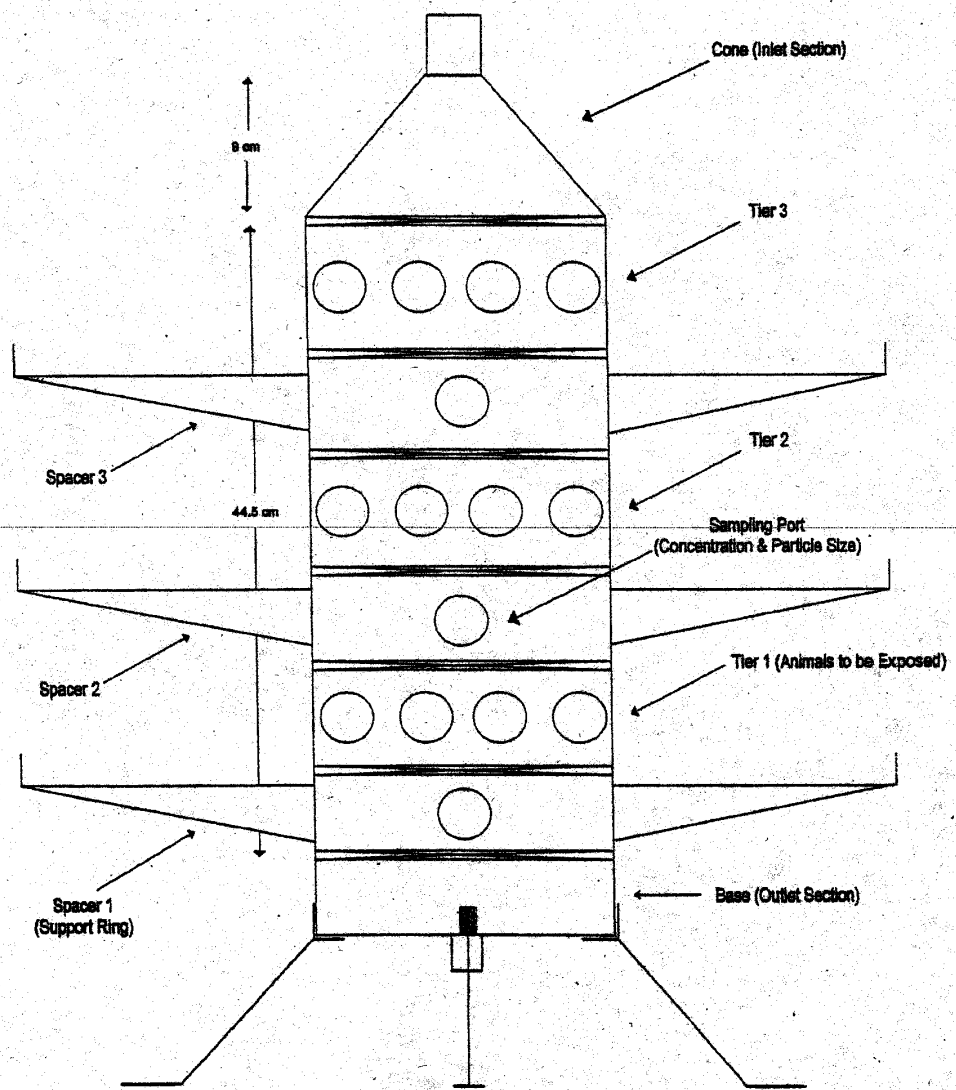

MULTI-STAGE 10 L NOSE ONLY INHALATION CHAMBER

Figure 1

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APPENDIX A

Preliminary Aerosol Generation Trials

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1. PRELIMINARY AEROSOL GENERATION TRIALS

Prior to experimental initiation, preliminary aerosol generation trials were conducted. These procedures were performed in order to determine the most efficient means of generating an aerosol of the test article. The type of equipment used during each aerosol trial procedure is presented in Trial Table 1. In each trial, attempts were made to generate the highest concentration of the test article while utilizing equipment that would minimize the aerodynamic particle size of the aerosol.

The results indicated that the equipment design/pump speed utilized during Trial #7 produced an analytical aerosol concentration $\geq$ 2.00 mg/L. Using the equipment design determined by the aerosol generation trials, the aerosol aerodynamic particle-size distribution was then determined utilizing the ITP 7 Stage Cascade Impactor. The aerodynamic particle size was acceptable. Therefore, this equipment design was used for the LC50 study exposure.

TRIAL NO.	EQUIPMENT USED	INPUT AIR (PSI)	TEST ARTICLE CONCEN-TRATION (%)	MAXIMUM ATTAINABLE CONCENTRATIONS (MG/L)	
				GRAVIMETRIC	ANALYTICAL
1	One Multistage 10L Nose-Only Chamber 5L Elutriator Master Flex Pump and Pump Heads 7523-30 and 77200-60 Spraying Systems, Pistol Air/Fluid Mixing Nozzle 4.0 mL/min pump speed 16 gauge tubing size	30	100	2.10	3.71
2	One Multistage 10L Nose-Only Chamber 5L Elutriator Master Flex Pump and Pump Heads 7523-30 and 77200-60 Spraying Systems, Pistol Air/Fluid Mixing Nozzle 4.0 mL/min pump speed 16 gauge tubing size	30	100	2.02	4.132
3	One Multistage 10L Nose-Only Chamber 5L Elutriator Master Flex Pump and Pump Heads 7523-30 and 77200-60 Spraying Systems, Pistol Air/Fluid Mixing Nozzle 0.8 mL/min pump speed 14 gauge tubing size	30	100	0.82	–
4	One Multistage 10L Nose-Only Chamber 5L Elutriator Master Flex Pump and Pump Heads 7523-30 and 77200-60 Spraying Systems, Pistol Air/Fluid Mixing Nozzle 0.5 mL/min pump speed 14 gauge tubing size	30	100	0.50	1.20

Note: Targeting ≥ 2.00 mg/L gravimetric concentration for Trials 1-2; ≥ 1.00 mg/L gravimetric concentration for Trial 3 and ≥ 0.50 mg/L gravimetric concentration for Trial 4.

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SLI STUDY NO.: 3596.4 TRIAL TABLE 1 PAGE 2
CLIENT: INL/A, US DEPARTMENT OF STATE PRELIMINARY AEROSOL GENERATION TRIALS

TRIAL NO.	EQUIPMENT USED	INPUT AIR (PSI)	TEST ARTICLE CONCEN-TRATION (%)	MAXIMUM ATTAINABLE CONCENTRATIONS (MG/L)	
				GRAVIMETRIC	ANALYTICAL
5	One Multistage 10L Nose-Only Chamber 5L Elutriator Master Flex Pump and Pump Heads 7523-30 and 77200-60 Spraying Systems, Pistol Air/Fluid Mixing Nozzle 0.5 mL/min pump speed 14 gauge tubing size	30	100	0.50	1.16
6	One Multistage 10L Nose-Only Chamber 5L Elutriator Master Flex Pump and Pump Heads 7523-30 and 77200-60 Spraying Systems, Pistol Air/Fluid Mixing Nozzle 1.0 mL/min pump speed 14 gauge tubing size	30	100	0.84	--
7	One Multistage 10L Nose-Only Chamber 5L Elutriator Master Flex Pump and Pump Heads 7523-30 and 77200-60 Spraying Systems, Pistol Air/Fluid Mixing Nozzle 2.5 mL/min pump speed 14 gauge tubing size	30	100	1.48	--

Note: Targeting ≥ 0.50 mg/L gravimetric concentration for Trial 5; ≥ 1.00 mg/L gravimetric concentration for Trial 6 and ≥ 1.50 mg/L gravimetric concentration for Trial 7.

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APPENDIX B

Analytical Chemistry Report

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1. SPRAY--ALPHA ANALYSIS

The analytical method for the analysis of the glyphosate component of Spray--Alpha was validated prior to the analytical chamber concentration analyses performed at Springborn Laboratories, Inc. This method was utilized to determine the inhalation chamber concentration during the Acute Nose -Only Inhalation Toxicity Study.

1.1. Experimental System

1.1.1. HPLC System

HPLC Model: Waters
Pump: Waters 600E
Injector: Waters WISP 717
Detector: Waters 2487
Data System: H-P 3396B Integrator
Precolumn: Phenomenex, SecurityGuard, C18, 4.0 x 3.0 mm ID
Column: Phenomenex, Spherex, C18, 5μ, 250 x 4.6 mm ID
Temperature: Ambient
Detection: 500 nm, 0.4000 AUFS
Mobile Phase: A: 0.05 M HCO_2NH_4, pH 3.6/5% ACN; B: 100% ACN
Gradient: 100% A hold for 6 minutes; linear change to 25% A/75% B over 1 minute; hold for 5 minutes; linear change to 100% A over 1 minute; hold at 100% A for 15 minutes.
Flow Rate: 1.0 mL/min
Injection Volume: 10 μL

1.1.2. Apparatus

Balance: Mettler AG 245, accuracy of 0.0001 gram
Glassware: Assorted volumetric glassware
Filters: Gelman, glass fiber; Millipore 0.2μ Nylon-66; Whatman Puradisc 25PP 0.45μm
Shaker: Labline, Multi-Wrist Shaker
Oven: Boekel Model 107905

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1.1.3. Solutions and Reagents

1.1.3.1. Reagents

Water, Fisher, HPLC Grade, Lot # 024948
Acetonitrile, Fisher, HPLC Grade, Lot # 011777
Methanol, Fisher, HPLC Grade, Lot # 011803
NBD Chloride, Aldrich, 98%, Lot #12214L1
Hydrochloric Acid, Fisher, ACS Grade, Lot # 012161
Potassium Tetraborate Tetrahydrate:, Aldrich, 99%, Lot # 15325D1
Formic Acid, Fisher, Laboratory Grade, Lot # 003630
Ammonium Formate, Fisher, Lot # 990125

1.1.3.2 Solutions

0.37 M Borate Solution: Prepared by dissolving approximately 11.44 g of potassium tetraborate tetrahydrate in 100 mL of water. The resulting solution was stable for 6 months under ambient storage conditions.

1.2 N HCl: Prepared by dissolving 10 mL of HCl in 90 mL of water. The resulting solution was stable for 6 months under ambient storage conditions.

25 mM NBD-Cl: Prepared by dissolving approximately 2.5 g of NBD-Cl in 500 mL of methanol. The resulting solution was stable for 6 months under ambient storage conditions.

Mobile Phase A: Prepared by dissolving approximately 1.58 g of ammonium formate in 950 mL of water. The pH was adjusted to approximately 3.6 with formic acid. Added 50 ml of acetonitrile. The resulting solution was mixed thoroughly, filtered through a 0.2μ Nylon-66 filter and degassed by helium sparging prior to use.

Mobile Phase B: Acetonitrile used 100% as received.

Diluent: All standards and samples were diluted in water.

Stock Standard Solution (Trial- mg/L): Prepared by dissolving 101.9 mg of the Spray--Alpha formulation in a 25 mL flask with diluent.

Stock Standard Solution (Exposure #1- mg/L): Prepared by dissolving 236.0 mg of Spray--Alpha formulation in a 25 mL flask with diluent.

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Standard Solutions: Prepared by serially diluting the stock standard solution with water. The final concentrations of the solutions were in the range of approximately 0.4 to 2.9 mg/mL (trial) and 0.9 to 4.7 mg/mL (Exposure # 1). These solutions were then further diluted in diluent at a ratio of 1:10 and filtered through Whatman Puradisc 25PP 0.45μm filters prior to derivatization.

Chamber Concentration Solutions: Prepared by placing the weighed glass fiber filter used for gravimetric concentration determination in a capped container with 10 mL of diluent. The solutions were then agitated mechanically for 5 minutes further diluted in diluent at a ratio of 1:10 and filtered through Whatman Puradisc 25PP 0.45 μm filters prior to derivatization.

Derivatization Procedure: In order to analyze the glyphosate component, a precolumn derivatization was performed by adding 1.2 mL of the appropriate control, standard, or sample solution to a labeled scintillation vial. Both 0.8 mL of the borate solution and 2.4 mL of the NBD-Cl solution were added to each vial. The vials were then capped and shaken by hand prior to being heated in an oven at 80° C for 30 minutes. After removal from the oven, the vials were allowed to cool for 10 minutes followed by the addition of 0.9 mL of the HCl solution. After the vials were again shaken by hand, they were allowed to stand for 10 minutes in order for incipient precipitation to occur. These solutions were then transferred to injection vials.

1.2. Analytical Procedures

1.2.1. Standard Curve Analysis

The peak area of the glyphosate acid component of each standard were determined, measured, combined, and plotted as a function of concentration to generate a standard curve. The actual values used for the calculations are shown in Chemistry Tables 1 and 2.

1.2.2. Sample Analysis

The peak areas of the glyphosate acid component of each sample were measured and combined and then the concentration was determined by linear fit to the standard curve. The actual values used for the calculations are shown in Chemistry Tables 1 and 2.

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1.3. Results and Conclusions

1.3.1. Analytical Chamber Concentration

The actual sample results of the trial work are shown in Chemistry Table 1. The actual sample results of the analytical chamber analysis are shown in Chemistry Table 2.

_____Date _____

M. Gardner Clemons, B.A.
Manager of Analytical Chemistry
And Pharmacy.

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Chemistry Table 1

Standard Curve and Sample Analysis Values for Trial Work

Sample No.	Theoretical Conc. (mg/L)	Peak Area	Analytical Chamber Conc. (mg/L)
Std 1	0.8152	25090	NA
Std 2	2.446	77738	NA
Std 3	4.076	131263	NA
Std 4	5.706	182542	NA
Trial # 1	NA	118551	3.707
Trial # 2	NA	132259	4.132
Trial # 4	NA	37811	1.204
Trial #5	NA	36312	1.158

* Correlation coefficient = 0.99997

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Chemistry Table 2

Standard Curve and Sample Analysis Values for Exposure #1

Sample No.	Theoretical Conc. (mg/L)	Peak Area	Analytical Chamber Conc. (mg/L)
Std 1	1.888	47622	NA
Std 2	3.776	114022	NA
Std 3	5.664	169206	NA
Std 4	7.552	225528	NA
Std 5	9.440	251583	NA
# 1	NA	111887	3.857
# 2	NA	107931	3.714
# 3	NA	90648	3.085
# 4	NA	93185	3.178
# 5	NA	92333	3.147
# 6	NA	89526	3.045
# 7	NA	94131	3.212
# 8	NA	97391	3.330
# 9	NA	91642	3.121
#10	NA	102623	3.521
#11	NA	100109	3.429

* Correlation coefficient = 0.991

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APPENDIX C

Individual Aerosol Generation and
Chamber Environmental Data

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3.27 mg/L Exposure Level

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AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS
CHAMBER ENVIRONMENTAL DATA
EXPOSURE: 3.27 MG/L

TIME (MIN.)	TEMPERATURE (°F)	RELATIVE HUMIDITY (%)	OXYGEN CONTENT (%)
0	72.6	69.3	20.9
30	72.8	65.7	20.9
60	72.7	67.6	20.9
90	72.9	68.0	20.9
120	73.4	66.7	20.9
150	73.1	67.5	20.9
180	73.5	67.6	20.9
210	73.5	67.7	20.9
240	73.7	67.3	20.9

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AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS
TIME WEIGHTED ANALYTICAL CONCENTRATION
ANALYTICAL EXPOSURE: 3.27 MG/L

Sample No.	Sample Time (min.)	Aerosol Concentration (mg/L)	Mean Concentration Per Interval (mg/L)	Interval Length (min.)	Time Weighted Concentration Per Interval
1	0	3.86			
			3.79	14.00	52.99
2	14	3.71			
			3.40	7.00	23.80
3	21	3.09			
			3.14	9.00	28.22
4	30	3.18			
			3.17	30.00	94.95
5	60	3.15			
			3.10	30.00	93.00
6	90	3.05			
			3.13	30.00	93.90
7	120	3.21			
			3.27	30.00	98.10
8	150	3.33			
			3.23	30.00	96.75
9	180	3.12			
			3.32	30.00	99.60
10	210	3.52			
			3.47	30.00	104.10
11	240	3.42			
TOTAL				240.00	785.41
TIME WEIGHTED MEAN ANALYTICAL CONCENTRATION (MG/L)					3.27

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AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS
AERODYNAMIC PARTICLE SIZE DATA
SAMPLE NO.: A
ANALYTICAL EXPOSURE: 3.27 MG/L

Stage	Effective Cutoff Diameter	Filter Weights (mg) Pre-sample	Filter Weights (mg) Post-sample	Difference Weights	% of Total	Cumulative % <ECD
1	10.00	102.4	102.4	0.0	0.0	100.0
2	6.11	102.7	102.9	0.2	5.4	94.6
3	3.70	102.7	103.3	0.6	16.2	78.4
4	2.22	103.2	104.7	1.5	40.5	37.8
5	1.39	103.6	104.6	1.0	27.0	10.8
6	0.79	104.4	104.8	0.4	10.8	0.0
7	0.50	103.4	103.4	0.0	0.0	0.0
Filter	-	102.6	102.6	0.0	0.0	
		Total of Difference Weights:		3.7		

Mass Median Aerodynamic Diameter = 2.6 microns
Geometric Standard Deviation = 1.67
Percentage ≤ 4.0 microns = 80 %

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SLI Study No. 3596.4

AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS
AERODYNAMIC PARTICLE SIZE DATA
SAMPLE NO.: B
ANALYTICAL EXPOSURE: 3.27 MG/L

Stage	Effective Cutoff Diameter	Filter Weights (mg) Pre-sample	Filter Weights (mg) Post-sample	Difference Weights	% of Total	Cumulative % <ECD
1	10.00	103.0	103.0	0.0	0.0	100.0
2	6.11	103.5	103.9	0.4	8.7	91.3
3	3.70	103.1	104.0	0.9	19.6	71.7
4	2.22	103.8	105.5	1.7	37.0	34.8
5	1.39	103.3	104.4	1.1	23.9	10.9
6	0.79	103.5	103.8	0.3	6.5	4.3
7	0.50	102.7	102.8	0.1	2.2	2.2
Filter	-	103.1	103.2	0.1	2.2	
		Total of Difference Weights:		4.6		

Mass Median Aerodynamic Diameter = 2.6 microns
Geometric Standard Deviation = 2.00
Percentage ≤ 4.0 microns = 74 %

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SLI Study No. 3596.4

AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS
AERODYNAMIC PARTICLE SIZE DATA
SAMPLE NO.: C
ANALYTICAL EXPOSURE: 3.27 MG/L

Stage	Effective Cutoff Diameter	Filter Weights (mg) Pre-sample	Filter Weights (mg) Post-sample	Difference Weights	% of Total	Cumulative % <ECD
1	10.00	103.4	103.4	0.0	0.0	100.0
2	6.11	103.5	103.8	0.3	7.5	92.5
3	3.70	103.3	104.1	0.8	20.0	72.5
4	2.22	103.7	105.1	1.4	35.0	37.5
5	1.39	103.2	104.1	0.9	22.5	15.0
6	0.79	103.4	103.9	0.5	12.5	2.5
7	0.50	103.3	103.4	0.1	2.5	0.0
Filter	-	104.2	104.2	0.0	0.0	
		Total of Difference Weights:		4.0		

Mass Median Aerodynamic Diameter = 2.6 microns
Geometric Standard Deviation = 1.82
Percentage ≤ 4.0 microns = 76 %

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SLI Study No. 3596.4

AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS
AERODYNAMIC PARTICLE SIZE DATA

ANALYTICAL EXPOSURE: 3.27 MG/L

Stage	Effective Cutoff Diameter	Cumulative % less than indicated size Sample A	Sample B	Sample C	
1	10.00	100.0	100.0	100.0	
2	6.11	94.6	91.3	92.5	
3	3.70	78.4	71.7	72.5	
4	2.22	37.8	34.8	37.5	
5	1.39	10.8	10.9	15.0	
6	0.79	0.0	4.3	2.5	
7	0.50	0.0	2.2	0.0	
					Mean
Mass Median Aerodynamic Diameter		2.6	2.6	2.6	2.6
Geometric Standard Deviation		1.67	2.00	1.82	1.83
Percentage ≤ 4.0 microns		80	74	76	77

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APPENDIX D

SLI Personnel Responsibilities

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SLI Study No. 3596.4

SLI PERSONNEL RESPONSIBILITIES

Kimberly L. Bonnette, M.S., LAGT	Study Director/Director, Acute Toxicologist
Dawn D. Rodabaugh, B.S.	Alternate Contact/Associate Toxicologist
Robert C. Springborn, Ph.D.	Chairman, President and CEO
Malcolm Blair, Ph.D.	Senior Vice President, Managing Director Emeritus
Joseph C. Siglin, Ph.D., DABT	Vice President, Managing Director
M. Gardner Clemons, B.A.	Manager of Analytical Chemistry and Pharmacy
Pamela S. Smith, ALAT	Supervisor of Acute Toxicology
Kevin V. Weitzel, A.S.	Primary Technician/Inhalation Team Leader
Delores P. Knippen	Supervisor of Pharmacy
Steven H. Magness, B.S., LATG	Senior Supervisor of Gross and Fetal Pathology
Anita M. Bosau, RQAP-GLP	Senior Director, Compliance Assurance
Deanna M. Talerico, RQAP-GLP	Senior Supervisor of Quality Assurance
J. Dale Thurman, D.V.M., M.S., DACVP	Senior Director, Pathology
Kathy M. Gasser	Supervisor of Archives

A DERMAL SENSITIZATION STUDY IN GUINEA PIGS WITH SPRAY--ALPHA MODIFIED BUEHLER DESIGN

FINAL REPORT

OPPTS Guidelines

870.2600

Author

Kimberly L. Bonnette, M.S., LATG

Study Completed on

September 3, 2002

Performing Laboratory

Springborn Laboratories, Inc. (SLI)
Ohio Research Center
640 North Elizabeth Street
Spencerville, Ohio 45887

SLI Study No.

3596.7

Submitted to

INL/A
U.S. Department of State
2201 C St. NW SA-4
Washington, DC 20520

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SLI Study No. 3596.7

1. STATEMENT OF *NO* DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B), or (C).

Company: _____

Company Agent: _____ Date: _____

_____ _____

Title Signature

SLI Study No. 3596.7 (3)

2. COMPLIANCE STATEMENT

This study was conducted in compliance with the Good Laboratory Practice Standards as described by the EPA (40 CFR Parts 160 and 792) with the following exception:

The dose preparations used during the range-finding study were not analyzed to confirm test article concentration, stability or homogeneity.

Kimberly L. Bonnette, M.S., LATG
Study Director/Author
Springborn Laboratories, Inc.

Date 9/3/02

Rogers Woolfolk
Senior Aviation Advisor
Sponsor/Submitter
INL/A
U.S. Department of State

Date 09/03/02

SLI Study No. 3596.7

3. QUALITY ASSURANCE STATEMENT

This study was inspected by the Quality Assurance Unit and reports were submitted to management and the Study Director in accordance with SLI's Standard Operating Procedures as follows:

Phase	Date
Protocol Review	04/25/02
Animal Receipt	06/06/02
Dermal Observations	06/21/02
Data Audit	09/01/02
Draft Report Review	09/01/02
Protocol Amendment Review	09/01/02
Final Report Review	09/03/02
Reports to Study Director and Management	06/06/02, 09/01/02, 09/03/02

The final report has been reviewed to assure that it accurately describes the materials and methods, and the reported results accurately reflect the raw data.

Rebecca A. Young
Rebecca A. Young
Quality Assurance Team Leader

Date 9/3/02

for Deanna M Salerico
Anita M. Bosau, RQAP-GLP
Senior Director, Compliance Assurance

Date 9/3/02

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6. SUMMARY

The dermal sensitization potential of Spray--Alpha was evaluated in Hartley-derived albino guinea pigs. Ten male and ten female guinea pigs were topically treated with 100% Spray--Alpha, once per week, for three consecutive weeks. Following an approximate two-week rest period, a challenge was performed whereby the twenty test and ten previously untreated (naive) challenge control guinea pigs were topically treated with 100% Spray--Alpha. Challenge responses in the test animals were compared with those of the challenge control animals.

6.1. Spray--Alpha

Following challenge with 100% Spray--Alpha, dermal reactions in the test and challenge control animals were limited to scores of 0. Group mean dermal scores were noted to be the same in the test animals as compared with the challenge control animals.

6.2. HCA

Using α-Hexylcinnamaldehyde (HCA) as a positive control, Springborn Laboratories, Inc., Spencerville, Ohio, has completed a study during the past six months which provided historical control data for contact sensitization to this agent utilizing the test system described herein (Modified Buehler Design). Following induction at 5% w/v HCA in ethanol and challenge at levels of 2.5% and 1% w/v HCA in acetone, a contact sensitization response was observed, thereby demonstrating the susceptibility of the test system to this sensitizing agent.

Based on the results of this study, Spray--Alpha is not considered to be a contact sensitizer in guinea pigs. The results of the HCA historical control study demonstrated that a valid test was performed and indicated that the test design would detect potential contact sensitizers.

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7. INTRODUCTION

This study was performed to assess the dermal sensitization potential (delayed contact hypersensitivity) of Spray--Alpha in Hartley-derived albino guinea pigs when administered by multiple topical applications. This study was intended to provide information on the potential health hazards of the test article with respect to dermal exposure. Data from this study may serve as a basis for classification and/or labeling of the test article. This study was performed in accordance with the US EPA, Health Effects Test Guidelines, OPPTS 870.2600, Skin Sensitization, August 1998. This study was performed at Springborn Laboratories, Inc., 553 North Broadway, Spencerville, Ohio. The protocol was signed by the Study Director on April 30, 2002 (GLP initiation date). The in-life phase of the main sensitization study was initiated with test article administration on June 13, 2002 (day 0), and concluded with final scoring on July 12, 2002.

Prior to initiation of the main sensitization study, a topical range-finding study was conducted in guinea pigs to aid in the selection of dosage levels. The in-life phase of the range-finding study was initiated with test article administration on June 10, 2002, and concluded on June 12, 2002. The experimental methods and results of the range-finding study are included in Appendix A.

8. MATERIALS AND METHODS

8.1. Test Article

The test article was received from the Sponsor and identified as follows:

Sponsor's ID	Assigned SLI ID	Physical Description	Receipt Date	Expiration Date
Spray—Alpha[a]	S02.001.3596	Light amber liquid	05/13/02	None Provided
Ingredients[b]				
Herbicide: Fuete-SL Lot No.: 02-01-02				None Provided
Surfactant: Cosmo Flux-411F Lot No.: 244301				10/2003

[a]Sample pooled at SLI from five different mixes of Spray--Alpha (top/middle/bottom).
[b]Ingredients used in the five Spray--Alpha mixes that were prepared by the Sponsor.

The test article was stored at room temperature. The Sponsor was responsible for any necessary evaluations related to identity, strength, purity, composition,

stability and method of synthesis of the test material according to 40 CFR 160.105, 40 CFR 792.105. Springborn Laboratories, Inc., analyzed the test article for the glyphosate (a.e.) which is presented in SLI Study No. 3596.1.

8.2. Retention Sample

An approximate 1 mL retention sample of each test article mixture sample (top/middle/bottom, maintained separately for a total of fifteen 1 mL samples) was taken and stored at SLI at room temperature. In addition, a 10 mL retention sample of the pooled test article samples (from the 5 test article mixtures) was collected and stored at SLI at room temperature. These samples serve as the retention samples for all studies conducted with this material.

8.3. Test Article Disposition

The remaining test article was returned to the Sponsor following completion of all studies with the test article.

8.4. Method of Test Article Preparation

The test article was utilized at 100% (induction and challenge). The test article was dispensed fresh on each day of dosing.

8.5. Animals and Animal Husbandry

8.5.1. Description, Identification and Housing

Young adult, Hartley-derived albino guinea pigs were received from Hilltop Lab Animals, Inc., Scottdale, PA. Upon receipt, plastic ear tags displaying unique identification numbers were used to individually identify the animals. Cage cards displaying at least the study number, animal number and sex were affixed to each cage. The animals were housed individually in suspended stainless steel cages. All housing and care were based on the standards recommended by the Guide for the Care and Use of Laboratory Animals [1].

8.5.2. Environment

The animal room temperature and relative humidity ranges were 63-74°F (17-23°C) and 48-82%, respectively. Environmental control equipment was monitored and adjusted as necessary to minimize fluctuations in the animal room environment. Light timers were set to maintain a 12-hour light/12-hour dark cycle and room ventilation was set to produce 10-15 air changes/hour. The room temperature and relative humidity were recorded a minimum of once daily.

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8.5.3. Food

PMI Certified Guinea Pig Chow #5026 (Purina Mills, Inc.) was provided ad libitum to the animals throughout the study. The lot number and expiration date of each batch of diet used during the study were recorded. The feed was analyzed and certified by the supplier for nutritional components and environmental contaminants. Dietary limitations for various environmental contaminants, including heavy metals, pesticides, polychlorinated biphenyls and total aflatoxin are set by the manufacturer. Within these limits, contaminants which may have been present were not expected to compromise the purpose of this study. Results of the dietary analyses (Certificates of Analysis) are provided by the manufacturer for each lot of diet. These are maintained by SLI.

8.5.4. Water

Municipal tap water treated by reverse osmosis was available ad libitum throughout the study. The purified water was supplied by an automatic watering system. Monitoring of the drinking water for contaminants is conducted by SLI and the records are available for inspection. Within generally accepted limits, contaminants which may have been present were not expected to compromise the purpose of this study. The water meets the standards specified under the EPA National Drinking Water Regulations (40 CFR Part 141).

8.5.5. Acclimation

Upon receipt, the animals were removed randomly from the shipping cartons, examined by qualified personnel, identified with plastic ear tags and then acclimated to the laboratory conditions for a minimum of five days. The animals were observed daily for overt physical or behavioral abnormalities, general health/moribundity and mortality.

8.5.6. Animal Selection

The animals chosen for study use were arbitrarily selected from healthy stock animals to avoid potential bias. All animals received a detailed pretest observation prior to dosing. Only healthy animals were chosen for study use. Females were nulliparous and nonpregnant. The male animals were approximately 7 weeks of age and weighed 375-458 g on the day prior to Induction 1 dosing. The female animals were approximately 8 weeks of age and weighed 346-389 g on the day prior to Induction 1 dosing.

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9. EXPERIMENTAL PROCEDURES

9.1. Study Design

This study consisted of a topical range-finding group, a test group and a challenge control group [2]. A rechallenge control group was maintained on this study; however, the rechallenge procedure was not required since the challenge results were definitive.

9.2. Sensitization Study

9.2.1. Preliminary Procedures

On the day prior to each dose administration, the guinea pigs had the hair removed with a small animal clipper. Care was taken to avoid abrading the skin.

9.2.2. Dosing

A dose of 0.3 mL of the test article was placed on a 25 mm Hilltop chamber backed by adhesive tape (occlusive patch). The chambers were then applied to the clipped surface as quickly as possible.

Following chamber application, the trunk of the animal was wrapped with elastic wrap which was secured with adhesive tape to prevent removal of the chamber and the animal was returned to its cage.

9.2.2.1. Induction

On the day prior to the first induction dose administration (day -1), all test and control animals were weighed and the hair was removed from the left side of the test animals. On the day following clipping (day 0), chambers were applied as follows:

Group	Material	Induction No.	Concentration (%)	Test Site No.	No. of Animals Male	No. of Animals Female
Test	Spray--Alpha	1	100[a]	1	10	10
		2	100[a]	1		
		3	100[a]	1		

[a]Pooled test article.

The induction procedure was repeated on study day 7 and on study day 14 so that a total of three consecutive induction exposures were made to the test animals.

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9.2.2.2. Challenge

On the day prior to challenge dose administration, the test and challenge control animals were weighed and the hair was removed from the right side of the animals. On the day following clipping (day 27), chambers were applied as follows:

Group	Material	Concentration (%)	Test Site No.	No. of Animals Male	No. of Animals Female
Test	Spray--Alpha	100[a]	2	10	10
Challenge Control	Spray--Alpha	100[a]	2	5	5

[a]Pooled test article.

9.2.3. Test Article Removal

Approximately six hours after chamber application, the binding materials were removed. The test sites were wiped with gauze moistened in deionized water, followed by dry gauze, to remove test article residue. The animals were then returned to their cages.

9.2.4. Dermal Observations

The test sites were graded for irritation at approximately 24 and 48 hours following chamber application (induction) or chamber removal (challenge) using the Dermal Grading System presented in Appendix B.

9.2.5. Clinical Observations

Any unusual observations and mortality were recorded. The animals were observed for general health/mortality twice daily, once in the morning and once in the afternoon.

9.2.6. Body Weights

Individual body weights were obtained for all sensitization study animals on the day prior to the first induction (day -1) and for the appropriate test and challenge control animals on the day prior to challenge dosing.

9.2.7. Scheduled Euthanasia

All sensitization study animals were euthanized by carbon dioxide inhalation following each animal's final scoring interval. Gross necropsy examinations were not required for these animals.

9.3. Protocol Deviations

The animal room temperature and relative humidity ranges [63-74°F (17-23°C) and 48-82%] exceeded the preferred ranges [63-73°F (17-23°C) and 30-70%, respectively] but were corrected on the same day. These occurrences were considered to have had no adverse effect on the outcome of this study.

10. ANALYSIS OF DATA

The sensitization potential of the test article was based on the dermal responses observed on the test and control animals at challenge. Generally, dermal scores of ≥1 in the test animals with scores of 0 to ± noted in the controls are considered indicative of sensitization. Dermal scores of 1 in both the test and control animals are generally considered equivocal unless a higher dermal response (≥ grade 2) is noted in the test animals. Group mean dermal scores were calculated for challenge.

11. MAINTENANCE OF RAW DATA AND RECORDS

All original raw data, the final report and magnetically encoded records were transferred to the SLI archives for a period of 10 years. The Sponsor will be contacted prior to final disposition of these items.

12. RESULTS

12.1. Topical Range-Finding Study

Individual Topical Range-Finding Data: Appendix A

The results of the range-finding study indicated that a test article concentration of 100% was considered appropriate for induction and challenge since it was the highest possible concentration which was nonirritating.

12.2. Sensitization Study

Individual Data: Tables 1-2

Following challenge with 100% Spray--Alpha, dermal reactions in the test and challenge control animals were limited to scores of 0. Group mean dermal scores were noted to be the same in the test animals as compared with the challenge control animals.

SLI Study No. 3596.7

12.3. Clinical Observations/Body Weights

Individual Body Weight Data: Appendix C

The sensitization study animals gained weight during the test period and generally appeared in good health.

12.4. Historical Control

HCA Historical Control Data: Appendix D

Using α-Hexylcinnamaldehyde (HCA) as a positive control, Springborn Laboratories, Inc., Spencerville, Ohio, has completed a study during the past six months which provided historical control data for contact sensitization to this agent utilizing the test system described herein (Modified Buehler Design). Following induction at 5% w/v HCA in ethanol and challenge at levels of 2.5% and 1% w/v HCA in acetone, a contact sensitization response was observed, thereby demonstrating the susceptibility of the test system to this sensitizing agent.

13. CONCLUSION

Based on the results of this study, Spray--Alpha is not considered to be a contact sensitizer in guinea pigs. The results of the HCA historical control study demonstrated that a valid test was performed and indicated that the test design would detect potential contact sensitizers.

Kimberly L. Bonnette, M.S., LATG
Study Director

Date 9/3/02

14. REPORT REVIEW

Dawn D. Rodabaugh, B.S.
Associate Toxicologist

Date 9/3/02

SLI Study No. 3596.7

15. REFERENCES

1. Guide for the Care and Use of Laboratory Animals, DHHS Publication No. (NIH) 96-03, 1996.

2. E. V. Buehler, Delayed Contact Hypersensitivity in the Guinea Pig, Arch. Dermat., 91:171-177, 1965.

TABLE 1

SLI STUDY NO.: 3596.7 A DERMAL SENSITIZATION STUDY IN GUINEA PIGS PAGE 1

CLIENT: INL/A, U.S. DEPARTMENT OF STATE INDIVIDUAL INDUCTION DATA

(SPRAY--ALPHA)

Group	Animal No./ Sex	Induction 1 Dermal Scores 100%[a]		Induction 2 Dermal Scores 100%[a]		Induction 3 Dermal Scores 100%[a]	
		24 Hr	48 Hr	24 Hr	48 Hr	24 Hr	48 Hr
Test	G8143/M	0	0	0	0	0	0
	G8144/M	0	0	0	0	0	0
	G8145/M	0	0	0	0	0	0
	G8146/M	0	0	0	0	0	0
	G8147/M	0	0	0	0	0	0
	G8148/M	0	0	0	0	0	0
	G8149/M	0	0	0	0	0	0
	G8150/M	0	0	0	0	0	0
	G8151/M	0	0	0	0	0	0
	G8152/M	0	0	0	0	0	0
	G8270/F	0	0	0	0	0	0
	G8271/F	0	0	0	0	0	0
	G8272/F	0	0	0	0	0	0
	G8273/F	0	0	0	0	0	0
	G8274/F	0	0	0	0	0	0
	G8275/F	0	0	0	0	0	0
	G8276/F	0	0	0	0	0	0
	G8277/F	0	0	0	0	0	0
	G8278/F	0	0	0	0	0	0
	G8279/F	0	0	0	0	0	0

Note: See Appendix B for definition of codes.

[a]Pooled test article.

TABLE 2

SLI STUDY NO.: 3596.7 A DERMAL SENSITIZATION STUDY IN GUINEA PIGS PAGE 1

CLIENT: INL/A, U.S. DEPARTMENT OF STATE INDIVIDUAL CHALLENGE DATA

(SPRAY--ALPHA)

Group	Animal No./ Sex	Dermal Scores 100%[a] 24 Hr	48 Hr
Test	G8143/M	0	0
	G8144/M	0	0
	G8145/M	0	0
	G8146/M	0	0
	G8147/M	0	0
	G8148/M[b]	0	0
	G8149/M	0	0
	G8150/M	0	0
	G8151/M	0	0
	G8152/M	0	0
	G8270/F	0	0
	G8271/F	0	0
	G8272/F	0	0
	G8273/F	0	0
	G8274/F	0	0
	G8275/F	0	0
	G8276/F	0	0
	G8277/F	0	0
	G8278/F	0	0
	G8279/F	0	0
	Mean	0.0	0.0

Notes: See Appendix B for definition of codes.

[a]Pooled test article

[b]Animal found out of binding at the time of patch removal.

TABLE 2

SLI STUDY NO.: 3596.7 A DERMAL SENSITIZATION STUDY IN GUINEA PIGS PAGE 2

CLIENT: INL/A, U.S. DEPARTMENT OF STATE INDIVIDUAL CHALLENGE DATA

(SPRAY--ALPHA)

Group	Animal No./ Sex	Dermal Scores 100%[a] 24 Hr	48 Hr
Challenge Control	G8153/M	0	0
	G8154/M	0	0
	G8155/M	0	0
	G8156/M	0	0
	G8157/M	0[IT]	0
	G8280/F	0	0
	G8281/F	0	0
	G8282/F	0[IT]	0
	G8283/F	0	0
	G8284/F	0	
	Mean	0.0	0.0

Notes: See Appendix B for definition of codes.

[a]Pooled test article.

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SLI Study No. 3596.7

APPENDIX A

Topical Range-Finding Study

(20)

SLI Study No. 3596.7

1. TOPICAL RANGE-FINDING STUDY

This appendix provides the experimental procedures and results of a topical range-finding study in guinea pigs with Spray--Alpha. The procedures for animal husbandry were similar to those described for the main sensitization study animals. The male animals were approximately 7 weeks of age and weighed 405-458 g; the female animals were approximately 11 weeks of age and weighed 480-481 g on the day prior to dosing.

1.1. Method of Test Article Preparation

The test article was utilized at 100% and at 75%, 50% and 25% w/v in deionized for the range-finding study. The test article was prepared and dispensed fresh on the day of dosing. The dosing preparations were stirred continuously during dosing.

1.2. Dosing

On the day prior to dose administration, four topical range-finding guinea pigs were weighed and the hair removed from the right and left side of the animals with a small animal clipper. Care was taken to avoid abrading the skin during clipping procedures.

On the following day, four concentrations of the test article were prepared and each concentration was applied to the clipped area of each topical range-finding animal as indicated below:

Group	Material	Concentration (%)	Test Site No.	Amount Applied	Patch Design[a]
Topical Range-Finding	Spray--Alpha	100[b]	1	0.3 mL	25 mm Hilltop Chamber
		75[b, c]	2	0.3 mL	25 mm Hilltop Chamber
		50[b, c]	3	0.3 mL	25 mm Hilltop Chamber
		25[b, c]	4	0.3 mL	25 mm Hilltop Chamber

[a]Occlusive patch.
[b]Pooled test article
[c]The vehicle was deionized water.

The chambers were applied to the clipped surface as quickly as possible. The trunk of the animal was wrapped with elastic wrap which was secured with adhesive tape to prevent removal of the chambers and the animal was returned to its cage.

Approximately six hours after chamber application, the binding materials were removed. The test sites were then wiped with gauze moistened in deionized water, followed by dry gauze, to remove test article residue and the animals returned to their cages.

1.3. Dermal Observations

The test sites of the topical range-finding animals were graded for irritation at approximately 24 and 48 hours following chamber application using the Dermal Grading System in Appendix B.

1.4. Clinical Observations

Any unusual observations and mortality were recorded. The topical range-finding animals were observed for general health/mortality twice daily, once in the morning and once in the afternoon.

1.5. Body Weights

Individual body weights were obtained for the topical range-finding animals on the day prior to dosing.

1.6. Scheduled Euthanasia

Following the 48-hour scoring interval, all topical range-finding animals were euthanized by carbon dioxide inhalation. Gross necropsy examinations were not required for these animals.

1.7. Results

The results of the range-finding study indicated that a test article concentration of 100% was considered appropriate for induction and challenge since it was the highest possible concentration which was nonirritating.

A DERMAL SENSITIZATION STUDY IN GUINEA PIGS
TOPICAL RANGE-FINDING DATA
(SPRAY--ALPHA)

Group	Animal No./Sex Body Weight (g)	Range-Finding Dermal Scores							
		100%[a]		75%[a,b]		50%[a,b]		25%[a,b]	
		24 Hr	48 Hr	24 Hr	48 Hr	24 Hr	48 Hr	24 Hr	48 Hr
Range-Finding	G7961/M 458	0	0	0	0	0	0	0	0
	G7969/M 405	0	0	0	0	0	0	0	0
	G7449/F 481	0	0	0	0	0	0	0	0
	G7477/F 480	0	0	0	0	0	0	0	0

[a]Pooled test article
[b]The vehicle used was deionized water.
Note: See Appendix B for definition of codes.

APPENDIX B

Dermal Grading System

(24)

SLI Study No. 3596.7

DERMAL GRADING SYSTEM

ERYTHEMA AND EDEMA OBSERVATIONS		
OBSERVATION	DEFINITION	CODE
Erythema – Grade 0	No reaction	0
Erythema – Grade ±	Slight patchy erythema	±
Erythema – Grade 1	Slight, but confluent or moderate patchy erythema	1
Erythema – Grade 2	Moderate, confluent erythema	2
Erythema – Grade 3	Severe erythema with or without edema	3
Maximized Grade 3	Notable dermal lesions	M – 3 (see below)
Edema – Grade 1	Very slight edema (barely perceptible)	ED-1
Edema – Grade 2	Slight edema (edges of area well defined by definite raising)	ED-2
Edema – Grade 3	Moderate edema (raised approximately 1 millimeter)	ED-3
Edema – Grade 4	Severe edema (raised more than 1 millimeter and extends beyond the area of exposure)	ED-4
An erythema code was assigned to each test site. An edema code was assigned only if edema was present at the test site. If notable dermal lesion(s) (> grade 1) were present, then the "Maximized Grade 3" was assigned to the test site in place of the erythema score and the type of the notable dermal lesion(s) was noted (e.g., M-3^{ES-2}).		

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DERMAL GRADING SYSTEM

NOTABLE DERMAL LESIONS		
OBSERVATION	CODE	DEFINITION
Eschar – Grade 1	ES-1	Focal and/or pinpoint areas up to 10% of test site.
Eschar – Grade 2	ES-2	> 10% < 25% of test site.
Eschar – Grade 3	ES-3	> 25% < 50% of test site.
Eschar – Grade 4	ES-4	> 50% of test site.
Blanching – Grade 1	BLA-1	Focal and/or pinpoint areas up to 10% of test site.
Blanching – Grade 2	BLA-2	> 10% < 25% of test site.
Blanching – Grade 3	BLA-3	> 25% < 50% of test site.
Blanching – Grade 4	BLA-4	> 50% of test site.
Ulceration – Grade 1	U-1	Focal and/or pinpoint areas up to 10% of test site.
Ulceration – Grade 2	U-2	> 10% < 25% of test site.
Ulceration – Grade 3	U-3	> 25% < 50% of test site.
Ulceration – Grade 4	U-4	> 50% of test site.
Necrosis – Grade 1	NEC-1 *(color)*	Focal and/or pinpoint areas up to 10% of test site (note color of necrosis).
Necrosis – Grade 2	NEC-2 *(color)*	> 10% < 25% of test site (Note color of necrosis).
Necrosis – Grade 3	NEC-3 *(color)*	> 25% < 50% of test site (Note color of necrosis).
Necrosis – Grade 4	NEC-4 *(color)*	> 50% of test site (Note color of necrosis).

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DERMAL GRADING SYSTEM

ADDITIONAL DERMAL FINDINGS		
OBSERVATION	DEFINITION	CODE
Desquamation	Characterized by scaling or flaking of dermal tissue or without denuded areas.	DES
Fissuring	Characterized by cracking of the skin with or without moist exudate. Fissuring should be checked prior to removing the animal from the cage and manipulating the test site.	FIS
Eschar Exfoliation	The process by which areas of eschar flake off the test site.	EXF
Test Site Staining	Skin located at test site appears to be discolored, possibly due to test article (note color of staining).	TSS (*color*)
Erythema Extends Beyond the Test Site	The erythema extends beyond the test site. Note: A study director should be contacted for erythema extending beyond the test site.	ERB
Superficial Lightening	Characterized by pale area(s) (almost a burn-like appearance) in the test site. However, erythema may still be observed through the pale area. Note: This observation may affect the overall erythema score of the test site. This observation may progress to other observations resulting in notable dermal lesions, but SL itself will not be considered a notable dermal lesion that will result in a dermal score to be maximized since it does not result in any in-depth injury. To be coded using an area designation (see below).	--
Superficial Lightening - Grade 1	Focal and/or pinpoint areas up to 10% of the test site	SL-1
Superficial Lightening - Grade 2	> 10% < 25% of test site	SL-2
Superficial Lightening - Grade 3	> 25% < 50% of test site	SL-3
Superficial Lightening - Grade 4	> 50% of test site	SL-4
Dermal Irritation - Outside of the Test Site	Noticeable irritation outside of test site probably due to the binding tape material. This notation will only be made for reactions greater than what are normally observed from tape removal which do not interfere with the scoring of the test site.	IT

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APPENDIX C

Individual Body Weight Data

Group	Animal No./Sex	Day -1	Day 26
Test	G8143/M	434	661
	G8144/M	394	573
	G8145/M	375	586
	G8146/M	419	597
	G8147/M	443	708
	G8148/M	412	633
	G8149/M	458	655
	G8150/M	441	675
	G8151/M	375	576
	G8152/M	399	625
	G8270/F	373	546
	G8271/F	389	547
	G8272/F	350	498
	G8273/F	386	608
	G8274/F	349	514
	G8275/F	355	512
	G8276/F	389	560
	G8277/F	382	553
	G8278/F	387	564
	G8279/F	366	508

		Body Weights	
Group	Animal No./Sex	Day -1	Day 26
Challenge Control	G8153/M	378	690
	G8154/M	398	678
	G8155/M	383	576
	G8156/M	436	617
	G8157/M	391	583
	G8280/F	357	480
	G8281/F	368	520
	G8282/F	356	497
	G8283/F	358	581
	G8284/F	378	511
Rechallenge Control[a]	G8158/M	431	–
	G8159/M	430	–
	G8160/M	415	–
	G8161/M	439	–
	G8162/M	436	–
	G8285/F	366	–
	G8286/F	346	–
	G8287/F	365	–
	G8288/F	364	–
	G8289/F	376	–

[a]A rechallenge control group was maintained on this study, but was not utilized since the results from challenge were conclusive.

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SLI Study No. 3596.7

APPENDIX D

HCA Historical Control Data

(31)

SLI Study No. 3596.7

SPRINGBORN LABORATORIES, INC.
MODIFIED BUEHLER HISTORICAL CONTROL DATA
USING α-HEXYLCINNAMALDEHYDE
(SLI Study No. 999.171)

1. OBJECTIVE

This study was performed to assess the dermal sensitization potential of α-Hexylcinnamaldehyde (HCA) when administered by multiple topical applications. This study may be used to provide information on the ability of the test system to detect potential contact sensitizers and to update the historical positive control of the testing facility. The protocol was signed by the Study Director on February 6, 2002 (GLP initiation date). The in-life phase of the study was initiated with test article administration on March 13, 2002, and concluded with final scoring on April 12, 2002.

2. TEST ARTICLE

The test article was received from the manufacturer, TCI America, and identified as follows:

Supplier's ID	Assigned SLI ID	Physical Description	Receipt Date	SLI Assigned Expiration Date
HCA Lot No.: GF01	S01.008.N	Clear yellow liquid	08/21/01	08/21/03

The bulk compound was stored desiccated, protected from light, at room temperature. The manufacturer provided a Certificate of Analysis for the test article which is presented as Attachment 1 of this Appendix.

The HCA was mixed with ethanol or acetone to produce the appropriate concentrations for dose administration. For the sensitization study, the test article concentrations utilized were 5% w/v in ethanol (induction) and 1% and 2.5% w/v in acetone (challenge).

SLI Study No. 3596.7

3. EXPERIMENTAL PROCEDURES [1]

Young adult Hartley-derived albino guinea pigs were received on March 7, 2002, from Hilltop Lab Animals, Inc., Scottdale, PA. The guinea pigs were uniquely identified by ear tag, individually housed in suspended stainless steel cages and received Purina Certified Guinea Pig Chow #5026 and water purified by reverse osmosis ad libitum. The animals were acclimated for a minimum of 5 days prior to experimental initiation. The male guinea pigs were approximately 7 weeks of age and weighed 370-463 g; the female guinea pigs were approximately 8 weeks of age and weighed 336-396 g on the day prior to Induction I dosing.

On the day prior to the first induction dose administration (day -1), the hair was removed from the left side of the twenty test animals. On the following day, 0.3 mL of 5% w/v HCA in ethanol was placed on a Hilltop chamber and applied to the clipped area of each animal=s back. The trunk of each animal was wrapped with elastic wrap which was secured with adhesive tape to prevent removal of the chamber. Approximately six hours after chamber application, the binding materials were removed. The test sites were wiped with gauze moistened with deionized water, followed by dry gauze, to remove test article residue. The test sites were graded for irritation at approximately 24 and 48 hours following chamber application using the Dermal Grading System. The induction procedure was repeated on study day 7 and on study day 14 so that a total of three induction exposures were made to the animals.

On the day prior to challenge dose administration, the hair was removed from the right side of the twenty test and ten challenge control animals. On the following day (day 28), 0.3 mL of 1% and 2.5% w/v HCA in acetone was placed on a 25 mm Hilltop chamber and applied to the clipped area of each animal=s back. Wrapping, unwrapping and rinsing procedures were the same as those utilized for the induction phase. The test sites were graded for irritation at approximately 24 and 48 hours following chamber removal.

Any unusual observations and/or mortality were recorded. Body weights were recorded for the test, challenge control and rechallenge control animals on the day prior to first induction (day -1) and for the test and challenge control animals on the day prior to challenge dosing. All sensitization study animals were euthanized by carbon dioxide inhalation following each animal's final scoring interval. Gross necropsy examinations were not required for these animals.

Note: The temperature and relative humidity of the animal room [64-75°F (18-24°C)] exceeded the preferred ranges [63-73°F (17-23°C) and 30-70%] during

this study. These occurrences were considered to have had no adverse effect on the outcome of this study.

4. RESULTS

Individual Data: Tables 1-2

Following challenge with 2.5% w/v HCA in acetone, dermal scores of 1 were noted in 8/20 test animals at the 24-hour scoring interval. At the 48-hour scoring interval, dermal scores of 1 were noted in 4/20 test animals. Dermal reactions in the remaining test and challenge control animals were limited to scores of 0 to ±. Group mean dermal scores were noted to be higher in the test animals as compared with the challenge control animals.

Following challenge with 1% w/v HCA in acetone, dermal scores of 1 were noted in 5/20 test animals at the 24-hour scoring interval. At the 48-hour scoring interval, dermal scores of 1 were noted in 2/20 test animals. Dermal reactions in the remaining test and challenge control animals were limited to scores of 0 to ±. Group mean dermal scores were noted to be higher in the test animals as compared with the challenge control animals.

5. CONCLUSION

The results of this α-Hexylcinnamaldehyde positive control study demonstrated that a valid test was performed and indicated that the test design would detect potential contact sensitizers. Based on the results of this study, α-Hexylcinnamaldehyde is considered to be a contact sensitizer in guinea pigs.

6. REFERENCE

1. E.V. Buehler, Occlusive Patch Method for Skin Sensitization in Guinea Pigs: The Buehler Method, Fd. Chem. Toxic., Vol. 32, No. 2, pp. 97-101, 1994.

SLI HISTORICAL CONTROL
STUDY NO.: 999.171

TABLE 1
A DERMAL SENSITIZATION STUDY IN GUINEA PIGS
INDIVIDUAL INDUCTION DATA
(α-HEXYLCINNAMALDEHYDE)

Group	Animal No./ Sex	Induction 1 Dermal Scores 5%[a] 24 Hr	Induction 1 Dermal Scores 5%[a] 48 Hr	Induction 2 Dermal Scores 5%[a] 24 Hr	Induction 2 Dermal Scores 5%[a] 48 Hr
Test	G5787/M	1 ED-1, BLA-1, DES	± BLA-1, DES,	2 ED-2, BLA-1, SL-1, DES	2 ED-2, BLA-1, DES
	G5788/M	1 ED-1, DES	± DES	2 ED-1, DES	2 ED-1, DES
	G5789/M	± ED-1, DES, IT	± DES	2 ED-1, BLA-1, DES	2 ED-1, BLA-1, DES
	G5790/M	2 ED-1, SL-4	1 ED-1, DES	M-3 ED-2, BLA-2, DES	M-3 ED-1, BLA-2, NEC-1 (BK), DES
	G5791/M	± ED-1, BLA-1, DES	± BLA-1, DES	2 ED-2, BLA-1, DES	2 ED-1, BLA-1, DES
	G5792/M	1 ED-1, BLA-1, DES	± BLA-1, DES	M-3 ED-2, NEC-2 (BK), BLA-1, DES	M-3 ED-1, BLA-1, ES-2, DES
	G5793/M	1 ED-1, BLA-1, DES	± ED-1, BLA-1, DES	M-3 ED-2, BLA-2, SL-1, DES	M-3 ED-1, BLA-2, DES
	G5794/M	1 ED-1, DES	± DES	2 ED-2, ES-1, DES	2 ED-1, ES-1, DES
	G5795/M	1 ED-1, BLA-1, DES	± ED-1, BLA-1, DES	2 ED-2, BLA-1, SL-3, DES	2 ED-1, BLA-1, DES
	G5796/M	2 ED-1, BLA-1, DES	1 BLA-1, DES	2 ED-2, BLA-1, DES	1 ED-1, BLA-1, DES
	G5894/F	± ED-1, DES, IT	± DES	2 ED-2, DES	1 ED-1, DES
	G5895/F	1 ED-1, DES, IT	± DES	2 ED-2, BLA-1, SL-1, DES	1 ED-1, BLA-1, DES
	G5896/F	± DES, IT	± DES	2 ED-2, BLA-1, ES-1, DES	M-3 ED-2, ES-2, DES
	G5897/F	1 ED-1, DES, IT	± DES	1 ED-1, DES, IT	± DES
	G5898/F	± DES, IT	± DES	± DES, IT	± DES
	G5899/F	± DES, IT	0 DES	2 ED-2, BLA-1, DES	2 ED-1, BLA-1, DES
	G5900/F	1 ED-1, BLA-1, DES	± ED-1, BLA-1, DES	2 ED-2, BLA-1, DES	2 ED-2, BLA-1, DES
	G5901/F	1 ED-1, DES, IT	± DES	2 ED-2, SL-4, DES, IT	2 ED-2, BLA-1, DES
	G5902/F	± DES	± DES	2 ED-2, SL-1, DES	2 ED-1, SL-1, DES
	G5903/F	0 IT	0	2 ED-2, DES	1 ED-1, DES

[a]The vehicle was ethanol.
Notes: See Appendix B for definition of codes. BK = black.

SLI HISTORICAL CONTROL
STUDY NO.: 999.171

TABLE 1
A DERMAL SENSITIZATION STUDY IN GUINEA PIGS
INDIVIDUAL INDUCTION DATA
(α-HEXYLCINNAMALDEHYDE)

Group	Animal No./ Sex	Induction 3 Dermal Scores 5%[a] 24 Hr	48 Hr
Test	G5787/M	2 ED-2, DES	2 ED-1
	G5788/M	2 ED-2, BLA-1	2 ED-2, BLA-1
	G5789/M	2 ED-2	2 ED-1, SL-1
	G5790/M	2 ED-2, SL-4, DES	2 ED-1, SL-4
	G5791/M	2 ED-2, DES	2 ED-1
	G5792/M	2 ED-2, SL-1, DES	2 ED-1, SL-1
	G5793/M	2 ED-2, DES	2 ED-1, DES
	G5794/M	2 ED-2, SL-2, DES	2 ED-2, SL-2, DES
	G5795/M	2 ED-2, SL-2, DES	2 ED-1, BLA-1, SL-2
	G5796/M	2 ED-2, SL-2, DES	2 ED-1, BLA-1, SL-1
	G5894/F	1 ED-1, DES	1 ED-1
	G5895/F	1 ED-1, DES	1 ED-1
	G5896/F	2 ED-2, SL-1, DES, IT	2 ED-2, SL-1
	G5897/F	1 ED-1, DES	1 ED-1
	G5898/F	± ED-1, DES	± ED-1
	G5899/F	2 ED-2, SL-4, DES	2 ED-2, SL-4
	G5900/F	2 ED-2, SL-2, DES	2 ED-1, SL-2
	G5901/F	2 ED-2, SL-4, DES	2 ED-1, SL-4
	G5902/F	2 ED-2, SL-4, DES	2 ED-1, SL-4
	G5903/F	1 ED-1, BLA-1, DES	1 ED-1, BLA-1, SL-1

[a]The vehicle was ethanol.
Note: See Appendix B for definition of codes.

SLI HISTORICAL CONTROL
STUDY NO.: 999.171

TABLE 2
A DERMAL SENSITIZATION STUDY IN GUINEA PIGS
INDIVIDUAL CHALLENGE DATA
(α-HEXYLCINNAMALDEHYDE)

Group	Animal No./ Sex	Dermal Scores			
		2.5%[a]		1%[a]	
		24 Hr	48 Hr	24 Hr	48 Hr
Test	G5787/M	1[IT]	±	1[IT]	±[IT]
	G5788/M	±	±	±	±
	G5789/M	±	0	±	0
	G5790/M	1[ED-1]	1	1	1
	G5791/M	1	1	±[IT]	±[IT]
	G5792/M	±	0	±	0
	G5793/M	±	±	±	±
	G5794/M	1	1	1	±
	G5795/M	1	±	±	0
	G5796/M	±[IT]	±	±	±
	G5894/F	±	0	±	0
	G5895/F	1	±	1[IT]	±
	G5896/F	1	±	±	±
	G5897/F	±	0	±[IT]	0[IT]
	G5898/F	±	±	0	0
	G5899/F	1	1	1[IT]	1[IT]
	G5900/F	±[IT]	0	±[IT]	0
	G5901/F	±	0	±	0
	G5902/F	±	±	±	0
	G5903/F	±	±	±	0
	Mean	0.7	0.5	0.6	0.3

[a]The vehicle was acetone.
Notes: For the purpose of calculation, ± = 0.5. See Appendix B for definition of codes.

SLI HISTORICAL CONTROL
STUDY NO.: 999.171

TABLE 2
A DERMAL SENSITIZATION STUDY IN GUINEA PIGS
INDIVIDUAL CHALLENGE DATA
(α-HEXYLCINNAMALDEHYDE)

Group	Animal No./ Sex	Dermal Scores 2.5%[a] 24 Hr	2.5%[a] 48 Hr	1%[a] 24 Hr	1%[a] 48 Hr
Challenge	G5797/M	0	0	0^{IT}	0
	G5798/M	0	0	0^{IT}	0
	G5799/M	0	0	$\pm^{IT}$	$\pm^{IT}$
	G5800/M	0	0	0	0
	G5801/M	0^{IT}	0	0^{IT}	0^{IT}
	G5904/F	0^{IT}	0^{IT}	0^{IT}	0
	G5905/F	0^{IT}	0	0^{IT}	0
	G5906/F	0^{IT}	0	0^{IT}	0
	G5907/F	0	0	0	0
	G5908/F	0^{IT}	0^{IT}	0^{IT}	0
	Mean	0.0	0.0	0.1	0.1

[a]The vehicle was acetone.

Notes: For the purpose of calculation, ± = 0.5. See Appendix B for definition of codes.

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SLI Study No. 3596.7

ATTACHMENT 1

Certificate of Analysis
(Provided by the Manufacturer)

SLI Study No. 3596.7
Dow Study No. 021090

(39)

TCI AMERICA

CERTIFICATE OF ANALYSIS

H0685
Lot# GF01
CAS# 101-86-0

ALPHA-N-HEXYLCINNAMALDEHYDE

Appearance:	Yellow clear liquid
SG(20/20):	0.96
n(20/D):	1.55
Assay(GC):	92%

9211N. Harborgate St. Portland, OR 97203 Phone: (503)283-1681 (800)423-8616 Fax: (503)283-1987

APPENDIX E

SLI Personnel Responsibilities

(41)

SLI Study No. 3596.7

SLI PERSONNEL RESPONSIBILITIES

Kimberly L. Bonnette, M.S., LATG	Study Director/Director, Acute Toxicology
Dawn D. Rodabaugh, B.S.	Alternate Contact/Associate Toxicologist
Robert C. Springborn, P h.D.	Chairman, President and CEO
Malcolm Blair, Ph.D.	Senior Vice President, Managing Director Emeritus
Joseph C. Siglin, Ph.D., DABT	Vice President, Managing Director
Jason W. Smedley, B.S.	Assistant Toxicologist
Pamela S. Smith, ALAT	Primary Technician/Supervisor of Acute Toxicology
Delores P. Knippen	Supervisor of Pharmacy
Steven H. Magness, B.S., LATG	Senior Supervisor of Gross and Fetal Pathology
Anita M. Bosau, RQAP-GLP	Senior Director, Compliance Assurance
Deanna M. Talerico, RQAP-GLP	Senior Supervisor of Quality Assurance
J. Dale Thurman, D.V.M., M.S., DACVP	Senior Director, Pathology
Kathy M. Gasser	Supervisor of Archives

A PRIMARY EYE IRRITATION STUDY IN RABBITS WITH SPRAY--ALPHA

FINAL REPORT

OPPTS Guideline

870.2400

Author

Kimberly L. Bonnette, M.S., LATG

Study Completed on

August 28, 2002

Performing Laboratory

Springborn Laboratories, Inc. (SLI)
Ohio Research Center
640 North Elizabeth Street
Spencerville, Ohio 45887

SLI Study No.

3596.5

Submitted to

INL/A
U.S. Department of State
2201 C St. NW SA-4
Washington, DC 20520

SLI Study No. 3596.5 (2)

1. STATEMENT OF *NO* DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B), or (C).

Company: _____

Company Agent: _____ Date: _____

_____ _____

Title Signature

AUG 16 2002

2. COMPLIANCE STATEMENT

This study was conducted in compliance with the Good Laboratory Practice Standards as described by the EPA (40 CFR Parts 160 and 792).

Kimberly L. Bonnette, M.S., LATG
Study Director/Author
Springborn Laboratories, Inc.

Date 8/20/02

Rogers Woolfolk
Senior Aviation Advisor
Sponsor/Submitter
INL/A
U.S. Department of State

Date 12 Aug 02

3. QUALITY ASSURANCE STATEMENT

This study was inspected by the Quality Assurance Unit and reports were submitted to management and the Study Director in accordance with SLI's Standard Operating Procedures as follows:

Phase	Date
Protocol Review	03/31/02
Ocular Observations	06/12/02
Data Audit	07/15/02
Draft Report Review	07/15/02
Protocol Amendment Review	07/25/02
Final Report Review	08/28/02
Reports to Study Director and Management	07/15/02, 08/28/02

The final report has been reviewed to assure that it accurately describes the materials and methods, and the reported results accurately reflect the raw data.

Jennifer D. McGue Date 8/28/02
Jennifer D. McGue
Quality Assurance Auditor

Anita M. Bosau Date 8/28/02
Anita M. Bosau, RQAP-GLP
Senior Director, Compliance Assurance

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SLI Study No. 3596.5 (7)

6. SUMMARY

The potential irritant and/or corrosive effects of Spray--Alpha were evaluated on the eyes of New Zealand White rabbits. Each of three rabbits received a 0.1 mL dose of the test article in the conjunctival sac of the right eye. The contralateral eye of each animal remained untreated and served as a control. Test and control eyes were examined for signs of irritation for up to 7 days following dosing.

Exposure to the test article produced conjunctivitis (redness, swelling and discharge) in 3/3 test eyes at the 1-hour scoring interval. The conjunctival irritation resolved completely in all test eyes by study day 7. An additional ocular finding of slight dulling of normal luster of the cornea was noted in 2/3 test eyes.

Based on the no rinse group, Spray--Alpha is considered to be a mild irritant to the ocular tissue of the rabbit.

SLI Study No. 3596.5 (8)

7. INTRODUCTION

This study was performed to assess the irritant and/or corrosive effects of Spray--Alpha in New Zealand White rabbits when administered by a single ocular dose. This study was intended to provide information on the potential health hazards of the test article with respect to ocular exposure. Data from this study may serve as a basis for classification and/or labeling of the test article. This study was conducted in accordance with the US EPA, Health Effects Test Guidelines, OPPTS 870.2400, Acute Eye Irritation, August 1998. This study was performed at Springborn Laboratories, Inc., 553 North Broadway, Spencerville, Ohio. The protocol was signed by the Study Director on April 30, 2002 (GLP initiation date). The in-life phase of the study was initiated with test article administration on June 11, 2002 (day 0), and concluded with final scoring on June 18, 2002.

8. MATERIALS AND METHODS

8.1. Test Article

The test article was received from the Sponsor and identified as follows:

Sponsor's ID	Assigned SLI ID	Physical Description	Receipt Date	Expiration Date
Spray—Alpha[a]	S02.001.3596	Light amber liquid	05/13/02	None Provided
Ingredients[b]				
Herbicide: Fuete-SL Lot No.: 02-01-02				None Provided
Surfactant: Cosmo Flux-411F Lot No.: 244301				10/2003

[a]Sample pooled at SLI from five different mixes of Spray--Alpha (top/middle/bottom).
[b]Ingredients used in the five Spray--Alpha mixes that were prepared by the Sponsor.

The test article was stored at room temperature. The Sponsor was responsible for any necessary evaluations related to identity, strength, purity, composition, stability and method of synthesis of the test material according to 40 CFR 160.105 and 40 CFR 792.105. Springborn Laboratories, Inc., analyzed the test article for the glyphosate (a.e.) which is presented in SLI Study No. 3596.1.

8.2. Retention Sample

An approximate 1 mL retention sample of each test article mixture sample (top/middle/bottom, maintained separately for a total of fifteen 1 mL samples) was taken and stored at SLI at room temperature. In addition, a 10 mL retention sample of the pooled test article samples (from the 5 test article mixtures) was collected and stored at SLI at room temperature. These samples serve as the retention samples for all studies conducted with this material.

8.3. Test Article Disposition

The remaining test article was returned to the Sponsor at the completion of all studies with the test article.

8.4. Method of Test Article Preparation

The test article was administered as received from the Sponsor and dispensed fresh on the day of dosing.

8.5. Animals and Animal Husbandry

8.5.1. Description, Identification and Housing

Adult, New Zealand White rabbits were received from Myrtle's Rabbitry, Thompson Station, TN. Upon receipt, plastic ear tags displaying unique identification numbers were used to individually identify the animals. Cage cards displaying at least the study number, animal number and sex were affixed to each cage. The animals were housed individually in suspended stainless steel cages. All housing and care were based on the standards recommended by the Guide for the Care and Use of Laboratory Animals [1].

8.5.2. Environment

The animal room temperature and relative humidity ranges were 71-74°F (22-23°C) and 41-75%, respectively. Environmental control equipment was monitored and adjusted as necessary to minimize fluctuations in the animal room environment. Light timers were set to maintain a 12-hour light/12-hour dark cycle and room ventilation was set to produce 10-15 air changes/hour. The animal room temperature and relative humidity were recorded a minimum of once daily.

SLI Study No. 3596.5 (10)

8.5.3. Food

PMI Certified Rabbit Chow #5322 (Purina Mills, Inc.) was provided ad libitum to the animals throughout the study. The lot number and expiration date of each batch of diet used during the study were recorded. The feed was analyzed and certified by the supplier for nutritional components and environmental contaminants. Dietary limitations for various environmental contaminants, including heavy metals, pesticides, polychlorinated biphenyls and total aflatoxin are set by the manufacturer. Within these limits, contaminants which may have been present were not expected to compromise the purpose of this study. Results of the dietary analyses (Certificates of Analysis) are provided by the manufacturer for each lot of diet. These are maintained by SLI.

8.5.4. Water

Municipal tap water treated by reverse osmosis was available ad libitum throughout the study. The purified water was supplied by an automatic watering system. Monitoring of the drinking water for contaminants is conducted by SLI and the records are available for inspection. Within generally accepted limits, contaminants which may have been present were not expected to compromise the purpose of this study. The water meets the standards specified under the EPA National Drinking Water Regulations (40 CFR Part 141).

8.5.5. Acclimation

Upon receipt, the animals were removed randomly from the shipping cartons, examined by qualified personnel, identified with plastic ear tags and then acclimated to the laboratory conditions for a minimum of five days. The animals were observed daily for overt physical or behavioral abnormalities, general health/moribundity and mortality.

8.5.6. Animal Selection

The animals chosen for study use were arbitrarily selected from healthy stock animals to avoid potential bias. All animals received a detailed pretest observation prior to dosing. Only healthy animals were chosen for study use. The male animals were approximately 16 weeks of age and weighed 3.6 kg prior to dosing.

SLI Study No. 3596.5 (11)

9. EXPERIMENTAL PROCEDURES

9.1. Preliminary Examination

On day 0 prior to dosing, both eyes of each animal provisionally selected for test use were examined macroscopically for ocular irritation with the aid of an auxiliary light source. In addition, the corneal surface was examined using fluorescein sodium dye. One drop of a fluorescein/physiological saline mixture was gently dropped onto the superior sclera of each eye. Following an approximate 15 second exposure, the eyes were thoroughly rinsed with physiological saline. The corneal surface was then examined for dye retention under a long-wave UV light source. Animals exhibiting ocular irritation, preexisting corneal injury or fluorescein dye retention were not used on study. All animals found to be acceptable for test use were returned to their cages until dosing.

9.2. Dosing

A minimum of one hour after preliminary ocular examination, the test article was instilled as follows:

	Concentration		No. of Animals
Group	(%)	Amount Instilled	Male
No Rinse	100[a]	0.1 mL	3

[a]Pooled test article.

The test article was instilled into the conjunctival sac of the right eye of each animal after gently pulling the lower lid away from the eye. Following instillation, the eyelids were gently held together for approximately one second in order to limit test article loss and the animal was returned to its cage. The contralateral eye remained untreated to serve as a control.

9.3. Ocular Observations

The eyes were macroscopically examined with the aid of an auxiliary light source for signs of irritation at 1, 24, 48 and 72 hours and up to 7 days after dosing according to the Ocular Grading System presented in Appendix A which is based on Draize [2]. Following macroscopic observations at the 24-hour scoring interval, the fluorescein examination procedure was repeated on all test and control eyes and any residual test article was gently rinsed from the eye at this time (if possible) using physiological saline. If any fluorescein findings were

noted at 24 hours, a fluorescein exam was conducted on the affected eyes at each subsequent interval until a negative response was obtained and/or until all corneal opacity had cleared, or as directed by the Study Director.

9.4. Clinical Observations

Any unusual observations and/or mortality were recorded. General health/mortality checks were performed twice daily (in the morning and in the afternoon).

9.5. Body Weights

Individual body weights were obtained for each animal prior to dosing on day 0.

9.6. Scheduled Euthanasia

Each animal was euthanized by an intravenous injection of sodium pentobarbital following its final observation interval. Gross necropsy examinations were not required for these animals.

9.7. Protocol Deviations

On one occasion, the animal room temperature and relative humidity ranges (71-74°F and 41-75%) exceeded the preferred ranges (63-73°F and 30-70% respectively) during this study. These occurrences are considered to have had no adverse effect on the outcome of this study.

10. ANALYSIS OF DATA

For each group, the ocular irritation score for each parameter (i.e., corneal opacity x area, iritis and conjunctival redness + swelling + discharge) was multiplied by the appropriate factor (i.e., corneal injury x 5, iritis x 5, conjunctivitis x 2) and the totals added for each animal/interval. The group mean irritation score was then calculated for each scoring interval based on the number of animals initially dosed in each group. The calculated group mean ocular irritation scores for each interval were used to classify the test article according to the Ocular Evaluation Criteria [3] presented in Appendix B.

11. MAINTENANCE OF RAW DATA AND RECORDS

All original raw data, the final report and magnetically encoded records were transferred to the SLI archives for a period of 10 years. The Sponsor will be contacted prior to final disposition of these items.

SLI Study No. 3596.5 (13)

12. RESULTS

12.1. Ocular Observations

Individual Data: Table 1

Exposure to the test article produced conjunctivitis (redness, swelling and discharge) in 3/3 test eyes at the 1-hour scoring interval. The conjunctival irritation resolved completely in all test eyes by study day 7. An additional ocular finding of slight dulling of normal luster of the cornea was noted in 2/3 test eyes.

No corneal opacity, iritis or conjunctivitis was observed in the control eyes.

13. CONCLUSION

Based on the no rinse group, Spray--Alpha is considered to be a mild irritant to the ocular tissue of the rabbit.

Kimberly L. Bonnette, M.S., LATG
Study Director

Date 8/28/02

14. REPORT REVIEW

Dawn D. Rodabaugh, B.S.
Associate Toxicologist

Date 8/28/02

15. REFERENCES

1. Guide for the Care and Use of Laboratory Animals, DHHS Publication No. (NIH) 96-03, 1996.

2. Draize, J.H., Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics, The Association of Food and Drug Officials of the United States, 49-51, 1959.

3. Kay, J.H. and Calandra, J.C., “Interpretation of Eye Irritation Tests”, Journal of the Society of Cosmetic Chemists, 13, 281-289, 1962.

TABLE 1

SLI STUDY NO.: 3596.5 A PRIMARY EYE IRRITATION STUDY IN RABBITS PAGE 1

CLIENT: INL/A, US DEPARTMENT OF STATE INDIVIDUAL OCULAR IRRITATION SCORES

(NO RINSE GROUP)

Animal No./Sex Body Weight (kg)	Scoring Interval	Cornea			Iris		Conjunctivae				Total	Test Eye*		Control Eye*	
		O	A	OxAx5	I	Ix5	R	S	D	(R+S+D)2		Fluorescein Examination	Secondary Ocular Findings	Fluorescein Examination	Secondary Ocular Findings
R2097/M	1 Hour	0	0	0	0	0	2	2	1	10	10				
3.625	24 Hours	0	0	0	0	0	2	2	2	12	12	[-]		[-]	
	48 Hours	0	0	0	0	0	2	2	0	8	8				
	72 Hours	0	0	0	0	0	1	0	0	2	2				
	7 Days	0	0	0	0	0	0	0	0	0	0				
R2101/M	1 Hour	0	0	0	0	0	2	2	1	10	10		SDL		
3.583	24 Hours	0	0	0	0	0	2	2	0	8	8	[-]		[-]	
	48 Hours	0	0	0	0	0	2	1	0	6	6				
	72 Hours	0	0	0	0	0	1	0	0	2	2				
	7 Days	0	0	0	0	0	0	0	0	0	0				
R2102/M	1 Hour	0	0	0	0	0	2	2	1	10	10		SDL		
3.617	24 Hours	0	0	0	0	0	2	2	0	8	8	[-]		[-]	
	48 Hours	0	0	0	0	0	2	2	0	8	8				
	72 Hours	0	0	0	0	0	1	0	0	2	2				
	7 Days	0	0	0	0	0	0	0	0	0	0				

*See Appendix A for definition of codes.

TABLE 1

SLI STUDY NO.: 3596.5 A PRIMARY EYE IRRITATION STUDY IN RABBITS PAGE 2

CLIENT: INL/A, US DEPARTMENT OF STATE INDIVIDUAL OCULAR IRRITATION SCORES

(NO RINSE GROUP)

Mean Ocular Scores		
1 Hour	-	10.00
24 Hours	-	9.33
48 Hours	-	7.33
72 Hours	-	2.00
7 Days	-	0.00

Mild Irritant

SLI Study No. 3596.5 (17)

APPENDIX A

Ocular Grading System

SLI Study No. 3596.5 (18)

OCULAR GRADING SYSTEM

(O) CORNEAL OPACITY—DEGREE OF DENSITY (AREA MOST DENSE TAKEN FOR READING)	
OBSERVATION	CODE
No ulceration or opacity	0
Scattered or diffuse areas of opacity (other than slight dulling of normal luster), details of iris clearly visible	1*
Easily discernible translucent area, details of iris slightly obscured	2*
Nacreous (opalescent) area, no details of iris visible, size of pupil barely discernible	3*
Opaque cornea, iris not discernible through opacity	4*

(A) AREA OF CORNEA INVOLVED (TOTAL AREA EXHIBITING ANY OPACITY, REGARDLESS OF DEGREE)	
OBSERVATION	CODE
No ulceration or opacity	0
One quarter (or less) but not zero	1
Greater than one quarter, but less than half	2
Greater than half, but less than three quarters	3
Greater than three quarters, up to whole area	4

Cornea Score = O x A x 5 Total Maximum = 80

(I) IRITIS	
OBSERVATION	CODE
Normal	0
Markedly deepened rugae (folds above normal), congestion, swelling, moderate circumcorneal hyperemia or injection, any or all of these or combination of any thereof, iris is still reacting to light (sluggish reaction is positive)	1*
No reaction to light, hemorrhage, gross destruction (any or all of these)	2*

Iris Score = I x 5 Total Maximum = 10

*Starred figures indicate positive effect.

SLI Study No. 3596.5 (19)

OCULAR GRADING SYSTEM

(R) CONJUNCTIVAL REDNESS (REFERS TO PALPEBRAL AND BULBAR CONJUNCTIVAE EXCLUDING CORNEA AND IRIS)	
OBSERVATION	CODE
Blood vessels normal	0
Some blood vessels definitely hyperemic (injected) above normal (slight erythema)	1
Diffuse, crimson color, individual vessels not easily discernible (moderate erythema)	2*
Diffuse beefy red (marked erythema)	3*

(S) CONJUNCTIVAL SWELLING (LIDS AND/OR NICTITATING MEMBRANE)	
OBSERVATION	CODE
No swelling	0
Any swelling above normal (includes nictitating membrane, slightly swollen)	1
Obvious swelling with partial eversion of lids	2*
Swelling with lids about half closed	3*
Swelling with lids more than half closed	4*

(D) CONJUNCTIVAL DISCHARGE	
OBSERVATION	CODE
No discharge	0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)	1
Discharge with moistening of the lids and hairs just adjacent to lids	2
Discharge with moistening of the lids and hairs and considerable area around the eye	3

Conjunctival Score = (R + S + D) x 2 Total Maximum = 20

*Starred figures indicate positive effect.

SLI Study No. 3596.5 (20)

OCULAR GRADING SYSTEM

CORNEAL NEOVASCULARIZATION		
OBSERVATION	CODE	DEFINITION
Neovascularization – Very Slight	VAS-1	Total area of vascularized corneal tissue is < 10% of corneal surface
Neovascularization – Mild	VAS-2	Total area of vascularized corneal tissue is > 10% but < 25% of corneal surface
Neovascularization – Moderate	VAS-3	Total area of vascularized corneal tissue is > 25% but < 50% of corneal surface
Neovascularization – Severe	VAS-4	Total area of vascularized corneal tissue is > 50% of corneal surface

SECONDARY OCULAR FINDINGS		
OBSERVATION	CODE	DEFINITION
Sloughing of the corneal epithelium	SCE	Corneal epithelial tissue is observed to be peeling off the corneal surface.
Corneal bulging	CB	The entire corneal surface appears to be protruding outward further than normal.
Slight dulling of normal luster of the cornea	SDL	The normal shiny surface of the cornea has a slightly dulled appearance.
Raised area on the corneal surface	RAC	A defined area on the corneal surface that is raised above the rest of the cornea. This area is generally associated with neovascularization and has an off-white to yellow color.
Corneal edema	CE	The cornea has a swollen appearance.
Test article present in eye	TAE	Apparent residual test article is observed on the eye or in the conjunctival sac/inner canthus.
Observation confirmed by slit lamp	OCS	A slit lamp examination was performed to confirm the initial observation.
Corneal mineralization	CM	Small white or off-white crystals that are observed in the corneal tissue.

SLI Study No. 3596.5 (21)

OCULAR GRADING SYSTEM

FLUORESCEIN EXAMINATION OF CORNEA	
OBSERVATION	CODE
Fluorescein Dye Retention Fluorescein dye retention associated with the area of corneal opacity Fluorescein dye retention is not associated with any other finding	 FAO FNF
Negative Results No fluorescein retention is observed	 (-)
Secondary Ocular Findings Superficial mechanical abrasion to the cornea observed during the fluorescein examination period Fine stippling on the cornea observed during the fluorescein examination procedure	 MI ST

POST-DOSE CLINICAL OBSERVATIONS	
OBSERVATION	CODE
Animal vocalized following dosing	VOC
Animal excessively pawed test eye following dosing	PAW
Animal exhibited excessive hyperactivity following dosing	HYP
Animal exhibited excessive head tilt following dosing	HT
Animal exhibited excessive squinting of test eye following dosing	SQ

APPENDIX B

Ocular Evaluation Criteria

SLI Study No. 3596.5 (23)

OCULAR EVALUATION CRITERIA

Maximum Mean Score (Days 0-3)	Maximum Mean Score	Persistence of Individual Scores	Descriptive Rating and Class	
0.00 – 0.49	24 hours = 0		Non-Irritating	1
	24 hours > 0		Practically Non-irritating	2
0.50 – 2.49	24 hours = 0		Non-Irritating	1
	24 hours > 0		Practically Non-irritating	2
2.50 – 14.99	48 hours = 0		Slight Irritant	3
	48 hours > 0		Mild Irritant	4
15.00 – 24.99	72 hours = 0		Mild Irritant	4
	72 hours > 0		Moderate Irritant	5
25.00 – 49.99	7 day ≤ 20	> half of day 7 scores ≤ 10	Moderate Irritant	5
		> half of day 7 scores > 10, but no score > 20	Moderate Irritant	5
		> half of day 7 scores > 10, and any score > 20	Severe Irritant	6
	7 day > 20		Severe Irritant	6
50.00 – 79.99	7 day ≤ 40	> half of day 7 scores ≤ 30	Severe Irritant	6
		> half of day 7 scores > 30, but no score > 60	Severe Irritant	6
		> half of day 7 scores > 30, and any score > 60	Very Severe Irritant	7
	7 day > 40		Very Severe Irritant	7
80.00 – 99.99	7 day ≤ 80	> half of day 7 scores ≤ 60	Very Severe Irritant	7
		> half of day 7 scores > 60, but no score > 100	Very Severe Irritant	7
		> half of day 7 scores > 60, and any score > 100	Extremely Severe Irritant	8
	7 day > 80		Extremely Severe Irritant	8
100.00 – 110.00	7 day ≤ 80		Very Severe Irritant	7
	7 day > 80		Extremely Severe Irritant	8

SLI Study No. 3596.5 (24)

APPENDIX C

SLI Personnel Responsibilities

SLI Study No. 3596.5 (25)

SLI PERSONNEL RESPONSIBILITIES

Kimberly L. Bonnette, M.S., LATG	Study Director/Director, Acute Toxicology
Dawn D. Rodabaugh, B.S.	Alternate Contact/Associate Toxicologist
Robert C. Springborn, Ph.D.	Chairman, President and CEO
Malcolm Blair, Ph.D.	Senior Vice President, Managing Director Emeritus
Joseph C. Siglin, Ph.D., DABT	Vice President, Managing Director
Christopher W. Wilson, B.S.	Associate Toxicologist
Pamela S. Smith, ALAT	Supervisor of Acute Toxicology
Kevin V. Weitzel, A.S.	Primary Technician/Inhalation Team Leader
Delores P. Knippen	Supervisor of Pharmacy
Steven H. Magness, B.S., LATG	Senior Supervisor of Gross and Fetal Pathology
Anita M. Bosau, RQAP-GLP	Senior Director, Compliance Assurance
Deanna M. Talerico, RQAP-GLP	Senior Supervisor of Quality Assurance
J. Dale Thurman, D.V.M., M.S., DACVP	Senior Director, Pathology
Kathy M. Gasser	Supervisor of Archives

AN ACUTE ORAL TOXICITY STUDY IN RATS WITH SPRAY--ALPHA

FINAL REPORT

OPPTS Guideline

870.1100

Author

Kimberly L. Bonnette, M.S., LATG

Study Completed on

September 3, 2002

Performing Laboratory

Springborn Laboratories, Inc. (SLI)
Ohio Research Center
640 North Elizabeth Street
Spencerville, Ohio 45887

SLI Study No.

3596.2

Submitted to

INL/A
U.S. Department of State
2201 C St. NW SA-4
Washington, DC 20520

SLI Study No. 3596.2 (2)

1. STATEMENT OF *NO* DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B), or (C).

Company: _____

Company Agent: _____ Date _____

_____ _____

Title Signature

2. COMPLIANCE STATEMENT

This study was conducted in compliance with the Good Laboratory Practice Standards as described by the EPA (40 CFR Parts 160 and 792).

Kimberly L. Bonnette, M.S., LATG
Study Director/Author
Springborn Laboratories, Inc.

Date 9/3/02

Rogers Woolfolk
Senior Aviation Advisor
Sponsor/Submitter
INL/A
U.S. Department of State

Date 12 Aug 02

3. QUALITY ASSURANCE STATEMENT

This study was inspected by the Quality Assurance Unit and reports were submitted to management and the Study Director in accordance with SLI's Standard Operating Procedures as follows:

Phase	Date
Protocol Review	03/31/02
Necropsy	06/18/02
Data Audit	07/16/02
Draft Report Review	07/16/02
Protocol Amendment Review	07/25/02
Final Report Review	09/03/02
Reports to Study Director and Management	07/16/02, 09/03/02

The final report has been reviewed to assure that it accurately describes the materials and methods, and the reported results accurately reflect the raw data.

Jennifer D. McGue Date 9/3/02
Jennifer D. McGue
Quality Assurance Auditor

for Deanna M. Talerico Date 9/3/02
Anita M. Bosau, RQAP-GLP
Senior Director, Compliance Assurance

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SLI Study No. 3596.2 (7)

6. SUMMARY

The single-dose oral toxicity of Spray--Alpha was evaluated in Sprague Dawley rats. A limit test was performed in which one group of five male and five female rats received a single oral administration of the test article at a dose of 5000 mg/kg body weight. Following dosing, the limit test rats were observed daily and weighed weekly. A gross necropsy examination was performed on all limit test animals at the time of scheduled euthanasia (day 14).

No mortality occurred during the limit test. No significant dinical abnormalities were observed during the study. Body weight gain was noted for all animals during the test period. No significant gross internal findings were observed at necropsy on study day 14.

Under the conditions of this test, the acute oral LD50 of Spray--Alpha was estimated to be greater than 5000 mg/kg in the rat.

SLI Study No. 3596.2 (8)

7. INTRODUCTION

This study was performed to assess the short-term toxicity of Spray--Alpha in Sprague Dawley rats when administered by gavage as a single oral dose. This study was intended to provide information on the potential health hazards of the test article with respect to oral exposure. Data from this study may serve as a basis for classification and/or labeling of the test article. This study was performed in accordance with the US EPA, Health Effects Test Guidelines, OPPTS 870.1100, Acute Oral Toxicity, August 1998. This study was performed at Springborn Laboratories, Inc., 553 North Broadway, Spencerville, Ohio. The protocol was signed by the Study Director on April 30, 2002 (GLP initiation date). The in-life phase of the study was initiated with test article administration on June 4, 2002 (day 0) and concluded with necropsy on June 18, 2002.

8. MATERIALS AND METHODS

8.1. Test Article

The test article was received from the Sponsor and identified as follows:

Sponsor's ID	Assigned SLI ID	Physical Description	Receipt Date	Expiration Date
Spray—Alpha[a]	S02.001.3596	Light amber liquid	05/13/02	None provided
Ingredients[b]				
Herbicide: Fuete-SL Lot No.: 02-01-02				None Provided
Surfactant: Cosmo Flux-411F Lot No.: 244301				10/2003

[a]Sample pooled at SLI from five different mixes of Spray--Alpha (top/middle/bottom).
[b]Ingredients used in the five Spray--Alpha mixes that were prepared by the Sponsor.

The test article was stored at room temperature. The Sponsor was responsible for any necessary evaluations related to identity, strength, purity, composition, stability and method of synthesis of the test material according to 40 CFR 160.105 and 40 CFR 792.105. Springborn Laboratories, Inc., analyzed the test article for the glyphosate (a.e.) which is presented in SLI Study No. 3596.1.

8.2. Retention Sample

An approximate 1 mL retention sample of each test article mixture sample (top/middle/bottom, maintained separately for a total of fifteen 1 mL samples) was taken and stored at SLI at room temperature. In addition, a 10 mL retention sample of the pooled test article samples (from the 5 test article mixtures) was collected and stored at SLI at room temperature. These samples serve as the retention samples for all studies conducted with this material.

8.3. Test Article Disposition

The remaining test article was returned to the Sponsor following completion of all studies with the test article.

8.4. Method of Test Article Preparation

The test article was administered as received from the Sponsor and dispensed fresh on the day of dosing. The density of the test article was determined to be 1.08 g/mL.

8.5. Animals and Animal Husbandry

8.5.1. Description, Identification and Housing

Young adult, Hsd: Sprague Dawley® SD® rats were received from Harlan Sprague Dawley, Inc., Indianapolis, IN. Upon receipt, metal ear tags displaying unique identification numbers were used to individually identify the animals. Cage cards displaying at least the study number, animal number and sex were affixed to each cage. The animals were housed individually in suspended stainless steel cages. All housing and care were based on the standards recommended by the Guide for the Care and Use of Laboratory Animals [1].

8.5.2. Environment

The animal room temperature and relative humidity ranges were 61-74°F (16-23°C) and 35-61%, respectively. Environmental control equipment was monitored and adjusted as necessary to minimize fluctuations in the animal room environment. Light timers were set to maintain a 12-hour light/12-hour dark cycle and room ventilation was set to produce 10-15 air changes/hour. The animal room temperature and relative humidity were recorded a minimum of once daily.

SLI Study No. 3596.2 (10)

8.5.3. Food

PMI Certified Rodent Chow #5002 (Purina Mills, Inc.) was provided ad libitum to the animals throughout the study (except during fasting). The lot number and expiration date of each batch of diet used during the study were recorded. The feed was analyzed and certified by the supplier for nutritional components and environmental contaminants. Dietary limitations for various environmental contaminants, including heavy metals, pesticides, polychlorinated biphenyls and total aflatoxin are set by the manufacturer. Within these limits, contaminants which may have been present were not expected to compromise the purpose of this study. Results of the dietary analyses (Certificates of Analysis) are provided by the manufacturer for each lot of diet. These are maintained by SLI.

8.5.4. Water

Municipal tap water treated by reverse osmosis was available ad libitum throughout the study. The purified water was supplied by an automatic watering system. Monitoring of the drinking water for contaminants is conducted by SLI and the records are available for inspection. Within generally accepted limits, contaminants which may have been present were not expected to compromise the purpose of this study. The water meets the standards specified under the EPA National Drinking Water Regulations (40 CFR Part 141).

8.5.5. Acclimation

Upon receipt, the animals were removed randomly from the shipping cartons, examined by qualified personnel, identified with metal ear tags and then acclimated to the laboratory conditions for a minimum of five days. The animals were observed daily for overt physical or behavioral abnormalities, general health/moribundity and mortality.

8.5.6. Animal Selection

The animals chosen for study use were randomly selected from healthy stock animals using a computerized (Alpha DS-10) random numbers table to avoid potential bias. All animals received a detailed pretest observation prior to dosing. Only healthy animals were chosen for study use. Females were nulliparous and nonpregnant. The male animals were approximately 10 weeks of age and weighed 249-259 g prior to fasting. The female animals were approximately 12 weeks of age and weighed 218-242 g prior to fasting.

SLI Study No. 3596.2 (11)

9. EXPERIMENTAL PROCEDURES

9.1. Dosing

On day -1, the animals chosen for the limit test were weighed and fasted overnight. On day 0, the test article was administered orally as a single dose using a ball tipped stainless steel gavage needle attached to a syringe at the following level:

Dose Level (mg/kg)	Dose Volume (mL/kg)	Concentration (mg/mL)	No. of Animals	
			Male	Female
5000	4.63	1000[a]	5	5

[a]Pooled test article.

Individual doses were calculated based on the animal's fasted (day 0) body weight. Animals were returned to ad libitum feeding after dosing.

9.2. Clinical Observations

The animals were observed for clinical abnormalities two times on study day 0 (post-dose) and daily thereafter (days 1-14). A general health/mortality check was performed twice daily (in the morning and in the afternoon).

9.3. Body Weights

Individual body weights were obtained for the animals prior to fasting (day -1), prior to dosing on day 0 and on days 7 and 14.

9.4. Gross Necropsy

All animals were euthanized by carbon dioxide inhalation at study termination (day 14) and necropsied. Body cavities (cranial, thoracic, abdominal and pelvic) were opened and examined. No tissues were retained.

9.5. Protocol Deviations

On one occasion, the animal room temperature range (61-74°F) exceeded the preferred range (66-77°F) during this study. This occurrence is considered to have had no adverse effect on the outcome of this study.

10. ANALYSIS OF DATA

Data from the study were analyzed and an LD50 value estimated as follows:

< 50% Mortality: LD50 was estimated as greater than the administered dose.
= 50% Mortality: LD50 was estimated as equal to the administered dose.
> 50% Mortality: LD50 was estimated as less than the administered dose.

Body weight means and standard deviations were calculated separately for males and females.

11. MAINTENANCE OF RAW DATA AND RECORDS

All original raw data, the final report and magnetically encoded records were transferred to the SLI archives for a period of 10 years. The Sponsor will be contacted prior to final disposition of these items.

12. RESULTS

12.1. Mortality

Individual Data: Table 1

No mortality occurred during the limit test.

12.2. Clinical Observations

Individual Data: Table 1

No significant clinical abnormalities were observed during the study.

12.3. Body Weight Data

Individual Data: Table 2

Body weight gain was noted for all animals during the test period.

12.4. Gross Necropsy

Individual Data: Table 3

No significant gross internal findings were observed at necropsy on study day 14.

13. CONCLUSION

Under the conditions of this test, the acute oral LD50 of Spray--Alpha was estimated to be greater than 5000 mg/kg in the rat.

Kimberly L. Bonnette, M.S., LATG
Study Director

Date 9/3/02

14. REPORT REVIEW

Dawn D. Rodabaugh, B.S.
Associate Toxicologist

Date 9/3/02

15. REFERENCE

1. Guide for the Care and Use of Laboratory Animals, DHHS Publication No. (NIH) 96-03, 1996.

STUDY NO.: 3596.2 PAGE 1

INL/A
U.S. DEPARTMENT OF STATE

TABLE 1

AN ACUTE ORAL TOXICITY STUDY IN RATS

INDIVIDUAL CLINICAL OBSERVATIONS
(POSITIVE FINDINGS)

MALES 5000 MG/KG

		DAY OF STUDY													
MALE#	OBSERVATIONS	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A5246	SCHEDULED EUTHANASIA															P
A5248	SCHEDULED EUTHANASIA															P
A5256	SCHEDULED EUTHANASIA															P
A5259	SCHEDULED EUTHANASIA															P
A5260	SCHEDULED EUTHANASIA															P
	CONGESTED BREATHING	P	P	P	P											

GRADE CODE: 1=SLIGHT 2=MODERATE 3=SEVERE P=PRESENT L=LEFT R=RIGHT B=BILATERAL

TABLE 1

AN ACUTE ORAL TOXICITY STUDY IN RATS

INDIVIDUAL CLINICAL OBSERVATIONS
(POSITIVE FINDINGS)

FEMALES 5000 MG/KG

FEMALE#	OBSERVATIONS	DAY OF STUDY 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
A5099	SCHEDULED EUTHANASIA	P
A5113	SCHEDULED EUTHANASIA	P
A5123	SCHEDULED EUTHANASIA	P
A5111	SCHEDULED EUTHANASIA	P
A5107	SCHEDULED EUTHANASIA	P

GRADE CODE: 1=SLIGHT 2=MODERATE 3=SEVERE P=PRESENT L=LEFT R=RIGHT B=BILATERAL

STUDY NO.: 3596.2 PAGE 1

INL/A
U.S. DEPARTMENT OF STATE

TABLE 2

AN ACUTE ORAL TOXICITY STUDY IN RATS

INDIVIDUAL BODY WEIGHTS (GRAMS)

MALES 5000 MG/KG

ANIMAL#	DAY OF STUDY -1	0	7	14	AT DEATH (DAY)
A5246	258	234	289	325	
A5248	259	236	301	314	
A5256	249	228	288	312	
A5259	249	230	286	306	
A5260	255	227	296	340	
MEAN	254	231	292	319	
S.D.	4.8	3.9	6.3	13.4	
N	5	5	5	5	

TABLE 2

AN ACUTE ORAL TOXICITY STUDY IN RATS

INDIVIDUAL BODY WEIGHTS (GRAMS)

FEMALES 5000 MG/KG

	DAY OF STUDY			
ANIMAL#	-1	0	7	14 AT DEATH (DAY)
A5099	218	206	237	238
A5113	219	206	225	235
A5123	230	211	242	243
A5111	223	206	234	256
A5107	242	221	242	250
MEAN	226	210	236	244
S.D.	9.9	6.5	7.0	8.6
N	5	5	5	5

STUDY NO.: 3596.2 PAGE 1

INL/A
U.S. DEPARTMENT OF STATE

TABLE 3

AN ACUTE ORAL TOXICITY STUDY IN RATS

INDIVIDUAL GROSS NECROPSY OBSERVATIONS

MALES 5000 MG/KG

ANIMAL#	DAY OF DEATH	STUDY DAY	OBSERVATION	FATE
A5246	18-JUN-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A5248	18-JUN-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A5256	18-JUN-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A5259	18-JUN-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A5260	18-JUN-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA

TABLE 3

AN ACUTE ORAL TOXICITY STUDY IN RATS

INDIVIDUAL GROSS NECROPSY OBSERVATIONS

FEMALES 5000 MG/KG

ANIMAL#	DAY OF DEATH	STUDY DAY	OBSERVATION	FATE
A5099	18-JUN-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A5113	18-JUN-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A5123	18-JUN-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A5111	18-JUN-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A5107	18-JUN-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA

(21)

SLI Study No. 3596.2

APPENDIX A

SLI Personnel Responsibilities

(22)

SLI Study No. 3596.2

SLI PERSONNEL RESPONSIBILITIES

Kimberly L. Bonnette, M.S., LATG	Study Director/Director, Acute Toxicology
Dawn D. Rodabaugh, B.S.	Alternate Contact/Associate Toxicologist
Robert C. Springborn, Ph.D.	Chairman, President and CEO
Malcolm Blair, Ph.D.	Senior Vice President, Managing Director Emeritus
Joseph C. Siglin, Ph.D., DABT	Vice President, Managing Director
Christopher W. Wilson, B.S.	Associate Toxicologist
Pamela S. Smith, ALAT	Supervisor of Acute Toxicology
Kevin V. Weitzel, A.S.	Primary Technician/Inhalation Team Leader
Delores P. Knippen	Supervisor of Pharmacy
Steven H. Magness, B.S., LATG	Senior Supervisor of Gross and Fetal Pathology
Anita M. Bosau, RQAP-GLP	Senior Director, Compliance Assurance
Deanna M. Talerico, RQAP-GLP	Senior Supervisor of Quality Assurance
J. Dale Thurman, D.V.M., M.S., DACVP	Senior Director, Pathology
Kathy M. Gasser	Supervisor of Archives

A PRIMARY SKIN IRRITATION STUDY IN RABBITS WITH SPRAY--ALPHA

FINAL REPORT

OPPTS Guideline

870.2500

Author

Kimberly L. Bonnette, M.S., LATG

Study Completed on

September 3, 2002

Performing Laboratory

Springborn Laboratories, Inc. (SLI)
Ohio Research Center
640 North Elizabeth Street
Spencerville, Ohio 45887

SLI Study No.

3596.6

Submitted to

INL/A
U.S. Department of State
2201 C St. NW SA-4
Washington, DC 20520

SLI Study No. 3596.6 (2)

1. STATEMENT OF *NO* DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B), or (C).

Company: _____

Company Agent: _____ Date _____

_____ _____

Title Signature

SLI Study No. 3596.6 (3)

2. COMPLIANCE STATEMENT

This study was conducted in compliance with the Good Laboratory Practice Standards as described by the EPA (40 CFR Parts 160 and 792).

Kimberly L. Bonnette, M.S., LATG
Study Director/Author
Springborn Laboratories, Inc.

Date 9/3/02

Rogers Woolfolk
Senior Aviation Advisor
Sponsor/Submitter
INL/A
U.S. Department of State

Date 12 Aug 02

3. QUALITY ASSURANCE STATEMENT

This study was inspected by the Quality Assurance Unit and reports were submitted to management and the Study Director in accordance with SLI's Standard Operating Procedures as follows:

Phase	Date
Protocol Review	03/31/02
Body Weights	06/06/02
Data Audit	07/15/02
Draft Report Review	07/15/02
Protocol Amendment Review	07/25/02
Final Report Review	09/03/02
Reports to Study Director and Management	07/15/02, 9/03/02

The final report has been reviewed to assure that it accurately describes the materials and methods, and the reported results accurately reflect the raw data.

Jennifer D. McGue Date 9/3/02
Jennifer D. McGue
Quality Assurance Auditor

Deanna M. Talenco Date 9/3/02
for Anita M. Bosau, RQAP-GLP
Senior Director, Compliance Assurance

4. TABLE OF CONTENTS

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6. SUMMARY

The potential irritant and/or corrosive effects of Spray--Alpha were evaluated on the skin of New Zealand White rabbits. Each of three rabbits received a 0.5 mL dose of the test article as a single dermal application. The dose was held in contact with the skin under a semi-occlusive binder for an exposure period of four hours. Following the exposure period, the binder was removed and the remaining test article was wiped from the skin using gauze moistened with deionized water followed by dry gauze. Test sites were subsequently examined and scored for dermal irritation for up to 7 days following patch application.

Exposure to the test article produced very slight erythema and very slight edema on 2/3 and 1/3 test sites, respectively, at the 1-hour scoring interval. The dermal irritation resolved on 2/3 test sites by the 24 hour scoring interval and the remaining test site by study day 7.

Under the conditions of the test, Spray--Alpha is considered to be a slight irritant to the skin of the rabbit. The calculated Primary Irritation Index for the test article was 0.50.

SLI Study No. 3596.6 (8)

7. INTRODUCTION

This study was performed to assess the potential irritant and/or corrosive effects of Spray--Alpha in New Zealand White rabbits when administered by a single dermal dose. This study was intended to provide information on the potential health hazards of the test article with respect to dermal exposure. Data from this study may serve as a basis for classification and/or labeling of the test article. This study was conducted in accordance with the US EPA, Health Effects Test Guidelines, OPPTS 870.2500, Acute Dermal Irritation, August 1998. This study was performed at Springborn Laboratories, Inc., 553 North Broadway, Spencerville, Ohio. The protocol was signed by the Study Director on April 30, 2002 (GLP initiation date). The in-life phase of the study was initiated with test article administration on June 6, 2002 (day 0) and concluded with final scoring on June 13, 2002.

8. MATERIALS AND METHODS

8.1. Test Article

The test article was received from the Sponsor and identified as follows:

Sponsor's ID	Assigned SLI ID	Physical Description	Receipt Date	Expiration Date
Spray—Alpha[a]	S02.001.3596	Light amber liquid	05/13/02	None Provided
Ingredients[b]				
Herbicide:Fuete-SL Lot No.: 02-01-02				None Provided
Surfactant: Cosmo Flux-411F Lot No.: 244301				10/2003

[a]Sample pooled at SLI from five different mixes of Spray--Alpha (top/middle/bottom).
[b]Ingredients used in the five Spray--Alpha mixes that were prepared by the Sponsor.

The test article was stored at room temperature. The Sponsor was responsible for any necessary evaluations related to identity, strength, purity, composition, stability and method of synthesis of the test material according to 40 CFR 160.105 and 40 CFR 792.105. Springborn Laboratories, Inc., analyzed the test article for the glyphosate (a.e.) which is presented in SLI Study No. 3596.1.

8.2. Retention Sample

An approximate 1 mL retention sample of each test article mixture sample (top/middle/bottom, maintained separately for a total of fifteen 1 mL samples) was taken and stored at SLI at room temperature. In addition, a 10 mL retention sample of the pooled test article samples (from the 5 test article mixtures) was collected and stored at SLI at room temperature. These samples serve as the retention samples for all studies conducted with this material.

8.3. Test Article Disposition

The remaining test article was returned to the Sponsor following completion of all studies with the test article.

8.4. Method of Test Article Preparation

The test article was administered as received from the Sponsor and dispensed fresh on the day of dosing.

8.5. Animals and Animal Husbandry

8.5.1. Description, Identification and Housing

Adult, New Zealand White rabbits were received from Myrtle's Rabbitry, Thompson Station, TN. Upon receipt, plastic ear tags displaying unique identification numbers were used to individually identify the animals. Cage cards displaying at least the study number, animal number and sex were affixed to each cage. The animals were housed individually in suspended stainless steel cages. All housing and care were based on the standards recommended by the Guide for the Care and Use of Laboratory Animals [1].

8.5.2. Environment

The animal room temperature and relative humidity ranges were 70-74°F (21-23°C) and 42-75%, respectively. Environmental control equipment was monitored and adjusted as necessary to minimize fluctuations in the animal room environment. Light timers were set to maintain a 12-hour light/12-hour dark cycle and room ventilation was set to produce 10-15 air changes/hour. The animal room temperature and relative humidity were recorded a minimum of once daily.

8.5.3. Food

PMI Certified Rabbit Chow #5322 (Purina Mills, Inc.) was provided ad libitum to the animals throughout the study. The lot number and expiration date of each batch of diet used during the study were recorded. The feed was analyzed and certified by the supplier for nutritional components and environmental contaminants. Dietary limitations for various environmental contaminants, including heavy metals, pesticides, polychlorinated biphenyls and total aflatoxin are set by the manufacturer. Within these limits, contaminants which may have been present were not expected to compromise the purpose of this study. Results of the dietary analyses (Certificates of Analysis) were provided by the manufacturer for each lot of diet. These are maintained by SLI.

8.5.4. Water

Municipal tap water treated by reverse osmosis was available ad libitum throughout the study. The purified water was supplied by an automatic watering system. Monitoring of the drinking water for contaminants is conducted by SLI and the records are available for inspection. Within generally accepted limits, contaminants which may have been present were not expected to compromise the purpose of this study. The water meets the standards specified under the EPA National Drinking Water Regulations (40 CFR Part 141).

8.5.5. Acclimation

Upon receipt, the animals were removed randomly from the shipping cartons, examined by qualified personnel, identified with plastic ear tags and then acclimated to the laboratory conditions for a minimum of five days. The animals were observed daily for overt physical or behavioral abnormalities, general health/moribundity and mortality.

8.5.6. Animal Selection

The animals chosen for study use were arbitrarily selected from healthy stock animals to avoid potential bias. All animals received a detailed pretest observation prior to dosing. Only healthy animals were chosen for study use. The male animals were approximately 12 weeks of age and weighed 2.6-2.9 kg prior to dosing.

SLI Study No. 3596.6 (11)

9. EXPERIMENTAL PROCEDURES

9.1. Preliminary Procedures

On day -1, the animals chosen for use on the primary skin irritation study had the fur removed from the dorsal area of the trunk using an animal clipper. Care was taken to avoid abrading the skin during the clipping procedure.

9.2. Dosing

On the following day (day 0), the test article was applied to a small area of intact skin on each test animal (approximately 1 inch x 1 inch) as indicated below:

Concentration (%)	Amount Applied	Patch Design	No. of Animals Male
100[a]	0.5 mL	~1" x 1" square 4-ply gauze patch	3

[a]Pooled test article.

The test article was administered under the gauze patch. The gauze patch was held in contact with the skin at the cut edges with a nonirritating tape. Removal and ingestion of the test article was prevented by placing an elastic wrap over the trunk and test area (semi-occlusive binding). The elastic wrap was then further secured with adhesive tape around the trunk at the cranial and caudal ends. After dosing, collars were placed on each animal and remained in place until removal on day 3. After a four-hour exposure period, the binding materials were removed from each animal and the corners of the test site delineated using a marker. Residual test article was removed using gauze moistened with deionized water, followed by dry gauze.

9.3. Dermal Observations

Animals were examined for signs of erythema and edema and the responses scored at 1 hour after patch removal and 24, 48 and 72 hours and up to 7 days after patch application according to the Macroscopic Dermal Grading System presented in Appendix A which is based on Draize [2]. The dermal test sites were reclipped as necessary to allow clear visualization of the skin.

9.4. Clinical Observations

Any unusual observations and/or mortality were recorded. General health/mortality checks were performed twice daily (in the morning and in the afternoon).

9.5. Body Weights

Individual body weights were obtained for each animal prior to dosing on day 0.

9.6. Scheduled Euthanasia

Each animal was euthanized by an intravenous injection of sodium pentobarbital following its final scoring interval. Gross necropsy examinations were not required for these animals.

9.7. Protocol Deviations

On one occasion, the animal room temperature and relative humidity ranges (70-74°F and 42-75%, respectively) exceeded the preferred ranges (63-73°F and 30-70%, respectively) during this study. These occurrences are considered to have had no adverse effect on the outcome of this study.

10. ANALYSIS OF DATA

The 1-, 24-, 48- and 72-hour erythema and edema scores for all animals were added and the total divided by the number of test sites x 4. The calculated Primary Irritation Index (P.I.I.) was classified according to the Dermal Evaluation Criteria [3] presented in Appendix B.

11. MAINTENANCE OF RAW DATA AND RECORDS

All original raw data, the final report and magnetically encoded records were transferred to the SLI archives for a period of 10 years. The Sponsor will be contacted prior to final disposition of these items.

12. RESULTS

12.1. Dermal Observations

Individual Data: Table 1

Exposure to the test article produced very slight erythema and very slight edema on 2/3 and 1/3 test sites, respectively, at the 1-hour scoring interval. The dermal irritation resolved on 2/3 test sites by the 24 hour scoring interval and the remaining test site by study day 7.

13. CONCLUSION

Under the conditions of the test, Spray--Alpha is considered to be a slight irritant to the skin of the rabbit. The calculated Primary Irritation Index for the test article was 0.50.

Kimberly L. Bonnette, M.S., LATG
Study Director

Date 9/3/02

14. REPORT REVIEW

Dawn D. Rodabaugh, B.S.
Associate Toxicologist

Date 9/3/02

15. REFERENCES

1. Guide for the Care and Use of Laboratory Animals, DHHS Publication No. (NIH) 96-03, 1996.

2. Draize, J.H., Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics, The Association of Food and Drug Officials of the United States, 49-51, 1959.

3. Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals-Addendum 3 on Data Reporting, US EPA, 1988.

TABLE 1

SLI STUDY NO.: 3596.6 — A PRIMARY SKIN IRRITATION STUDY IN RABBITS — PAGE 1

CLIENT: INL/A, US DEPARTMENT OF STATE — INDIVIDUAL DERMAL IRRITATION SCORES

(SPRAY--ALPHA)

Animal No./Sex Body Weight (kg)	Scoring Interval	Erythema	Edema	Comments
R2176/M	1 Hour	1	0	
2.595	24 Hours	0	0	
	48 Hours	0	0	
	72 Hours	0	0	
R2161/M	1 Hour	1	1	
2.877	24 Hours	1	0	
	48 Hours	1	0	
	72 Hours	1	0	
	7 Days	0	0	
R2165/M	1 Hour	0	0	
2.915	24 Hours	0	0	
	48 Hours	0	0	
	72 Hours	0	0	

Note: See Appendix A for definition of codes.

Primary Irritation Index

0.50 = Slight Irritant

APPENDIX A

Macroscopic Dermal Grading System

SLI Study No. 3596.6 (17)

MACROSCOPIC DERMAL GRADING SYSTEM

ERYTHEMA AND EDEMA OBSERVATIONS		
OBSERVATION	DEFINITION	CODE
Erythema – Grade 0	No erythema	0
Erythema – Grade 1	Very slight erythema (barely perceptible)	1
Erythema – Grade 2	Well-defined erythema	2
Erythema – Grade 3	Moderate to severe erythema	3
Erythema – Grade 4	Severe erythema (beet redness)	4
Maximized Grade 4	Notable dermal lesions (see below)	M – 4 (see below)
Edema – Grade 0	No edema	0
Edema – Grade 1	Very slight edema (barely perceptible)	1
Edema – Grade 2	Slight edema (edges of area well defined by definite raising)	2
Edema – Grade 3	Moderate edema (raised approximately 1 millimeter)	3
Edema – Grade 4	Severe edema (raised more than 1 millimeter and extends beyond the area of exposure)	4
NOTE: Each animal was assigned an erythema and edema score. The most severely affected area within the test site was graded. If eschar, blanching, ulceration and/or necrosis greater than grade 1 was observed, then the “Maximized Grade 4" was assigned to the test site in place of the erythema score and the type of notable dermal lesion(s) (e.g., eschar - grade 2, blanching - grade 3, ulceration - grade 4, etc.) was noted. The presence of any other dermal changes (e.g., desquamation, fissuring, eschar exfoliation, etc.) was also recorded.		

SLI Study No. 3596.6 (18)

MACROSCOPIC DERMAL GRADING SYSTEM

NOTABLE DERMAL LESIONS		
OBSERVATION	CODE	DEFINITION
Eschar – Grade 1	ES-1	Focal and/or pinpoint areas up to 10% of test site.
Eschar – Grade 2	ES-2	> 10% < 25% of test site.
Eschar – Grade 3	ES-3	> 25% < 50% of test site.
Eschar – Grade 4	ES-4	> 50% of test site.
Blanching – Grade 1	BLA-1	Focal and/or pinpoint areas up to 10% of test site.
Blanching – Grade 2	BLA-2	> 10% < 25% of test site.
Blanching – Grade 3	BLA-3	> 25% < 50% of test site.
Blanching – Grade 4	BLA-4	> 50% of test site.
Ulceration – Grade 1	U-1	Focal and/or pinpoint areas up to 10% of test site.
Ulceration – Grade 2	U-2	> 10% < 25% of test site.
Ulceration – Grade 3	U-3	> 25% < 50% of test site.
Ulceration – Grade 4	U-4	> 50% of test site.
Necrosis – Grade 1	NEC-1 *(color)*	Focal and/or pinpoint areas up to 10% of test site (note color of necrosis).
Necrosis – Grade 2	NEC-2 *(color)*	> 10% < 25% of test site (note color of necrosis).
Necrosis – Grade 3	NEC-3 *(color)*	> 25% < 50% of test site (note color of necrosis).
Necrosis – Grade 4	NEC-4 *(color)*	> 50% of test site (note color of necrosis).

SLI Study No. 3596.6 (19)

MACROSCOPIC DERMAL GRADING SYSTEM

ADDITIONAL DERMAL FINDINGS		
OBSERVATION	DEFINITION	CODE
Desquamation	Characterized by scaling or flaking of dermal tissue or without denuded areas.	DES
Fissuring	Characterized by cracking of the skin with or without moist exudate. Fissuring should be checked prior to removing the animal from the cage and manipulating the test site.	FIS
Eschar Exfoliation	The process by which areas of eschar flake off the test site.	EXF
Test Site Staining	Skin located at test site appears to be discolored, possibly due to test article (note color of staining).	TSS (*color*)
Erythema Extends Beyond the Test Site	The erythema extends beyond the test site. Note: A study director should be contacted for erythema extending beyond the test site.	ERB
Superficial Lightening	Characterized by pale area(s) (almost a burn-like appearance) in the test site. However, erythema may still be observed through the pale area. Note: This observation may affect the overall erythema score of the test site. This observation may progress to other observations resulting in notable dermal lesions, but SL itself will not be considered a notable dermal lesion that will result in a dermal score to be maximized since it does not result in any in-depth injury. To be coded using an area designation (see below).	--
Superficial Lightening - Grade 1	Focal and/or pinpoint areas up to 10% of the test site	SL-1
Superficial Lightening - Grade 2	> 10% < 25% of test site	SL-2
Superficial Lightening - Grade 3	> 25% < 50% of test site	SL-3
Superficial Lightening - Grade 4	> 50% of test site	SL-4
Dermal Irritation - Outside of the Test Site	Noticeable irritation outside of test site probably due to the binding tape material. This notation will only be made for reactions greater than what are normally observed from tape removal which does not interfere with the scoring of the test site.	IT

APPENDIX B

Dermal Evaluation Criteria

SLI Study No. 3596.6 (21)

DERMAL EVALUATION CRITERIA	
Primary Irritation Index (P.I.I.)	Irritation Rating
0.00	Nonirritant
0.01 - 1.99	Slight Irritant
2.00 - 5.00	Moderate Irritant
5.01 - 8.00	Severe Irritant

APPENDIX C

SLI Personnel Responsibilities

SLI Study No. 3596.6 (23)

SLI PERSONNEL RESPONSIBILITIES

Kimberly L. Bonnette, M.S., LATG	Study Director/Director, Acute Toxicology
Dawn D. Rodabaugh, B.S.	Alternate Contact/Associate Toxicologist
Robert C. Springborn, Ph.D.	Chairman, President and CEO
Malcolm Blair, Ph.D.	Senior Vice President, Managing Director Emeritus
Joseph C. Siglin, Ph.D., DABT	Vice President, Managing Director
Rusty E. Rush, M.S., LAT, DABT	Director, Neurotoxicity and Transgenics
Christopher W. Wilson, B.S.	Associate Toxicologist
Pamela S. Smith, ALAT	Supervisor of Acute Toxicology
Kathy A. Pugh, ALAT	Primary Technician/Team Leader
Delores P. Knippen	Supervisor of Pharmacy
Steven H. Magness, B.S., LATG	Senior Supervisor of Gross and Fetal Pathology
Anita M. Bosau, RQAP-GLP	Senior Director, Compliance Assurance
Deanna M. Talerico, RQAP-GLP	Senior Supervisor of Quality Assurance
J. Dale Thurman, D.V.M., M.S., DACVP	Senior Director, Pathology
Kathy M. Gasser	Supervisor of Archives

PURITY ANALYSIS FOR GLYPHOSATE OF SPRAY--ALPHA (ACTIVE INGREDIENT)

FINAL REPORT

Author

Kimberly L. Bonnette, M.S., LATG

Study Completed on

October 3 , 2002

Performing Laboratory

Springborn Laboratories, Inc. (SLI)
Ohio Research Center
640 North Elizabeth Street
Spencerville, Ohio 45887

SLI Study No.

3596.1

Submitted to

INL/A
U.S. Department of State
2201 C St. NW SA-4
Washington, DC 20520

(2)

SLI Study No. 3596.1

1. STATEMENT OF *NO* DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B), or (C).

Company: _____

Company Agent: _____ Date _____

_____ _____

Title Signature

SLI Study No. 3596.1 (3)

SEP 30 2002

2. COMPLIANCE STATEMENT

This study was conducted in compliance with the Good Laboratory Practice Standards as described by the EPA (40 CFR Parts 160 and 792) with the following exception:

Since the test article mixtures were prepared in the field, the test article mixtures and the sample collection by the Sponsor were not performed according to GLP guidelines.

Kimberly L. Bonnette, M.S., LATG
Study Director/Author
Springborn Laboratories, Inc.

Date 10/3/02

Rogers Woolfolk
Senior Aviation Advisor
Sponsor/Submitter
INL/A
U.S. Department of State

Date 15 Sep 02

SLI Study No. 3596.1

3. QUALITY ASSURANCE STATEMENT

This study was inspected by the Quality Assurance Unit and reports were submitted to management and the Study Director in accordance with SLI's Standard Operating Procedures as follows:

Phase	Date
Protocol Review	03/31/02
Analytical Chemistry – Solution Preparation	05/08/02
Analytical Chemistry – Derivatization Procedure	05/22/02
Data Audit	09/06/02
Draft Report Review	09/06/02
Protocol Amendment Review	09/06/02
Final Report Review	10/03/02
Reports to Study Director and Management	09/06/02, 10/03/02

The final report has been reviewed to assure that it accurately describes the materials and methods, and the reported results accurately reflect the raw data.

Rebecca A. Young
Quality Assurance Team Leader

Date 10/3/02

Anita M. Bosau, RQAP-GLP
Senior Director, Compliance Assurance

Date 10/3/02

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SLI Study No. 3596.1

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6. SUMMARY

The objective of this study was to assess the concentration(s) of glyphosate (active ingredient) in the Spray--Alpha formulation.

Five test article mixtures were prepared in the field by the Sponsor. Three 500 mL samples of each mixture were collected at the top/middle/bottom (or beginning/middle/end) of Hoppers PNC 3065 (Test Article Mixtures 1 and 4), PNC 2070 (Test Article Mixtures 2 and 5), and PNC 3077 (Test Article Mixture 3). Test Article mixtures were prepared as follows:

Ingredient	Amount Added (gallons)
Herbicide:	
Fuente-SL (MON 2139)	87.9
Surfactant:	
Cosmo Flux-411F	2.0
Well water	110.1
Mixing time: 10 minutes in flight.	

Test article mixtures were prepared on two separate days (May 2, 2002, for Test Article Mixtures 1 and 2, and May 3, 2002 for Test Article Mixtures 3, 4, and 5).

The overall concentration of the Spray--Alpha was 16.3 [in terms of % glyphosate (a.e.)] before use at SLI and 15.5 [in terms of % glyphosate (a.e.)] after use at SLI, indicating that the test material was stable during use at SLI.

The overall result (~16.3% glyphosate a.e.) was slightly higher than the anticipated 14.80% glyphosate (a.e.), but well within acceptable error of mixing conditions in the field. Therefore, since the results of the analysis were appropriate (and would provide conservative results for toxicity, irritation and sensitization since they were slightly higher than expected), approximately 400 mL of each sample were pooled into a single container for use in the remaining studies.

7. INTRODUCTION

This study was performed to assess the concentrations of glyphosate (active ingredient) in Spray--Alpha. This study was performed to support studies conducted under the US EPA, Health Effects Test Guidelines. This study was performed at Springborn Laboratories, Inc., 553 North Broadway, Spencerville, Ohio. The protocol was signed by the Study Director on April 17, 2002 (GLP initiation date). The test article mixtures were analyzed for glyphosate (a.e.) initially on May 22, 2002, prior to all other studies and again on August 12, 2002, after all studies were complete for purposes of stability.

8. MATERIALS AND METHODS

8.1. Test Article

The test article was received from the Sponsor and identified as follows:

Sponsor's ID	Assigned SLI ID	Physical Description	Receipt Date	Expiration Date
Spray—Alpha[a]	S02.001.3596	Light amber liquid	05/13/02	None Provided
Ingredients[b]				
Herbicide: Fuete-SL Lot No.: 02-01-02				None Provided
Surfactant: Cosmo Flux-411F Lot No.: 244301				10/2003

[a]Sample pooled at SLI from five different mixes of Spray--Alpha (top/middle/bottom).
[b]Ingredients used in the five Spray--Alpha mixes that were prepared by the Sponsor.

The test article was stored at room temperature. The Sponsor was responsible for any necessary evaluations related to identity, strength, purity, composition, stability and method of synthesis of the test material according to 40 CFR 160.105 and 40 CFR 792.105.

8.2. Retention Sample

An approximate 1 mL retention sample of each test article mixture sample (top/middle/bottom, maintained separately for a total of fifteen 1 mL samples) was taken and stored at SLI at room temperature. In addition, a 10 mL retention sample of the pooled test article samples (from the 5 test article mixtures) was

collected and stored at SLI at room temperature. These samples serve as the retention samples for all studies conducted with this material.

8.3. Test Article Disposition

The test article was returned to the Sponsor following completion of all studies with the test article.

8.4. Method of Test Article Preparation

The test article containers were hand shaken and dispensed fresh on the day of analysis. The samples were stirred continuously until diluted for analysis.

9. EXPERIMENTAL PROCEDURE

9.1. Sample Collection

Samples were collected from the prepared test article mix using pre-labeled containers provided by SLI as follows:

Test Article Mix 1	500 mL 500 mL 500 mL	Beginning Middle End
Test Article Mix 2	500 mL 500 mL 500 mL	Beginning Middle End
Test Article Mix 3	500 mL 500 mL 500 mL	Beginning Middle End
Test Article Mix 4	500 mL 500 mL 500 mL	Beginning Middle End
Test Article Mix 5	500 mL 500 mL 500 mL	Beginning Middle End

Five test article mixtures were prepared in the field by the Sponsor. Three 500 mL samples of each mixture were collected from the top/middle/bottom (or beginning/middle/end) of Hoppers PNC 3065 (Test Article Mixtures 1 and 4), PNC 2070 (Test Article Mixtures 2 and 5), and PNC 3077 (Test Article Mixture 3). The Test Article mixtures were prepared as follows:

Ingredient	Amount Added (gallons)
Herbicide:	
Fuente-SL (MON 2139)	87.9
Surfactant:	
Cosmo Flux-411F	2.0
Well water	110.1
Mixing time: 10 minutes in flight.	

Test article mixtures were prepared on two separate days (May 2, 2002, for Test Article Mixtures 1 and 2, and May 3, 2002 for Test Article Mixtures 3, 4, and 5).

A total of fifteen 500 mL samples were collected. The individual (Robert Derosier, (Fixed Wing Standards Pilot, American Embassy, Bogota, Unit 5127, APO AA 34038) collecting samples completed the SLI provided form upon collection including signature and date when collected at San Jose del Guaviare, Columbia. Samples were maintained under ambient conditions.

10. ANALYTICAL CHEMISTRY

The samples were analyzed in terms of the active ingredient for concentration determination prior to any dosing (Before Use-Purity) and again after completion of all studies for stability determination (After-Use Purity). All analytical dilutions were performed in duplicate (either the same day or over two days).

The analytical method was a previously validated method for the analysis of glyphosate in solution. Purity analysis of the test article was performed in duplicate by comparison of the test article with supplied reference standards of known concentrations.

11. SPRAY--ALPHA ANALYSIS

The analytical method for the analysis of the glyphosate component of Spray--Alpha was validated prior to the purity analyses performed at Springborn Laboratories, Inc. This method was utilized to determine both the purity and the stability of the Spray--Alpha test material before and after use at SLI.

11.1. Experimental System

11.1.1. High Performance Liquid Chromatography (HPLC) System

HPLC Model: Waters
Pump: Waters 600E
Injector: Waters WISP 717

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Detector: Waters 2487
Data System: H-P 3396B Integrator
Precolumn: Phenomenex, SecurityGuard, C18, 4.0 x 3.0 mm ID
Column: Phenomenex, Spherex, C18, 5μ, 250 x 4.6 mm ID
Temperature: Ambient
Detection: 500 nm, 0.4000 AUFS
Mobile Phase: A: 0.05 M HCO_2NH_4, pH 3.6/5% ACN; B: 100% ACN
Gradient: 100% A hold for 6 minutes; linear change to 25% A/75% B over 1 minute; hold for 5 minutes; linear change to 100% A over 1 minute; hold at 100% A for 15 minutes.
Flow Rate: 1.0 mL/min
Injection Volume: 10 μL

11.1.2. Apparatus

Balance: Mettler AG 245, accuracy of 0.0001 gram
Glassware: Assorted volumetric glassware
Filters: Gelman, glass fiber; Millipore 0.2μ Nylon-66; Whatman Puradisc 25PP 0.45μm
Shaker: Labline, Multi-Wrist Shaker
Oven: Boekel Model 107905

11.1.3. Solutions and Reagents

11.1.3.1. Reagents

Water, Fisher, HPLC Grade, Lot # 024471, 025012
Acetonitrile, Fisher, HPLC Grade, Lot # 011777
Acetonitrile, J.T. Baker, HPLC Grade, Lot # M13828
Methanol, Fisher, HPLC Grade, Lot # 011803
NBD Chloride, Aldrich, 98%, Lot #12214L1
Hydrochloric Acid, Fisher, ACS Grade, Lot # 012161
Potassium Tetraborate Tetrahydrate:, Aldrich, 99%, Lot # 15325D1
Formic Acid, Fisher, Laboratory Grade, Lot # 003630
Ammonium Formate, Fisher, Lot # 990125
Glyphosate, Sigma, Lot # 71K36491

11.1.3.2. Solutions

0.37 M Borate Solution: Prepared by dissolving approximately 11.44 g of potassium tetraborate tetrahydrate in 100 mL of water. The resulting solution was stable for 6 months under ambient storage conditions.

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1.2 N HCl: Prepared by dissolving 10 mL of HCl in 90 mL of water. The resulting solution was stable for 6 months under ambient storage conditions.

25 mM NBD-Cl: Prepared by dissolving approximately 2.5 g of NBD-Cl in 500 mL of methanol. The resulting solution was stable for 6 months under ambient storage conditions.

Mobile Phase A: Prepared by dissolving approximately 2.36 g of ammonium formate in 1425 mL of water. The pH was adjusted to approximately 3.6 with formic acid prior to the addition of 75 ml of acetonitrile. The resulting solution was mixed thoroughly, filtered through a 0.2μ Nylon-66 filter and degassed by helium sparging prior to use. Larger volumes were also prepared using the same ratio of components.

Mobile Phase B: Acetonitrile used 100% as received.

Diluent: All standards and samples were diluted in water.

Stock Standard Solution: Prepared by dissolving approximately 30 mg of glyphosate standard in a 100 mL flask with diluent.

Standard Solutions: Prepared by serially diluting the stock standard solution with water. The final concentrations of the solutions were in the range of approximately 0.02 to 0.14 mg/mL. These solutions were sonicated and then further diluted in diluent at a ratio of 3:10 and filtered through Whatman Puradisc 25PP 0.45μm filters prior to derivatization.

Purity Solutions: Prepared by diluting 1.2 mL aliquots of each sample to a final volume of 100 mL with diluent. The solutions were further diluted in diluent first at a ratio of 4:100 and then at a ratio of 4:10. The resulting solutions were then filtered through Whatman Puradisc 25PP 0.45 μm filters prior to derivatization. These preparations were performed in duplicate for each sample.

Derivatization Procedure: In order to analyze the glyphosate component, a precolumn derivatization was performed by adding 1.2 mL of the appropriate control, standard, or sample solution to a labeled scintillation vial. Both 0.8 mL of the borate solution and 2.4 mL of the NBD-Cl solution were added to each vial. The vials were then capped and shaken by hand prior to being heated in an oven at 80° C for 30 minutes. After removal from the oven, the vials were allowed to cool for 10 minutes followed by the addition of 0.9 mL of the HCl solution. After the vials were again shaken by hand, they were allowed to stand for 10 minutes in order for incipient precipitation to occur. These solutions were then transferred to injection vials.

11.2. Analytical Procedures

11.2.1. Standard Curve Analysis

The peak area of the glyphosate acid component of each standard were determined, measured, combined, and plotted as a function of concentration to generate a standard curve. The actual values used for the calculations are shown in Chemistry Tables 1 and 2.

11.2.2. Sample Analysis

The peak areas of the glyphosate acid component of each sample were measured and combined and then the concentration was determined by linear fit to the standard curve. The actual values used for the calculations are shown in Chemistry Tables 1 and 2.

12. STATISTICAL ANALYSIS

A statistical analysis was conducted on the average results of the % glyphosate (a.e.) for each test article mixture as compared to the theoretical value [14.80% glyphosate (a.e.) as calculated by the Sponsor] and for the combined results of all test article mixture samples as compared to the theoretical value using one way analysis of variance (ANOVA).

13. PROTOCOL DEVIATIONS

No protocol deviations occurred during this study.

14. MAINTENANCE OF RAW DATA AND RECORDS

All original raw data, the final report and magnetically encoded records were transferred to the SLI archives for a period of 10 years. The Sponsor will be contacted prior to final disposition of these items.

15. RESULTS

15.1. Analytical Chemistry Results

Individual Data: Tables 1-5

The actual sample results of the initial purity analyses are shown in Chemistry Tables 1, 2 and 3. These samples were analyzed over two separate days (Before-Use Purity). The actual sample results of the final purity analyses (After-

Use Purity for stability purposes) are shown in Chemistry Tables 4 and 5. These samples were all analyzed on the same day. All concentration values are reported in terms of the acid equivalent (a.e.) of the glyphosate. The overall concentration of Spray--Alpha was 16.3 [in terms of % glyphosate (a.e.)] before use at SLI and 15.5 [in terms of % glyphosate (a.e.)] after use at SLI, indicating that the test material was stable during use at SLI. The average % error for Before-Use (and After-Use) indicate that the Test Article Mix 3 was significantly higher in concentration then the other 4 mixes.

15.2. Statistical Analysis

Individual Data: Appendix A

Results of the Before-Use statistical analysis indicate that Test Article Mixture 4 (18.4% glyphosate a.e.) and test article mixture 2 (16.2% glyphosate a.e.) were significantly higher than the theoretical value (14.8% glyphosate a.e.). However, since these values were within the possible error rate of field mixing and since these samples were to be part of a pooled sample for dosing the remaining studies, these samples were included. Overall, the results of all mixtures for the pooled sample (16.3% glyphosate a.e.) were significantly higher than the theoretical value (14.8% glyphosate a.e.). This was considered within possible field mixing error and would provide a conservative estimate of toxicity, irritation and sensitization for the remaining studies. Therefore, the pooled sample was considered to be acceptable for use.

16. CONCLUSION

The overall result (~16.3% glyphosate a.e.) was slightly higher than the anticipated 14.80% glyphosate (a.e.), but well within acceptable error of mixing conditions in the field. Therefore, since the results of the analysis were appropriate (and would provide conservative results for toxicity, irritation and sensitization since they were slightly higher than expected), approximately 400 mL of each sample were pooled into a single container for use in the remaining studies.

_____ Date _____

Kimberly L. Bonnette, M.S., LATG
Study Director

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17. REPORT REVIEW

Dawn D. Rodabaugh, B.S.
Toxicologist

Date 10/3/02

M. Gardner Clemons, B.S.
Senior Supervisor of Analytical Chemistry
and Pharmacy

Date 10.3.2002

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Chemistry Table 1

Standard Curve and Sample Analysis Values for the Before Use Purity Analyses (5/22/2002)

Sample Type.	Theoretical Conc. (mg/mL)	Peak Area	Actual Conc. [% Glyphosate (a.e.)]
Std 1	0.008580	36729	NA
Std 2	0.01716	74954	NA
Std 3	0.02574	110393	NA
Std 4	0.03432	152099	NA
Std 5	0.04290	191914	NA
Test Mix # 1, B	NA	134276	15.84
Test Mix # 1, M	NA	139682	16.46
Test Mix # 1, E	NA	133783	15.77
Test Mix # 2, B	NA	122717	14.50
Test Mix # 2, M	NA	177523	13.90
Test Mix # 2, E	NA	115833	13.71
Test Mix # 3, B	NA	146078	17.20
Test Mix # 3, M	NA	149827	17.63
Test Mix # 3, E	NA	142745	16.81
Test Mix # 3, B*	NA	140800	18.26
Test Mix # 3, M*	NA	145972	18.92
Test Mix # 3, E*	NA	151078	19.56
Test Mix # 4, B**	NA	114166	14.91
Test Mix # 4, M	NA	112720	13.35
Test Mix # 4, E	NA	116564	13.79
Test Mix # 5, B	NA	118306	13.99
Test Mix # 5, M	NA	122335	14.46
Test Mix # 5, E	NA	116804	13.82

Correlation coefficient = 0.9996

Note: B = Beginning; M = Middle; E = End; NA = Not Applicable

* These samples were re-analyzed on 5/23/2002 to verify the original results.

** The original value generated for this sample on 5/22/2002 was not reported due to it's deviation from the mean.

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Chemistry Table 2

Standard Curve and Sample Analysis Values for the Before Use Purity Analyses
(5/23/2002)
(Duplicate Samples)

Sample Type.	Theoretical Conc. (mg/mL)	Peak Area	Actual Conc. [% Glyphosate (a.e.)]
Std 1	0.008550	32585	NA
Std 2	0.01710	65919	NA
Std 3	0.02565	99885	NA
Std 4	0.03420	136969	NA
Std 5	0.04275	173829	NA
Test Mix # 1, B'	NA	140334	18.21
Test Mix # 1, M'	NA	138656	17.99
Test Mix # 1, E'	NA	132930	17.27
Test Mix # 2, B'	NA	122491	15.96
Test Mix # 2, M'	NA	118147	15.41
Test Mix # 2, E'	NA	123855	16.13
Test Mix # 3, B'	NA	151318	19.59
Test Mix # 3, M'	NA	147145	19.07
Test Mix # 3, E'	NA	145996	18.92
Test Mix # 4, B'	NA	113519	14.83
Test Mix # 4, M'	NA	117864	15.38
Test Mix # 4, E'	NA	118768	15.49
Test Mix # 5, B'	NA	122705	15.99
Test Mix # 5, M'	NA	118657	15.48
Test Mix # 5, E'	NA	136909	17.77

Correlation coefficient = 0.9997
' = Duplicate
Note: B = Beginning; M = Middle; E = End; NA = Not Applicable

Chemistry Table 3

Sample Analysis Value and % Error Based on Theoretical Value (Before Use-Purity)

Test Mix No.	Sample Type	% Glyphosate (a.e.)	Average % Glyphosate (a.e.) by	Average % Glyphosate (a.e.) by Test	Overall Average % Glyphosate (a.e.)	Theoretical Value % Error[a]	Average % Error by Type[a]	Average % Error by Test Mix[a]	Date of Analysis
1	Beginning	15.84			16.04	7.0			5/22/2002
1	Beginning'	18.21	17.03			23.0	15.0		5/23/2002
1	Middle	16.46				11.2			5/22/2002
1	Middle'	17.99	17.23			21.6	16.4		5/23/2002
1	End	15.77				6.6			5/22/2002
1	End'	17.27	16.52	16.92		16.7	11.6	14.3	5/23/2002
2	Beginning	14.50				2.0			5/22/2002
2	Beginning'	15.96	15.23			7.8	4.9		5/23/2002
2	Middle	13.90				6.1			5/22/2002
2	Middle'	15.41	14.66			4.1	5.1		5/23/2002
2	End	13.71				7.4			5/22/2002
2	End'	16.13	14.92	14.94		9.0	8.2	6.1	5/23/2002
3	Beginning	17.20				16.2			5/22/2002
3	Beginning'	19.59				32.4			5/23/2002
3*	Beginning	18.26	18.35			23.4	24.0		5/23/2002
3	Middle	17.63				19.1			5/22/2002
3	Middle'	19.07				28.9			5/23/2002
3*	Middle	18.92	18.54			27.8	25.3		5/23/2002
3	End	16.81				13.6			5/22/2002
3	End'	18.92				27.8			5/23/2002
3*	End	19.56	18.43	18.44		32.2	24.5	24.6	5/23/2002
4*	Beginning	14.91				0.7			5/23/2002
4	Beginning'	14.83	14.87			0.2	0.5		5/23/2002
4	Middle	13.35				9.8			5/22/2002
4	Middle'	15.38	14.37			3.9	6.9		5/23/2002
4	End	13.79				6.8			5/22/2002
4	End'	15.49	14.64	14.63		4.7	5.7	4.4	5/23/2002
5	Beginning	13.99				5.5			5/22/2002
5	Beginning'	15.99	14.99			8.0	6.8		5/23/2002
5	Middle	14.46				2.3			5/22/2002
5	Middle'	15.48	14.97			4.6	3.4		5/23/2002
5	End	13.82				6.6			5/22/2002
5	End'	17.77	15.80	15.25		20.1	13.3	7.8	5/23/2002

' = Duplicate

*Re-run of initial sample to verify results

[a]Percent error determined based on result compared to theoretical value (14.80%).

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Chemistry Table 4
Standard Curve and Sample Analysis Values for the After-Use Purity (for Stability) Analyses
(8/12/2002)

Sample Type.	Theoretical Conc. (mg/mL)	Peak Area	Actual Conc. [% Glyphosate (a.e.)]
Std 1	0.008778	35758	NA
Std 2	0.01756	52370	NA
Std 3	0.02633	105625	NA
Std 4	0.03511	149415	NA
Std 5	0.04389	198319	NA
Test Mix # 1, B	NA	128284	15.54
Test Mix # 1, B'	NA	136144	16.43
Test Mix # 1, M	NA	135922	16.40
Test Mix # 1, M'	NA	131126	15.86
Test Mix # 1, E	NA	135464	16.35
Test Mix # 1, E'	NA	139284	16.79
Test Mix # 2, B	NA	123800	15.03
Test Mix # 2, B'	NA	118776	14.46
Test Mix # 2, M	NA	123293	14.97
Test Mix # 2, M'	NA	120982	14.71
Test Mix # 2, E	NA	125297	15.20
Test Mix # 2, E'	NA	122015	14.83
Test Mix # 3, B	NA	148552	17.84
Test Mix # 3, B'	NA	149797	17.98
Test Mix # 3, M	NA	149962	18.00
Test Mix # 3, M'	NA	146301	17.58
Test Mix # 3, E	NA	150692	18.08
Test Mix # 3, E'	NA	152330	18.27
Test Mix # 4, B	NA	114245	13.95
Test Mix # 4, B'	NA	118361	14.41
Test Mix # 4, M	NA	116396	14.19
Test Mix # 4, M'	NA	112566	13.75
Test Mix # 4, E	NA	115074	14.04
Test Mix # 4, E'	NA	114163	13.94
Test Mix # 5, B	NA	120549	14.66
Test Mix # 5, B'	NA	116356	14.19
Test Mix # 5, M	NA	121537	14.77
Test Mix # 5, M'	NA	115371	14.07
Test Mix # 5, E	NA	119116	14.50
Test Mix # 5, E'	NA	119244	14.51

Correlation coefficient = 0.996
Note: B = Beginning; M = Middle; E = End; NA = Not Applicable
' = Duplicate Sample

SLI Study No. 3596.1 Chemistry Table 5

Sample Analysis Value and % Error Based on Theoretical Value (After Use-Purity for Stability)

Test Mix No.	Sample Type	% Glyphosate (a.e.)	Average % Glyphosate (a.e.) by	Average % Glyphosate (a.e.) by Test	Overall Average % Glyphosate (a.e.)	Theoretical Value % Error[a]	Average % Error by Type[a]	Average % Error by Test Mix[a]	Date of Analysis
1	Beginning	15.54			15.51	5.0			8/12/2002
1	Beginning'	16.43	15.99			11.0	8.0		8/12/2002
1	Middle	16.40				10.8			8/12/2002
1	Middle'	15.86	16.13			7.2	9.0		8/12/2002
1	End	16.35				10.5			8/12/2002
1	End'	16.79	16.57	16.23		13.4	12.0	9.7	8/12/2002
2	Beginning	15.03				1.6			8/12/2002
2	Beginning'	14.46	14.75			2.3	1.9		8/12/2002
2	Middle	14.97				1.1			8/12/2002
2	Middle'	14.71	14.84			0.6	0.9		8/12/2002
2	End	15.20				2.7			8/12/2002
2	End'	14.83	15.02	14.87		0.2	1.5	1.4	8/12/2002
3	Beginning	17.84				20.5			8/12/2002
3	Beginning'	17.98	17.91			21.5	21.0		8/12/2002
3	Middle	18.00				21.6			8/12/2002
3	Middle'	17.58	17.79			18.8	20.2		8/12/2002
3	End	18.08				22.2			8/12/2002
3	End'	18.27	18.18	17.96		23.4	22.8	21.3	8/12/2002
4	Beginning	13.95				5.7			8/12/2002
4	Beginning'	14.41	14.18			2.6	4.2		8/12/2002
4	Middle	14.19				4.1			8/12/2002
4	Middle'	13.75	13.97			7.1	5.6		8/12/2002
4	End	14.04				5.1			8/12/2002
4	End'	13.94	13.99	14.05		5.8	5.5	5.1	8/12/2002
5	Beginning	14.66				0.9			8/12/2002
5	Beginning'	14.19	14.43			4.1	2.5		8/12/2002
5	Middle	14.77				0.2			8/12/2002
5	Middle'	14.07	14.42			4.9	2.6		8/12/2002
5	End	14.50				2.0			8/12/2002
5	End'	14.51	14.51	14.45		2.0	2.0	2.4	8/12/2002

' = Duplicate

*Re-run of initial sample to verify results

**Not used in calculation of average. Refer to statement dated 5/28/2002.

[a]Percent error determined based on result compared to theoretical value (14.8%).

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APPENDIX A

Statistical Analysis

SLI STUDY NO. 3596.1 PURITY ANALYSIS FOR GLYPHOSATE (ACTIVE INGREDIENT) PAGE 1

BEFORE USE PURITY
% GLYPHOSATE (a.e.)
RAW DATA LISTING
TREATMENTS

OBSERVATIONS	CONTROL (THEORETICAL VALUE)	TEST ARTICLE MIXTURE NO.: 1	2	3a	4	5
GROUP	1	2	3	4	5	6
1	14.800	15.840	14.500	17.200	14.910	13.990
2	14.800	18.210	15.960	19.590	14.830	15.990
3	14.800	16.460	13.900	18.260	13.350	14.460
4	14.800	17.990	15.410	17.630	15.380	15.480
5	14.800	15.770	13.710	19.070	13.790	13.820
6	14.800	17.270	16.130	18.920	15.490	17.770
7				16.810		
8				18.920		
9				19.560		

aNOTE: ADDITIONAL REPLICATE SAMPLE ANALYZED FOR TOP/MIDDLE/BOTTOM TO VERIFY HIGHER RESULTS.

A N A L Y S I S OF V A R I A N C E 3596.1 BEFORE USE PURITY

SOURCE OF VARIATION	DF	SUM OF SQUARES	MEAN SQUARE
BETWEEN CLASSES		88.8117	17.7623
WITHIN CLASSES	33	34.3867	1.0420
TOTAL	38	123.1984	

F = 17.05, DF= 5/ 33, P=0.0000

GROUP:	1	2	3	4
MEANS:	14.8	16.9	14.9	18.4
S.D. :	0.00	1.06	1.05	1.02

TUKEYS TEST (2-tailed)

GROUP	DF	PROB	T
1 VS 2	33	0.0120	5.095
1 VS 3	33	0.9999	0.324
1 VS 4	33	0.0000	9.568
1 VS 5	33	0.9997	0.420
1 VS 6	33	0.9713	1.084
2 VS 3	33	0.0215	4.771
2 VS 4	33	0.0792	3.987
2 VS 5	33	0.0055	5.515
2 VS 6	33	0.0763	4.011
3 VS 4	33	0.0000	9.213
3 VS 5	33	0.9947	0.744
3 VS 6	33	0.9942	0.760
4 VS 5	33	0.0000	10.028
4 VS 6	33	0.0000	8.381
5 VS 6	33	0.8922	1.504

2	*	0.0120
3		0.9999
4	#	0.0000
5		0.9997
6		0.9713

* SIGNIFICANT AT .05
** SIGNIFICANT AT .01
\# SIGNIFICANT AT .001

SLI STUDY NO. 3596.1 PURITY ANALYSIS FOR GLYPHOSATE (ACTIVE INGREDIENT) PAGE 1

BEFORE USE PURITY
% GLYPHOSATE (a.e.)
RAW DATA LISTING
TREATMENTS

GROUP OBSERVATIONS	CONTROL (THEORETICAL VALUE) 1	COMBINED RESULTS (FOR POOLED SAMPLE) 2
1	14.800	15.840
2	14.800	18.210
3	14.800	16.460
4	14.800	17.990
5	14.800	15.770
6	14.800	17.270
7	14.800	14.500
8	14.800	15.960
9	14.800	13.900
10	14.800	15.410
11	14.800	13.710
12	14.800	16.130
13	14.800	17.200
14	14.800	19.590
15	14.800	18.260
16	14.800	17.630
17	14.800	19.070
18	14.800	18.920
19	14.800	16.810
20	14.800	18.920
21	14.800	19.560
22	14.800	14.910
23	14.800	14.830
24	14.800	13.350
25	14.800	15.380
26	14.800	13.790
27	14.800	15.490
28	14.800	13.990
29	14.800	15.990
30	14.800	14.460
31	14.800	15.480
32	14.800	13.820
33	14.800	17.770

A N A L Y S I S OF V A R I A N C E BEFORE USE PURITY

SOURCE OF VARIATION	DF	SUM OF SQUARES	MEAN SQUARE
BETWEEN CLASSES	1	34.8655	34.8655
WITHIN CLASSES	64	112.4706	7574
TOTAL	65	147.3360	

F = 19.84, DF= 1/ 64, P=0.0000

GROUP:	1	2
MEANS:	14.8	16.3
S.D. :	0.00	1.87

TUKEYS TEST (2-tailed)

GROUP	DF	PROB	T
1 VS 2	64	0.0000	6.299
2 #		0.0000	

* SIGNIFICANT AT .05
** SIGNIFICANT AT .01
\# SIGNIFICANT AT .001

SLI STUDY NO. 3596.1 PURITY ANALYSIS FOR GLYPHOSATE (ACTIVE INGREDIENT) PAGE 1

AFTER USE PURITY (STABILITY)
% GLYPHOSATE (a.e.)
RAW DATA LISTING
TREATMENTS

OBSERVATIONS	CONTROL (THEORETICAL VALUE)	TEST ARTICLE MIXTURE NO.: 1	2	3	4	5
GROUP	1	2	3	4	5	6
	14.800	15.540	15.030	17.840	13.950	14.660
	14.800	16.430	14.460	17.980	14.410	14.190
	14.800	16.400	14.970	18.000	14.190	14.770
	14.800	15.860	14.710	17.580	13.750	14.070
	14.800	16.350	15.200	18.080	14.040	14.500
	14.800	16.790	14.830	18.270	13.940	14.510

A N A L Y S I S OF V A R I A N C E 3596.1 AFTER USE PURITY (STABILITY)

SOURCE OF VARIATION	DF	SUM OF SQUARES	MEAN SQUARE
BETWEEN CLASSES	5	63.6555	12.7311
WITHIN CLASSES	30	2.2468	0.0749
TOTAL	35	65.9023	

F =169.99, DF= 5/ 30, P=0.0000

GROUP:	1	2	3	4	5
MEANS:	14.8	16.2	14.9	18.0	14.0
S.D. :	0.00	0.45	0.26	0.23	0.23

TUKEYS TEST (2-tailed)

GROUP	DF	PROB	T
1 VS 2	30	0.0000	12.784
1 VS 3	30	0.9981	0.597
1 VS 4	30	0.0000	28.269
1 VS 5	30	0.0006	6.743
1 VS 6	30	0.2607	3.133
2 VS 3	30	0.0000	12.188
2 VS 4	30	0.0000	15.484
2 VS 5	30	0.0000	19.527
2 VS 6	30	0.0000	15.917
3 VS 4	30	0.0000	27.672
3 VS 5	30	0.0002	7.339
3 VS 6	30	0.1193	3.729
4 VS 5	30	0.0000	35.012
4 VS 6	30	0.0000	31.402
5 VS 6	30	0.1410	3.610

2	#	0.0000
3		0.9981
4	#	0.0000
5	#	0.0006
6		0.2607

* SIGNIFICANT AT .05
** SIGNIFICANT AT .01
\# SIGNIFICANT AT .001

AFTER USE PURITY (STABILITY)
% GLYPHOSATE (a.e.)
RAW DATA LISTING
TREATMENTS

GROUP OBSERVATIONS	CONTROL (THEORETICAL VOLUME) 1	COMBINED RESULTS (FOR POOLED SAMPLE) 2
1	14.800	15.540
2	14.800	16.430
3	14.800	16.400
4	14.800	15.860
5	14.800	16.350
6	14.800	16.790
7	14.800	15.030
8	14.800	14.460
9	14.800	14.970
10	14.800	14.710
11	14.800	15.200
12	14.800	14.830
13	14.800	17.840
14	14.800	17.980
15	14.800	18.000
16	14.800	17.580
17	14.800	18.080
18	14.800	18.270
19	14.800	13.950
20	14.800	14.410
21	14.800	14.190
22	14.800	13.750
23	14.800	14.040
24	14.800	13.940
25	14.800	14.660
26	14.800	14.190
27	14.800	14.770
28	14.800	14.070
29	14.800	14.500
30	14.800	14.510

A N A L Y S I S OF V A R I A N C E AFTER USE PURITY (STABILITY)

SOURCE OF VARIATION	DF	SUM OF SQUARES	MEAN SQUARE
BEIWEEN CLASSES	1	7.5615	7.5615
WITHIN CLASSES	58	63.3818	1.0928
TOTAL	59	70.9433	

F = 6.92, DF= 1/ 58, P=0.0109

GROUP:	1	2
MEANS:	14.8	15.5
S.D. :	0.00	1.48

TUKEYS TEST (2-tailed)

GROUP	DF	PROB	T
1 VS 2	58	0.0109	3.720
2 *		0.0109	

* SIGNIFICANT AT .05
** SIGNIFICANT AT .01
\# SIGNIFICANT AT .001

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SLI Study No. 3596.1

APPENDIX B

SLI Personnel Responsibilities

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SLI Study No. 3596.1

SLI PERSONNEL RESPONSIBILITIES

Kimberly L. Bonnette, M.S., LATG	Study Director/Director, Acute Toxicology
Dawn D. Rodabaugh, B.S.	Alternate Contact/Toxicologist
Robert C. Springborn, Ph.D.	Chairman, President and CEO
Malcolm Blair, Ph.D.	Senior Vice President, Managing Director Emeritus
Joseph C. Siglin, Ph.D., DABT	Vice President, Managing Director
Jason W. Smedley, B.S.	Assistant Toxicologist
M. Gardner Clemons, B.S.	Senior Supervisor of Analytical Chemistry and Pharmacy
Delores P. Knippen	Supervisor of Pharmacy
Anita M. Bosau, RQAP-GLP	Senior Director, Compliance Assurance
Deanna M. Talerico, RQAP-GLP	Senior Supervisor of Quality Assurance
Kathy M. Gasser	Supervisor of Archives

Annex 56-C

Six Acute Toxicity Studies with Spray-Bravo, SLI Study N° 3596.10, 4 September 2002

(United States Embassy in Bogotá, 2011)

AN ACUTE DERMAL TOXICITY STUDY IN RATS WITH SPRAY--BRAVO

FINAL REPORT

OPPTS Guideline

870.1200

Author

Kimberly L. Bonnette, M.S., LATG

Study Completed on

September 4, 2002

Performing Laboratory

Springborn Laboratories, Inc. (SLI)
Ohio Research Center
640 North Elizabeth Street
Spencerville, Ohio 45887

SLI Study No.

3596.10

Submitted to

INL/A
U.S. Department of State
2201 C St. NW SA-4
Washington, DC 20520

Page 1 of 28

(2)

SLI Study No. 3596.10

1. STATEMENT OF *NO* DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B), or (C).

Company: _____

Company Agent: Date _____ Date _____

_____ _____

Title Signature

SLI Study No. 3596.10 (3)

AUG 2 9 2002

2. COMPLIANCE STATEMENT

This study was conducted in compliance with the Good Laboratory Practice Standards as described by the EPA (40 CFR Parts 160 and 792).

Kimberly L. Bonnette, M.S., LATG
Study Director/Author
Springborn Laboratories, Inc.

Date 9/4/02

Rogers Woolfolk
Senior Aviation Advisor
Sponsor/Submitter
INL/A
U.S. Department of State

Date 28 Aug 02

SLI Study No. 3596.10

3. QUALITY ASSURANCE STATEMENT

This study was inspected by the Quality Assurance Unit and reports were submitted to management and the Study Director in accordance with SLI's Standard Operating Procedures as follows:

Phase	Date
Protocol Review	04/25/02
Necropsy	07/15/02
Data Audit	08/23/02
Draft Report Review	08/23/02
Protocol Amendment Review	08/23/02
Final Report Review	09/04/02
Reports to Study Director and Management	08/23/02, 09/04/02

The final report has been reviewed to assure that it accurately describes the materials and methods, and the reported results accurately reflect the raw data.

Rebecca A. Young
Rebecca A. Young
Quality Assurance Team Leader

Date 9/4/02

Joanna M Tolerico
for Anita M. Bosau, RQAP-GLP
Senior Director, Compliance Assurance

Date 9/4/02

(5)

SLI Study No. 3596.10

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SLI Study No. 3596.10

6. SUMMARY

The single-dose dermal toxicity of Spray--Bravo was evaluated in Sprague Dawley rats. A limit test was performed in which one group of five male and five female rats received a single dermal administration of the test article at a dose of 5000 mg/kg body weight. Following dosing, the limit test rats were observed daily and weighed weekly. A gross necropsy examination was performed on all animals at the time of scheduled euthanasia (day 14).

No mortality occurred during the limit test. Clinical abnormalities observed during the study included dark material around the facial area and urine stain. Minor/transient dermal irritation was noted at the site of test article application. Body weight loss was noted in two male and two females during the study day 0 to 7 body weight interval which is routinely observed in this study type due to experimental manipulation. Body weight gain was noted for all other animals during the test period. All animals exceeded their initial body weight by study termination (day 14). No significant gross internal findings were observed at necropsy on study day 14.

Under the conditions of this test, the acute dermal LD50 of Spray--Bravo was estimated to be greater than 5000 mg/kg in the rat.

7. INTRODUCTION

This study was performed to assess the short-term toxicity of Spray--Bravo in Sprague Dawley rats when administered by a single dermal dose. This study was intended to provide information on the potential health hazards of the test article with respect to dermal exposure. Data from this study may serve as a basis for classification and/or labeling of the test article. This study was performed in accordance with the US EPA, Health Effects Test Guidelines, OPPTS 870.1200, Acute Dermal Toxicity, August 1998. This study was performed at Springborn Laboratories, Inc., 553 North Broadway, Spencerville, Ohio. The protocol was signed by the Study Director on April 26, 2002 (GLP initiation date). The in-life phase of the study was initiated with test article administration on July 1, 2002 (day 0), and concluded with necropsy on July 15, 2002.

8. MATERIALS AND METHODS

8.1. Test Article

The test article was received from the Sponsor and identified as follows:

Sponsor's ID	Assigned SLI ID	Physical Description	Receipt Date	Expiration Date
Spray--Bravo[a]	S02.002.3596	Cloudy pale amber liquid	05/31/02	None provided
Ingredients:[b]				
Herbicide: Roundup SL Lot No.: 4010/4212 4397/4272 4333/4340 4379/4076 4397/4333				None provided
Surfactant: Cosmo Flux-411F Lot No.: Unknown				None provided

[a]Sample pooled at SLI from five different mixes of Spray--Bravo (top/middle/bottom).
[b]Ingredients used in the five Spray--Bravo mixes that were prepared by the Sponsor.

The test article was stored at room temperature. The Sponsor was responsible for any necessary evaluations related to identity, strength, purity, composition, stability and method of synthesis of the test material according to 40 CFR 160.105 and 40 CFR 792.105. Springborn Laboratories, Inc., analyzed the test article for the glyphosate (a.e.) which is presented in SLI Study No. 3596.8.

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SLI Study No. 3596.10

8.2. Retention Sample

An approximate 1 mL retention sample of each test article mixture (top/middle/bottom, maintained separately for a total of fifteen 1 mL samples) was taken and stored at SLI at room temperature. In addition, a 10 mL retention sample of the pooled test article samples (from the 5 test article mixtures) was collected and stored at SLI at room temperature. These samples serve as the retention samples for all studies conducted with this material.

8.3. Test Article Disposition

The test article was returned to the Sponsor following completion of all studies with the test article.

8.4. Method of Test Article Preparation

The test article was administered as received from the Sponsor and dispensed fresh on the day of dosing. The density of the test article was determined to be 1.08 g/mL.

8.5. Animals and Animal Husbandry

8.5.1. Description, Identification and Housing

Adult, Hsd: Sprague Dawley® SD® rats were received from Harlan Sprague Dawley, Inc., Indianapolis, IN. Upon receipt, metal ear tags displaying unique identification numbers were used to individually identify the animals. Cage cards displaying at least the study number, animal number and sex were affixed to each cage. The animals were housed individually in suspended stainless steel cages. All housing and care were based on the standards recommended by the Guide for the Care and Use of Laboratory Animals [1].

8.5.2. Environment

The animal room temperature and relative humidity ranges were 69-75°F (21-24°C) and 37-58%, respectively. Environmental control equipment was monitored and adjusted as necessary to minimize fluctuations in the animal room environment. Light timers were set to maintain a 12-hour light/12-hour dark cycle and room ventilation was set to produce 10-15 air changes/hour. The animal room temperature and relative humidity were recorded a minimum of once daily.

8.5.3. Food

PMI Certified Rodent Chow #5002 (Purina Mills, Inc.) was provided ad libitum to the animals throughout the study. The lot number and expiration date of each batch of diet used during the study were recorded. The feed was analyzed and certified by the supplier for nutritional components and environmental

contaminants. Dietary limitations for various environmental contaminants, including heavy metals, pesticides, polychlorinated biphenyls and total aflatoxin are set by the manufacturer. Within these limits, contaminants which may have been present were not expected to compromise the purpose of this study. Results of the dietary analyses (Certificates of Analysis) were provided by the manufacturer for each lot of diet. These are maintained by SLI.

8.5.4. Water

Municipal tap water treated by reverse osmosis was available ad libitum throughout the study. The purified water was supplied by an automatic watering system. Monitoring of the drinking water for contaminants is conducted by SLI and the records are available for inspection. Within generally accepted limits, contaminants which may have been present were not expected to compromise the purpose of this study. The water meets the standards specified under the EPA National Drinking Water Regulations (40 CFR Part 141).

8.5.5. Acclimation

Upon receipt, the animals were removed randomly from the shipping cartons, examined by qualified personnel, identified with metal ear tags and then acclimated to the laboratory conditions for a minimum of five days. The animals were observed daily for overt physical or behavioral abnormalities, general health/moribundity and mortality.

8.5.6. Animal Selection

The animals chosen for study use were randomly selected from healthy stock animals using a computerized (Alpha DS-10 AcuTox) random numbers table to avoid potential bias. All animals received a detailed pretest observation prior to dosing. Only healthy animals were chosen for study use. Females were nulliparous and nonpregnant. The male animals were approximately 11 weeks of age and weighed 360-391 g prior to dosing. The female animals were approximately 11 weeks of age and weighed 212-235 g prior to dosing.

9. EXPERIMENTAL PROCEDURES

9.1. Preliminary Procedures

On day -1, the fur was removed from the dorsal trunk area of the animals chosen for the limit test using an animal clipper. The clipped area was approximately 10% of the animal's body surface area (BSA). The region included the scapula (shoulder) to the wing of the ilium (hipbone) and half way down the flank on each side of the animal. Care was taken to avoid abrading the skin during the clipping procedure.

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SLI Study No. 3596.10

9.2. Dosing

On the following day (day 0), the test article was administered dermally to approximately 10% of the body surface area. The four corners of this area were delineated in the clipped area with an indelible marker. The test article was then spread evenly over the delineated test area and held in contact with the skin with an appropriately sized 4-ply porous gauze dressing backed with a plastic wrap which was placed over the gauze dressing (occlusive binding). Removal and ingestion of the test article was prevented by placing an elastic wrap over the trunk and test area. The elastic wrap was further secured with a tape harness on the cranial end of the trunk and then secured with adhesive tape around the trunk at the caudal end.

The test article was administered at the following level:

Dose Level (mg/kg)	Dose Volume (mL/kg)	Concentration (%)	No. of Animals	
			Male	Female
5000	4.63[a]	100[b]	5	5

[a]Adjusted based on a density of 1.08 g/mL.
[b]Pooled test article.

Individual doses were calculated based on the animal's day 0 body weight. After an approximate 24-hour exposure period, the binding materials were removed and the corners of the test site were re-delineated using a marker. Residual test article was removed using gauze moistened with deionized water followed by dry gauze.

9.3. Dermal Observations

The test animals were examined for erythema and edema following patch removal and the responses scored on study day 1 and daily thereafter (days 2-14) according to the Macroscopic Dermal Grading System provided in Appendix A which is based on Draize [2]. The dermal test sites were reclipped as necessary to allow clear visualization of the skin.

9.4. Clinical Observations

The animals were observed for clinical abnormalities a minimum of two times on study day 0 (postdose) and daily thereafter (days 1-14). A mortality check was performed twice daily, in the morning and afternoon.

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9.5. Body Weights

Individual body weights were obtained for the animals prior to dosing on day 0 and on days 7 and 14.

9.6. Gross Necropsy

All animals were euthanized by carbon dioxide inhalation at study termination (day 14) and necropsied. Body cavities (cranial, thoracic, abdominal and pelvic) were opened and examined. No tissues were retained.

9.7. Protocol Deviations

No protocol deviations occurred during this study.

10. ANALYSIS OF DATA

Data from the study were analyzed and an LD50 value estimated as follows:

< 50% Mortality: LD50 was estimated as greater than the administered dose.
= 50% Mortality: LD50 was estimated as equal to the administered dose.
> 50% Mortality: LD50 was estimated as less than the administered dose.

Body weight means and standard deviations were calculated separately for males and females.

11. MAINTENANCE OF RAW DATA AND RECORDS

All original raw data, the final report and magnetically encoded records were transferred to the SLI archives for a period of 10 years. The Sponsor will be contacted prior to final disposition of these items.

12. RESULTS

12.1. Mortality

Individual Data: Table 1

No mortality occurred during the limit test.

SLI Study No. 3596.10

12.2. Clinical/Dermal Observations

Individual Data: Table 1

Clinical abnormalities observed during the study included dark material around the facial area and urine stain. Minor/transient dermal irritation was noted at the site of test article application.

12.3. Body Weight Data

Individual Data: Table 2

Body weight loss was noted in two males and two females during the study day 0 to 7 body weight interval which is routinely observed in this study type due to experimental manipulation. Body weight gain was noted for all other animals during the test period. All animals exceeded their initial body weight by study termination (day 14).

12.4. Gross Necropsy

Individual Data: Table 3

No significant gross internal findings were observed at necropsy on study day 14.

13. CONCLUSION

Under the conditions of this test, the acute dermal LD50 of Spray--Bravo was estimated to be greater than 5000 mg/kg in the rat.

Kimberly L. Bonnette, M.S., LATG
Study Director

Date 9/4/02

14. REPORT REVIEW

Dawn D. Rodabaugh, B.S.
Associate Toxicologist

Date 9/4/02

15. REFERENCES

1. Guide for the Care and Use of Laboratory Animals, DHHS Publication No. (NIH) 96-03, 1996.

2. Draize, J.H., Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics, The Association of Food and Drug Officials of the United States, 49-51, 1959.

INL/A, U.S. DEPARTMENT OF STATE

TABLE 1

AN ACUTE DERMAL TOXICITY STUDY IN RATS

INDIVIDUAL CLINICAL OBSERVATIONS
(POSITIVE FINDINGS)

MALES 5000 MG/KG

		DAY OF STUDY														
MALE#	OBSERVATIONS	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A5315	SCHEDULED EUTHANASIA															P
	ERYTHEMA GRADE O		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	EDEMA GRADE 0		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	DARK MATERIAL AROUND EYE(S)		P													
	DARK MATERIAL AROUND NOSE	P														
A5316	SCHEDULED EUTHANASIA															P
	ERYTHEMA GRADE O			P	P	P	P	P	P	P	P	P	P	P	P	P
	EDEMA GRADE 0		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	DARK MATERIAL AROUND EYE(S)		P		P											
	DARK MATERIAL AROUND NOSE	P														
	ERYTHEMA GRADE 1		P													
A5317	SCHEDULED EUTHANASIA															P
	ERYTHEMA GRADE O		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	EDEMA GRADE 0		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	DARK MATERIAL AROUND EYE(S)	P														
	DARK MATERIAL AROUND NOSE	P	P	P												
	DARK MATERIAL AROUND MOUTH	P														
A5307	SCHEDULED EUTHANASIA															P
	ERYTHEMA GRADE O		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	EDEMA GRADE 0		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	DARK MATERIAL AROUND EYE(S)		P													
	DARK MATERIAL AROUND NOSE	P	P	P	P											
A5314	SCHEDULED EUTHANASIA															P
	ERYTHEMA GRADE O		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	EDEMA GRADE 0		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	DARK MATERIAL AROUND EYE(S)		P													
	DARK MATERIAL AROUND NOSE	P														

GRADE CODE: 1=SLIGHT 2=MODERATE 3=SEVERE P=PRESENT L=LEFT R=RIGHT B=BILATERAL

TABLE 1

AN ACUTE DERMAL TOXICITY STUDY IN RATS

INDIVIDUAL CLINICAL OBSERVATIONS
(POSITIVE FINDINGS)

FEMALES 5000 MG/KG

FEMALE#	OBSERVATIONS	DAY OF STUDY 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A5348	SCHEDULED EUTHANASIA															P
	URINE STAIN	1	1													
	UNKEMPT APPEARANCE			P												
	ERYTHEMA GRADE O		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	EDEMA GRADE 0		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	DARK MATERIAL AROUND EYE(S)	P	P													
	DARK MATERIAL AROUND NOSE	P														
	DARK MATERIAL AROUND MOUTH	P														
A5330	SCHEDULED EUTHANASIA															P
	URINE STAIN	1														
	ERYTHEMA GRADE O			P	P	P	P	P	P	P	P	P	P	P	P	P
	EDEMA GRADE 0		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	DARK MATERIAL AROUND EYE(S)		P													
	DARK MATERIAL AROUND NOSE	P														
	DARK MATERIAL AROUND MOUTH	P														
	ERYTHEMA GRADE 1		P													
A5338	SCHEDULED EUTHANASIA															P
	ERYTHEMA GRADE O		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	EDEMA GRADE 0		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	DARK MATERIAL AROUND EYE(S)	P														
	DARK MATERIAL AROUND NOSE	P														
A5331	SCHEDULED EUTHANASIA															P
	ERYTHEMA GRADE O			P	P	P	P	P	P	P	P	P	P	P	P	P
	EDEMA GRADE 0		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	ERYTHEMA GRADE 1		P													

GRADE CODE: 1=SLIGHT 2=MODERATE 3=SEVERE P=PRESENT L=LEFT R=RIGHT B=BILATERAL

TABLE 1

AN ACUTE DERMAL TOXICITY STUDY IN RATS

INDIVIDUAL CLINICAL OBSERVATIONS
(POSITIVE FINDINGS)

FEMALES 5000 MG/KG

FEMALE#	OBSERVATIONS	DAY OF STUDY 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A5344	SCHEDULED EUTHANASIA															P
	URINE STAIN	1	1													
	ERYTHEMA GRADE O			P	P	P	P	P	P	P	P	P	P	P	P	P
	EDEMA GRADE 0		P	P	P	P	P	P	P	P	P	P	P	P	P	P
	DARK MATERIAL AROUND EYE(S)		P													
	DARK MATERIAL AROUND NOSE	P	P													
	ERYTHEMA GRADE 1		P													

GRADE CODE: 1=SLIGHT 2=MODERATE 3=SEVERE P=PRESENT L=LEFT R=RIGHT B=BILATERAL

STUDY NO.: 3596.10 PAGE 1

INL/A, U.S. DEPARTMENT OF STATE

TABLE 2

AN ACUTE DERMAL TOXICITY STUDY IN RATS

INDIVIDUAL BODY WEIGHTS (GRAMS)

MALES 5000 MG/KG

	DAY OF STUDY			
ANIMAL#	0	7	14	AT DEATH (DAY)
A5315	376	377	409	
A5316	377	379	405	
A5317	389	389	408	
A5307	360	356	382	
A5314	391	387	406	
MEAN	379	378	402	
S.D.	12.4	13.1	11.3	
N	5	5	5	

TABLE 2

AN ACUTE DERMAL TOXICITY STUDY IN RATS

INDIVIDUAL BODY WEIGHTS (GRAMS)

FEMALES 5000 MG/KG

	DAY OF STUDY		
ANIMAL#	0	7	14 AT DEATH (DAY)
A5348	225	218	237
A5330	212	209	216
A5338	226	226	234
A5331	235	241	246
A5344	228	234	242
MEAN	225	226	235
S.D.	8.3	12.7	11.6
N	5	5	5

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INL/A, U.S. DEPARTMENT OF STATE

TABLE 3

AN ACUTE DERMAL TOXICITY STUDY IN RATS

INDIVIDUAL GROSS NECROPSY OBSERVATIONS

MALES 5000 MG/KG

ANIMAL#	DAY OF DEATH	STUDY DAY	OBSERVATION	FATE
A5315	15-JUL-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A5316	15-JUL-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A5317	15-JUL-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A5307	15-JUL-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A5314	15-JUL-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA

TABLE 3

AN ACUTE DERMAL TOXICITY STUDY IN RATS

INDIVIDUAL GROSS NECROPSY OBSERVATIONS

FEMALES 5000 MG/KG

ANIMAL#	DAY OF DEATH	STUDY DAY	OBSERVATION	FATE
A5348	15-JUL-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A5330	15-JUL-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A5338	15-JUL-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A5331	15-JUL-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A5344	15-JUL-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA

APPENDIX A

Macroscopic Dermal Grading System

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SLI Study No. 3596.10

MACROSCOPIC DERMAL GRADING SYSTEM

ERYTHEMA AND EDEMA OBSERVATIONS		
OBSERVATION	DEFINITION	CODE
Erythema – Grade 0	No erythema	0
Erythema – Grade 1	Very slight erythema (barely perceptible)	1
Erythema – Grade 2	Well-defined erythema	2
Erythema – Grade 3	Moderate to severe erythema	3
Erythema – Grade 4	Severe erythema (beet redness)	4
Maximized Grade 4	Notable dermal lesions (see below)	M – 4 (see below)
Edema – Grade 0	No edema	0
Edema – Grade 1	Very slight edema (barely perceptible)	1
Edema – Grade 2	Slight edema (edges of area well defined by definite raising)	2
Edema – Grade 3	Moderate edema (raised approximately 1 millimeter)	3
Edema – Grade 4	Severe edema (raised more than 1 millimeter and extends beyond the area of exposure)	4
NOTE: Each animal was assigned an erythema and edema score. The most severely affected area within the test site was graded. If eschar, blanching, ulceration and/or necrosis greater than grade 1 was observed, then the “Maximized Grade 4" was assigned to the test site in place of the erythema score and the type of notable dermal lesion(s) (e.g., eschar - grade 2, blanching - grade 3, ulceration - grade 4, etc.) was noted. The presence of any other dermal changes (e.g., desquamation, fissuring, eschar exfoliation, etc.) was also recorded.		

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SLI Study No. 3596.10

MACROSCOPIC DERMAL GRADING SYSTEM

NOTABLE DERMAL LESIONS		
OBSERVATION	CODE	DEFINITION
Eschar – Grade 1	ES-1	Focal and/or pinpoint areas up to 10% of test site.
Eschar – Grade 2	ES-2	> 10% < 25% of test site.
Eschar – Grade 3	ES-3	> 25% < 50% of test site.
Eschar – Grade 4	ES-4	> 50% of test site.
Blanching – Grade 1	BLA-1	Focal and/or pinpoint areas up to 10% of test site.
Blanching – Grade 2	BLA-2	> 10% < 25% of test site.
Blanching – Grade 3	BLA-3	> 25% < 50% of test site.
Blanching – Grade 4	BLA-4	> 50% of test site.
Ulceration – Grade 1	U-1	Focal and/or pinpoint areas up to 10% of test site.
Ulceration – Grade 2	U-2	> 10% < 25% of test site.
Ulceration – Grade 3	U-3	> 25% < 50% of test site.
Ulceration – Grade 4	U-4	> 50% of test site.
Necrosis – Grade 1	NEC-1 *(color)*	Focal and/or pinpoint areas up to 10% of test site (Note color of necrosis).
Necrosis – Grade 2	NEC-2 *(color)*	> 10% < 25% of test site (Note color of necrosis).
Necrosis – Grade 3	NEC-3 *(color)*	> 25% < 50% of test site (Note color of necrosis).
Necrosis – Grade 4	NEC-4 *(color)*	> 50% of test site (Note color of necrosis).

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SLI Study No. 3596.10

MACROSCOPIC DERMAL GRADING SYSTEM

ADDITIONAL DERMAL FINDINGS		
OBSERVATION	DEFINITION	CODE
Desquamation	Characterized by scaling or flaking of dermal tissue with or without denuded areas.	DES
Fissuring	Characterized by cracking of the skin with or without moist exudate. Fissuring should be checked prior to removing the animal from the cage and manipulating the test site.	FIS
Eschar Exfoliation	The process by which areas of eschar flake off the test site.	EXF
Test Site Staining	Skin located at test site appears to be discolored, possibly due to test article (note color of staining).	TSS *(color)*
Erythema Extends Beyond the Test Site	The erythema extends beyond the test site. Note: A study director should be contacted for erythema extending beyond the test site.	ERB
Superficial Lightening	Characterized by pale area(s) (almost a burn-like appearance) in the test site. However, erythema may still be observed through the pale area. Note: This observation may affect the overall erythema score of the test site. This observation may progress to other observations resulting in notable dermal lesions, but SL itself will not be considered a notable dermal lesion that will result in a dermal score to be maximized since it does not result in any in-depth injury. To be coded using an area designation (see below).	--
Superficial Lightening - Grade 1	Focal and/or pinpoint areas up to 10% of the test site	SL-1
Superficial Lightening - Grade 2	> 10% < 25% of test site	SL-2
Superficial Lightening - Grade 3	> 25% < 50% of test site	SL-3
Superficial Lightening - Grade 4	> 50% of test site	SL-4

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MACROSCOPIC DERMAL GRADING SYSTEM

ADDITIONAL FINDINGS		
OBSERVATION	DEFINITION	CODE
Dermal Irritation - Outside of the Test Site	Noticeable irritation outside of test site probably due to the binding tape material. This notation will only be made for reactions greater than what are normally observed from tape removal which do not interfere with the scoring of the test site.	IT

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APPENDIX B

SLI Personnel Responsibilities

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SLI Study No. 3596.10

SLI PERSONNEL RESPONSIBILITIES

Kimberly L. Bonnette, M.S., LATG	Study Director/Director, Acute Toxicology
Dawn D. Rodabaugh, B.S.	Alternate Contact/Associate Toxicologist
Robert C. Springborn, Ph.D.	Chairman, President and CEO
Malcolm Blair, Ph.D.	Senior Vice President, Managing Director Emeritus
Joseph C. Siglin, Ph.D., DABT	Vice President, Managing Director
Jason W. Smedley, B.S.	Assistant Toxicologist
Pamela S. Smith, ALAT	Supervisor of Acute Toxicology
Kevin V. Weitzel, A.S.	Primary Technician/Inhalation Team Leader
Delores P. Knippen	Supervisor of Pharmacy
Steven H. Magness, B.S., LATG	Senior Supervisor of Gross and Fetal Pathology
Anita M. Bosau, RQAP-GLP	Senior Director, Compliance Assurance
Deanna M. Talerico, RQAP-GLP	Senior Supervisor of Quality Assurance
J. Dale Thurman, D.V.M., M.S., DACVP	Senior Director, Pathology
Kathy M. Gasser	Supervisor of Archives

AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS WITH SPRAY--BRAVO

FINAL REPORT

OPPTS Guideline

870.1300

Author

Kimberly L. Bonnette, M.S., LATG

Study Completed on

January 7, 2003

Performing Laboratory

Springborn Laboratories, Inc. (SLI)
Ohio Research Center
640 North Elizabeth Street
Spencerville, Ohio 45887

SLI Study No.

3596.11

Submitted to

INL/A
U.S. Department of State
2201 C St. NW SA-4
Washington, DC 20520

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SLI Study No. 3596.11

1. STATEMENT OF *NO* DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B), or (C).

Company: _____

Company Agent: _____ Date_____

_____ _____
Title Signature

SLI Study No. 3596.11

NOV 2 1 2002

2. COMPLIANCE STATEMENT

This study was conducted in compliance with the Good Laboratory Practice Standards as described by the EPA (40 CFR Parts 160 and 792).

Kimberly L. Bonnette, M.S., LATG
Study Director/Author
Springborn Laboratories, Inc.

Date 1/7/03

Rogers Woolfolk
Senior Aviation Advisor
Sponsor/Submitter
INL/A
U.S. Department of State

Date 20 Nov 02

SLI Study No. 3596.11

3. QUALITY ASSURANCE STATEMENT

This study was inspected by the Quality Assurance Unit and reports were submitted to management and the Study Director in accordance with SLI's Standard Operating Procedures as follows:

Phase	Date
Protocol Review	04/25/02
Body Weights	08/02/02, 08/15/02
Data Audit	11/18/02
Draft Report Review	11/18/02
Protocol Amendment Review	11/18/02
Final Report Review	01/07/03
Reports to Study Director and Management	11/18/02, 01/07/03

The final report has been reviewed to assure that it accurately describes the materials and methods, and the reported results accurately reflect the raw data.

Troy O. Joseph
Quality Assurance Auditor

Date 1/7/03

Anita M. Bosau, RQAP-GLP
Senior Director, Compliance Assurance

Date 1/7/03

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SLI Study No. 3596.11

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6. SUMMARY

The four-hour nose-only inhalation toxicity of Spray--Bravo was evaluated in Sprague Dawley rats. A limit test was performed in which a group of five male and five female rats received a four-hour nose-only inhalation exposure to a time-weighted average aerosol concentration (analytically determined) of 2.40 mg/L. Following the exposure, the limit test rats were observed daily and weighed weekly. A gross necropsy examination was performed on all limit test animals at the time of death or scheduled euthanasia (day 14).

Mortality occurred during the limit test as follows:

Dose Level (mg/L)	No. Dead/No. Dosed		
	Males	Female	Combined
2.40	2/5	0/5	2/10

All mortality occurred by study day 1. Although mortality was observed in 2/5 males the LD50 is still estimated to be greater than 2.40 mg/L, which is well above the EPA required 2.00 mg/L. The most notable clinical abnormalities observed during the study included decreased activity, breathing abnormalities, decreased defecation, rough haircoat, nasal discharge and dark material around the facial area. A slight body weight loss was noted for two males during the day 0 to 7 body weight interval. Body weight gain/maintenance was noted for all other surviving animals during the test period. The most notable gross internal findings were observed in the animals that died and included dark red lobes of the lung and abnormal content in the small intestine. No significant gross internal findings were observed at necropsy on study day 14.

Under the conditions of this test, the acute inhalation LC50 of Spray--Bravo was estimated to be greater than 2.40 mg/L in the rat (which was well above the EPA required 2.00 mg/L).

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SLI Study No. 3596.11

7. INTRODUCTION

This study was performed to assess the short-term toxicity of Spray--Bravo in Sprague Dawley rats when administered by a four-hour nose-only inhalation exposure. This study was intended to provide information on the potential health hazards of the test article with respect to inhalation exposure. Data from this study may serve as a basis for classification and/or labeling of the test article. This study was conducted in accordance with the US EPA, Health Effects Test Guidelines OPPTS 870.1300, Acute Inhalation Toxicity, August 1998. This study was performed at Springborn Laboratories, Inc., 553 North Broadway, Spencerville, Ohio. The protocol was signed by the Study Director on April 26, 2002, (GLP initiation date). The in-life phase of the study was initiated with test article administration on August 1, 2002 (day 0), and concluded with terminal euthanasia on August 15, 2002.

8. MATERIALS AND METHODS

8.1. Test Article

The test article was received from the Sponsor and identified as follows:

Sponsor's ID	Assigned SLI ID	Physical Description	Receipt Date	Expiration Date
Spray--Bravo[a]	S02.002.3596	Cloudy pale amber liquid	05/31/02	None provided
Ingredients:[b]				
Herbicide: Roundup SL Lot No.: 4010/4212 4397/4272 4333/4340 4379/4076 4397/4333				None provided
Surfactant: Cosmo Flux-411F Lot No.: Unknown				None provided

[a]Sample pooled at SLI from five different mixes of Spray--Bravo (top/middle/bottom).
[b]Ingredients used in the five Spray--Bravo mixes that were prepared by the Sponsor.

The test article was stored at room temperature. The Sponsor was responsible for any necessary evaluations related to identity, strength, purity, composition, stability and method of synthesis of the test material according to 40 CFR 160.105 and 40 CFR 792.105. Springborn Laboratories, Inc., analyzed the test article for the glyphosate (a.e.) which is presented in SLI Study No. 3596.8.

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SLI Study No. 3596.11

8.2. Retention Sample

An approximate 1 mL retention sample of each test article mixture (top/middle/bottom, maintained separately for a total of fifteen 1 mL samples) was taken and stored at SLI at room temperature. In addition, a 10 mL retention sample of the pooled test article samples (from the 5 test article mixtures) was collected and stored at SLI at room temperature. These samples serve as the retention samples for all studies conducted with this material.

8.3. Test Article Disposition

The remaining test article was returned to the Sponsor following completion of all studies with the test article.

8.4. Method of Test Article Preparation

The test article was utilized as received from the Sponsor and dispensed fresh on the day of dosing. The test article was stirred prior to and continuously during exposure.

8.5. Animals and Animal Husbandry

8.5.1. Description, Identification and Housing

Young adult, Hsd: Sprague Dawley® SD® rats were received from Harlan Sprague Dawley, Inc., Indianapolis, IN. Upon receipt, metal ear tags displaying unique identification numbers were used to individually identify the animals. Cage cards displaying at least the study number, animal number and sex were affixed to each cage. The animals were housed individually in suspended stainless steel cages. All housing and care were based on the standards recommended by the Guide for the Care and Use of Laboratory Animals [1].

8.5.2. Environment

The animal room temperature and relative humidity ranges were 69-78°F (21-26°C) and 34-60%, respectively. Environmental control equipment was monitored and adjusted as necessary to minimize fluctuations in the animal room environment. Light timers were set to maintain a 12-hour light/12-hour dark cycle and room ventilation was set to produce 10-15 air changes/hour. The animal room temperature and relative humidity were recorded a minimum of once daily.

8.5.3. Food

PMI Certified Rodent Chow #5002 (Purina Mills, Inc.) was provided ad libitum to the animals throughout the study (except during the time that the animals were acclimated to the exposure tubes and maintained in the inhalation room for the

exposure procedure). The lot number and expiration date of each batch of diet used during the study were recorded. The feed was analyzed and certified by the supplier for nutritional components and environmental contaminants. Dietary limitations for various environmental contaminants, including heavy metals, pesticides, polychlorinated biphenyls and total aflatoxin are set by the manufacturer. Within these limits, contaminants which may have been present were not expected to compromise the purpose of this study. Results of the dietary analyses (Certificates of Analysis) are provided by the manufacturer for each lot of diet. These are maintained by SLI.

8.5.4. Water

Municipal tap water treated by reverse osmosis was available ad libitum throughout the study (except during the time that the animals were acclimated to the exposure tubes and maintained in the inhalation room for the exposure procedure). The purified water was supplied by an automatic watering system. Monitoring of the drinking water for contaminants is conducted by SLI and the records are available for inspection. Within generally accepted limits, contaminants which may have been present were not expected to compromise the purpose of this study. The water meets the standards specified under the EPA National Drinking Water Regulations (40 CFR Part 141).

8.5.5. Acclimation

Upon receipt, the animals were removed randomly from the shipping cartons, examined by qualified personnel, identified with metal ear tags and then acclimated to the laboratory conditions for a minimum of five days. The animals were observed daily for overt physical or behavioral abnormalities, general health/moribundity and mortality.

8.5.6. Animal Selection

The animals chosen for study use were randomly selected from healthy stock animals using a computerized (Alpha DS-10 AcuTox) random numbers table to avoid potential bias. All animals received a detailed pretest observation prior to dosing. Only healthy animals were chosen for study use. Females were nulliparous and nonpregnant. The male animals were approximately 10 weeks of age and weighed 305-324 g on the day of exposure. The female animals were approximately 9 weeks of age and weighed 191-200 g on the day of exposure.

9. EXPERIMENTAL PROCEDURES

9.1. Preliminary Procedures

9.1.1. Test Article Volatility Determination

The volatility of the test article relative to a distilled water standard was determined prior to experimental initiation. This procedure was performed in order to determine if the test article had sufficiently low volatility to allow for an accurate gravimetric determination of the aerosol concentration. A known quantity of the test article was placed on a preweighed filter disk and was allowed to evaporate for a total of ten minutes. The test article weight was determined each minute and the amount of evaporation of the test article was then determined. The results of this volatility trial indicated that the test article evaporation rate (0.52 mg/minute) was comparable to the SLI determined distilled water evaporation rate (0.55 mg/minute); therefore was considered to not be volatile.

9.1.2. Preliminary Aerosol Generation Trials

Prior to experimental initiation, preliminary aerosol generation trials were conducted. These trials were performed in order to determine the most efficient means of generating an aerosol of the appropriate concentration while utilizing equipment that would reduce the aerodynamic particle size. Data obtained during the preliminary aerosol generation trials are presented in Appendix A.

9.2. Limit Test

9.2.1. Aerosol Generation Equipment

The test aerosol was generated with a Pistol Spraying System and a Master Flex Pump and Pump Heads 77200-60 and 7523-30. Conditioned high pressure external air was used in generating the test atmosphere. The aerosol was blown through a 5L Elutriator, the nose-only inhalation chamber and then vented from the chamber to an air treatment system which consisted of a prefilter, a HEPA filter, a charcoal bed and a water scrubbing tower (see Figure 1).

9.2.2. Dosing

On day 0, the animals chosen for the limit test were weighed, placed in a nose-only exposure tube and allowed to acclimate to the exposure tube for at least one hour. Animals that appeared to have been acclimated to the exposure tube (i.e., minimal struggling and no inversion) were considered to be acceptable and removed from the exposure tube and returned to their cages until initiation of the

aerosol exposure. Animals that did not appear to acclimate to the exposure tube were not acceptable and were removed from the exposure tube and returned to their cages.

The acceptable animals were then placed in exposure tubes and the tubes inserted into the Multistage 10L nose-only inhalation chamber and the test article aerosolized at the following level:

Exposure Level (mg/L)	No. of Animals	
	Male	Female
2.40	5	5

The aerosol exposure consisted of a 4-minute T99 equilibration period, a 240-minute exposure period and a 4-minute de-equilibration period equal to the T99 equilibration period. After each aerosol exposure, animals were removed from the exposure tubes and residual test article was removed from the animal's exterior surfaces (where practical) by wiping the haircoat with a towel. The animals were then returned to ad libitum feed and water. The following parameters were measured during the exposure.

9.2.2.1. Chamber Air Flow

Air flow readings were recorded at the initiation of the T99 equilibration period, at approximate 30-minute intervals during the aerosol exposure and at the conclusion of the de-equilibration period.

9.2.2.2. Aerosol Concentration

For the analytical concentration, the test article aerosol concentration was collected in the inhalation chamber utilizing impinger glassware containing 20 mL of methanol per tube. Three impingers were placed in tandem and the aerosol atmosphere was drawn through the three sample tubes to collect the test article. Three impingers were utilized in order to ensure that all test article was collected in the initial tube and none had escaped into the second or third (last) tube. A 2 L sample of the aerosol was drawn from the breathing zone of the chamber for two minutes (4 L of atmosphere). The aerosol concentration was measured at the beginning of the aerosol exposure (after equilibration), then hourly during the exposure and at the conclusion of the aerosol exposure (before de-equilibration) for a total of five samples. However, the initial sampling collection procedure did not produce a viable sample (confirmed by analytical chemistry to not contain any test article) due to a probable loose connection tube. Therefore, the second sample collected was considered the aerosol concentration during the entire first hour. The samples were analyzed by Springborn Laboratories, Inc., for glyphosate, a non-volatile component of the

test article. These analyses were performed in order to determine the analytical (actual) concentrations of the aerosol in the chamber for each sampling period. The average time weighted analytical concentration of the test atmosphere was then calculated for the exposure. Chemistry methods and results are detailed in the Analytical Chemistry Report (Appendix B).

Note: There were no changes in air flow nor test article flow over this time period to the second sampling.

9.2.2.3. Chamber Temperature and Humidity

The chamber temperature and humidity were measured electronically and recorded at approximate 30-minute intervals during the aerosol exposure.

9.2.2.4. Aerosol Aerodynamic Particle-Size Distribution

The aerosol aerodynamic particle-size distribution was determined three times during the aerosol exposure using the ITP 7 Stage Cascade Impactor. Each stage of the impactor was fitted with a preweighed glass fiber filter. Five liters per minute of the chamber air were drawn through the impactor and the change in weight of each filter was then determined and recorded. The mean particle-size distribution was subsequently plotted using an Excel computer adaptation of the manual method. The Mass Median Aerodynamic Diameter, Geometric Standard Deviation and percentage of particles $\leq$ 4.0 μ were then determined. At least one hour passed between each aerosol particle-size analysis.

9.2.2.5. Chamber Oxygen

Chamber oxygen content was measured and recorded at approximate 30-minute intervals during the aerosol exposure.

9.2.3. Clinical Observations

The limit test animals were observed for clinical abnormalities during the aerosol exposure, a minimum of two times on study day 0 (post-exposure) and daily thereafter (days 1-14). A general health/mortality check was performed twice daily (in the morning and in the afternoon).

9.2.4. Body Weights

Individual body weights were obtained for the limit test animals prior to dosing on day 0 and for all surviving animals on days 7 and 14. Animals found dead after day 0 were also weighed.

9.2.5. Gross Necropsy

All limit test animals that died spontaneously during the study or were euthanized by carbon dioxide inhalation at study termination (day 14) were necropsied. Body cavities (cranial, thoracic, abdominal and pelvic) were opened and examined. No tissues were retained.

9.3. Protocol Deviations

The temperature of the animal room [69-78°F (21-26°C)] exceeded the preferred range [66-77°F (19-25°C)] during this study. This occurrence was considered to have had no adverse effect on the outcome of this study.

10. ANALYSIS OF DATA

Data from the limit tests were analyzed and an LC50 value estimated as follows:

< 50% Mortality: LC50 was estimated as greater than the administered dose.
= 50% Mortality: LC50 was estimated as equal to the administered dose.
> 50% Mortality: LC50 was estimated as less than the administered dose.

Body weight means and standard deviations were calculated separately for males and females. The aerodynamic particle-size distribution of the test article aerosol was plotted using an Excel computer adaptation of the three cycle logarithmic probability paper as per the ITP Cascade Impactor instruction manual. The Mass Median Aerodynamic Diameter, Geometric Standard Deviation and particles $\leq$ 4.0 µ was determined based on the plotted distribution.

11. MAINTENANCE OF RAW DATA AND RECORDS

All original raw data, the final report and magnetically encoded records were transferred to the SLI archives for a period of 10 years. The Sponsor will be contacted prior to final disposition of these items.

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12. RESULTS

12.1. Aerosol Generation and Chamber Environmental Data

12.1.1. Aerosol Generation Data

Individual Data: Table 1

The average time-weighted analytical concentration for the aerosol exposure was determined to be 2.40 mg/L. The mass median aerodynamic diameter and geometric standard deviation of the sampled particles were 3.2 μ ± 1.96. The percentage of particles ≤ 4.0 μ was determined to be 63%.

12.1.2. Chamber Environmental Data

Individual Data: Table 1

Chamber temperature and relative humidity for the aerosol exposure ranged from 74.9-77.0°F and 57.1-60.6%, respectively. Oxygen content was maintained at 21% throughout the exposure.

12.2. Limit Test Data

12.2.1. Mortality

Individual Data: Table 2

All mortality occurred by study day 1.

12.2.2. Clinical Observations

Individual Data: Table 2

The most notable clinical abnormalities observed during the study included transient incidences of decreased activity, breathing abnormalities, decreased defecation, rough haircoat, nasal discharge and dark material around the facial area. No positive findings were noted at the time of observation during the 4-hour exposure period.

12.2.3. Body Weight Data

Individual Data: Table 3

A slight body weight loss was noted for two males during the day 0 to 7 body weight interval. Body weight gain/maintenance was noted for all other surviving animals during the test period.

12.2.4. Gross Necropsy

Individual Data: Table 4

The most notable gross internal findings were observed in the animals that died and included dark red lobes of the lung and abnormal content in the small intestine. No significant gross internal findings were observed at necropsy on study day 14.

13. CONCLUSION

Under the conditions of this test, the acute inhalation LC50 of Spray-Bravo was estimated to be greater than 2.40 mg/L in the rat (which was well above the EPA required 2.00 mg/L).

Kimberly L. Bonnette, M.S., LATG
Study Director

Date 1/7/03

14. REPORT REVIEW

Dawn D. Rodabaugh, B.S.
Toxicologist

Date 1/7/03

15. REFERENCE

1. Guide for the Care and Use of Laboratory Animals, DHHS Publication No. (NIH) 96-03, 1996.

TABLE 1

SLI STUDY NO.: 3596.11 AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS PAGE 1

CLIENT: INL/A, U.S. DEPARMENT OF STATE SUMMARY OF AEROSOL GENERATION AND CHAMBER ENVIRONMENTAL DATA

	EXPOSURE LEVEL (MG/L)
	2.40
CHAMBER AND EXPOSURE DATA	
CHAMBER VOLUME (L):	10
ELUTRIATOR VOLUME (L):	5
MEAN AIR FLOW RATE (L/MIN):	22
MEAN AIR CHANGES PER HOUR:	88.24
T99 EQUILIBRATION PERIOD (MIN.):	4
EXPOSURE TIME (MIN):	240
DE-EQUILIBRATION PERIOD (MIN):	4
AEROSOL CONCENTRATIONS	
CALCULATED NOMINAL CONCENTRATION (MG/L):	297.69
TIME-WEIGHTED MEAN ANALYTICAL CONCENTRATION (MG/L):	2.40
AEROSOL PARTICLE-SIZE ANALYSIS	
MASS MEDIAN AERODYNAMIC DIAMETER (μ):	3.2
GEOMETRIC STANDARD DEVIATION:	±1.96
PERCENTAGE OF PARTICLES ≤ 4.0 μ (%):	63
CHAMBER ENVIRONMENTAL DATA	
TEMPERATURE RANGE (°F):	74.9-77.0
HUMIDITY RANGE (%):	57.1-60.6
OXYGEN CONTENT (%):	21

INL/A, U.S. DEPARTMENT OF STATE

TABLE 2

AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS

INDIVIDUAL CLINICAL OBSERVATIONS
(POSITIVE FINDINGS)

MALES 2.40 MG/L

		DAY OF STUDY														
MALE#	OBSERVATIONS	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A5619	FOUND DEAD		P													
	CONGESTED BREATHING	P														
	LABORED BREATHING	P														
	RALES	P														
	NASAL DISCHARGE-CLEAR	P														
	DARK MATERIAL AROUND NOSE	P														
A5621	SCHEDULED EUTHANASIA															P
	CONGESTED BREATHING	P	P	P												
A5622	SCHEDULED EUTHANASIA															P
	ACTIVITY DECREASED		P													
	CONGESTED BREATHING	P	P	P	P	P	P	P	P	P	P	P				
	LABORED BREATHING		P													
	RALES		P	P		P		P	P	P	P					
	FEW FECES			P	P											
	DARK MATERIAL AROUND EYE(S)											P	P			
	NASAL DISCHARGE-CLEAR	P	P	P												
	DARK MATERIAL AROUND NOSE	P	P													
A5620	SCHEDULED EUTHANASIA															P
	CONGESTED BREATHING		P	P	P											
	RALES		P	P												
	FEW FECES		P													
	ABNORMAL COLORED URINE:RED													P		
A5624	FOUND DEAD		P													
	ACTIVITY DECREASED		P													
	CONGESTED BREATHING	P	P													
	LABORED BREATHING		P													
	RALES	P	P													
	GASPING		P													

GRADE CODE: 1=SLIGHT 2=MODERATE 3=SEVERE P=PRESENT L=LEFT R=RIGHT B=BILATERAL

TABLE 2

AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS

INDIVIDUAL CLINICAL OBSERVATIONS
(POSITIVE FINDINGS)

MALES 2.40 MG/L

MALE#	OBSERVATIONS	DAY OF STUDY 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A5624	(CONTINUED)															
	FEW FECES		P													
	ROUGH COAT		P													
	COOL TO THE TOUCH		P													
	NASAL DISCHARGE-CLEAR		P													
	DARK MATERIAL AROUND NOSE		P													
	DARK MATERIAL AROUND MOUTH		P													

GRADE CODE: 1=SLIGHT 2=MODERATE 3=SEVERE P=PRESENT L=LEFT R=RIGHT B=BILATERAL

TABLE 2

AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS

INDIVIDUAL CLINICAL OBSERVATIONS
(POSITIVE FINDINGS)

FEMALES 2.40 MG/L

FEMALE#	OBSERVATIONS	DAY OF STUDY 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A5747	SCHEDULED EUTHANASIA															P
	CONGESTED BREATHING	P	P	P	P	P										
	LABORED BREATHING			P												
	RALES	P		P												
	GASPING			P												
	FEW FECES		P	P	P											
	SOFT STOOL											P				
A5748	SCHEDULED EUTHANASIA															P
	CONGESTED BREATHING	P	P	P												
	LABORED BREATHING	P	P													
	RALES	P	P													
	FEW FECES		P	P												
	DARK MATERIAL AROUND NOSE	P														
A5750	SCHEDULED EUTHANASIA															P
	CONGESTED BREATHING	P	P	P	P											
	LABORED BREATHING	P														
	RALES	P														
	FEW FECES		P													
	DARK MATERIAL AROUND EYE(S)			P												
A5751	SCHEDULED EUTHANASIA															P
	CONGESTED BREATHING	P	P	P						P	P					
	LABORED BREATHING	P	P													
	RALES	P	P	P						P						
	FEW FECES		P													
	NO FECES			P												
	ROUGH COAT		P													
	URINE STAIN		1													

GRADE CODE: 1=SLIGHT 2=MODERATE 3=SEVERE P=PRESENT L=LEFT R=RIGHT B=BILATERAL

TABLE 2

AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS

INDIVIDUAL CLINICAL OBSERVATIONS
(POSITIVE FINDINGS)

FEMALES 2.40 MG/L

		DAY OF STUDY														
FEMALE#	OBSERVATIONS	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A5752	SCHEDULED EUTHANASIA															P
	CONGESTED BREATHING	P	P	P												
	FEW FECES			P												

GRADE CODE: 1=SLIGHT 2=MODERATE 3=SEVERE P=PRESENT L=LEFT R=RIGHT B=BILATERAL

TABLE 3

AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS

INDIVIDUAL BODY WEIGHTS (GRAMS)

MALES 2.40 MG/L

	DAY OF STUDY			
ANIMAL#	0	7	14	AT DEATH (DAY)
A5619	305			271 (1)
A5621	324	329	354	
A5622	322	312	353	
A5620	305	296	316	
A5624	314			262 (1)
MEAN	314	312	341	
S.D.	9.0	16.5	21.7	
N	5	3	3	

TABLE 3

AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS

INDIVIDUAL BODY WEIGHTS (GRAMS)

FEMALES 2.40 MG/L

	DAY OF STUDY		
ANIMAL#	0	7	14 AT DEATH (DAY)
A5747	191	191	193
A5748	196	222	236
A5750	200	219	235
A5751	199	211	221
A5752	194	204	208
MEAN	196	209	219
S.D.	3.7	12.5	18.3
N	5	5	5

TABLE 4

AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS

INDIVIDUAL GROSS NECROPSY OBSERVATIONS

MALES 2.40 MG/L

ANIMAL#	DAY OF DEATH	STUDY DAY	OBSERVATION	FATE
A5619	2-AUG-02	1	HAIRCOAT: DARK MATERIAL; PRESENT AROUND NOSE, RED TESTES: SMALL; PRESENT LEFT, APPROXIMATELY 50% SMALLER THAN NORMAL EPIDIDYMIDES: MISSHAPEN; PRESENT LEFT, CORPUS ELONGATED; CAPUT IS UNATTACHED TO TESTIS LUNG: DARK RED; PRESENT ALL LOBES SMALL INTESTINE: CONTENT ABNORMAL; PRESENT ENTIRE TRACT, YELLOW MUCOID MATERIAL TO REDDISH-YELLOW MUCOID MATERIAL	FOUND DEAD
A5621	15-AUG-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A5622	15-AUG-02	14	HAIRCOAT: DARK MATERIAL; PRESENT AROUND LEFT EYE, RED	SCHEDULED EUTHANASIA
A5620	15-AUG-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A5624	2-AUG-02	1	HAIRCOAT: DARK MATERIAL; PRESENT AROUND NOSE, MOUTH AND FOREPAWS; RED HAIRCOAT: WET MATTING; PRESENT VENTRAL THORAX, CLEAR COLORLESS; ANOGENITAL AND UROGENITAL AREAS, YELLOW SMALL INTESTINE: CONTENT ABNORMAL; PRESENT ENTIRE TRACT, YELLOW TO RED MUCOID MATERIAL LUNG: DARK RED; PRESENT ALL LOBES	SCHEDULED EUTHANASIA

TABLE 4

AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS

INDIVIDUAL GROSS NECROPSY OBSERVATIONS

FEMALES 2.40 MG/L

ANIMAL#	DAY OF DEATH	STUDY DAY	OBSERVATION	FATE
A5747	15-AUG-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A5748	15-AUG-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A5750	15-AUG-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A5751	15-AUG-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A5752	15-AUG-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA

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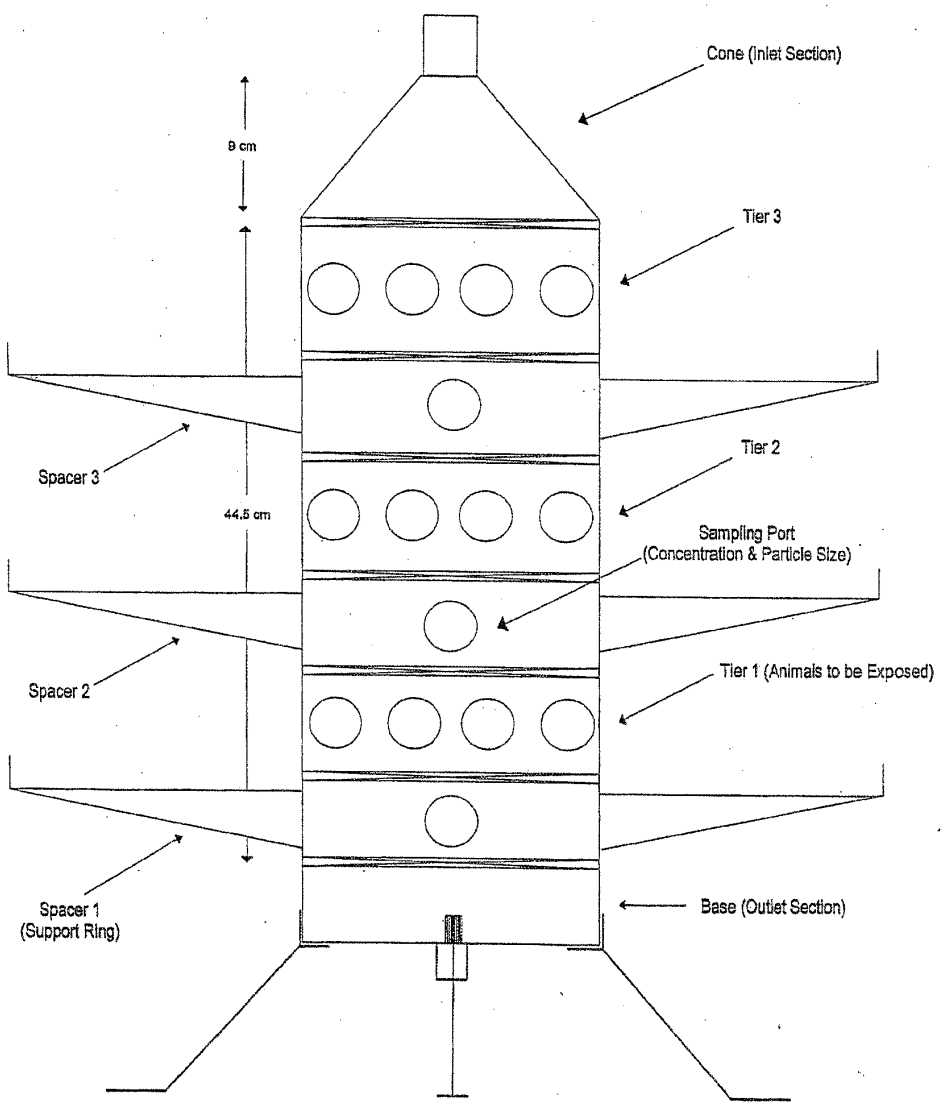

MULTI-STAGE 10 L NOSE ONLY INHALATION CHAMBER

Figure 1

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APPENDIX A

Preliminary Aerosol Generation Trials

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1. PRELIMINARY AEROSOL GENERATION TRIALS

Prior to experimental initiation, preliminary aerosol generation trials were conducted. These procedures were performed in order to determine the most efficient means of generating an aerosol of the test article. The type of equipment used during each aerosol trial procedure is presented in Trial Table 1. In each trial, attempts were made to generate the highest concentration of the test article while utilizing equipment that would minimize the aerodynamic particle size of the aerosol.

The analytical chemistry was initially attempted by extracting the active (glyphosate) from the glass fiber filters. However, for this material, the results were inaccurate and the collection procedure changed to collect the atmosphere test article sample directly into a liquid (using 20 mL methanol in an impinger). Four impingers were utilized in tandem to insure that all of the test article was trapped. Based on these results of less than 10% test article in the second, third and fourth impingers, no more than two impingers were needed for the main study. However, three impingers were utilized as a precaution. In addition, the sample collection procedure was the same as utilized for Trial #2 (2 L of atmosphere drawn through the impingers for 2 minutes for a total of 4 L of atmosphere). In order to ensure a $\geq$ 2.00 target dose, the test article flow rate was increased to 5.0 mL/minute.

Using the equipment design determined by the aerosol generation trials, preliminary results from previous trial work indicated the aerosol aerodynamic particle-size distribution would be acceptable.

SLI STUDY NO.: 3596.11 TRIAL TABLE 1 PAGE 1
CLIENT: INL/A, U.S. DEPARTMENT OF STATE PRELIMINARY AEROSOL GENERATION TRIALS

TRIAL NO.	EQUIPMENT USED	INPUT AIR (PSI)	TEST ARTICLE CONCEN-TRATION (%)[a]	MAXIMUM ATTAINABLE CONCENTRATIONS (MG/L) IMPINGERS			
				a	b	c	d
1	One Multistage 10L Nose-Only Chamber 5L Elutriator Master Flex Pump and Pump Heads 7523-30 and 77200-60 Spraying Systems, Pistol Air/Fluid Mixing Nozzle 4.0 mL/min pump speed 14 gauge tubing size Sample Collection: 2 L of atmosphere for 5 minutes (2 L x 5 min).	30	100	1.07	0.06	0.06	ND
2	One Multistage 10L Nose-Only Chamber 5L Elutriator Master Flex Pump and Pump Heads 7523-30 and 77200-60 Spraying Systems, Pistol Air/Fluid Mixing Nozzle 4.0 mL/min pump speed 14 gauge tubing size Sample Collection: 2 L of atmosphere for 2 minutes (2 L x 2 min).	30	100	1.63	0.06	0.06	ND
3	One Multistage 10L Nose-Only Chamber 5L Elutriator Master Flex Pump and Pump Heads 7523-30 and 77200-60 Spraying Systems, Pistol Air/Fluid Mixing Nozzle 4.0 mL/min pump speed 14 gauge tubing size Sample Collection: 1 L of atmosphere for 5 minutes (1 L x 5 min).	30	100	1.31	0.02	0.02	ND
4	One Multistage 10L Nose-Only Chamber 5L Elutriator Master Flex Pump and Pump Heads 7523-30 and 77200-60 Spraying Systems, Pistol Air/Fluid Mixing Nozzle 4.0 mL/min pump speed 14 gauge tubing size Sample Collection: 1 L of atmosphere for 5 minutes (1 L x 5 min).	30	100	1.51	0.05	0.02	ND

[a]Pooled test article.
Note: Targeting ≥ 2.00 mg/L analytical concentration for Trials 1-4. ND = None Detected.

TRIAL NO.	EQUIPMENT USED	INPUT AIR (PSI)	TEST ARTICLE CONCEN-TRATION (%)[a]	MAXIMUM ATTAINABLE CONCENTRATIONS (MG/L) IMPINGERS						
				a	b	c	d	e	f	g
5	One Multistage 10L Nose-Only Chamber 5L Elutriator Master Flex Pump and Pump Heads 7523-30 and 77200-60 Spraying Systems, Pistol Air/Fluid Mixing Nozzle 4.0 mL/min pump speed 14 gauge tubing size Sample Collection: 1 L of atmosphere for 1 minute (1 L x 1 min).	30	100	1.65	0.13	ND	ND	ND	ND	ND
6	One Multistage 10L Nose-Only Chamber 5L Elutriator Master Flex Pump and Pump Heads 7523-30 and 77200-60 Spraying Systems, Pistol Air/Fluid Mixing Nozzle 4.0 mL/min pump speed 14 gauge tubing size Sample Collection: 1 L of atmosphere for 1 minute (1 L x 2 min).	30	100	1.31	0.20	ND	ND	ND	ND	ND

[a]Pooled test article.

Note: Targeting ≥ 2.00 mg/L analytical concentration for Trials 5-6. ND = None Detected.

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APPENDIX B

Analytical Chemistry Report

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1. SPRAY--BRAVO ANALYSIS

The analytical method for the analysis of the glyphosate component of Spray--Bravo was validated prior to the analytical chamber concentration analyses performed at Springborn Laboratories, Inc. This method was utilized to determine the inhalation chamber concentration during the Acute Nose -Only Inhalation Toxicity Study.

1.1. Experimental System

1.1.1. HPLC System

HPLC Model:	Waters
Pump:	Waters 600E
Injector:	Waters WISP 717
Detector:	Waters 2487
Data System:	H-P 3396B Integrator
Precolumn:	Phenomenex, SecurityGuard, C18, 4.0 x 3.0 mm ID
Column:	Phenomenex, Spherex, C18, 5μ, 250 x 4.6 mm ID
Temperature:	Ambient
Detection:	500 nm, 0.4000 AUFS
Mobile Phase:	A: 0.05 M HCO_2NH_4, pH 3.6/5% Acetonitrile (ACN); B: 100% ACN
Gradient:	100% A hold for 6 minutes; linear change to 25% A/75% B over 1 minute; hold for 5 minutes; linear change to 100% A over 1 minute; hold at 100% A for 15 minutes.
Flow Rate:	1.0 mL/min
Injection Volume:	10 μL

1.1.2. Apparatus

Balance:	Mettler AG 245, accuracy of 0.0001 gram
Glassware:	Assorted volumetric glassware
Filters:	Gelman, glass fiber; Millipore 0.2μ Nylon-66; Whatman Puradisc 25PP 0.45μm
Shaker:	Labline, Multi-Wrist Shaker
Oven:	Boekel Model 107905
Pipet:	Mettler, VoluMate, 200-1000 μL

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1.1.3. Solutions and Reagents

1.1.3.1. Reagents

Water, Fisher, HPLC Grade, Lot # 024948, 025012
Acetonitrile, Baker, HPLC Grade, Lot # M15811
Methanol, Fisher, HPLC Grade, Lot # 011803, 023006
NBD Chloride, Aldrich, 98%, Lot #12214L1
Hydrochloric Acid, Fisher, ACS Grade, Lot # 012161
Potassium Tetraborate Tetrahydrate: Aldrich, 99%, Lot # 15325D1
Formic Acid, Fisher, Laboratory Grade, Lot # 003630
Ammonium Formate, Fisher, Certified, Lot # 990125

1.1.3.2. Solutions

0.37 M Borate Solution: Prepared by dissolving approximately 11.44 g of potassium tetraborate tetrahydrate in 100 mL of water. The resulting solution was stable for 6 months under ambient storage conditions.

1.2 N HCl: Prepared by dissolving 10 mL of HCl in 90 mL of water. The resulting solution was stable for 6 months under ambient storage conditions.

25 mM NBD-Cl: Prepared by dissolving approximately 2.5 g of NBD-Cl in 500 mL of methanol. The resulting solution was stable for 6 months under ambient storage conditions.

Mobile Phase A: Prepared by dissolving approximately 3.153 g of ammonium formate in 1900 mL of water. The pH was adjusted to approximately 3.6 with formic acid. Then added 100 mL of acetonitrile. The resulting solution was mixed thoroughly, filtered through a 0.2μ Nylon-66 filter and degassed by helium sparging prior to use. Different volumes were used using the same ratio of components.

Mobile Phase B: Acetonitrile used 100% as received.

Diluent: All standards and samples were diluted in methanol.

Stock Standard Solution (Impinger Trial, mg/L): For the 2 $\times$ 5L trial, prepared by dissolving 65.8 mg of the Spray Bravo formulation in a 25 mL flask with diluent. For the 2 $\times$ 2L trial, prepared by dissolving 13.4 mg of the Spray

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Bravo formulation in a 25 mL flask with diluent. For the 1 × 5L trial, prepared by dissolving 22.5 mg of the Spray Bravo formulation in a 25 mL flask with diluent. For the 1 × 1L trial, prepared by dissolving 7.8 mg of the Spray Bravo formulation in a 200 mL flask with diluent.

Stock Standard Solution (Exposure #1): Prepared by dissolving 13.2 mg of Spray Bravo formulation in a 25 mL flask with diluent.

Standard Solutions (Impinger Trial): Prepared by serially diluting the stock standard solution with methanol. The final concentrations of the solutions were in the range of approximately 0.10 to 0.52 mg/mL (2 min × 5 L); 0.053 to 0.26 mg/mL (2 min × 2 L); 0.09 to 0.45 mg/mL (1 min × 5 L); and 0.0039 to 0.019 mg/mL (1 min × 1 L). The 2 min × 5 L solutions were then further diluted in diluent at a ratio of 4:10 prior to derivatization, due to the higher concentration.

Standard Solutions (Exposure #1): Prepared by serially diluting the stock standard solution with methanol. The final concentrations of the solutions were in the range of approximately 0.26 to 1.3 mg/mL.

Chamber Concentration Solutions (Exposure # 1): Prepared by passing the analytical chamber sample through three impingers, each filled with 20 mL of diluent. The diluent from each impinger was collected and derivatized separately.

Derivatization Procedure: In order to analyze the glyphosate component, a precolumn derivatization was performed by adding 1.2 mL of the appropriate control, standard, or sample solution to a labeled scintillation vial. Both 0.8 mL of the borate solution and 2.4 mL of the NBD-Cl solution were added to each vial. The vials were then capped and shaken by hand prior to being heated in an oven at 80° C for 30 minutes. After removal from the oven, the vials were allowed to cool for 10 minutes followed by the addition of 0.9 mL of the HCl solution. After the vials were again shaken by hand, they were allowed to stand for 10 minutes in order for incipient precipitation to occur. These solutions were then transferred to injection vials.

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1.1.4. Analytical Procedures

1.1.5. Standard Curve Analysis

The peak area of the glyphosate acid component of each standard were determined, measured, combined, and plotted as a function of concentration to generate a standard curve. The actual values used for the calculations are shown in Chemistry Tables 1, 2, 3, 4, and 5.

1.1.6. Sample Analysis

The peak areas of the glyphosate acid component of each sample were measured and combined and then the concentration was determined by linear fit to the standard curve. The actual values used for the calculations are shown in Chemistry Tables 1, 2, 3, 4, and 5.

1.2. Results and Conclusions

1.2.1. Analytical Chamber Concentration

The actual sample results of the trial work are shown in Chemistry Tables 1, 2, 3, and 4. The actual sample results of the analytical chamber analysis are shown in Chemistry Table 5.

M. Gardner Clemons

M. Gardner Clemons, B.A.
Manager of Analytical Chemistry
and Pharmacy.

1.7.2003
Date

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Chemistry Table 1

Standard Curve and Sample Analysis Values for Impinger Trial Work for 2 × 5 L

Sample No.	Theoretical Conc. (mg/L)	Peak Area	Analytical Chamber Conc. (mg/L)
Std 1A	0.2632	45363	NA
Std 2A	0.5264	108136	NA
Std 3A	0.7896	144205	NA
Std 4A	1.053	198178	NA
Std 5A	1.316	259386	NA
Trial # 1a	NA	304141	1.567
Trial # 1b	NA	8136	0.06353
Trial # 1c	NA	6969	0.05760
Trial # 1d	NA	ND	ND

Correlation coefficient = 0.997; NA = Not applicable; ND = Not Detected.

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Chemistry Table 2

Standard Curve and Sample Analysis Values for Impinger Trial Work for 2 × 2 L

Sample No.	Theoretical Conc. (mg/L)	Peak Area	Analytical Chamber Conc. (mg/L)
Std 1B	0.1340	26211	NA
Std 2B	0.2680	54882	NA
Std 3B	0.4020	85616	NA
Std 4B	0.5360	115986	NA
Std 5B	0.6700	131941	NA
Trial # 2a	NA	331783	1.625
Trial # 2b	NA	13774	0.06202
Trial # 2c	NA	12332	0.05493
Trial # 2d	NA	ND	ND

Correlation coefficient = 0.997; NA = Not applicable; ND = Not Detected.

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Chemistry Table 3

Standard Curve and Sample Analysis Values for Impinger Trial Work 1 × 5 L

Sample No.	Theoretical Conc. (mg/L)	Peak Area	Analytical Chamber Conc. (mg/L)
Std 1C	0.1800	40947	NA
Std 2C	0.3600	86151	NA
Std 3C	0.5400	133858	NA
Std 4C	0.7200	182217	NA
Std 5C	0.9000	250029	NA
Trial # 3a	NA	358270	1.309
Trial # 3b	NA	19872	0.1243
Trial # 3c	NA	21161	0.1288
Trial # 3d	NA	ND	ND
Trial # 4a	NA	415221	1.508
Trial # 4b	NA	26568	0.1477
Trial # 4c	NA	17339	0.1154
Trial # 4d	NA	ND	ND

Correlation coefficient = 0.997; NA = Not Applicable; ND = Not Detected

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Chemistry Table 4

Standard Curve and Sample Analysis Values for Impinger Trial Work 1 × 1 L

Sample No.	Theoretical Conc. (mg/L)	Peak Area	Analytical Chamber Conc. (mg/L)
Std 1D	0.03900	ND	NA
Std 2D	0.07800	3520	NA
Std 3D	0.1170	5630	NA
Std 4D	0.1560	6869	NA
Std 5D	0.1950	8931	NA
Trial # 5a	NA	74105	1.651
Trial # 5b	NA	6043	0.1322
Trial # 5c	NA	ND	ND
Trial # 5d	NA	ND	ND
Trial # 5e	NA	ND	ND
Trial # 5f	NA	ND	ND
Trial # 5g	NA	ND	ND
Trial # 6a	NA	58780	1.309
Trial # 6b	NA	9271	0.2042
Trial # 6c	NA	ND	ND
Trial # 6d	NA	ND	ND
Trial # 6e	NA	ND	ND
Trial # 6f	NA	ND	ND
Trial # 6g	NA	ND	ND

* Correlation coefficient = 0.995; NA = Not Applicable; ND = Not Detected

(41)

SLI Study No. 3596.11

APPENDIX C

Individual Aerosol Generation and
Chamber Environmental Data

(42)

SLI Study No. 3596.11

2.40 mg/L Exposure Level

(43)

SLI Study No. 3596.11

AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS
CHAMBER ENVIRONMENTAL DATA
EXPOSURE: 2.40 MG/L

TIME (MIN.)	TEMPERATURE (°F)	RELATIVE HUMIDITY (%)	OXYGEN CONTENT (%)
0	77.0	57.1	21
30	74.9	60.2	21
60	75.1	60.6	21
90	76.0	58.2	21
120	75.6	59.8	21
150	75.6	59.6	21
180	75.6	59.8	21
210	75.9	59.5	21
240	75.6	59.8	21

(44)

SLI Study No. 3596.11

Standard Curve and Sample Analysis Values for Impinger Exposure #1

Sample No.	Theoretical Conc. (mg/L)	Peak Area	Analytical Chamber Conc. (mg/L)
Std 1	0.1320	22300	NA
Std 2	0.2640	41117	NA
Std 3	0.3960	74124	NA
Std 4	0.5280	87613	NA
Std 5	0.6600	110814	NA
1A	NA	ND	ND
1B	NA	ND	ND
1C	NA	ND	ND
2A	NA	344241	2.032
2B	NA	8366	0.04860[a]
2C	NA	8105	0.04706[a]
3A	NA	324116	1.913
3B	NA	11740	0.06852[a]
3C	NA	8177	0.04748[a]
4A	NA	510006	3.011
4B	NA	20840	0.1223[a]
4C	NA	7258	0.04206[a]
5A	NA	566238	3.343
5B	NA	8150	0.04732[a]
5C	NA	9333	0.05431[a]

* Correlation coefficient = 0.995; NA = Not Applicable; ND = Not Detected
[a]Less than 10%; therefore, not utilized in determining chamber concentration.

(45)

SLI Study No. 3596.11

AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS
AERODYNAMIC PARTICLE SIZE DATA
SAMPLE NO.: A
EXPOSURE: 2.40 MG/L

Stage	Effective Cutoff Diameter	Filter Weights (mg) Pre-sample	Post-sample	Difference Weights	% of Total	Cumulative % <ECD
1	10.00	103.3	103.5	0.2	2.6	97.4
2	6.11	102.8	103.6	0.8	10.5	86.8
3	3.70	102.6	104.6	2.0	26.3	60.5
4	2.22	103.2	106.1	2.9	38.2	22.4
5	1.39	102.7	104.0	1.3	17.1	5.3
6	0.79	103.5	103.8	0.3	3.9	1.3
7	0.50	102.9	102.9	0.0	0.0	1.3
Filter	-	103.4	103.5	0.1	1.3	
		Total of Difference Weights:		7.6		

Mass Median Aerodynamic Diameter = 3.1 microns
Geometric Standard Deviation = 1.90
Percentage ≤ 4.0 microns = 66 %

(46)

SLI Study No. 3596.11

AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS
AERODYNAMIC PARTICLE SIZE DATA
SAMPLE NO.: B
EXPOSURE: 2.40 MG/L

Stage	Effective Cutoff Diameter	Filter Weights (mg) Pre-sample	Post-sample	Difference Weights	% of Total	Cumulative % <ECD
1	10.00	103.1	103.2	0.1	1.2	98.8
2	6.11	102.7	103.9	1.2	14.5	84.3
3	3.70	102.4	104.4	2.0	24.1	60.2
4	2.22	102.9	105.8	2.9	34.9	25.3
5	1.39	102.5	103.9	1.4	16.9	8.4
6	0.79	102.8	103.3	0.5	6.0	2.4
7	0.50	103.3	103.3	0.0	0.0	2.4
Filter	-	102.9	103.1	0.2	2.4	
		Total of Difference Weights:		8.3		

Mass Median Aerodynamic Diameter = 2.8 microns
Geometric Standard Deviation = 1.93
Percentage ≤ 4.0 microns = 70 %

(47)

SLI Study No. 3596.11

AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS
AERODYNAMIC PARTICLE SIZE DATA
SAMPLE NO.: C
EXPOSURE: 2.40 MG/L

Stage	Effective Cutoff Diameter	Filter Weights (mg) Pre-sample	Post-sample	Difference Weights	% of Total	Cumulative % <ECD
1	10.00	103.4	104.1	0.7	8.3	91.7
2	6.11	102.2	103.5	1.3	15.5	76.2
3	3.70	103.0	105.0	2.0	23.8	52.4
4	2.22	102.5	105.2	2.7	32.1	20.2
5	1.39	101.7	103.1	1.4	16.7	3.6
6	0.79	102.0	102.2	0.2	2.4	1.2
7	0.50	102.0	102.0	0.0	0.0	1.2
Filter	-	102.5	102.6	0.1	1.2	
		Total of Difference Weights:		8.4		

Mass Median Aerodynamic Diameter = 3.7 microns
Geometric Standard Deviation = 2.06
Percentage ≤ 4.0 microns = 54 %

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SLI Study No. 3596.11

AN ACUTE NOSE-ONLY INHALATION TOXICITY STUDY IN RATS
AERODYNAMIC PARTICLE SIZE DATA

EXPOSURE: 2.40 MG/L

Stage	Effective Cutoff Diameter	Cumulative % less than indicated size Sample A	Sample B	Sample C	
1	10.00	97.4	98.8	91.7	
2	6.11	86.8	84.3	76.2	
3	3.70	60.5	60.2	52.4	
4	2.22	22.4	25.3	20.2	
5	1.39	5.3	8.4	3.6	
6	0.79	1.3	2.4	1.2	
7	0.50	1.3	2.4	1.2	
					Mean
Mass Median Aerodynamic Diameter		3.1	2.8	3.7	3.2
Geometric Standard Deviation		1.90	1.93	2.06	1.96
Percentage ≤ 4.0 microns		66	70	54	63

(49)

SLI Study No. 3596.11

APPENDIX D

SLI Personnel Responsibilities

(50)

SLI Study No. 3596.11

SLI PERSONNEL RESPONSIBILITIES

Kimberly L. Bonnette, M.S., LATG	Study Director/Director, Acute Toxicology
Dawn D. Rodabaugh, B.S.	Alternate Contact/Toxicologist
Robert C. Springborn, Ph.D.	Chairman, President and CEO
Malcolm Blair, Ph.D.	Senior Vice President, Managing Director Emeritus
Joseph C. Siglin, Ph.D., DABT	Vice President, Managing Director
Rusty E. Rush, M.S., LAT, DABT	Director, Neurotoxicity and Transgenics
Jason W. Smedley, B.S.	Assistant Toxicologist
Pamela S. Smith, ALAT	Supervisor of Acute Toxicology
Kevin V. Weitzel, A.S.	Primary Technician/Inhalation Team Leader
Delores P. Knippen	Supervisor of Pharmacy
Steven H. Magness, B.S., LATG	Senior Supervisor of Gross and Fetal Pathology
Anita M. Bosau, RQAP-GLP	Senior Director, Compliance Assurance
Deanna M. Talerico, RQAP-GLP	Senior Supervisor of Quality Assurance
J. Dale Thurman, D.V.M., M.S., DACVP	Senior Director, Pathology
Kathy M. Gasser	Supervisor of Archives

A DERMAL SENSITIZATION STUDY IN GUINEA PIGS WITH SPRAY--BRAVO MODIFIED BUEHLER DESIGN

FINAL REPORT

OPPTS Guidelines

870.2600

Author

Kimberly L. Bonnette, M.S., LATG

Study Completed on

October 4, 2002

Performing Laboratory

Springborn Laboratories, Inc. (SLI)
Ohio Research Center
640 North Elizabeth Street
Spencerville, Ohio 45887

SLI Study No.

3596.14

Submitted to

INL/A
U.S. Department of State
2201 C St. NW SA-4
Washington, DC 20520

(2)

SLI Study No. 3596.14

1. STATEMENT OF *NO* DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B), or (C).

Company: _____

Company Agent: _____ Date: _____

_____ _____

Title Signature

SLI Study No. 3596.14 (3)

OCT 1 2002

2. COMPLIANCE STATEMENT

This study was conducted in compliance with the Good Laboratory Practice Standards as described by the EPA (40 CFR Parts 160 and 792) with the following exception:

The dose preparations used during the range-finding study were not analyzed to confirm test article concentration, stability or homogeneity.

Kimberly L. Bonnette, M.S., LATG
Study Director/Author
Springborn Laboratories, Inc.

Date 10/4/02

Rogers Woolfolk
Senior Aviation Advisor
Sponsor/Submitter
INL/A
U.S. Department of State

Date 28 Sep 02

SLI Study No. 3596.14

3. QUALITY ASSURANCE STATEMENT

This study was inspected by the Quality Assurance Unit and reports were submitted to management and the Study Director in accordance with SLI's Standard Operating Procedures as follows:

Phase	Date
Protocol Review	04/25/02
Dosing	07/08/02
Data Audit	09/19/02
Draft Report Review	09/19/02
Protocol Amendment Review	09/24/02
Final Report Review	10/04/02
Reports to Study Director and Management	09/19/02, 10/04/02

The final report has been reviewed to assure that it accurately describes the materials and methods, and the reported results accurately reflect the raw data.

Jennifer D. McGue — Date 10/4/02
Jennifer D. McGue
Quality Assurance Auditor

Anita M Bosau — Date 10/4/02
Anita M. Bosau, RQAP-GLP
Senior Director, Compliance Assurance

(5)

SLI Study No. 3596.14

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6. SUMMARY

The dermal sensitization potential of Spray--Bravo was evaluated in Hartley-derived albino guinea pigs. Ten male and ten female guinea pigs were topically treated with 100% Spray--Bravo, once per week, for three consecutive weeks. Following a two-week rest period, a challenge was performed whereby the twenty test and ten previously untreated (naive) challenge control guinea pigs were topically treated with 100% Spray--Bravo. Challenge responses in the test animals were compared with those of the challenge control animals.

6.1. Spray--Bravo

Following challenge with 100% Spray--Bravo, dermal reactions in the test and challenge control animals were limited to scores of 0. Group mean dermal scores were noted to be the same in the test animals as compared with the challenge control animals.

6.2. HCA

Using α-Hexylcinnamaldehyde (HCA) as a positive control, Springborn Laboratories, Inc., Spencerville, Ohio, has completed a study during the past six months which provided historical control data for contact sensitization to this agent utilizing the test system described herein (Modified Buehler Design). Following induction at 5% w/v HCA in ethanol and challenge at levels of 2.5% and 1% w/v HCA in acetone, a contact sensitization response was observed, thereby demonstrating the susceptibility of the test system to this sensitizing agent.

6.3. Conclusion

Based on the results of this study, Spray--Bravo is not considered to be a contact sensitizer in guinea pigs. The results of the HCA historical control study demonstrated that a valid test was performed and indicated that the test design would detect potential contact sensitizers.

7. INTRODUCTION

This study was performed to assess the dermal sensitization potential (delayed contact hypersensitivity) of Spray--Bravo in Hartley-derived albino guinea pigs when administered by multiple topical applications. This study was intended to provide information on the potential health hazards of the test article with respect to dermal exposure. Data from this study may serve as a basis for classification and/or labeling of the test article. This study was performed in accordance with the US EPA, Health Effects Test Guidelines, OPPTS 870.2600, Skin Sensitization, August 1998. This study was performed at Springborn Laboratories, Inc., 553 North Broadway, Spencerville, Ohio. The protocol was signed by the Study Director on April 26, 2002 (GLP initiation date). The in-life phase of the main sensitization study was initiated with test article administration on July 8, 2002 (day 0) and concluded with final scoring on August 7, 2002.

Prior to initiation of the main sensitization study, a topical range-finding study was conducted in guinea pigs to aid in the selection of dosage levels. The in-life phase of the range-finding study was initiated with test article administration on July 1, 2002, and concluded on July 3, 2002. The experimental methods and results of the range-finding study are included in Appendix A.

8. MATERIALS AND METHODS

8.1. Test Article

The test article was received from the Sponsor and identified as follows:

Sponsor's ID	Assigned SLI ID	Physical Description	Receipt Date	Expiration Date
Spray—Bravo[a]	S02.002.3596	Cloudy pale amber liquid	05/31/02	None Provided
Ingredients[b]				
Herbicide: Roundup SL				None
Lot Nos.: 4010/4212				Provided
4397/4272				
4333/4340				
4379/4076				
4397/4333				
Surfactant: Cosmo Flux-411F				None
Lot No.: Unknown				provided

[a]Sample pooled at SLI from five different mixes of Spray--Bravo (top/middle/bottom).
[b]Ingredients used in the five Spray--Bravo mixes that were prepared by the Sponsor.

The test article was stored at room temperature. The Sponsor was responsible for any necessary evaluations related to identity, strength, purity, composition, stability and method of synthesis of the test material according to 40 CFR 160.105, 40 CFR 792.105. Springborn Laboratories, Inc., analyzed the test article for the glyphosate (a.e.) which is presented in SLI Study No. 3596.8.

8.2. Retention Sample

An approximate 1 mL retention sample of each test article mixture sample (top/middle/bottom, maintained separately for a total of fifteen 1 mL samples) was taken and stored at SLI at room temperature. In addition, a 10 mL retention sample of the pooled test article samples (from the 5 test article mixtures) was collected and stored at SLI at room temperature. These samples serve as the retention samples for all studies conducted with this material.

8.3. Test Article Disposition

The remaining test article was returned to the Sponsor following completion of all studies with the test article.

8.4. Method of Test Article Preparation

The test article was utilized at 100% (induction and challenge). The test article was dispensed fresh on each day of dosing.

8.5. Animals and Animal Husbandry

8.5.1. Description, Identification and Housing

Young adult, Hartley-derived albino guinea pigs were received from Hilltop Lab Animals, Inc., Scottdale, PA. Upon receipt, plastic ear tags displaying unique identification numbers were used to individually identify the animals. Cage cards displaying at least the study number, animal number and sex were affixed to each cage. The animals were housed individually in suspended stainless steel cages. All housing and care were based on the standards recommended by the Guide for the Care and Use of Laboratory Animals [1].

8.5.2. Environment

The animal room temperature and relative humidity ranges were 64-74°F (18-23°C) and 34-72%, respectively. Environmental control equipment was monitored and adjusted as necessary to minimize fluctuations in the animal room environment. Light timers were set to maintain a 12-hour light/12-hour dark cycle and room ventilation was set to produce 10-15 air changes/hour. The room temperature and relative humidity were recorded a minimum of once daily.

8.5.3. Food

PMI Certified Guinea Pig Chow #5026 (Purina Mills, Inc.) was provided ad libitum to the animals throughout the study. The lot number and expiration date of each batch of diet used during the study were recorded. The feed was analyzed and certified by the supplier for nutritional components and environmental contaminants. Dietary limitations for various environmental contaminants, including heavy metals, pesticides, polychlorinated biphenyls and total aflatoxin are set by the manufacturer. Within these limits, contaminants which may have been present were not expected to compromise the purpose of this study. Results of the dietary analyses (Certificates of Analysis) are provided by the manufacturer for each lot of diet. These are maintained by SLI.

8.5.4. Water

Municipal tap water treated by reverse osmosis was available ad libitum throughout the study. The purified water was supplied by an automatic watering system. Monitoring of the drinking water for contaminants is conducted by SLI and the records are available for inspection. Within generally accepted limits, contaminants which may have been present were not expected to compromise the purpose of this study. The water meets the standards specified under the EPA National Drinking Water Regulations (40 CFR Part 141).

8.5.5. Acclimation

Upon receipt, the animals were removed randomly from the shipping cartons, examined by qualified personnel, identified with plastic ear tags and then acclimated to the laboratory conditions for a minimum of five days. The animals were observed daily for overt physical or behavioral abnormalities, general health/moribundity and mortality.

8.5.6. Animal Selection

The animals chosen for study use were arbitrarily selected from healthy stock animals to avoid potential bias. All animals received a detailed pretest observation prior to dosing. Only healthy animals were chosen for study use. Females were nulliparous and nonpregnant. The male animals were approximately 7 weeks of age and weighed 410-483 g on the day prior to Induction I dosing. The female animals were approximately 9 weeks of age and weighed 364-453 g on the day prior to Induction 1 dosing.

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SLI Study No. 3596.14

9. EXPERIMENTAL PROCEDURES

9.1. Study Design

This study consisted of a topical range-finding group, a test group and a challenge control group [2]. A rechallenge control group was maintained on this study; however, the rechallenge procedure was not required since the challenge results were definitive.

9.2. Sensitization Study

9.2.1. Preliminary Procedures

On the day prior to each dose administration, the guinea pigs had the hair removed with a small animal clipper. Care was taken to avoid abrading the skin.

9.2.2. Dosing

A dose of 0.3 mL of the test article was placed on a 25 mm Hilltop chamber backed by adhesive tape (occlusive patch). The chambers were then applied to the clipped surface as quickly as possible.

Following chamber application, the trunk of the animal was wrapped with elastic wrap which was secured with adhesive tape to prevent removal of the chamber and the animal was returned to its cage.

9.2.2.1. Induction

On the day prior to the first induction dose administration (day -1), all test and control animals were weighed and the hair was removed from the left side of the test animals. On the day following clipping (day 0), chambers were applied as follows:

Group	Material	Induction No.	Concentration (%)	Test Site No.	No. of Animals Male	No. of Animals Female
Test	Spray--Bravo	1	100[a]	1	10	10
		2	100[a]	1		
		3	100[a]	1		

[a]Pooled test article.

The induction procedure was repeated on study day 7 and on study day 14 so that a total of three consecutive induction exposures were made to the test animals.

9.2.2.2. Challenge

On the day prior to challenge dose administration, the test and challenge control animals were weighed and the hair was removed from the right side of the animals. On the day following clipping (day 28), chambers were applied as follows:

Group	Material	Concentration (%)	Test Site No.	No. of Animals Male	No. of Animals Female
Test	Spray--Bravo	100[a]	2	10	10
Challenge Control	Spray--Bravo	100[a]	2	5	5

[a]Pooled test article.

9.2.3. Test Article Removal

Approximately six hours after chamber application, the binding materials were removed. The test sites were wiped with gauze moistened in deionized water, followed by dry gauze, to remove test article residue. The animals were then returned to their cages.

9.2.4. Dermal Observations

The test sites were graded for irritation at approximately 24 and 48 hours following chamber application (induction) or chamber removal (challenge) using the Dermal Grading System presented in Appendix B.

9.2.5. Clinical Observations

Any unusual observations and mortality were recorded. The animals were observed for general health/mortality twice daily, once in the morning and once in the afternoon.

9.2.6. Body Weights

Individual body weights were obtained for all sensitization study animals on the day prior to the first induction (day -1) and for the appropriate test and challenge control animals on the day prior to challenge dosing.

9.2.7. Scheduled Euthanasia

All sensitization study animals were euthanized by carbon dioxide inhalation following each animal's final scoring interval. Gross necropsy examinations were not required for these animals.

9.3. Protocol Deviations

On one occasion each, the animal room temperature and relative humidity ranges [64-74°F (17-23°C) and 34-72%] exceeded the preferred ranges [63-73°F (17-23°C) and 30-70%, respectively] during this study. These occurrences were considered to have had no adverse effect on the outcome of this study.

10. ANALYSIS OF DATA

The sensitization potential of the test article was based on the dermal responses observed on the test and control animals at challenge. Generally, dermal scores of ≥1 in the test animals with scores of 0 to ± noted in the controls are considered indicative of sensitization. Dermal scores of 1 in both the test and control animals are generally considered equivocal unless a higher dermal response (≥ grade 2) is noted in the test animals. Group mean dermal scores were calculated for challenge.

11. MAINTENANCE OF RAW DATA AND RECORDS

All original raw data, the final report and magnetically encoded records were transferred to the SLI archives for a period of 10 years. The Sponsor will be contacted prior to final disposition of these items.

12. RESULTS

12.1. Topical Range-Finding Study

Individual Topical Range-Finding Data: Appendix A

The results of the range-finding study indicated that a test article concentration of 100% was considered appropriate for induction and challenge since it was the highest possible concentration which was nonirritating.

12.2. Sensitization Study

Individual Data: Tables 1-2

Following challenge with 100% Spray--Bravo, dermal reactions in the test and challenge control animals were limited to scores of 0. Group mean dermal scores were noted to be the same in the test animals as compared with the challenge control animals.

SLI Study No. 3596.14

12.3. Clinical Observations/Body Weights

Individual Clinical Observations: Appendix C
Individual Body Weight Data: Appendix D

The sensitization study animals gained weight during the test period and generally appeared in good health.

12.4. Historical Control

HCA Historical Control Data: Appendix E

Using α-Hexylcinnamaldehyde (HCA) as a positive control, Springborn Laboratories, Inc., Spencerville, Ohio, has completed a study during the past six months which provided historical control data for contact sensitization to this agent utilizing the test system described herein (Modified Buehler Design). Following induction at 5% w/v HCA in ethanol and challenge at levels of 2.5% and 1% w/v HCA in acetone, a contact sensitization response was observed, thereby demonstrating the susceptibility of the test system to this sensitizing agent.

13. CONCLUSION

Based on the results of this study, Spray-Bravo is not considered to be a contact sensitizer in guinea pigs. The results of the HCA historical control study demonstrated that a valid test was performed and indicated that the test design would detect potential contact sensitizers.

Kimberly L. Bonnette, M.S., LATG
Study Director

Date 10/4/02

14. REPORT REVIEW

Dawn D. Rodabaugh, B.S.
Toxicologist

Date 10/4/02

15. REFERENCES

1. Guide for the Care and Use of Laboratory Animals, DHHS Publication No. (NIH) 96-03, 1996.

2. E. V. Buehler, Delayed Contact Hypersensitivity in the Guinea Pig, Arch. Dermat., 91:171-177, 1965.

TABLE 1

SLI STUDY NO.: 3596.14 A DERMAL SENSITIZATION STUDY IN GUINEA PIGS PAGE 1

CLIENT: INL/A, U.S. DEPARTMENT OF STATE INDIVIDUAL INDUCTION DATA

(SPRAY--BRAVO)

Group	Animal No./ Sex	Induction 1 Dermal Scores 100%[a]		Induction 2 Dermal Scores 100%[a]		Induction 3 Dermal Scores 100%[a]	
		24 Hr	48 Hr	24 Hr	48 Hr	24 Hr	48 Hr
Test	G8744/M	0	0	0	0	0	0
	G8754/M	0	0	0	0	0	0
	G8748/M	0	0	0	0	0	0
	G8749/M	0	0	0	0	0	0
	G8759/M	0	0	0	0	0	0
	G8753/M	0	0	0[IT]	0	0	0
	G8745/M	0	0	0	0	0	0
	G8746/M	0	0	0	0	0	0
	G8747/M	0	0	0	0	0	0
	G8750/M	0	0	0	0	0	0
	G8836/F	0	0	0	0	0	0
	G8837/F	0	0	0	0	0	0
	G8838/F	0	0	0	0	0	0
	G8839/F	0	0	0	0	0	0
	G8840/F	0	0	0	0	0	0
	G8841/F	0	0	0	0	0	0
	G8842/F	0	0	0	0	0	0
	G8843/F	0	0	0	0	0	0
	G8844/F	0	0	0	0	0	0
	G8845/F	0	0	0	0	0	0

Note: See Appendix B for definition of codes.

[a]Pooled test article.

TABLE 2

SLI STUDY NO.: 3596.14 A DERMAL SENSITIZATION STUDY IN GUINEA PIGS PAGE 1

CLIENT: INL/A, U.S. DEPARTMENT OF STATE INDIVIDUAL CHALLENGE DATA

(SPRAY--BRAVO)

Group	Animal No./ Sex	Dermal Scores 100%[a] 24 Hr	48 Hr
Test	G8744/M	0	0
	G8754/M	0	0
	G8748/M	0	0
	G8749/M	0	0
	G8759/M	0	0
	G8753/M	0	0
	G8745/M	0	0
	G8746/M	0	0
	G8747/M	0	0
	G8750/M	0	0
	G8836/F	0	0
	G8837/F	0	0
	G8838/F	0	0
	G8839/F	0	0
	G8840/F	0	0
	G8841/F	0	0
	G8842/F	0	0
	G8843/F	0	0
	G8844/F	0	0
	G8845/F	0	0
	Mean	0.0	0.0

Notes: See Appendix B for definition of codes.

[a]Pooled test article.

TABLE 2

SLI STUDY NO.: 3596.14 A DERMAL SENSITIZATION STUDY IN GUINEA PIGS PAGE 2

CLIENT: INL/A, U.S. DEPARTMENT OF STATE INDIVIDUAL CHALLENGE DATA

(SPRAY--BRAVO)

Group	Animal No./ Sex	Dermal Scores 100%[a] 24 Hr	48 Hr
Challenge Control	G8751/M	0	0
	G8752/M	0	0
	G8755/M	0	0
	G8756/M	0	0
	G8757/M	0	0
	G8847/F	0	0
	G8848/F	0	0
	G8803/F	0	0
	G8826/F	0	0
	G8827/F	0	
	Mean	0.0	0.0

Notes: See Appendix B for definition of codes.

[a]Pooled test article.

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SLI Study No. 3596.14

APPENDIX A

Topical Range-Finding Study

(20)

SLI Study No. 3596.14

1. TOPICAL RANGE-FINDING STUDY

This appendix provides the experimental procedures and results of a topical range-finding study in guinea pigs with Spray--Bravo. The procedures for animal husbandry were similar to those described for the main sensitization study animals. The male animals were approximately 8 weeks of age and weighed 407-497 g; the female animals were approximately 10 weeks of age and weighed 479-498 g on the day prior to dosing.

1.1. Method of Test Article Preparation

The test article was utilized at 100% and at 75%, 50% and 25% w/v in deionized for the range-finding study. The test article was prepared and dispensed fresh on the day of dosing. The dosing preparations were stirred continuously during dosing.

1.2. Dosing

On the day prior to dose administration, four topical range-finding guinea pigs were weighed and the hair removed from the right and left side of the animals with a small animal clipper. Care was taken to avoid abrading the skin during clipping procedures.

On the following day, four concentrations of the test article were prepared and each concentration was applied to the clipped area of each topical range-finding animal as indicated below:

Group	Material	Concentration (%)	Test Site No.	Amount Applied	Patch Design[a]
Topical Range-Finding	Spray--Bravo	100[b]	1	0.3 mL	25 mm Hilltop Chamber
		75[c]	2	0.3 mL	25 mm Hilltop Chamber
		50[c]	3	0.3 mL	25 mm Hilltop Chamber
		25[c]	4	0.3 mL	25 mm Hilltop Chamber

[a]Occlusive patch.
[b]Pooled test article.
[c]The vehicle was deionized water.

The chambers were applied to the clipped surface as quickly as possible. The trunk of the animal was wrapped with elastic wrap which was secured with adhesive tape to prevent removal of the chambers and the animal was returned to its cage.

Approximately six hours after chamber application, the binding materials were removed. The test sites were then wiped with gauze moistened in deionized water, followed by dry gauze, to remove test article residue and the animals returned to their cages.

1.3. Dermal Observations

The test sites of the topical range-finding animals were graded for irritation at approximately 24 and 48 hours following chamber application using the Dermal Grading System in Appendix B.

1.4. Clinical Observations

Any unusual observations and mortality were recorded. The topical range-finding animals were observed for general health/mortality twice daily, once in the morning and once in the afternoon.

1.5. Body Weights

Individual body weights were obtained for the topical range-finding animals on the day prior to dosing.

1.6. Scheduled Euthanasia

Following the 48-hour scoring interval, all topical range-finding animals were euthanized by carbon dioxide inhalation. Gross necropsy examinations were not required for these animals.

1.7. Results

The results of the range-finding study indicated that a test article concentration of 100% was considered appropriate for induction and challenge since it was the highest possible concentration which was nonirritating.

SLI STUDY NO.: 3596.14
CLIENT: INL/A, U.S. DEPARTMENT OF STATE

A DERMAL SENSITIZATION STUDY IN GUINEA PIGS
TOPICAL RANGE-FINDING DATA
(SPRAY--BRAVO)

Group	Animal No./Sex Body Weight (g)	Range-Finding Dermal Scores							
		100%[a]		75%[a,b]		50%[a,b]		25%[a,b]	
		24 Hr	48 Hr	24 Hr	48 Hr	24 Hr	48 Hr	24 Hr	48 Hr
Range-Finding	G8349/M 407	0	0	0	0	0	0	0	0
	G8353/M 497	0	0	0	0	0	0	0	0
	G8506/F 479	0	0	0	0	0	0	0	0
	G8507/F 498	0	0	0	0	0	0	0	0

[a]Pooled test article.
[b]The vehicle used was deionized water.
Note: See Appendix B for definition of codes.

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SLI Study No. 3596.14

APPENDIX B

Dermal Grading System

(24)

SLI Study No. 3596.14

DERMAL GRADING SYSTEM

ERYTHEMA AND EDEMA OBSERVATIONS		
OBSERVATION	DEFINITION	CODE
Erythema – Grade 0	No reaction	0
Erythema – Grade ±	Slight patchy erythema	±
Erythema – Grade 1	Slight, but confluent or moderate patchy erythema	1
Erythema – Grade 2	Moderate, confluent erythema	2
Erythema – Grade 3	Severe erythema with or without edema	3
Maximized Grade 3	Notable dermal lesions	M – 3 (see below)
Edema – Grade 1	Very slight edema (barely perceptible)	ED-1
Edema – Grade 2	Slight edema (edges of area well defined by definite raising)	ED-2
Edema – Grade 3	Moderate edema (raised approximately 1 millimeter)	ED-3
Edema – Grade 4	Severe edema (raised more than 1 millimeter and extends beyond the area of exposure)	ED-4
An erythema code was assigned to each test site. An edema code was assigned only if edema was present at the test site. If notable dermal lesion(s) (> grade 1) were present, then the "Maximized Grade 3" was assigned to the test site in place of the erythema score and the type of the notable dermal lesion(s) was noted (e.g., M-3^{ES-2}).		

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DERMAL GRADING SYSTEM

NOTABLE DERMAL LESIONS		
OBSERVATION	CODE	DEFINITION
Eschar – Grade 1	ES-1	Focal and/or pinpoint areas up to 10% of test site.
Eschar – Grade 2	ES-2	> 10% < 25% of test site.
Eschar – Grade 3	ES-3	> 25% < 50% of test site.
Eschar – Grade 4	ES-4	> 50% of test site.
Blanching – Grade 1	BLA-1	Focal and/or pinpoint areas up to 10% of test site.
Blanching – Grade 2	BLA-2	> 10% < 25% of test site.
Blanching – Grade 3	BLA-3	> 25% < 50% of test site.
Blanching – Grade 4	BLA-4	> 50% of test site.
Ulceration – Grade 1	U-1	Focal and/or pinpoint areas up to 10% of test site.
Ulceration – Grade 2	U-2	> 10% < 25% of test site.
Ulceration – Grade 3	U-3	> 25% < 50% of test site.
Ulceration – Grade 4	U-4	> 50% of test site.
Necrosis – Grade 1	NEC-1 *(color)*	Focal and/or pinpoint areas up to 10% of test site (note color of necrosis).
Necrosis – Grade 2	NEC-2 *(color)*	> 10% < 25% of test site (Note color of necrosis).
Necrosis – Grade 3	NEC-3 *(color)*	> 25% < 50% of test site (Note color of necrosis).
Necrosis – Grade 4	NEC-4 *(color)*	> 50% of test site (Note color of necrosis).

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DERMAL GRADING SYSTEM

ADDITIONAL DERMAL FINDINGS		
OBSERVATION	DEFINITION	CODE
Desquamation	Characterized by scaling or flaking of dermal tissue or without denuded areas.	DES
Fissuring	Characterized by cracking of the skin with or without moist exudate. Fissuring should be checked prior to removing the animal from the cage and manipulating the test site.	FIS
Eschar Exfoliation	The process by which areas of eschar flake off the test site.	EXF
Test Site Staining	Skin located at test site appears to be discolored, possibly due to test article (note color of staining).	TSS (*color*)
Erythema Extends Beyond the Test Site	The erythema extends beyond the test site. Note: A study director should be contacted for erythema extending beyond the test site.	ERB
Superficial Lightening	Characterized by pale area(s) (almost a burn-like appearance) in the test site. However, erythema may still be observed through the pale area. Note: This observation may affect the overall erythema score of the test site. This observation may progress to other observations resulting in notable dermal lesions, but SL itself will not be considered a notable dermal lesion that will result in a dermal score to be maximized since it does not result in any in-depth injury. To be coded using an area designation (see below).	--
Superficial Lightening - Grade 1	Focal and/or pinpoint areas up to 10% of the test site	SL-1
Superficial Lightening - Grade 2	> 10% < 25% of test site	SL-2
Superficial Lightening - Grade 3	> 25% < 50% of test site	SL-3
Superficial Lightening - Grade 4	> 50% of test site	SL-4
Dermal Irritation - Outside of the Test Site	Noticeable irritation outside of test site probably due to the binding tape material. This notation will only be made for reactions greater than what are normally observed from tape removal which do not interfere with the scoring of the test site.	IT

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APPENDIX C

Individual Clinical Observations

SLI STUDY NO.: 3596.14 A DERMAL SENSITIZATION STUDY IN GUINEA PIGS PAGE 1
CLIENT: INL/A, U.S. DEPARTMENT OF STATE INDIVIDUAL CLINICAL OBSERVATIONS
(POSITIVE FINDINGS)

Group	Animal No./Sex	Clinical Observation
Test	G8836/F	Thin appearance: Days 6-7
	G8837F	Thin Appearance: Days 6-7

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APPENDIX D

Individual Body Weight Data

SLI STUDY NO.: 3596.14 A DERMAL SENSITIZATION STUDY IN GUINEA PIGS PAGE 1
CLIENT: INL/A, U.S. DEPARTMENT OF STATE INDIVIDUAL BODY WEIGHT DATA

Group	Animal No./Sex	Day -1	Day 27
Test	G8744/M	410	536
	G8754/M	483	711
	G8748/M	454	653
	G8749/M	453	672
	G8759/M	479	691
	G8753/M	451	672
	G8745/M	468	689
	G8746/M	456	657
	G8747/M	483	716
	G8750/M	413	579
	G8836/F	412	518
	G8837/F	404	526
	G8838/F	382	511
	G8839/F	382	505
	G8840/F	416	594
	G8841/F	367	521
	G8842/F	421	628
	G8843/F	367	513
	G8844/F	391	571
	G8845/F	453	632

		Body Weights	
Group	Animal No./Sex	Day -1	Day 27
Challenge Control	G8751/M	427	629
	G8752/M	458	684
	G8755/M	433	606
	G8756/M	453	656
	G8757/M	457	692
	G8847/F	381	540
	G8848/F	407	532
	G8803/F	376	526
	G8826/F	384	512
	G8827/F	370	507
Rechallenge Control[a]	G8760/M	446	--
	G8761/M	457	--
	G8762/M	444	--
	G8763/M	463	--
	G8758/M	421	--
	G8828/F	438	--
	G8829/F	373	--
	G8831/F	364	--
	G8832/F	369	--
	G8833/F	370	--

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SLI Study No. 3596.14

APPENDIX E

HCA Historical Control Data

(33)

SLI Study No. 3596.14

SPRINGBORN LABORATORIES, INC.
MODIFIED BUEHLER HISTORICAL CONTROL DATA
USING α-HEXYLCINNAMALDEHYDE
(SLI Study No. 999.171)

1. OBJECTIVE

This study was performed to assess the dermal sensitization potential of α-Hexylcinnamaldehyde (HCA) when administered by multiple topical applications. This study may be used to provide information on the ability of the test system to detect potential contact sensitizers and to update the historical positive control of the testing facility. The protocol was signed by the Study Director on February 6, 2002 (GLP initiation date). The in-life phase of the study was initiated with test article administration on March 13, 2002, and concluded with final scoring on April 12, 2002.

2. TEST ARTICLE

The test article was received from the manufacturer, TCI America, and identified as follows:

Supplier's ID	Assigned SLI ID	Physical Description	Receipt Date	SLI Assigned Expiration Date
HCA Lot No.: GF01	S01.008.N	Clear yellow liquid	08/21/01	08/21/03

The bulk compound was stored desiccated, protected from light, at room temperature. The manufacturer provided a Certificate of Analysis for the test article which is presented as Attachment 1 of this Appendix.

The HCA was mixed with ethanol or acetone to produce the appropriate concentrations for dose administration. For the sensitization study, the test article concentrations utilized were 5% w/v in ethanol (induction) and 1% and 2.5% w/v in acetone (challenge).

SLI Study No. 3596.14

3. EXPERIMENTAL PROCEDURES [1]

Young adult Hartley-derived albino guinea pigs were received on March 7, 2002, from Hilltop Lab Animals, Inc., Scottdale, PA. The guinea pigs were uniquely identified by ear tag, individually housed in suspended stainless steel cages and received Purina Certified Guinea Pig Chow #5026 and water purified by reverse osmosis ad libitum. The animals were acclimated for a minimum of 5 days prior to experimental initiation. The male guinea pigs were approximately 7 weeks of age and weighed 370-463 g; the female guinea pigs were approximately 8 weeks of age and weighed 336-396 g on the day prior to Induction I dosing.

On the day prior to the first induction dose administration (day -1), the hair was removed from the left side of the twenty test animals. On the following day, 0.3 mL of 5% w/v HCA in ethanol was placed on a Hilltop chamber and applied to the clipped area of each animal=s back. The trunk of each animal was wrapped with elastic wrap which was secured with adhesive tape to prevent removal of the chamber. Approximately six hours after chamber application, the binding materials were removed. The test sites were wiped with gauze moistened with deionized water, followed by dry gauze, to remove test article residue. The test sites were graded for irritation at approximately 24 and 48 hours following chamber application using the Dermal Grading System. The induction procedure was repeated on study day 7 and on study day 14 so that a total of three induction exposures were made to the animals.

On the day prior to challenge dose administration, the hair was removed from the right side of the twenty test and ten challenge control animals. On the following day (day 28), 0.3 mL of 1% and 2.5% w/v HCA in acetone was placed on a 25 mm Hilltop chamber and applied to the clipped area of each animal=s back. Wrapping, unwrapping and rinsing procedures were the same as those utilized for the induction phase. The test sites were graded for irritation at approximately 24 and 48 hours following chamber removal.

Any unusual observations and/or mortality were recorded. Body weights were recorded for the test, challenge control and rechallenge control animals on the day prior to first induction (day -1) and for the test and challenge control animals on the day prior to challenge dosing. All sensitization study animals were euthanized by carbon dioxide inhalation following each animal's final scoring interval. Gross necropsy examinations were not required for these animals.

Note: The temperature and relative humidity of the animal room [64-75°F (18-24°C)] exceeded the preferred ranges [63-73°F (17-23°C) and 30-70%] during

this study. These occurrences were considered to have had no adverse effect on the outcome of this study.

4. RESULTS

Individual Data: Tables 1-2

Following challenge with 2.5% w/v HCA in acetone, dermal scores of 1 were noted in 8/20 test animals at the 24-hour scoring interval. At the 48-hour scoring interval, dermal scores of 1 were noted in 4/20 test animals. Dermal reactions in the remaining test and challenge control animals were limited to scores of 0 to ±. Group mean dermal scores were noted to be higher in the test animals as compared with the challenge control animals.

Following challenge with 1% w/v HCA in acetone, dermal scores of 1 were noted in 5/20 test animals at the 24-hour scoring interval. At the 48-hour scoring interval, dermal scores of 1 were noted in 2/20 test animals. Dermal reactions in the remaining test and challenge control animals were limited to scores of 0 to ±. Group mean dermal scores were noted to be higher in the test animals as compared with the challenge control animals.

5. CONCLUSION

The results of this α-Hexylcinnamaldehyde positive control study demonstrated that a valid test was performed and indicated that the test design would detect potential contact sensitizers. Based on the results of this study, α-Hexylcinnamaldehyde is considered to be a contact sensitizer in guinea pigs.

6. REFERENCE

1. E.V. Buehler, Occlusive Patch Method for Skin Sensitization in Guinea Pigs: The Buehler Method, Fd. Chem. Toxic., Vol. 32, No. 2, pp. 97-101, 1994.

SLI HISTORICAL CONTROL
STUDY NO.: 999.171

TABLE 1
A DERMAL SENSITIZATION STUDY IN GUINEA PIGS
INDIVIDUAL INDUCTION DATA
(α-HEXYLCINNAMALDEHYDE)

Group	Animal No./ Sex	Induction 1 Dermal Scores 5%[a] 24 Hr	Induction 1 Dermal Scores 5%[a] 48 Hr	Induction 2 Dermal Scores 5%[a] 24 Hr	Induction 2 Dermal Scores 5%[a] 48 Hr
Test	G5787/M	1 ED-1, BLA-1, DES	± BLA-1, DES,	2 ED-2, BLA-1, SL-1, DES	2 ED-2, BLA-1, DES
	G5788/M	1 ED-1, DES	± DES	2 ED-1, DES	2 ED-1, DES
	G5789/M	± ED-1, DES, IT	± DES	2 ED-1, BLA-1, DES	2 ED-1, BLA-1, DES
	G5790/M	2 ED-1, SL-4	1 ED-1, DES	M-3 ED-2, BLA-2, DES	M-3 ED-1, BLA-2, NEC-1 (BK), DES
	G5791/M	± ED-1, BLA-1, DES	± BLA-1, DES	2 ED-2, BLA-1, DES	2 ED-1, BLA-1, DES
	G5792/M	1 ED-1, BLA-1, DES	± BLA-1, DES	M-3 ED-2, NEC -2 (BK), BLA-1, DES	M-3 ED-1, BLA-1, ES-2, DES
	G5793/M	1 ED-1, BLA-1, DES	± ED-1, BLA-1, DES	M-3 ED-2, BLA-2, SL-1, DES	M-3 ED-1, BLA-2, DES
	G5794/M	1 ED-1, DES	± DES	2 ED-2, ES-1, DES	2 ED-1, ES-1, DES
	G5795/M	1 ED-1, BLA-1, DES	± ED-1, BLA-1, DES	2 ED-2, BLA-1, SL-3, DES	2 ED-1, BLA-1, DES
	G5796/M	2 ED-1, BLA-1, DES	1 BLA-1, DES	2 ED-2, BLA-1, DES	1 ED-1, BLA-1, DES
	G5894/F	± ED-1, DES, IT	± DES	2 ED-2, DES	1 ED-1, DES
	G5895/F	1 ED-1, DES, IT	± DES	2 ED-2, BLA-1, SL-1, DES	1 ED-1, BLA-1, DES
	G5896/F	± DES, IT	± DES	2 ED-2, BLA-1, ES-1, DES	M-3 ED-2, ES-2, DES
	G5897/F	1 ED-1, DES, IT	± DES	1 ED-1, DES, IT	± DES
	G5898/F	± DES, IT	± DES	± DES, IT	± DES
	G5899/F	± DES, IT	0 DES	2 ED-2, BLA-1, DES	2 ED-1, BLA-1, DES
	G5900/F	1 ED-1, BLA-1, DES	± ED-1, BLA-1, DES	2 ED-2, BLA-1, DES	2 ED-2, BLA-1, DES
	G5901/F	1 ED-1, DES, IT	± DES	2 ED-2, SL-4, DES, IT	2 ED-2, BLA-1, DES
	G5902/F	± DES	± DES	2 ED-2, SL-1, DES	2 ED-1, SL-1, DES
	G5903/F	0 IT	0	2 ED-2, DES	1 ED-1, DES

[a]The vehicle was ethanol.
Notes: See Appendix B for definition of codes. BK = black.

TABLE 1
A DERMAL SENSITIZATION STUDY IN GUINEA PIGS
INDIVIDUAL INDUCTION DATA
(α-HEXYLCINNAMALDEHYDE)

Group	Animal No./ Sex	Induction 3 Dermal Scores 5%[a] 24 Hr	48 Hr
Test	G5787/M	$2^{ED-2, DES}$	2^{ED-1}
	G5788/M	$2^{ED-2, BLA-1}$	$2^{ED-2, BLA-1}$
	G5789/M	2^{ED-2}	$2^{ED-1, SL-1}$
	G5790/M	$2^{ED-2, SL-4, DES}$	$2^{ED-1, SL-4}$
	G5791/M	$2^{ED-2, DES}$	2^{ED-1}
	G5792/M	$2^{ED-2, SL-1, DES}$	$2^{ED-1, SL-1}$
	G5793/M	$2^{ED-2, DES}$	$2^{ED-1, DES}$
	G5794/M	$2^{ED-2, SL-2, DES}$	$2^{ED-2, SL-2, DES}$
	G5795/M	$2^{ED-2, SL-2, DES}$	$2^{ED-1, BLA-1, SL-2}$
	G5796/M	$2^{ED-2, SL-2, DES}$	$2^{ED-1, BLA-1, SL-1}$
	G5894/F	$1^{ED-1, DES}$	1^{ED-1}
	G5895/F	$1^{ED-1, DES}$	1^{ED-1}
	G5896/F	$2^{ED-2, SL-1, DES, IT}$	$2^{ED-2, SL-1}$
	G5897/F	$1^{ED-1, DES}$	1^{ED-1}
	G5898/F	$\pm^{ED-1, DES}$	$\pm^{ED-1}$
	G5899/F	$2^{ED-2, SL-4, DES}$	$2^{ED-2, SL-4}$
	G5900/F	$2^{ED-2, SL-2, DES}$	$2^{ED-1, SL-2}$
	G5901/F	$2^{ED-2, SL-4, DES}$	$2^{ED-1, SL-4}$
	G5902/F	$2^{ED-2, SL-4, DES}$	$2^{ED-1, SL-4}$
	G5903/F	$1^{ED-1, BLA-1, DES}$	$1^{ED-1, BLA-1, SL-1}$

[a]The vehicle was ethanol.
Note: See Appendix B for definition of codes.

SLI HISTORICAL CONTROL
STUDY NO.: 999.171

TABLE 2
A DERMAL SENSITIZATION STUDY IN GUINEA PIGS
INDIVIDUAL CHALLENGE DATA
(α-HEXYLCINNAMALDEHYDE)

Group	Animal No./ Sex	Dermal Scores 2.5%[a] 24 Hr	2.5%[a] 48 Hr	1%[a] 24 Hr	1%[a] 48 Hr
Test	G5787/M	1[IT]	±	1[IT]	±[IT]
	G5788/M	±	±	±	±
	G5789/M	±	0	±	0
	G5790/M	1[ED-1]	1	1	1
	G5791/M	1	1	±[IT]	±[IT]
	G5792/M	±	0	±	0
	G5793/M	±	±	±	±
	G5794/M	1	1	1	±
	G5795/M	1	±	±	0
	G5796/M	±[IT]	±	±	±
	G5894/F	±	0	±	0
	G5895/F	1	±	1[IT]	±
	G5896/F	1	±	±	±
	G5897/F	±	0	±[IT]	0[IT]
	G5898/F	±	±	0	0
	G5899/F	1	1	1[IT]	1[IT]
	G5900/F	±[IT]	0	±[IT]	0
	G5901/F	±	0	±	0
	G5902/F	±	±	±	0
	G5903/F	±	±	±	0
	Mean	0.7	0.5	0.6	0.3

[a]The vehicle was acetone.
Notes: For the purpose of calculation, ± = 0.5. See Appendix B for definition of codes.

SLI HISTORICAL CONTROL
STUDY NO.: 999.171

TABLE 2
A DERMAL SENSITIZATION STUDY IN GUINEA PIGS
INDIVIDUAL CHALLENGE DATA
(α-HEXYLCINNAMALDEHYDE)

Group	Animal No./ Sex	Dermal Scores 2.5%[a] 24 Hr	2.5%[a] 48 Hr	1%[a] 24 Hr	1%[a] 48 Hr
Challenge	G5797/M	0	0	0^{IT}	0
	G5798/M	0	0	0^{IT}	0
	G5799/M	0	0	$\pm^{IT}$	$\pm^{IT}$
	G5800/M	0	0	0	0
	G5801/M	0^{IT}	0	0^{IT}	0^{IT}
	G5904/F	0^{IT}	0^{IT}	0^{IT}	0
	G5905/F	0^{IT}	0	0^{IT}	0
	G5906/F	0^{IT}	0	0^{IT}	0
	G5907/F	0	0	0	0
	G5908/F	0^{IT}	0^{IT}	0^{IT}	0
	Mean	0.0	0.0	0.1	0.1

[a]The vehicle was acetone.
Notes: For the purpose of calculation, ± = 0.5. See Appendix B for definition of codes.

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SLI Study No. 3596.14

ATTACHMENT 1

Certificate of Analysis
(Provided by the Manufacturer)

(41)

TCI AMERICA

CERTIFICATE OF ANALYSIS

H0685
Lot# GF01
CAS# 101-86-0

ALPHA-N-HEXYLCINNAMALDEHYDE

Appearance:	Yellow clear liquid
SG(20/20):	0.96
n(20/D):	1.55
Assay(GC):	92%

9211N. Harborgate St. Portland, OR 97203 Phone: (503)283-1681 (800)423-8616 Fax: (503)283-1987

SLI Study No. 3596.14

APPENDIX F

SLI Personnel Responsibilities

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SLI Study No. 3596.14

SLI PERSONNEL RESPONSIBILITIES

Kimberly L. Bonnette, M.S., LATG	Study Director/Director, Acute Toxicology
Dawn D. Rodabaugh, B.S.	Alternate Contact/Associate Toxicologist
Robert C. Springborn, P h.D.	Chairman, President and CEO
Malcolm Blair, Ph.D.	Senior Vice President, Managing Director Emeritus
Joseph C. Siglin, Ph.D., DABT	Vice President, Managing Director
Jason W. Smedley, B.S.	Assistant Toxicologist
Pamela S. Smith, ALAT	Primary Technician/Supervisor of Acute Toxicology
Delores P. Knippen	Supervisor of Pharmacy
Steven H. Magness, B.S., LATG	Senior Supervisor of Gross and Fetal Pathology
Anita M. Bosau, RQAP-GLP	Senior Director, Compliance Assurance
Deanna M. Talerico, RQAP-GLP	Senior Supervisor of Quality Assurance
J. Dale Thurman, D.V.M., M.S., DACVP	Senior Director, Pathology
Kathy M. Gasser	Supervisor of Archives

A PRIMARY EYE IRRITATION STUDY IN RABBITS WITH SPRAY--BRAVO

FINAL REPORT

OPPTS Guideline

870.2400

Author

Kimberly L. Bonnette, M.S., LATG

Study Completed on

September 18, 2002

Performing Laboratory

Springborn Laboratories, Inc. (SLI)
Ohio Research Center
640 North Elizabeth Street
Spencerville, Ohio 45887

SLI Study No.

3596.12

Submitted to

INL/A
U.S. Department of State
2201 C St. NW SA-4
Washington, DC 20520

SLI Study No. 3596.12 (2)

1. STATEMENT OF *NO* DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B), or (C).

Company: _____

Company Agent: _____ Date: _____

_____ _____

Title Signature

SEP 0 5 2002

2. COMPLIANCE STATEMENT

This study was conducted in compliance with the Good Laboratory Practice Standards as described by the EPA (40 CFR Parts 160 and 792).

Kimberly L. Bonnette, M.S., LATG
Study Director/Author
Springborn Laboratories, Inc.

Date 9/18/02

Rogers Woolfolk
Senior Aviation Advisor
Sponsor/Submitter
INL/A
U.S. Department of State

Date 30 Aug 02

SLI Study No. 3596.12 (4)

3. QUALITY ASSURANCE STATEMENT

This study was inspected by the Quality Assurance Unit and reports were submitted to management and the Study Director in accordance with SLI's Standard Operating Procedures as follows:

Phase	Date
Protocol Review	04/25/02
Dose Preparation	06/28/02
Data Audit	08/26/02
Draft Report Review	08/26/02
Protocol Amendment Review	08/26/02
Final Report Review	09/18/02
Reports to Study Director and Management	08/26/02, 9/18/02

The final report has been reviewed to assure that it accurately describes the materials and methods, and the reported results accurately reflect the raw data.

Jennifer D. McGue Date 9/18/02
Jennifer D. McGue
Quality Assurance Auditor

Anita M Bosau Date 9/18/02
Anita M. Bosau, RQAP-GLP
Senior Director, Compliance Assurance

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SLI Study No. 3596.12 (7)

6. SUMMARY

The potential irritant and/or corrosive effects of Spray--Bravo were evaluated on the eyes of New Zealand White rabbits. Each of three rabbits received a 0.1 mL dose of the test article in the conjunctival sac of the right eye. The contralateral eye of each animal remained untreated and served as a control. Test and control eyes were examined for signs of irritation for up to 7 days following dosing.

Exposure to the test article produced iritis in 2/3 test eyes at the 1-hour scoring interval which resolved completely in all test eyes by the 24-hour scoring interval. Conjunctivitis (redness, swelling and discharge) was noted in 3/3 test eyes at the 1-hour scoring interval. The conjunctival irritation resolved completely in all test eyes by study day 7.

Based on the Kay and Calandra Evaluation, Spray--Bravo is considered to be a mild irritant to the ocular tissue of the rabbit.

SLI Study No. 3596.12 (8)

7. INTRODUCTION

This study was performed to assess the irritant and/or corrosive effects of Spray--Bravo in New Zealand White rabbits when administered by a single ocular dose. This study was intended to provide information on the potential health hazards of the test article with respect to ocular exposure. Data from this study may serve as a basis for classification and/or labeling of the test article. This study was conducted in accordance with the US EPA, Health Effects Test Guidelines, OPPTS 870.2400, Acute Eye Irritation, August 1998. This study was performed at Springborn Laboratories, Inc., 553 North Broadway, Spencerville, Ohio. The protocol was signed by the Study Director on April 26, 2002 (GLP initiation date). The in-life phase of the study was initiated with test article administration on June 28, 2002 (day 0), and concluded with final scoring on July 5, 2002.

8. MATERIALS AND METHODS

8.1. Test Article

The test article was received from the Sponsor and identified as follows:

Sponsor's ID	Assigned SLI ID	Physical Description	Receipt Date	Expiration Date
Spray—Bravo[a]	S02.002.3596	Cloudy pale amber liquid	05/31/02	None Provided
Ingredients[b]				
Herbicide: Roundup-SL Lot No.: 4010/4212				None Provided
Surfactant: Cosmo Flux-411F Lot No.: Unknown				None Provided

[a]Sample pooled at SLI from five different mixes of Spray--Bravo (top/middle/bottom).
[b]Ingredients used in the five Spray--Bravo mixes that were prepared by the Sponsor.

The test article was stored at room temperature. The Sponsor was responsible for any necessary evaluations related to identity, strength, purity, composition, stability and method of synthesis of the test material according to 40 CFR 160.105 and 40 CFR 792.105. Springborn Laboratories, Inc., analyzed the test article for the glyphosate (a.e.) which is presented in SLI Study No. 3596.8.

8.2. Retention Sample

An approximate 1 mL retention sample of each test article mixture sample (top/middle/bottom, maintained separately for a total of fifteen 1 mL samples) was taken and stored at SLI at room temperature. In addition, a 10 mL retention sample of the pooled test article samples (from the 5 test article mixtures) was collected and stored at SLI at room temperature. These samples serve as the retention samples for all studies conducted with this material.

8.3. Test Article Disposition

The remaining test article was returned to the Sponsor at the completion of all studies with the test article.

8.4. Method of Test Article Preparation

The test article was administered as received from the Sponsor and dispensed fresh on the day of dosing.

8.5. Animals and Animal Husbandry

8.5.1. Description, Identification and Housing

Adult, New Zealand White rabbits were received from Myrtle's Rabbitry, Thompson Station, TN. Upon receipt, plastic ear tags displaying unique identification numbers were used to individually identify the animals. Cage cards displaying at least the study number, animal number and sex were affixed to each cage. The animals were housed individually in suspended stainless steel cages. All housing and care were based on the standards recommended by the Guide for the Care and Use of Laboratory Animals [1].

8.5.2. Environment

The animal room temperature and relative humidity ranges were 69-72°F (21-22°C) and 46-61%, respectively. Environmental control equipment was monitored and adjusted as necessary to minimize fluctuations in the animal room environment. Light timers were set to maintain a 12-hour light/12-hour dark cycle and room ventilation was set to produce 10-15 air changes/hour. The animal room temperature and relative humidity were recorded a minimum of once daily.

SLI Study No. 3596.12 (10)

8.5.3. Food

PMI Certified Rabbit Chow #5322 (Purina Mills, Inc.) was provided ad libitum to the animals throughout the study. The lot number and expiration date of each batch of diet used during the study were recorded. The feed was analyzed and certified by the supplier for nutritional components and environmental contaminants. Dietary limitations for various environmental contaminants, including heavy metals, pesticides, polychlorinated biphenyls and total aflatoxin are set by the manufacturer. Within these limits, contaminants which may have been present were not expected to compromise the purpose of this study. Results of the dietary analyses (Certificates of Analysis) are provided by the manufacturer for each lot of diet. These are maintained by SLI.

8.5.4. Water

Municipal tap water treated by reverse osmosis was available ad libitum throughout the study. The purified water was supplied by an automatic watering system. Monitoring of the drinking water for contaminants is conducted by SLI and the records are available for inspection. Within generally accepted limits, contaminants which may have been present were not expected to compromise the purpose of this study. The water meets the standards specified under the EPA National Drinking Water Regulations (40 CFR Part 141).

8.5.5. Acclimation

Upon receipt, the animals were removed randomly from the shipping cartons, examined by qualified personnel, identified with plastic ear tags and then acclimated to the laboratory conditions for a minimum of five days. The animals were observed daily for overt physical or behavioral abnormalities, general health/moribundity and mortality.

8.5.6. Animal Selection

The animals chosen for study use were arbitrarily selected from healthy stock animals to avoid potential bias. All animals received a detailed pretest observation prior to dosing. Only healthy animals were chosen for study use. The female was nulliparous and nonpregnant. The male animals were approximately 16 weeks of age and weighed 3.4-3.5 kg prior to dosing. The female animal was approximately 14 weeks of age and weighed 3.3 kg prior to dosing.

SLI Study No. 3596.12 (11)

9. EXPERIMENTAL PROCEDURES

9.1. Preliminary Examination

On day 0 prior to dosing, both eyes of each animal provisionally selected for test use were examined macroscopically for ocular irritation with the aid of an auxiliary light source. In addition, the corneal surface was examined using fluorescein sodium dye. One drop of a fluorescein/physiological saline mixture was gently dropped onto the superior sclera of each eye. Following an approximate 15 second exposure, the eyes were thoroughly rinsed with physiological saline. The corneal surface was then examined for dye retention under a long-wave UV light source. Animals exhibiting ocular irritation, preexisting corneal injury or fluorescein dye retention were not used on study. All animals found to be acceptable for test use were returned to their cages until dosing.

9.2. Dosing

A minimum of one hour after preliminary ocular examination, the test article was instilled as follows:

Group	Concentration (%)	Amount Instilled	No. of Animals	
			Male	Female
No Rinse	100[a]	0.1 mL	2	1

[a]Pooled test article.

The test article was instilled into the conjunctival sac of the right eye of each animal after gently pulling the lower lid away from the eye. Following instillation, the eyelids were gently held together for approximately one second in order to limit test article loss and the animal was returned to its cage. The contralateral eye remained untreated to serve as a control.

9.3. Ocular Observations

The eyes were macroscopically examined with the aid of an auxiliary light source for signs of irritation at 1, 24, 48 and 72 hours and up to 7 days after dosing according to the Ocular Grading System presented in Appendix A which is based on Draize [2]. Following macroscopic observations at the 24-hour scoring interval, the fluorescein examination procedure was repeated on all test and control eyes and any residual test article was gently rinsed from the eye at this time (if possible) using physiological saline. If any fluorescein findings were

noted at 24 hours, a fluorescein exam was conducted on the affected eyes at each subsequent interval until a negative response was obtained and/or until all corneal opacity had cleared, or as directed by the Study Director.

9.4. Clinical Observations

Any unusual observations and/or mortality were recorded. General health/mortality checks were performed twice daily (in the morning and in the afternoon).

9.5. Body Weights

Individual body weights were obtained for each animal prior to dosing on day 0.

9.6. Scheduled Euthanasia

Each animal was euthanized by an intravenous injection of sodium pentobarbital following its final observation interval. Gross necropsy examinations were not required for these animals.

9.7. Protocol Deviations

No protocol deviations occurred during this study.

10. ANALYSIS OF DATA

For each group, the ocular irritation score for each parameter (i.e., corneal opacity x area, iritis and conjunctival redness + swelling + discharge) was multiplied by the appropriate factor (i.e., corneal injury x 5, iritis x 5, conjunctivitis x 2) and the totals added for each animal/interval. The group mean irritation score was then calculated for each scoring interval based on the number of animals initially dosed in each group. The calculated group mean ocular irritation scores for each interval were used to classify the test article according to the Ocular Evaluation Criteria [3] presented in Appendix B.

11. MAINTENANCE OF RAW DATA AND RECORDS

All original raw data, the final report and magnetically encoded records were transferred to the SLI archives for a period of 10 years. The Sponsor will be contacted prior to final disposition of these items.

12. RESULTS

12.1. Ocular/Clincial Observations

Individual Data: Table 1
Individual Clinical Observations: Appendix C

Exposure to the test article produced iritis in 2/3 test eyes at the 1-hour scoring interval which resolved completely in all test eyes by the 24-hour scoring interval. Conjunctivitis (redness, swelling and discharge) was noted in 3/3 test eyes at the 1-hour scoring interval. The conjunctival irritation resolved completely in all test eyes by study day 7.

A mechanical abrasion was observed in 1/3 control eyes during the fluorescein examination, but was not considered to be significant since it was not observed macroscopically and was transient in nature. No corneal opacity, iritis or conjunctivitis was observed in the control eyes.

Soft stools was observed in one animal on study day 1 only and was therefore not considered to be significant.

13. CONCLUSION

Based on the Kay and Calandra Evaluation, Spray--Bravo is considered to be a mild irritant to the ocular tissue of the rabbit.

Kimberly L. Bonnette, M.S., LATG
Study Director

Date 9/18/02

14. REPORT REVIEW

Dawn D. Rodabaugh, B.S.
Associate Toxicologist

Date 9/18/02

15. REFERENCES

1. Guide for the Care and Use of Laboratory Animals, DHHS Publication No. (NIH) 96-03, 1996.

2. Draize, J.H., Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics, The Association of Food and Drug Officials of the United States, 49-51, 1959.

3. Kay, J.H. and Calandra, J.C., “Interpretation of Eye Irritation Tests”, Journal of the Society of Cosmetic Chemists, 13, 281-289, 1962.

TABLE 1

SLI STUDY NO.: 3596.12 A PRIMARY EYE IRRITATION STUDY IN RABBITS

CLIENT: INL/A, US DEPARTMENT OF STATE INDIVIDUAL OCULAR IRRITATION SCORES

(SPRAY—BRAVO)

(NO RINSE GROUP)

Animal No./Sex Body Weight (kg)	Scoring Interval	Cornea			Iris		Conjunctivae				Total	Test Eye*		Control Eye*	
		O	A	OxAx5	I	Ix5	R	S	D	(R+S+D)2		Fluorescein Examination	Secondary Ocular Findings	Fluorescein Examination	Secondary Ocular Findings
R2257/F	1 Hour	0	0	0	1	5	2	2	1	10	15				
3.327	24 Hours	0	0	0	0	0	2	2	1	10	10	[-]		MI	
	48 Hours	0	0	0	0	0	1	1	0	4	4			[-]	
	72 Hours	0	0	0	0	0	1	0	0	2	2				
	7 Days	0	0	0	0	0	0	0	0	0	0				
R2167/M	1 Hour	0	0	0	0	0	1	2	1	8	8				
3.436	24 Hours	0	0	0	0	0	2	2	1	10	10	[-]		[-]	
	48 Hours	0	0	0	0	0	2	1	1	8	8				
	72 Hours	0	0	0	0	0	1	0	0	2	2				
	7 Days	0	0	0	0	0	0	0	0	0	0				
R2163/M	1 Hour	0	0	0	1	5	2	2	2	12	17				
3.451	24 Hours	0	0	0	0	0	2	2	2	12	12	[-]		[-]	
	48 Hours	0	0	0	0	0	2	1	1	8	8				
	72 Hours	0	0	0	0	0	1	1	0	4	4				
	7 Days	0	0	0	0	0	0	0	0	0	0				

*See Appendix A for definition of codes.

TABLE 1

SLI STUDY NO.: 3596.12 A PRIMARY EYE IRRITATION STUDY IN RABBITS

CLIENT: INL/A, US DEPARTMENT OF STATE INDIVIDUAL OCULAR IRRITATION SCORES

(SPRAY—BRAVO)

(NO RINSE GROUP)

Mean Ocular Scores		
1 Hour	-	13.33
24 Hours	-	10.67
48 Hours	-	6.67
72 Hours	-	2.67
7 Days	-	0.00

Mild Irritant

APPENDIX A

Ocular Grading System

SLI Study No. 3596.12 (18)

OCULAR GRADING SYSTEM

(O) CORNEAL OPACITY—DEGREE OF DENSITY (AREA MOST DENSE TAKEN FOR READING)	
OBSERVATION	CODE
No ulceration or opacity	0
Scattered or diffuse areas of opacity (other than slight dulling of normal luster), details of iris clearly visible	1*
Easily discernible translucent area, details of iris slightly obscured	2*
Nacreous (opalescent) area, no details of iris visible, size of pupil barely discernible	3*
Opaque cornea, iris not discernible through opacity	4*

(A) AREA OF CORNEA INVOLVED (TOTAL AREA EXHIBITING ANY OPACITY, REGARDLESS OF DEGREE)	
OBSERVATION	CODE
No ulceration or opacity	0
One quarter (or less) but not zero	1
Greater than one quarter, but less than half	2
Greater than half, but less than three quarters	3
Greater than three quarters, up to whole area	4

Cornea Score = O x A x 5 Total Maximum = 80

(I) IRITIS	
OBSERVATION	CODE
Normal	0
Markedly deepened rugae (folds above normal), congestion, swelling, moderate circumcorneal hyperemia or injection, any or all of these or combination of any thereof, iris is still reacting to light (sluggish reaction is positive)	1*
No reaction to light, hemorrhage, gross destruction (any or all of these)	2*

Iris Score = I x 5 Total Maximum = 10

*Starred figures indicate positive effect.

SLI Study No. 3596.12 (19)

OCULAR GRADING SYSTEM

(R) CONJUNCTIVAL REDNESS (REFERS TO PALPEBRAL AND BULBAR CONJUNCTIVAE EXCLUDING CORNEA AND IRIS)	
OBSERVATION	CODE
Blood vessels normal	0
Some blood vessels definitely hyperemic (injected) above normal (slight erythema)	1
Diffuse, crimson color, individual vessels not easily discernible (moderate erythema)	2*
Diffuse beefy red (marked erythema)	3*

(S) CONJUNCTIVAL SWELLING (LIDS AND/OR NICTITATING MEMBRANE)	
OBSERVATION	CODE
No swelling	0
Any swelling above normal (includes nictitating membrane, slightly swollen)	1
Obvious swelling with partial eversion of lids	2*
Swelling with lids about half closed	3*
Swelling with lids more than half closed	4*

(D) CONJUNCTIVAL DISCHARGE	
OBSERVATION	CODE
No discharge	0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)	1
Discharge with moistening of the lids and hairs just adjacent to lids	2
Discharge with moistening of the lids and hairs and considerable area around the eye	3

Conjunctival Score = (R + S + D) x 2 Total Maximum = 20

*Starred figures indicate positive effect.

SLI Study No. 3596.12 (20)

OCULAR GRADING SYSTEM

CORNEAL NEOVASCULARIZATION		
OBSERVATION	CODE	DEFINITION
Neovascularization – Very Slight	VAS-1	Total area of vascularized corneal tissue is < 10% of corneal surface
Neovascularization – Mild	VAS-2	Total area of vascularized corneal tissue is > 10% but < 25% of corneal surface
Neovascularization – Moderate	VAS-3	Total area of vascularized corneal tissue is > 25% but < 50% of corneal surface
Neovascularization – Severe	VAS-4	Total area of vascularized corneal tissue is > 50% of corneal surface

SECONDARY OCULAR FINDINGS		
OBSERVATION	CODE	DEFINITION
Sloughing of the corneal epithelium	SCE	Corneal epithelial tissue is observed to be peeling off the corneal surface.
Corneal bulging	CB	The entire corneal surface appears to be protruding outward further than normal.
Slight dulling of normal luster of the cornea	SDL	The normal shiny surface of the cornea has a slightly dulled appearance.
Raised area on the corneal surface	RAC	A defined area on the corneal surface that is raised above the rest of the cornea. This area is generally associated with neovascularization and has an off-white to yellow color.
Corneal edema	CE	The cornea has a swollen appearance.
Test article present in eye	TAE	Apparent residual test article is observed on the eye or in the conjunctival sac/inner canthus.
Observation confirmed by slit lamp	OCS	A slit lamp examination was performed to confirm the initial observation.
Corneal mineralization	CM	Small white or off-white crystals that are observed in the corneal tissue.

SLI Study No. 3596.12 (21)

OCULAR GRADING SYSTEM

FLUORESCEIN EXAMINATION OF CORNEA	
OBSERVATION	CODE
Fluorescein Dye Retention Fluorescein dye retention associated with the area of corneal opacity Fluorescein dye retention is not associated with any other finding	 FAO FNF
Negative Results No fluorescein retention is observed	 (-)
Secondary Ocular Findings Superficial mechanical abrasion to the cornea observed during the fluorescein examination period Fine stippling on the cornea observed during the fluorescein examination procedure	 MI ST

POST-DOSE CLINICAL OBSERVATIONS	
OBSERVATION	CODE
Animal vocalized following dosing	VOC
Animal excessively pawed test eye following dosing	PAW
Animal exhibited excessive hyperactivity following dosing	HYP
Animal exhibited excessive head tilt following dosing	HT
Animal exhibited excessive squinting of test eye following dosing	SQ

APPENDIX B

Ocular Evaluation Criteria
(Kay and Calandra)

SLI Study No. 3596.12 (23)

OCULAR EVALUATION CRITERIA

Maximum Mean Score (Days 0-3)	Maximum Mean Score	Persistence of Individual Scores	Descriptive Rating and Class	
0.00 – 0.49	24 hours = 0		Non-Irritating	1
	24 hours > 0		Practically Non-irritating	2
0.50 – 2.49	24 hours = 0		Non-Irritating	1
	24 hours > 0		Practically Non-irritating	2
2.50 – 14.99	48 hours = 0		Slight Irritant	3
	48 hours > 0		Mild Irritant	4
15.00 – 24.99	72 hours = 0		Mild Irritant	4
	72 hours > 0		Moderate Irritant	5
25.00 – 49.99	7 day $\leq$ 20	> half of day 7 scores $\leq$ 10	Moderate Irritant	5
		> half of day 7 scores > 10, but no score > 20	Moderate Irritant	5
		> half of day 7 scores > 10, and any score > 20	Severe Irritant	6
	7 day > 20		Severe Irritant	6
50.00 – 79.99	7 day $\leq$ 40	> half of day 7 scores $\leq$ 30	Severe Irritant	6
		> half of day 7 scores > 30, but no score > 60	Severe Irritant	6
		> half of day 7 scores > 30, and any score > 60	Very Severe Irritant	7
	7 day > 40		Very Severe Irritant	7
80.00 – 99.99	7 day $\leq$ 80	> half of day 7 scores $\leq$ 60	Very Severe Irritant	7
		> half of day 7 scores > 60, but no score > 100	Very Severe Irritant	7
		> half of day 7 scores > 60, and any score > 100	Extremely Severe Irritant	8
	7 day > 80		Extremely Severe Irritant	8
100.00 – 110.00	7 day $\leq$ 80		Very Severe Irritant	7
	7 day > 80		Extremely Severe Irritant	8

APPENDIX C

Individual Clinical Observations

(25)

A PRIMARY EYE IRRITATION STUDY IN RABBITS
INDIVIDUAL CLINICAL OBSERVATIONS
(POSITIVE FINDINGS)

SLI STUDY NO.: 3596.12
CLIENT: INL/A, US DEPARTMENT OF STATE

Animal No./Sex	Clinical Observations
R2257/F	Soft stools: Day 1

APPENDIX D

SLI Personnel Responsibilities

SLI Study No. 3596.12 (27)

SLI PERSONNEL RESPONSIBILITIES

Kimberly L. Bonnette, M.S., LATG	Study Director/Director, Acute Toxicology
Dawn D. Rodabaugh, B.S.	Alternate Contact/Associate Toxicologist
Robert C. Springborn, Ph.D.	Chairman, President and CEO
Malcolm Blair, Ph.D.	Senior Vice President, Managing Director Emeritus
Joseph C. Siglin, Ph.D., DABT	Vice President, Managing Director
Christopher W. Wilson, B.S.	Associate Toxicologist
Pamela S. Smith, ALAT	Supervisor of Acute Toxicology
Kathy A. Pugh, ALAT	Primary Technician/Team Leader
Delores P. Knippen	Supervisor of Pharmacy
Steven H. Magness, B.S., LATG	Senior Supervisor of Gross and Fetal Pathology
Anita M. Bosau, RQAP-GLP	Senior Director, Compliance Assurance
Deanna M. Talerico, RQAP-GLP	Senior Supervisor of Quality Assurance
J. Dale Thurman, D.V.M., M.S., DACVP	Senior Director, Pathology
Kathy M. Gasser	Supervisor of Archives

AN ACUTE ORAL TOXICITY STUDY IN RATS WITH SPRAY--BRAVO

FINAL REPORT

OPPTS Guideline

870.1100

Author

Kimberly L. Bonnette, M.S., LATG

Study Completed on

October 2, 2002

Performing Laboratory

Springborn Laboratories, Inc. (SLI)
Ohio Research Center
640 North Elizabeth Street
Spencerville, Ohio 45887

SLI Study No.

3596.9

Submitted to

INL/A
U.S. Department of State
2201 C St. NW SA-4
Washington, DC 20520

SLI Study No. 3596.9 (2)

1. STATEMENT OF *NO* DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B), or (C).

Company: _____

Company Agent: _____ Date _____

_____ _____

Title Signature

SLI Study No. 3596.9 (3)

SEP 3 0 2002

2. COMPLIANCE STATEMENT

This study was conducted in compliance with the Good Laboratory Practice Standards as described by the EPA (40 CFR Parts 160 and 792).

Kimberly L. Bonnette, M.S., LATG
Study Director/Author
Springborn Laboratories, Inc.

Date 10/2/02

Rogers Woolfolk
Senior Aviation Advisor
Sponsor/Submitter
INL/A
U.S. Department of State

Date 15 Sep 02

3. QUALITY ASSURANCE STATEMENT

This study was inspected by the Quality Assurance Unit and reports were submitted to management and the Study Director in accordance with SLI's Standard Operating Procedures as follows:

Phase	Date
Protocol Review	04/25/02
Body Weights	06/28/02
Protocol Amendment Review	08/23/02
Data Audit	08/26/02
Draft Report Review	08/26/02
Final Report Review	10/02/02
Reports to Study Director and Management	08/26/02, 10/02/02

The final report has been reviewed to assure that it accurately describes the materials and methods, and the reported results accurately reflect the raw data.

Jennifer D. McGue
Jennifer D. McGue
Quality Assurance Auditor

Date 10/2/02

Anita M. Bosau
Anita M. Bosau, RQAP-GLP
Senior Director, Compliance Assurance

Date 10/2/02

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SLI Study No. 3596.9 (7)

6. SUMMARY

The single-dose oral toxicity of Spray--Bravo was evaluated in Sprague Dawley rats. A limit test was performed in which one group of five male and five female rats received a single oral administration of the test article at a dose of 5000 mg/kg body weight. Following dosing, the limit test rats were observed daily and weighed weekly. A gross necropsy examination was performed on all limit test animals at the time of scheduled euthanasia (day 14).

No mortality occurred during the limit test. Clinical abnormalities observed during the study included transient incidences of congested breathing, few feces and feces small in size. Body weight gain was noted for all animals during the test period. No gross internal findings were observed at necropsy on study day 14.

Under the conditions of this test, the acute oral LD50 of Spray--Bravo was estimated to be greater than 5000 mg/kg in the rat.

7. INTRODUCTION

This study was performed to assess the short-term toxicity of Spray--Bravo in Sprague Dawley rats when administered by gavage as a single oral dose. This study was intended to provide information on the potential health hazards of the test article with respect to oral exposure. Data from this study may serve as a basis for classification and/or labeling of the test article. This study was performed in accordance with the US EPA, Health Effects Test Guidelines, OPPTS 870.1100, Acute Oral Toxicity, August 1998. This study was performed at Springborn Laboratories, Inc., 553 North Broadway, Spencerville, Ohio. The protocol was signed by the Study Director on April 26, 2002 (GLP initiation date). The in-life phase of the study was initiated with test article administration on June 28, 2002 (day 0) and concluded with necropsy on July 12, 2002.

8. MATERIALS AND METHODS

8.1. Test Article

The test article was received from the Sponsor and identified as follows:

Sponsor's ID	Assigned SLI ID	Physical Description	Receipt Date	Expiration Date
Spray—Bravo[a]	S02.002.3596	Cloudy pale amber liquid	05/31/02	None provided
Ingredients:[b]				
Herbicide: Roundup SL				None provided
Lot Nos.: 4010/4212				
4397/4272				
4333/4340				None provided
4379/4076				
4397/4333				
Surfactant: Cosmo Flux-411F				
Lot No.: Unknown				

[a]Sample pooled at SLI from five different mixes of Spray--Bravo (top/middle/bottom).
[b]Ingredients used in the five Spray--Bravo mixes that were prepared by the Sponsor.

The test article was stored at room temperature. The Sponsor was responsible for any necessary evaluations related to identity, strength, purity, composition, stability and method of synthesis of the test material according to 40 CFR 160.105 and 40 CFR 792.105. Springborn Laboratories, Inc., analyzed the test article for the glyphosate (a.e.) which is presented in SLI Study No. 3596.8.

8.2. Retention Sample

An approximate 1 mL retention sample of each test article mixture sample (top/middle/bottom, maintained separately for a total of fifteen 1 mL samples) was taken and stored at SLI at room temperature. In addition, a 10 mL retention sample of the pooled test article samples (from the 5 test article mixtures) was collected and stored at SLI at room temperature. These samples serve as the retention samples for all studies conducted with this material.

8.3. Test Article Disposition

The remaining test article was returned to the Sponsor following completion of all studies with the test article.

8.4. Method of Test Article Preparation

The test article was administered as received from the Sponsor and dispensed fresh on the day of dosing. The density of the test article was determined to be 1.08 g/mL.

8.5. Animals and Animal Husbandry

8.5.1. Description, Identification and Housing

Young adult, Hsd: Sprague Dawley® SD® rats were received from Harlan Sprague Dawley, Inc., Indianapolis, IN. Upon receipt, metal ear tags displaying unique identification numbers were used to individually identify the animals. Cage cards displaying at least the study number, animal number and sex were affixed to each cage. The animals were housed individually in suspended stainless steel cages. All housing and care were based on the standards recommended by the Guide for the Care and Use of Laboratory Animals [1].

8.5.2. Environment

The animal room temperature and relative humidity ranges were 69-75°F (21-24°C) and 37-58%, respectively. Environmental control equipment was monitored and adjusted as necessary to minimize fluctuations in the animal room environment. Light timers were set to maintain a 12-hour light/12-hour dark cycle and room ventilation was set to produce 10-15 air changes/hour. The animal room temperature and relative humidity were recorded a minimum of once daily.

8.5.3. Food

PMI Certified Rodent Chow #5002 (Purina Mills, Inc.) was provided ad libitum to the animals throughout the study (except during fasting). The lot number and expiration date of each batch of diet used during the study were recorded. The

feed was analyzed and certified by the supplier for nutritional components and environmental contaminants. Dietary limitations for various environmental contaminants, including heavy metals, pesticides, polychlorinated biphenyls and total aflatoxin are set by the manufacturer. Within these limits, contaminants which may have been present were not expected to compromise the purpose of this study. Results of the dietary analyses (Certificates of Analysis) are provided by the manufacturer for each lot of diet. These are maintained by SLI.

8.5.4. Water

Municipal tap water treated by reverse osmosis was available ad libitum throughout the study. The purified water was supplied by an automatic watering system. Monitoring of the drinking water for contaminants is conducted by SLI and the records are available for inspection. Within generally accepted limits, contaminants which may have been present were not expected to compromise the purpose of this study. The water meets the standards specified under the EPA National Drinking Water Regulations (40 CFR Part 141).

8.5.5. Acclimation

Upon receipt, the animals were removed randomly from the shipping cartons, examined by qualified personnel, identified with metal ear tags and then acclimated to the laboratory conditions for a minimum of five days. The animals were observed daily for overt physical or behavioral abnormalities, general health/moribundity and mortality.

8.5.6. Animal Selection

The animals chosen for study use were randomly selected from healthy stock animals using a computerized (Alpha DS-10 AcuTox) random numbers table to avoid potential bias. All animals received a detailed pretest observation prior to dosing. Only healthy animals were chosen for study use. Females were nulliparous and nonpregnant. The male animals were approximately 8 weeks of age and weighed 239-263 g prior to fasting. The female animals were approximately 8 weeks of age and weighed 172-202 g prior to fasting.

9. EXPERIMENTAL PROCEDURES

9.1. Dosing

On day -1, the animals chosen for the limit test were weighed and fasted overnight. On day 0, the test article was administered orally as a single dose using a ball tipped stainless steel gavage needle attached to a syringe at the following level:

SLI Study No. 3596.9 (11)

Dose Level (mg/kg)	Dose Volume (mL/kg)	Concentration (%)	No. of Animals	
			Male	Female
5000	4.63[a]	100[b]	5	5

[a]Adusted based on a density of 1.08 g/mL
[b]Pooled test article.

Individual doses were calculated based on the animal's fasted (day 0) body weight. Animals were returned to ad libitum feeding after dosing.

9.2. Clinical Observations

The animals were observed for clinical abnormalities a minimum of two times on study day 0 (post-dose) and daily thereafter (days 1-14). A general health/mortality check was performed twice daily (in the morning and in the afternoon).

9.3. Body Weights

Individual body weights were obtained for the animals prior to fasting (day -1), prior to dosing on day 0 and on days 7 and 14.

9.4. Gross Necropsy

All animals were euthanized by carbon dioxide inhalation at study termination (day 14) and necropsied. Body cavities (cranial, thoracic, abdominal and pelvic) were opened and examined. No tissues were retained.

9.5. Protocol Deviations

No protocol deviations occurred during this study.

10. ANALYSIS OF DATA

Data from the study were analyzed and an LD50 value estimated as follows:

< 50% Mortality: LD50 was estimated as greater than the administered dose.
= 50% Mortality: LD50 was estimated as equal to the administered dose.
> 50% Mortality: LD50 was estimated as less than the administered dose.

Body weight means and standard deviations were calculated separately for males and females.

11. MAINTENANCE OF RAW DATA AND RECORDS

All original raw data, the final report and magnetically encoded records were transferred to the SLI archives for a period of 10 years. The Sponsor will be contacted prior to final disposition of these items.

12. RESULTS

12.1. Mortality

Individual Data: Table 1

No mortality occurred during the limit test.

12.2. Clinical Observations

Individual Data: Table 1

Clinical abnormalities observed during the study included transient incidences of congested breathing, few feces and feces small in size.

12.3. Body Weight Data

Individual Data: Table 2

Body weight gain was noted for all animals during the test period.

12.4. Gross Necropsy

Individual Data: Table 3

No gross internal findings were observed at necropsy on study day 14.

13. CONCLUSION

Under the conditions of this test, the acute oral LD50 of Spray--Bravo was estimated to be greater than 5000 mg/kg in the rat.

Kimberly L. Bonnette, M.S., LATG
Study Director

Date 10/2/02

14. REPORT REVIEW

Dawn D. Rodabaugh, B.S.
Associate Toxicologist

Date 10/2/02

15. REFERENCE

1. Guide for the Care and Use of Laboratory Animals, DHHS Publication No. (NIH) 96-03, 1996.

INL/A, U.S DEPARTMENT OF STATE

TABLE 1

AN ACUTE ORAL TOXICITY STUDY IN RATS

INDIVIDUAL CLINICAL OBSERVATIONS
(POSITIVE FINDINGS)

MALES 5000 MG/KG

MALE#	OBSERVATIONS	DAY OF STUDY 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A5452	SCHEDULED EUTHANASIA															P
	CONGESTED BREATHING	P														
A5454	SCHEDULED EUTHANASIA															P
	SOFT STOOLS	P														
A5455	SCHEDULED EUTHANASIA															P
	FEW FECES		P													
A5456	SCHEDULED EUTHANASIA															P
	FEW FECES												P			
A5457	SCHEDULED EUTHANASIA															P
	RALES	P	P													
	CONGESTED BREATHING	P	P	P												

GRADE CODE: 1=SLIGHT 2=MODERATE 3=SEVERE P=PRESENT L=LEFT R=RIGHT B=BILATERAL

TABLE 1

AN ACUTE ORAL TOXICITY STUDY IN RATS

INDIVIDUAL CLINICAL OBSERVATIONS
(POSITIVE FINDINGS)

FEMALES 5000 MG/KG

		DAY OF STUDY														
FEMALE#	OBSERVATIONS	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A5471	SCHEDULED EUTHANASIA															P
	CONGESTED BREATHING	P														
	FECES SMALL IN SIZE		P													
A5472	SCHEDULED EUTHANASIA															P
A5474	SCHEDULED EUTHANASIA															P
A5475	SCHEDULED EUTHANASIA															P
A5476	SCHEDULED EUTHANASIA															P
	FECES SMALL IN SIZE		P													

GRADE CODE: 1=SLIGHT 2=MODERATE 3=SEVERE P=PRESENT L=LEFT R=RIGHT B=BILATERAL

INL/A, U.S DEPARTMENT OF STATE

TABLE 2

AN ACUTE ORAL TOXICITY STUDY IN RATS

INDIVIDUAL BODY WEIGHTS (GRAMS)

MALES 5000 MG/KG

	DAY OF STUDY				
ANIMAL#	-1	0	7	14	AT DEATH (DAY)
A5452	239	221	263	288	
A5454	257	232	282	304	
A5455	259	235	290	315	
A5456	263	240	297	332	
A5457	243	221	268	295	
MEAN	252	230	280	307	
S.D.	10.5	8.5	14.4	17.3	
N	5	5	5	5	

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INL/A, U.S DEPARTMENT OF STATE

TABLE 2

AN ACUTE ORAL TOXICITY STUDY IN RATS

INDIVIDUAL BODY WEIGHTS (GRAMS)

FEMALES 5000 MG/KG

	DAY OF STUDY				
ANIMAL#	-1	0	7	14	AT DEATH (DAY)
A5471	181	166	197	217	
A5472	178	161	191	200	
A5474	172	157	188	200	
A5475	202	184	223	244	
A5476	182	165	194	204	
MEAN	183	167	199	213	
S.D.	11.3	10.4	14.0	18.7	
N	5	5	5	5	

INL/A, U.S DEPARTMENT OF STATE

TABLE 3

AN ACUTE ORAL TOXICITY STUDY IN RATS

INDIVIDUAL GROSS NECROPSY OBSERVATIONS

MALES 5000 MG/KG

ANIMAL#	DAY OF DEATH	STUDY DAY	OBSERVATION	FATE
A5452	12-JUL-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A5454	12-JUL-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A5455	12-JUL-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A5456	12-JUL-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A5457	12-JUL-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA

TABLE 3

AN ACUTE ORAL TOXICITY STUDY IN RATS

INDIVIDUAL GROSS NECROPSY OBSERVATIONS

FEMALES 5000 MG/KG

ANIMAL#	DAY OF DEATH	STUDY DAY	OBSERVATION	FATE
A5471	12-JUL-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A5472	12-JUL-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A5474	12-JUL-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A5475	12-JUL-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA
A5476	12-JUL-02	14	ALL TISSUES WITHIN NORMAL LIMITS	SCHEDULED EUTHANASIA

APPENDIX A

SLI Personnel Responsibilities

SLI Study No. 3596.9 (22)

SLI PERSONNEL RESPONSIBILITIES

Kimberly L. Bonnette, M.S., LATG	Study Director/Director, Acute Toxicology
Dawn D. Rodabaugh, B.S.	Alternate Contact/Associate Toxicologist
Robert C. Springborn, Ph.D.	Chairman, President and CEO
Malcolm Blair, Ph.D.	Senior Vice President, Managing Director Emeritus
Joseph C. Siglin, Ph.D., DABT	Vice President, Managing Director
Jason W. Smedley, B.S.	Assistant Toxicologist
Pamela S. Smith, ALAT	Supervisor of Acute Toxicology
Christina L. Dutil, B.S.	Primary Technician/Acute Technician I
Delores P. Knippen	Supervisor of Pharmacy
Steven H. Magness, B.S., LATG	Senior Supervisor of Gross and Fetal Pathology
Anita M. Bosau, RQAP-GLP	Senior Director, Compliance Assurance
Deanna M. Talerico, RQAP-GLP	Senior Supervisor of Quality Assurance
J. Dale Thurman, D.V.M., M.S., DACVP	Senior Director, Pathology
Kathy M. Gasser	Supervisor of Archives

A PRIMARY SKIN IRRITATION STUDY IN RABBITS WITH SPRAY--BRAVO

FINAL REPORT

OPPTS Guideline

870.2500

Author

Kimberly L. Bonnette, M.S., LATG

Study Completed on

September 3, 2002

Performing Laboratory

Springborn Laboratories, Inc. (SLI)
Ohio Research Center
640 North Elizabeth Street
Spencerville, Ohio 45887

SLI Study No.

3596.13

Submitted to

INL/A
U.S. Department of State
2201 C St. NW SA-4
Washington, DC 20520

SLI Study No. 3596.13 (2)

1. STATEMENT OF *NO* DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B), or (C).

Company: _____

Company Agent: _____ Date _____

_____ _____

Title Signature

SLI Study No. 3596.13 (3)

AUG 28 2002

2. COMPLIANCE STATEMENT

This study was conducted in compliance with the Good Laboratory Practice Standards as described by the EPA (40 CFR Parts 160 and 792).

Kimberly L. Bonnette, M.S., LATG
Study Director/Author
Springborn Laboratories, Inc.

Date 9/3/02

Rogers Woolfolk
Senior Aviation Advisor
Sponsor/Submitter
INL/A
U.S. Department of State

Date 8/27/02

3. QUALITY ASSURANCE STATEMENT

This study was inspected by the Quality Assurance Unit and reports were submitted to management and the Study Director in accordance with SLI's Standard Operating Procedures as follows:

Phase	Date
Protocol Review	04/25/02
Body Weights	06/24/02
Data Audit	08/12/02
Draft Report Review	08/12/02
Protocol Amendment Review	08/20/02
Final Report Review	09/03/02
Reports to Study Director and Management	08/12/02, 9/03/02

The final report has been reviewed to assure that it accurately describes the materials and methods, and the reported results accurately reflect the raw data.

Rebecca A. Young Date 9/3/02
Rebecca A. Young
Quality Assurance Team Leader

for Deanna M. Salerno Date 9/3/02
Anita M. Bosau, RQAP-GLP
Senior Director, Compliance Assurance

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SLI Study No. 3596.13 (7)

6. SUMMARY

The potential irritant and/or corrosive effects of Spray--Bravo were evaluated on the skin of New Zealand White rabbits. Each of three rabbits received a 0.5 mL dose of the test article as a single dermal application. The dose was held in contact with the skin under a semi-occlusive binder for an exposure period of four hours. Following the exposure period, the binder was removed and the remaining test article was wiped from the skin using gauze moistened with deionized water followed by dry gauze. Test sites were subsequently examined and scored for dermal irritation for up to 7 days following patch application.

Exposure to the test article produced very slight erythema on 3/3 test sites at the 1-hour scoring interval. The dermal irritation resolved completely on all test sites by study day 7.

Under the conditions of the test, Spray--Bravo is considered to be a slight irritant to the skin of the rabbit. The calculated Primary Irritation Index for the test article was 0.83.

7. INTRODUCTION

This study was performed to assess the potential irritant and/or corrosive effects of Spray--Bravo in New Zealand White rabbits when administered by a single dermal dose. This study was intended to provide information on the potential health hazards of the test article with respect to dermal exposure. Data from this study may serve as a basis for classification and/or labeling of the test article. This study was conducted in accordance with the US EPA, Health Effects Test Guidelines, OPPTS 870.2500, Acute Dermal Irritation, August 1998. This study was performed at Springborn Laboratories, Inc., 553 North Broadway, Spencerville, Ohio. The protocol was signed by the Study Director on April 26, 2002 (GLP initiation date). The in-life phase of the study was initiated with test article administration on June 24, 2002 (day 0) and concluded with final scoring on July 1, 2002.

8. MATERIALS AND METHODS

8.1. Test Article

The test article was received from the Sponsor and identified as follows:

Sponsor's ID	Assigned SLI ID	Physical Description	Receipt Date	Expiration Date
Spray--Bravo[a]	S02.002.3596	Cloudy pale amber liquid	05/31/02	None provided
Ingredients:[b]				
Herbicide: Roundup SL				None
Lot No.: 4010/4212				provided
4397/4272				
4333/4340				
4379/4076				
4397/4333				
Surfactant: Cosmo Flux-411F				None
Lot No.: Unknown				provided

[a]Sample pooled at SLI from five different mixes of Spray--Bravo (top/middle/bottom).
[b]Ingredients used in the five Spray--Bravo mixes that were prepared by the Sponsor.

The test article was stored at room temperature. The Sponsor was responsible for any necessary evaluations related to identity, strength, purity, composition, stability and method of synthesis of the test material according to 40 CFR 160.105 and 40 CFR 792.105. Springborn Laboratories, Inc. analyzed the test article for the glyphosate (a.e.) which is presented in SLI Study No. 3596.8.

8.2. Retention Sample

An approximate 1 mL retention sample of each test article mixture sample (top/middle/bottom, maintained separately for a total of fifteen 1 mL samples) was taken and stored at SLI at room temperature. In addition, a 10 mL retention sample of the pooled test article samples (from the 5 test article mixtures) was collected and stored at SLI at room temperature. These samples serve as the retention samples for all studies conducted with this material.

8.3. Test Article Disposition

The remaining test article was returned to the Sponsor following completion of all studies with the test article.

8.4. Method of Test Article Preparation

The test article was administered as received from the Sponsor and dispensed fresh on the day of dosing.

8.5. Animals and Animal Husbandry

8.5.1. Description, Identification and Housing

Adult, New Zealand White rabbits were received from Myrtle's Rabbitry, Thompson Station, TN. Upon receipt, plastic ear tags displaying unique identification numbers were used to individually identify the animals. Cage cards displaying at least the study number, animal number and sex were affixed to each cage. The animals were housed individually in suspended stainless steel cages. All housing and care were based on the standards recommended by the Guide for the Care and Use of Laboratory Animals [1].

8.5.2. Environment

The animal room temperature and relative humidity ranges were 71-76°F (22-24°C) and 43-61%, respectively. Environmental control equipment was monitored and adjusted as necessary to minimize fluctuations in the animal room environment. Light timers were set to maintain a 12-hour light/12-hour dark cycle and room ventilation was set to produce 10-15 air changes/hour. The animal room temperature and relative humidity were recorded a minimum of once daily.

8.5.3. Food

PMI Certified Rabbit Chow #5322 (Purina Mills, Inc.) was provided ad libitum to the animals throughout the study. The lot number and expiration date of each batch of diet used during the study were recorded. The feed was analyzed and certified by the supplier for nutritional components and environmental

contaminants. Dietary limitations for various environmental contaminants, including heavy metals, pesticides, polychlorinated biphenyls and total aflatoxin are set by the manufacturer. Within these limits, contaminants which may have been present were not expected to compromise the purpose of this study. Results of the dietary analyses (Certificates of Analysis) were provided by the manufacturer for each lot of diet. These are maintained by SLI.

8.5.4. Water

Municipal tap water treated by reverse osmosis was available ad libitum throughout the study. The purified water was supplied by an automatic watering system. Monitoring of the drinking water for contaminants is conducted by SLI and the records are available for inspection. Within generally accepted limits, contaminants which may have been present were not expected to compromise the purpose of this study. The water meets the standards specified under the EPA National Drinking Water Regulations (40 CFR Part 141).

8.5.5. Acclimation

Upon receipt, the animals were removed randomly from the shipping cartons, examined by qualified personnel, identified with plastic ear tags and then acclimated to the laboratory conditions for a minimum of five days. The animals were observed daily for overt physical or behavioral abnormalities, general health/moribundity and mortality.

8.5.6. Animal Selection

The animals chosen for study use were arbitrarily selected from healthy stock animals to avoid potential bias. All animals received a detailed pretest observation prior to dosing. Only healthy animals were chosen for study use. The male animals were approximately 17-18 weeks of age and weighed 3.4-3.7 kg prior to dosing.

9. EXPERIMENTAL PROCEDURES

9.1. Preliminary Procedures

On day -1, the animals chosen for use on the primary skin irritation study had the fur removed from the dorsal area of the trunk using an animal clipper. Care was taken to avoid abrading the skin during the clipping procedure.

9.2. Dosing

On the following day (day 0), the test article was applied to a small area of intact skin on each test animal (approximately 1 inch x 1 inch) as indicated below:

SLI Study No. 3596.13 (11)

Concentration (%)	Amount Applied	Patch Design	No. of Animals Male
100[a]	0.5 mL	~1" x 1" square 4-ply gauze patch	3

[a]Pooled test article

The test article was administered under the gauze patch. The gauze patch was held in contact with the skin at the cut edges with a nonirritating tape. Removal and ingestion of the test article was prevented by placing an elastic wrap over the trunk and test area (semi-occlusive binding). The elastic wrap was then further secured with adhesive tape around the trunk at the cranial and caudal ends. After dosing, collars were placed on each animal and remained in place until removal on day 3. After a four-hour exposure period, the binding materials were removed from each animal and the corners of the test site delineated using a marker. Residual test article was removed using gauze moistened with deionized water, followed by dry gauze.

9.3. Dermal Observations

Animals were examined for signs of erythema and edema and the responses scored at 1 hour after patch removal and 24, 48 and 72 hours and up to 7 days after patch application according to the Macroscopic Dermal Grading System presented in Appendix A which is based on Draize [2].

9.4. Clinical Observations

Any unusual observations and/or mortality were recorded. General health/mortality checks were performed twice daily (in the morning and in the afternoon).

9.5. Body Weights

Individual body weights were obtained for each animal prior to dosing on day 0.

9.6. Scheduled Euthanasia

Each animal was euthanized by an intravenous injection of sodium pentobarbital following its final scoring interval. Gross necropsy examinations were not required for these animals.

9.7. Protocol Deviations

On two occasions, the animal room temperature range [71-76°F (22-24°C)] exceeded the preferred range [63-73°F (17-23°C)] during this study. This occurrence was considered to have had no adverse effect on the outcome of this study.

10. ANALYSIS OF DATA

The 1-, 24-, 48- and 72-hour erythema and edema scores for all animals were added and the total divided by the number of test sites x 4. The calculated Primary Irritation Index (P.I.I.) was classified according to the Dermal Evaluation Criteria [3] presented in Appendix B.

11. MAINTENANCE OF RAW DATA AND RECORDS

All original raw data, the final report and magnetically encoded records were transferred to the SLI archives for a period of 10 years. The Sponsor will be contacted prior to final disposition of these items.

12. RESULTS

12.1. Dermal/Clinical Observations

Individual Data: Table 1
Individual Clinical Observations: Appendix C

Exposure to the test article produced very slight erythema on 3/3 test sites at the 1-hour scoring interval. The dermal irritation resolved completely on all test sites by study day 7.

Transient clinical observations of few feces, decreased food consumption and feces small in size were observed in one animal during the study and were not considered to be test article-related.

13. CONCLUSION

Under the conditions of the test, Spray–Bravo is considered to be a slight irritant to the skin of the rabbit. The calculated Primary Irritation Index for the test article was 0.83.

Kimberly L. Bonnette, M.S., LATG
Study Director

Date 9/3/02

14. REPORT REVIEW

Dawn D. Rodabaugh, B.S.
Associate Toxicologist

Date 9/3/02

15. REFERENCES

1. Guide for the Care and Use of Laboratory Animals, DHHS Publication No. (NIH) 96-03, 1996.

2. Draize, J.H., Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics, The Association of Food and Drug Officials of the United States, 49-51, 1959.

3. Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals-Addendum 3 on Data Reporting, US EPA, 1988.

TABLE 1

SLI STUDY NO.: 3596.13 — A PRIMARY SKIN IRRITATION STUDY IN RABBITS — PAGE 1

CLIENT: INL/A, US DEPARTMENT OF SATE — INDIVIDUAL DERMAL IRRITATION SCORES

(SPRAY--BRAVO)

Animal No./Sex Body Weight (kg)	Scoring Interval	Erythema	Edema	Comments
R2111/M	1 Hour	1	0	
3.567	24 Hours	1	0	
	48 Hours	1	0	
	72 Hours	0	0	
R2122/M	1 Hour	1	0	IT
3.364	24 Hours	1	0	
	48 Hours	1	0	
	72 Hours	1	0	
	7 Days	0	0	
R2126/M	1 Hour	1	0	IT
3.650	24 Hours	1	0	
	48 Hours	1	0	
	72 Hours	0	0	

Note: See Appendix A for definition of codes.

Primary Irritation Index

0.83 = Slight irritant

SLI Study No. 3596.13 (16)

APPENDIX A

Macroscopic Dermal Grading System

SLI Study No. 3596.13 (17)

MACROSCOPIC DERMAL GRADING SYSTEM

ERYTHEMA AND EDEMA OBSERVATIONS		
OBSERVATION	DEFINITION	CODE
Erythema – Grade 0	No erythema	0
Erythema – Grade 1	Very slight erythema (barely perceptible)	1
Erythema – Grade 2	Well-defined erythema	2
Erythema – Grade 3	Moderate to severe erythema	3
Erythema – Grade 4	Severe erythema (beet redness)	4
Maximized Grade 4	Notable dermal lesions (see below)	M – 4 (see below)
Edema – Grade 0	No edema	0
Edema – Grade 1	Very slight edema (barely perceptible)	1
Edema – Grade 2	Slight edema (edges of area well defined by definite raising)	2
Edema – Grade 3	Moderate edema (raised approximately 1 millimeter)	3
Edema – Grade 4	Severe edema (raised more than 1 millimeter and extends beyond the area of exposure)	4
NOTE: Each animal was assigned an erythema and edema score. The most severely affected area within the test site was graded. If eschar, blanching, ulceration and/or necrosis greater than grade 1 was observed, then the “Maximized Grade 4" was assigned to the test site in place of the erythema score and the type of notable dermal lesion(s) (e.g., eschar - grade 2, blanching - grade 3, ulceration - grade 4, etc.) was noted. The presence of any other dermal changes (e.g., desquamation, fissuring, eschar exfoliation, etc.) was also recorded.		

SLI Study No. 3596.13 (18)

MACROSCOPIC DERMAL GRADING SYSTEM

NOTABLE DERMAL LESIONS		
OBSERVATION	CODE	DEFINITION
Eschar – Grade 1	ES-1	Focal and/or pinpoint areas up to 10% of test site.
Eschar – Grade 2	ES-2	> 10% < 25% of test site.
Eschar – Grade 3	ES-3	> 25% < 50% of test site.
Eschar – Grade 4	ES-4	> 50% of test site.
Blanching – Grade 1	BLA-1	Focal and/or pinpoint areas up to 10% of test site.
Blanching – Grade 2	BLA-2	> 10% < 25% of test site.
Blanching – Grade 3	BLA-3	> 25% < 50% of test site.
Blanching – Grade 4	BLA-4	> 50% of test site.
Ulceration – Grade 1	U-1	Focal and/or pinpoint areas up to 10% of test site.
Ulceration – Grade 2	U-2	> 10% < 25% of test site.
Ulceration – Grade 3	U-3	> 25% < 50% of test site.
Ulceration – Grade 4	U-4	> 50% of test site.
Necrosis – Grade 1	NEC-1 *(color)*	Focal and/or pinpoint areas up to 10% of test site (note color of necrosis).
Necrosis – Grade 2	NEC-2 *(color)*	> 10% < 25% of test site (note color of necrosis).
Necrosis – Grade 3	NEC-3 *(color)*	> 25% < 50% of test site (note color of necrosis).
Necrosis – Grade 4	NEC-4 *(color)*	> 50% of test site (note color of necrosis).

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MACROSCOPIC DERMAL GRADING SYSTEM

ADDITIONAL DERMAL FINDINGS		
OBSERVATION	DEFINITION	CODE
Desquamation	Characterized by scaling or flaking of dermal tissue or without denuded areas.	DES
Fissuring	Characterized by cracking of the skin with or without moist exudate. Fissuring should be checked prior to removing the animal from the cage and manipulating the test site.	FIS
Eschar Exfoliation	The process by which areas of eschar flake off the test site.	EXF
Test Site Staining	Skin located at test site appears to be discolored, possibly due to test article (note color of staining).	TSS (*color*)
Erythema Extends Beyond the Test Site	The erythema extends beyond the test site. Note: A study director should be contacted for erythema extending beyond the test site.	ERB
Superficial Lightening	Characterized by pale area(s) (almost a burn-like appearance) in the test site. However, erythema may still be observed through the pale area. Note: This observation may affect the overall erythema score of the test site. This observation may progress to other observations resulting in notable dermal lesions, but SL itself will not be considered a notable dermal lesion that will result in a dermal score to be maximized since it does not result in any in-depth injury. To be coded using an area designation (see below).	--
Superficial Lightening - Grade 1	Focal and/or pinpoint areas up to 10% of the test site	SL-1
Superficial Lightening - Grade 2	> 10% < 25% of test site	SL-2
Superficial Lightening - Grade 3	> 25% < 50% of test site	SL-3
Superficial Lightening - Grade 4	> 50% of test site	SL-4
Dermal Irritation - Outside of the Test Site	Noticeable irritation outside of test site probably due to the binding tape material. This notation will only be made for reactions greater than what are normally observed from tape removal which does not interfere with the scoring of the test site.	IT

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APPENDIX B

Dermal Evaluation Criteria

SLI Study No. 3596.13 (21)

DERMAL EVALUATION CRITERIA	
Primary Irritation Index (P.I.I.)	Irritation Rating
0.00	Nonirritant
0.01 - 1.99	Slight Irritant
2.00 - 5.00	Moderate Irritant
5.01 - 8.00	Severe Irritant

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APPENDIX C

Individual Clinical Observations

Animal No./Sex	Clinical Observations
R2122/M	Few feces: Day 3 Decreased food consumption: Days 3, 5 Feces small in size: Day 4

SLI Study No. 3596.13 (24)

APPENDIX D

SLI Personnel Responsibilities

SLI Study No. 3596.13 (25)

SLI PERSONNEL RESPONSIBILITIES

Kimberly L. Bonnette, M.S., LATG	Study Director/Director, Acute Toxicology
Dawn D. Rodabaugh, B.S.	Alternate Contact/Associate Toxicologist
Robert C. Springborn, Ph.D.	Chairman, President and CEO
Malcolm Blair, Ph.D.	Senior Vice President, Managing Director Emeritus
Joseph C. Siglin, Ph.D., DABT	Vice President, Managing Director
Rusty E. Rush, M.S., LAT, DABT	Director, Neurotoxicity and Transgenics
Jason W. Smedley, B.S.	Assistant Toxicologist
Pamela S. Smith, ALAT	Supervisor of Acute Toxicology
Lyndsay K. Simindinger, A.S.	Primary Technician/Acute Technician I
Delores P. Knippen	Supervisor of Pharmacy
Steven H. Magness, B.S., LATG	Senior Supervisor of Gross and Fetal Pathology
Anita M. Bosau, RQAP-GLP	Senior Director, Compliance Assurance
Deanna M. Talerico, RQAP-GLP	Senior Supervisor of Quality Assurance
J. Dale Thurman, D.V.M., M.S., DACVP	Senior Director, Pathology
Kathy M. Gasser	Supervisor of Archives

PURITY ANALYSIS FOR GLYPHOSATE OF SPRAY--BRAVO (ACTIVE INGREDIENT)

FINAL REPORT

Author

Kimberly L. Bonnette, M.S., LATG

Study Completed on

January 9, 2003

Performing Laboratory

Springborn Laboratories, Inc. (SLI)
Ohio Research Center
640 North Elizabeth Street
Spencerville, Ohio 45887

SLI Study No.

3596.8

Submitted to

INL/A
U.S. Department of State
2201 C St. NW SA-4
Washington, DC 20520

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1. STATEMENT OF *NO* DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B), or (C).

Company: _____

Company Agent: _____ Date _____

_____ _____

Title Signature

NOV 2 1 2002

2. COMPLIANCE STATEMENT

This study was conducted in compliance with the Good Laboratory Practice Standards as described by the EPA (40 CFR Parts 160 and 792) with the following exception:

Since the test article mixtures were prepared in the field, the test article mixtures and the sample collection by the Sponsor were not performed according to GLP guidelines.

Kimberly L. Bonnette, M.S., LATG
Study Director/Author
Springborn Laboratories, Inc.

Date 1/9/03

Rogers Woolfolk
Senior Aviation Advisor
Sponsor/Submitter
INL/A
U.S. Department of State

Date 20 Nov 02

SLI Study No. 3596.8

3. QUALITY ASSURANCE STATEMENT

This study was inspected by the Quality Assurance Unit and reports were submitted to management and the Study Director in accordance with SLI's Standard Operating Procedures as follows:

Phase	Date
Protocol Review	04/25/02
Purity Analysis	06/11/02
Data Audit	11/11/02
Draft Report Review	11/11/02
Protocol Amendment Review	11/11/02
Final Report Review	01/09/03
Reports to Study Director and Management	11/11/02, 01/09/03

The final report has been reviewed to assure that it accurately describes the materials and methods, and the reported results accurately reflect the raw data.

Stephanie K. Clemons Date 1/9/03
Stephanie K. Clemons
Quality Assurance Auditor II

Anita M Bosau Date 1/9/03
Anita M. Bosau, RQAP-GLP
Senior Director, Compliance Assurance

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6. SUMMARY

The objective of this study was to assess the concentration(s) of glyphosate (active ingredient) in the Spray--Bravo formulation.

Five test article mixtures were prepared in the field by the Sponsor. Three 500 mL samples of each mixture were collected from the top/middle/bottom (or beginning/middle/end) of Aircraft 3077 (Test Article Mixtures 1 and 5), Aircraft 3064 (Test Article Mixtures 2 and 4) and Aircraft - unknown (Test Article Mixture 3 – aircraft not documented). Test Article mixtures were prepared as follows:

Ingredient	Amount Added (gallons)
Herbicide: Roundup SL	88
Surfactant: Cosmo Flux-411F	2
Well water	110
Mixing time: 10-15 minutes in flight.	

Test article mixtures were prepared on two separate days (May 26, 2002, for Test Article Mixtures 1, 2 and 3; and May 28, 2002 for Test Article Mixtures 4 and 5).

The overall concentration of the Spray--Bravo was 16.33 [in terms of % glyphosate (a.e.)] before use at SLI and 17.04 [in terms of % glyphosate (a.e.)] after use at SLI, indicating that the test material was stable during use at SLI.

The overall result (~16.33% glyphosate a.e.) was slightly higher than the anticipated 14.80% glyphosate (a.e.), but well within acceptable error of mixing conditions in the field. Therefore, since the results of the analysis were appropriate (and would provide conservative results for toxicity, irritation and sensitization since they were slightly higher than expected), approximately 400 mL of each sample were pooled into a single container for use in the remaining studies.

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7. INTRODUCTION

This study was performed to assess the concentrations of glyphosate (active ingredient) in Spray--Bravo. This study was performed to support studies conducted under the US EPA, Health Effects Test Guidelines. This study was performed at Springborn Laboratories, Inc., 553 North Broadway, Spencerville, Ohio. The protocol was signed by the Study Director on April 25, 2002 (GLP initiation date). The test article mixtures were analyzed for glyphosate (a.e.) initially on June 11, 2002, prior to all other studies and again on August 21, 2002, after all studies were complete for purposes of stability.

8. MATERIALS AND METHODS

8.1. Test Article

The test article was received from the Sponsor and identified as follows:

Sponsor's ID	Assigned SLI ID	Physical Description	Receipt Date	Expiration Date
Spray—Bravo[a]	S02.002.3596	Cloudy pale amber liquid	05/31/02	None provided
Ingredients:[b]				
Herbicide: Roundup SL Lot Nos.: 4010/4212 4397/4272 4333/4340 4379/4076 4397/4333				None provided
Surfactant: Cosmo Flux-411F Lot No.: Unknown				None provided

[a]Sample pooled at SLI from five different mixes of Spray--Bravo (top/middle/bottom).
[b]Ingredients used in the five Spray--Bravo mixes that were prepared by the Sponsor.

The test article was stored at room temperature. The Sponsor was responsible for any necessary evaluations related to identity, strength, purity, composition, stability and method of synthesis of the test material according to 40 CFR 160.105 and 40 CFR 792.105.

8.2. Retention Sample

An approximate 1 mL retention sample of each test article mixture sample (top/middle/bottom, maintained separately for a total of fifteen 1 mL samples)

was taken and stored at SLI at room temperature. In addition, a 10 mL retention sample of the pooled test article samples (from the 5 test article mixtures) was collected and stored at SLI at room temperature. These samples serve as the retention samples for all studies conducted with this material.

8.3. Test Article Disposition

The test article was returned to the Sponsor following completion of all studies with the test article.

8.4. Method of Test Article Preparation

The test article containers were hand shaken and dispensed fresh on the day of analysis. The samples were stirred continuously until diluted for analysis.

9. EXPERIMENTAL PROCEDURE

9.1. Sample Collection

Samples were collected from the prepared test article mix using pre-labeled containers provided by SLI as follows:

Test Article Mix 1	500 mL 500 mL 500 mL	Beginning Middle End
Test Article Mix 2	500 mL 500 mL 500 mL	Beginning Middle End
Test Article Mix 3	500 mL 500 mL 500 mL	Beginning Middle End
Test Article Mix 4	500 mL 500 mL 500 mL	Beginning Middle End
Test Article Mix 5	500 mL 500 mL 500 mL	Beginning Middle End

Five test article mixtures were prepared in the field by the Sponsor. Three 500 mL samples of each mixture were collected from the top/middle/bottom (or beginning/middle/end) Aircraft 3077 (Test Article Mixtures 1 and 5), Aircraft 3064

(Test Article Mixtures 2 and 4) and Aircraft - unknown (Test Article Mixture 3 – aircraft not documented). The Test Article mixtures were prepared as follows:

Ingredient	Amount Added (gallons)
Herbicide: Roundup SL	88
Surfactant: Cosmo Flux-411F	2
Well water	110
Mixing time: 10 (Test mixture 4) -15 (Test mixtures 1, 2, 3 and 5) minutes in flight.	

Test article mixtures were prepared on two separate days (May 26, 2002, for Test Article Mixtures 1, 2 and 3; and May 28, 2002 for Test Article Mixtures 4 and 5).

A total of fifteen 500 mL samples were collected. The individual (Brad Carter, Assistant Operations Manager, Embajada Americana, Carrera 45, No. 22D-45, Bogota, Columbia, South America) collecting samples completed the SLI provided form upon collection including signature and date when collected at San Jose del Guaviare, Columbia. Samples were maintained under ambient conditions.

10. ANALYTICAL CHEMISTRY

The samples were analyzed in terms of the active ingredient for concentration determination prior to any dosing (Before Use-Purity) and again after completion of all studies for stability determination (After Use-Purity). All analytical dilutions were performed in duplicate (all dilutions were performed on the same day).

The analytical method was a previously validated method for the analysis of glyphosate in solution. Purity analysis of the test article was performed in duplicate by comparison of the test article with supplied reference standards of known concentrations.

11. SPRAY--BRAVO ANALYSIS

The analytical method for the analysis of the glyphosate component of Spray--Bravo was validated prior to the purity analyses performed at Springborn Laboratories, Inc. This method was utilized to determine both the purity and the stability of the Spray--Bravo test material before and after use at SLI.

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11.1. Experimental System

11.1.1. HPLC System

HPLC Model: Waters
Pump: Waters 600E
Injector: Waters WISP 717
Detector: Waters 2487
Data System: H-P 3396B Integrator
Precolumn: Phenomenex, SecurityGuard, C18, 4.0 x 3.0 mm ID
Column: Phenomenex, Spherex, C18, 5 μ, 250 x 4.6 mm ID
Temperature: Ambient
Detection: 500 nm, 0.4000 AUFS
Mobile Phase: A: 0.05 M HCO_2NH_4, pH 3.6/5% ACN (Acetonitrile);
B: 100% ACN
Gradient: 100% A hold for 6 minutes; linear change to 25% A/75% B over 1 minute; hold for 5 minutes; linear change to 100% A over 1 minute; hold at 100% A for 15 minutes.
Flow Rate: 1.0 mL/min
Injection Volume: 10 μL

11.1.2. Apparatus

Balance: Mettler AG 245, accuracy of 0.0001 gram
Glassware: Assorted volumetric glassware
Filters: Millipore 0.2 μ Nylon-66; Whatman Puradisc 25PP 0.45μm
Oven: Boekel Model 107905
Pipet:: Mettler VoluMate, 200-1000 μL

11.1.3. Solutions and Reagents

11.1.3.1. Reagents

Water, Fisher, HPLC Grade, Lot # 024948, 025012
Acetonitrile, Baker, HPLC Grade, Lot # M15811
NBD Chloride, Aldrich, 98%, Lot #12214L1
Hydrochloric Acid, Fisher, ACS Grade, Lot # 012161
Potassium Tetraborate Tetrahydrate, Aldrich, 99%, Lot # 15325D1
Formic Acid, Fisher, Laboratory Grade, 90%, Lot # 003630
Ammonium Formate, Fisher, Certified, Lot # 990125
Glyphosate, Sigma, 95%, Lot # 71K36491

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1.1.3.2 Solutions

0.37 M Borate Solution: Prepared by dissolving approximately 11.44 g of potassium tetraborate tetrahydrate in 100 mL of water. The resulting solution was stable for 6 months under ambient storage conditions.

1.2 N HCl: Prepared by dissolving 10 mL of HCl in 90 mL of water. The resulting solution was stable for 6 months under ambient storage conditions.

25 mM NBD-Cl: Prepared by dissolving approximately 2.5 g of NBD-Cl in 500 mL of methanol. The resulting solution was stable for 6 months under ambient storage conditions.

Mobile Phase A: Prepared by dissolving approximately 3.153 g of ammonium formate in 1900 mL of water. The pH was adjusted to approximately 3.6 with formic acid prior to the addition of 100 mL of acetonitrile. The resulting solution was mixed thoroughly, filtered through a 0.2μ Nylon-66 filter and degassed by helium sparging prior to use.

Mobile Phase B: Acetonitrile used 100% as received.

Diluent: All standards and samples were diluted in water.

Stock Standard Solution: Prepared by dissolving approximately 30 mg of glyphosate standard in a 100 mL flask with diluent.

Standard Solutions: Prepared by serially diluting the stock standard solution with water. The final concentrations of the solutions were in the range of approximately 0.02 to 0.14 mg/mL. These solutions were then further diluted in diluent at a ratio of 3:10 and filtered through Whatman Puradisc 25PP 0.45μm filters prior to derivatization.

Purity Solutions: Prepared by diluting 1.0 mL aliquots of each sample to a final volume of 100 mL with diluent. The solutions were then further diluted in diluent first at a ratio of 2:50 and then at a ratio of 4:10. The resulting solutions were then filtered through Whatman Puradisc 25PP 0.45 μm filters prior to derivatization. These preparations were performed in duplicate for each sample.

Derivatization Procedure: In order to analyze the glyphosate component, a precolumn derivatization was performed by adding 1.2 mL of the appropriate control, standard, or sample solution to a labeled scintillation vial. Both 0.8 mL of the borate solution and 2.4 mL of the NBD-Cl solution were added to each vial. The vials were then capped and shaken by hand prior to being heated in an oven

at 80° C for 30 minutes. After removal from the oven, the vials were allowed to cool for 10 minutes followed by the addition of 0.9 mL of the HCl solution. After the vials were again shaken by hand, they were allowed to stand for 10 minutes in order for incipient precipitation to occur. These solutions were then transferred to injection vials.

11.2. Analytical Procedures

11.2.1. Standard Curve Analysis

The peak areas of the glyphosate acid component of each standard were determined, measured, combined, and plotted as a function of concentration to generate a standard curve. The actual values used for the calculations are shown in Chemistry Tables 1 and 3.

11.2.2. Sample Analysis

The peak areas of the glyphosate acid component of each sample were measured and combined and then the concentration was determined by linear fit to the standard curve. The actual values used for the calculations are shown in Chemistry Tables 1 and 3.

12. STATISTICAL ANALYSIS

A statistical analysis was conducted on the average results of the % glyphosate (a.e.) for each test article mixture as compared to the theoretical value [14.80% glyphosate (a.e.) as calculated by the Sponsor] and for the combined results of all test article mixture samples as compared to the theoretical value using one way analysis of variance (ANOVA).

13. PROTOCOL DEVIATIONS

No protocol deviations occurred during this study.

14. MAINTENANCE OF RAW DATA AND RECORDS

All original raw data, the final report and magnetically encoded records were transferred to the SLI archives for a period of 10 years. The Sponsor will be contacted prior to final disposition of these items.

15. RESULTS

15.1. Analytical Chemistry Results

Individual Data: Tables 1-4

The actual sample results of the before use purity analyses are shown in Chemistry Table 1. The % errors of the results of the before use purity analyses are shown in Chemistry Table 2. The actual sample results of the after use purity (stability) analyses are shown in Chemistry Table 3. The % errors of the results of the after use purity (stability) analyses are shown in Chemistry Table 4. All concentration values are reported in terms of the acid equivalent (a.e.) of the glyphosate. The overall concentration of the Spray Bravo was 16.33 [in terms of % glyphosate (a.e.)] before use at SLI and 17.04 [in terms of % glyphosate (a.e.)] after use at SLI, indicating that the test material was stable during use at SLI. The average % error (based upon a comparison between the analyzed value and the theoretical value) for the before use purity analysis was between 4.8 and 20.1%. The average % error (based upon a comparison between the analyzed value and the theoretical value) for the after use purity (stability) analysis was between 7.1 and 30.7%.

15.2. Statistical Analysis

Individual Data: Appendix A

Results of the Before-Use statistical analysis indicate that Test Article Mixtures 2, 3 and 5 (17.07, 17.78 and 17.35% glyphosate a.e.) were significantly higher than the theoretical value (14.8% glyphosate a.e.). However, since these values were within the possible error rate of field mixing and since these samples were to be part of a pooled sample for dosing the remaining studies, these samples were included. Overall, the results of all mixtures for the pooled sample (16.33% glyphosate a.e.) were significantly higher than the theoretical value (14.8% glyphosate a.e.). Again, this result was considered within possible field mixing error and would provide a conservative estimate of toxicity, irritation and sensitization for the remaining studies. Therefore, the pooled sample was considered to be acceptable for use.

16. CONCLUSION

The overall result (~16.33% glyphosate a.e.) was slightly higher than the anticipated 14.80% glyphosate (a.e.), but well within acceptable error of mixing conditions in the field. Therefore, since the results of the analysis were appropriate (and would provide conservative results for toxicity, irritation and sensitization since they were slightly higher than expected), approximately 400 mL of each sample were pooled into a single container for use in the remaining studies.

Kimberly L. Bonnette, M.S., LATG
Study Director

Date 1/9/03

17. REPORT REVIEW

Dawn D. Rodabaugh, B.S.
Toxicologist

Date 1/9/03

M. Gardner Clemons, B.A.
Manager of Analytical Chemistry
and Pharmacy

Date 1.9.2003

SLI Study No. 3596.8 (16)

Chemistry Table 1

Standard Curve and Sample Analysis Values for the Before Use-Purity Analysis (6/11/2002)

Sample Type	Theoretical Conc. (mg/L)	Peak Area	Actual Conc. [% Glyphosate (a.e.)]
Std 1	0.008637	35543	NA
Std 2	0.01727	73477	NA
Std 3	0.02591	110900	NA
Std 4	0.03456	154704	NA
Std 5	0.04320	193670	NA
Test Mix # 1, B	NA	112077	15.98
Test Mix # 1, B'	NA	112767	16.08
Test Mix # 1, M	NA	114677	16.34
Test Mix # 1, M'	NA	118352	16.84
Test Mix # 1, E	NA	126172	17.90
Test Mix # 1, E'	NA	136131	19.25
Test Mix # 2, B	NA	128331	18.19
Test Mix # 2, B'	NA	129222	18.31
Test Mix # 2, M	NA	133033	18.83
Test Mix # 2, M'	NA	129348	18.33
Test Mix # 2, E	NA	117614	16.74
Test Mix # 2, E'	NA	114082	16.26
Test Mix # 3, B	NA	106042	15.16
Test Mix # 3, B'	NA	109377	15.61
Test Mix # 3, M	NA	108735	15.53
Test Mix # 3, M'	NA	108624	15.51
Test Mix # 3, E	NA	110508	15.77
Test Mix # 3, E'	NA	108454	15.49
Test Mix # 4, B	NA	119612	17.01
Test Mix # 4, B'	NA	120670	17.15
Test Mix # 4, M	NA	125863	17.86
Test Mix # 4, M'	NA	122465	17.39
Test Mix # 4, E	NA	119981	17.06
Test Mix # 4, E'	NA	124304	17.64
Test Mix # 5, B	NA	98279	14.11
Test Mix # 5, B'	NA	99554	14.28
Test Mix # 5, M	NA	96188	13.83
Test Mix # 5, M'	NA	93828	13.50
Test Mix # 5, E	NA	98206	14.10
Test Mix # 5, E'	NA	96311	13.84

Correlation coefficient = 0.9996; NA = Not Applicable
Note: B = Beginning; M = Middle; E = End; ' = Replicate sample

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Chemistry Table 2
Sample Analysis Values and % Error Based on Theoretical Value
(Before Use-Purity)

Test Mix No.	Sample Type	% Glyphosate (a.e.)	Average % Glyphosate (a.e.) by Sample Type	Average % Glyphosate (a.e.) by Test Mix	Overall Average % Glyphosate (a.e.)	% Error	Average % Error by Type	Average % Error by Test Mix	Date of Analysis
1	Beginning	15.98			16.33	8.0			6/11/2002
1	Beginning'	16.08	16.03			8.6	8.3		6/11/2002
1	Middle	16.34				10.4			6/11/2002
1	Middle'	16.84	16.59			13.8	12.1		6/11/2002
1	End	17.90				20.9			6/11/2002
1	End'	19.25	18.58	17.07		30.1	25.5	15.3	6/11/2002
2	Beginning	18.19				22.9			6/11/2002
2	Beginning'	18.31	18.25			23.7	23.3		6/11/2002
2	Middle	18.83				27.2			6/11/2002
2	Middle'	18.33	18.58			23.9	25.5		6/11/2002
2	End	16.74				13.1			6/11/2002
2	End'	16.26	16.50	17.78		9.9	11.5	20.1	6/11/2002
3	Beginning	15.16				2.4			6/11/2002
3	Beginning'	15.61	15.39			5.5	4.0		6/11/2002
3	Middle	15.53				4.9			6/11/2002
3	Middle'	15.51	15.52			4.8	4.9		6/11/2002
3	End	15.77				6.6			6/11/2002
3	End'	15.49	15.63	15.51		4.7	5.6	4.8	6/11/2002
4	Beginning	17.01				14.9			6/11/2002
4	Beginning'	17.15	17.08			15.9	15.4		6/11/2002
4	Middle	17.86				20.7			6/11/2002
4	Middle'	17.39	17.63			17.5	19.1		6/11/2002
4	End	17.06				15.3			6/11/2002
4	End'	17.64	17.35	17.35		19.2	17.2	17.2	6/11/2002
5	Beginning	14.11				4.7			6/11/2002
5	Beginning'	14.28	14.20			3.5	4.1		6/11/2002
5	Middle	13.83				6.6			6/11/2002
5	Middle'	13.50	13.67			8.8	7.7		6/11/2002
5	End	14.10				4.7			6/11/2002
5	End'	13.84	13.97	13.94		6.5	5.6	5.8	6/11/2002

SLI Study No. 3596.8 (18)

Chemistry Table 3

Standard Curve and Sample Analysis Values for the
After Use-Purity Analysis for (Stability) (8/21/2002)

Sample Type	Theoretical Conc. (mg/L)	Peak Area	Actual Conc. (mg/mL)
Std 1	0.008580	29599	NA
Std 2	0.01716	64382	NA
Std 3	0.02574	94096	NA
Std 4	0.03432	124119	NA
Std 5	0.04290	147270	NA
Test Mix # 1, B	NA	95077	16.67
Test Mix # 1, B'	NA	94928	16.64
Test Mix # 1, M	NA	94778	16.61
Test Mix # 1, M'	NA	85965	15.01
Test Mix # 1, E	NA	92202	16.14
Test Mix # 1, E'	NA	106892	18.81
Test Mix # 2, B	NA	110867	19.54
Test Mix # 2, B'	NA	110275	19.43
Test Mix # 2, M	NA	107060	18.84
Test Mix # 2, M'	NA	107748	18.97
Test Mix # 2, E	NA	101906	17.91
Test Mix # 2, E'	NA	98293	17.25
Test Mix # 3, B	NA	97602	17.13
Test Mix # 3, B'	NA	97729	17.15
Test Mix # 3, M	NA	90909	15.91
Test Mix # 3, M'	NA	89923	15.73
Test Mix # 3, E	NA	93383	16.36
Test Mix # 3, E'	NA	90589	15.85
Test Mix # 4, B	NA	111212	19.60
Test Mix # 4, B'	NA	113409	20.00
Test Mix # 4, M	NA	113974	20.10
Test Mix # 4, M'	Na	107497	18.93
Test Mix # 4, E	NA	112424	19.82
Test Mix # 4, E'	NA	100144	17.59
Test Mix # 5, B	NA	90451	15.83
Test Mix # 5, B'	NA	86161	15.04
Test Mix # 5, M	NA	84031	14.66
Test Mix # 5, M'	NA	71194	12.33
Test Mix # 5, E	NA	83091	14.49
Test Mix # 5, E'	NA	73311	12.71

Correlation coefficient = 0.998; NA = Not Applicable
Note: B = Beginning; M = Middle; E = End; ' = Replicate sample

'

SLI Study No. 3596.8

Chemistry Table 4
Sample Analysis Values and % Error Based on Theoretical Value
(After Use- Purity for Stability)

Test Mix No.	Sample Type	% Glyphosate (a.e.)	Average % Glyphosate (a.e.) by Sample Type	Average % Glyphosate (a.e.) by Test Mix	Overall Average % Glyphosate (a.e.)	% Error	Average % Error by Type	Average % Error by Test Mix	Date of Analysis
1	Beginning	16.67			17.04	12.6			8/21/2002
1	Beginning'	16.64	16.66			12.4	12.5		8/21/2002
1	Middle	16.61				12.2			8/21/2002
1	Middle'	15.01	15.81			1.4	6.8		8/21/2002
1	End	16.14				9.1			8/21/2002
1	End'	18.81	17.48	16.65		27.1	18.1	12.5	8/21/2002
2	Beginning	19.54				32.0			8/21/2002
2	Beginning'	19.43	19.49			31.3	31.7		8/21/2002
2	Middle	18.84				27.3			8/21/2002
2	Middle'	18.97	18.91			28.2	27.7		8/21/2002
2	End	17.91				21.0			8/21/2002
2	End'	17.25	17.58	18.66		16.6	18.8	26.1	8/21/2002
3	Beginning	17.13				15.7			8/21/2002
3	Beginning'	17.15	17.14			15.9	15.8		8/21/2002
3	Middle	15.91				7.5			8/21/2002
3	Middle'	15.73	15.82			6.3	6.9		8/21/2002
3	End	16.36				10.5			8/21/2002
3	End'	15.85	16.11	16.36		7.1	8.8	10.5	8/21/2002
4	Beginning	19.60				32.4			8/21/2002
4	Beginning'	20.00	19.80			35.1	33.8		8/21/2002
4	Middle	20.10				35.8			8/21/2002
4	Middle'	18.93	19.52			27.9	31.9		8/21/2002
4	End	19.82				33.9			8/21/2002
4	End'	17.59	18.71	19.34		18.9	26.4	30.7	8/21/2002
5	Beginning	15.83				7.0			8/21/2002
5	Beginning'	15.04	15.44			1.6	4.3		8/21/2002
5	Middle	14.66				0.9			8/21/2002
5	Middle'	12.33	13.50			16.7	8.8		8/21/2002
5	End	14.49				2.1			8/21/2002
5	End'	12.71	13.60	14.18		14.1	8.1	7.1	8/21/2002

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SLI Study No. 3596.8

APPENDIX A

Statistical Analysis

BEFORE USE PURITY
(% GLYPHSATE (a.e.)
RAW DATA LISTING
TREATMENTS

GROUP	CONTROL (THEORECTICAL VALUE) 1	COMBINED RESULTS (FOR POOLED SAMPLE) 2 OBSERVATIONS
1	14.800	15.980
2	14.800	16.080
3	14.800	16.340
4	14.800	16.840
5	14.800	17.900
6	14.800	19.250
7	14.800	18.190
8	14.800	18.310
9	14.800	18.830
10	14.800	18.330
11	14.800	16.740
12	14.800	16.260
13	14.800	15.160
14	14.800	15.610
15	14.800	15.530
16	14.800	15.510
17	14.800	15.770
18	14.800	15.490
19	14.800	17.010
20	14.800	17.150
21	14.800	17.860
22	14.800	17.390
23	14.800	17.060
24	14.800	17.640
25	14.800	14.110
26	14.800	14.280
27	14.800	13.830
28	14.800	13.500
29	14.800	14.100
30	14.800	13.840

A N A L Y S I S OF V A R I A N C E PURITY BEFORE USE

SOURCE OF VARIATION	DF	SUM OF SQUARES	MEAN SQUARE
BETWEEN CLASSES	1	35.0982	35.0982
WITHIN CLASSES	58	74.8695	1.2909
TOTAL	59	109.9677	

F = 27.19, DF= 1/ 58, P=0.0000

GROUP: 1 2 MEANS: 14.80 16.33 S.D. : 0.000 1.607

TUKEYS TEST (2-tailed) ----------

GROUP	DF	PROB	T
1 VS 2	58	0.0000	7.374
2 #		0.0000	

* SIGNIFICANT AT .05
** SIGNIFICANT AT .01
\# SIGNIFICANT AT .001

BEFORE USE PURITY
% GLYPHOSATE (a.e.)
RAW DATA LISTING
TREATMENTS

	CONTROL (THEORECTICAL VALUE)	TEST ARTICLE MIXTURE NO.: OBSERVATIONS								
GROUP	1	1	2	3	4	5	6			
1	14.800	15.980	18.190	15.160	17.010	14.110				
2	14.800	16.080	18.310	15.610	17.150	14.280				
3	14.800	16.340	18.830	15.530	17.860	13.830				
4	14.800	16.840	18.330	15.510	17.390	13.500				
5	14.800	17.900	16.740	15.770	17.060	14.100				
6	14.800	19.250	16.260	15.490	17.640	13.840				

A N A L Y S I S OF V A R I A N C E BEFORE USE PURITY

SOURCE OF VARIATION	DF	SUM OF SQUARES	MEAN SQUARE
BETWEEN CLASSES	5	71.9557	14.3911
WITHIN CLASSES	30	14.6132	0.4871
TOTAL	35	86.5689	

F = 29.54, DF= 5/ 30, P=0.0000

GROUP: 1 2 3 4 5 6 MEANS: 14.80 17.06 17.78 15.51 17.35 13.94 S.D. : 0.000 1.280 1.024 0.200 0.342 0.278

TUKEYS TEST (2-tailed) ----------

GROUP	DF	PROB	T
1 VS 2	30	0.0001	7.949
1 VS 3	30	0.0000	10.447
1 VS 4	30	0.5015	2.498
1 VS 5	30	0.0000	8.955
1 VS 6	30	0.3017	3.007
2 VS 3	30	0.5015	2.498
2 VS 4	30	0.0068	5.452
2 VS 5	30	0.9791	1.006
2 VS 6	30	0.0000	10.956
3 VS 4	30	0.0001	7.949
3 VS 5	30	0.8951	1.492
3 VS 6	30	0.0000	13.454
4 VS 5	30	0.0010	6.458
4 VS 6	30	0.0062	5.504
5 VS 6	30	0.0000	11.962

2	#	0.0001
3	#	0.0000
4		0.5015
5	#	0.0000
6		0.3017

* SIGNIFICANT AT .05
** SIGNIFICANT AT .01
\# SIGNIFICANT AT .001

AFTER USE PURITY (STABILITY)
% GLYPHOSATE (a.e.)
RAW DATA LISTING
TREATMENTS

GROUP	CONTROL (THEORECTICAL VOLUME) 1	COMBINED RESULTS (FOR POOLED SAMPLE) 2 OBSERVATIONS
1	14.800	16.670
2	14.800	16.640
3	14.800	16.610
4	14.800	15.010
5	14.800	16.140
6	14.800	18.810
7	14.800	19.540
8	14.800	19.430
9	14.800	18.840
10	14.800	18.970
11	14.800	17.910
12	14.800	17.250
13	14.800	17.130
14	14.800	17.150
15	14.800	15.910
16	14.800	15.730
17	14.800	16.360
18	14.800	15.850
19	14.800	19.600
20	14.800	20.000
21	14.800	20.100
22	14.800	18.930
23	14.800	19.820
24	14.800	17.590
25	14.800	15.830
26	14.800	15.040
27	14.800	14.660
28	14.800	12.330
29	14.800	14.490
30	14.800	12.710

A N A L Y S I S OF V A R I A N C E AFTER USE PURITY (STABILITY)

SOURCE OF VARIATION	DF	SUM OF SQUARES	MEAN SQUARE
BETWEEN CLASSES	1	74.9284	74.9284
WITHIN CLASSES	58	128.0104	2.2071
TOTAL	59	202.9387	

F = 33.95, DF= 1/ 58, P=0.0000

GROUP: 1 2 MEANS: 14.80 17.03 S.D. : 0.000 2.101

TUKEYS TEST (2-tailed) ----------

GROUP	DF	PROB	T
1 VS 2	58	0.0000	8.240
2 #		0.0000	

* SIGNIFICANT AT .05
** SIGNIFICANT AT .01
\# SIGNIFICANT AT .001

AFTER USE PURITY (STABILITY)
% GLYPHOSATE (a.e.)
RAW DATA LISTING
TREATMENTS

OBSERVATIONS	CONTROL (THEORECTICAL VALUE) GROUP 1	TEST ARTICLE MIXTURE NO.: 1 2	2 3	3 4	4 5	5 6
1	14.800	16.670	19.540	17.130	19.600	15.830
2	14.800	16.640	19.430	17.150	20.000	15.040
3	14.800	16.610	18.840	15.910	20.100	14.660
4	14.800	15.010	18.970	15.730	18.930	12.330
5	14.800	16.140	17.910	16.360	19.820	14.490
6	14.800	18.810	17.250	15.850	17.590	12.710

A N A L Y S I S OF V A R I A N C E AFTER USE PURITY (STABILITY)

SOURCE OF VARIATION	DF	SUM OF SQUARES	MEAN SQUARE
BETWEEN CLASSES	5	125.3327	25.0665
WITHIN CLASSES	30	27.6537	0.9218
TOTAL	35	152.9865	

F = 27.19, DF= 5/ 30, P=0.0000

GROUP: 1 2 3 4 5 6 MEANS: 14.80 16.65 18.66 16.35 19.34 14.18 S.D. : 0.000 1.234 0.900 0.644 0.953 1.369

TUKEYS TEST (2-tailed) ----------

GROUP	DF	PROB	T
1 VS 2	30	0.0254	4.711
1 VS 3	30	0.0000	9.839
1 VS 4	30	0.0841	3.967
1 VS 5	30	0.0000	11.583
1 VS 6	30	0.8673	1.590
2 VS 3	30	0.0123	5.128
2 VS 4	30	0.9947	0.744
2 VS 5	30	0.0005	6.871
2 VS 6	30	0.0014	6.302
3 VS 4	30	0.0031	5.872
3 VS 5	30	0.8174	1.743
3 VS 6	30	0.0000	11.430
4 VS 5	30	0.0001	7.616
4 VS 6	30	0.0056	5.558
5 VS 6	30	0.0000	13.173

2	*	0.0254
3	#	0.0000
4		0.0841
5	#	0.0000
6		0.8673

* SIGNIFICANT AT .05 ** SIGNIFICANT AT .01
\# SIGNIFICANT AT .001

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SLI Study No. 3596.8

APPENDIX B

SLI Personnel Responsibilities

(30)

SLI Study No. 3596.8

SLI PERSONNEL RESPONSIBILITIES

Kimberly L. Bonnette, M.S., LATG	Study Director/Director, Acute Toxicology
Dawn D. Rodabaugh, B.S.	Alternate Contact/Toxicologist
Robert C. Springborn, Ph.D.	Chairman, President and CEO
Malcolm Blair, Ph.D.	Senior Vice President, Managing Director Emeritus
Joseph C. Siglin, Ph.D., DABT	Vice President, Managing Director
Jason W. Smedley, B.S.	Assistant Toxicologist
M. Gardner Clemons, B.A.	Manager of Analytical Chemistry and Pharmacy
Delores P. Knippen	Supervisor of Pharmacy
Anita M. Bosau, RQAP-GLP	Senior Director, Compliance Assurance
Deanna M. Talerico, RQAP-GLP	Senior Supervisor of Quality Assurance
Kathy M. Gasser	Supervisor of Archives

Annex 57

Letter by Ms Rebecca L. Puskas to the United States Environmental Protection Agency, 11 November 2008

(United States Embassy in Bogotá, 2011)

FOLEY HOAG LLP

Seaport World Trade Center West
155 Seaport Boulevard
Boston, MA 02210-2600

617 832 1000 *main*
617 832 7000 *fax*

Rebecca L. Puskas
Boston Office
617 832 3039

November 11, 2008

OIA
OPPTS
HQ-RIN-00275-09
Due: 12/17/08

Via Certified Mail #7001-0320-0002-1246-3449

U.S. Environmental Protection Agency
HQ FOIA Operations Staff (2822T)
Ariel Rios Building
1200 Pennsylvania Avenue, NW
Washington, DC 20460

Re: Freedom of Information Act Request – Office of Pesticide Programs

Dear Sir or Madam:

This is a request pursuant to the Freedom of Information Act (5 U.S.C. § 552). I am writing to request certain documents in the possession of the Environmental Protection Agency (EPA) Office of Pesticide Programs (OPP) regarding herbicide use for the aerial eradication of illicit coca in Colombia.

Please provide the following documents[1] or other materials described below:

(1) Any and all documents referenced or otherwise relied upon in the EPA's consultations with the U.S. Department of State (DoS) in connection with DoS approval of the aerial eradication program pursuant to the Andean Counterdrug Initiative section of the Foreign Operations, Export Financing, and Related Programs Appropriations Act. These consultations include, but are not limited to: "U.S. Environmental Protection Agency Office of Pesticide Programs Details of the Consultation for Department of State: Use of Pesticide for Coca Eradication Program in Colombia, August 2002" (EPA 2002 Analysis),[2] "U.S. Environmental Protection Agency Office of Pesticide Programs Details of the 2003 Consultation for the Department of State: Use of Pesticide for Coca and Poppy Eradication

[1] As used in this request, the term "documents" includes, without limitation, the following: studies, reports, memoranda, decision documents, records of decision, assessments, comments, consent decrees, notes, letters, telecopier transmissions, contracts, leases, tapes (audio or video), or any other written, recorded (including on disk or other computer format) or transcribed matter, including drafts.

[2] Available online at: http://www.state.gov/p/inl/rls/rpt/aeicc/13237.htm

NOV 1 [illegible]

Program in Colombia," June 2003 (EPA 2003 Analysis),[3] and "Letter and Consultation Report from EPA Administrator Leavitt," November 17, 2004 (EPA 2004 Analysis).[4]

(2) Any and all documentation of EPA consultations with DoS regarding the aerial eradication program between 2004 and the present.[5]

(3) Any and all documents regarding the composition of the chemical spray mix used in the aerial eradication program.

(4) Any and all documents considering the expected or actual impacts of the spraying program on human health and livelihoods, including, but not limited to, the impacts of direct human contact with off-target spray, impacts on human water supplies, crops and domesticated animals, and the effect of the spraying program on indigenous peoples.

(5) Any and all documents considering the expected or actual impacts of the spraying program on the environment, including, but not limited to, impacts on non-target plants, waterbodies, wildlife, biodiversity, endemic or endangered species, protected areas, soil health, and ecosystem-level effects.

(6) Any and all documents considering the expected or actual impacts of the spraying program on neighboring countries, including Ecuador and Venezuela.

(7) "Department of State (DoS) Presentation, DoS Coca Eradication Program, 4/18/02."[6]

(8) "Description of Use of Glyphosate in Coca Eradication in Colombia in attachment to a letter from Secretary of State Colin Powell to Environmental Protection Agency Administrator Governor Christine Whitman."[7]

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[3] Available online at: http://www.state.gov/documents/organization/27516.pdf

[4] Available online at: http://www.state.gov/p/inl/rls/rpt/aeicc/44455.htm

[5] There is no documentation available online regarding EPA's consultations with DoS about the spraying program since 2004 yet it appears the consultations have continued: "In 2006, the Secretary of State determined and certified to Congress identical conditions concerning human health and environmental safety issues, including endemic species. These certifications were based on, among other information ... verbal and written consultations on the spray program with USDA and EPA." DoS 2007, Memorandum of Justification Concerning the Secretary of State's 2007 Certification of Conditions Related to the Aerial Eradication of Illicit Coca in Colombia, available online at: http://www.state.gov/p/inl/rls/rpt/aeicc/111210.htm.

[6] This presentation is described as one of two key sources for the EPA 2002 Analysis.

[7] Cited in EPA 2002 Analysis, Section 1.

(9) Any and all reports, assessments and other documents of the Hazard Identification Assessment Review Committee (HIARC) regarding the human health effects of the spray mixture used in Colombia, including but not limited to, "HIARC Report for Glyphosate (TXR No. 0050428, W. Dykstra, 22-JAN-2002)."[8]

(10) Any and all documents concerning the EPA's approval under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug, and Cosmetic Act (FFDCA) of the inert ingredients in the glyphosate formulation used in Colombia. Without limiting the foregoing, please provide any non-exempt data submitted by the registrant for product approval under these statutes.[9]

(11) Any and all documents concerning the EPA's approval under the FIFRA and the FFDCA of the components of the adjuvant Cosmo-Flux 11F used in Colombia. Without limiting the foregoing, please provide any non-exempt data submitted by the registrant for product approval under these statutes and the letter cited in the EPA 2002 Analysis, "Letter from R.Forrest/EPA, to R.Woolfolk/DoS, 7/30/2001."[10]

(12) A June 28, 2002 memorandum entitled "Description of Glyphosate Use in the U.S. for Comparison to Use in Colombia for Coca Eradication from Virginia Werling and Timothy Kiely to Jay Ellenberger."[11]

(13) A report from the Department of Narino, Municipality of El Tablon De Gomez entitled "A Study of Health Complaints Related to Aerial Eradication in Colombia" and dated September 2001. This report was commissioned by the U.S. Embassy in Bogotá, Colombia.[12]

(14) Any and all environmental fate studies relied upon to produce the environmental fate assessment in the EPA 2002 Analysis.[13]

(15) Any and all documentation of inputs to the AgDrift model used to estimate the potential spray drift of glyphosate, including, but not limited to, default inputs.[14]

[8] Cited in EPA 2002 Analysis, Section 2.

[9] EPA 2002 Analysis, Section 2.

[10] EPA 2002 Analysis, Section 2.

[11] EPA 2002 Analysis, Section 2.

[12] EPA 2002 Analysis, Section 3.

[13] The EPA 2002 Analysis states that: "[t]he present environmental fate assessment is based on regulatory fate studies submitted to the Agency to support the registration of glyphosate salts and their formulated pesticide products." EPA 2002 Analysis, Section 4.

[14] EPA 2002 Analysis, Section 4.

(16) Any and all video tape recordings of spraying operations.[15]

(17) Any and all herbicide, formulant, or adjuvant labels, including but not limited to, the label for Cosmo-Flux 411F.[16]

(18) Any and all acute toxicity tests on the tank mix used in the aerial eradication program, including, but not limited to, "Evaluation of 6 acute toxicity studies conducted on test material identified as Spray–Charlie. (DP Barcode: D289806, 13-MAY-2003)."[17]

(19) "Interagency Soil and Water Sampling Field Study Report: Glyphosate Persistence in and Effects on the Soil and Bodies of Water."[18]

(20) Any and all documents related to herbicide runoff simulations conducted by the EPA to evaluate the potential impacts of the spraying program.[19]

Please contact me immediately at 617-832-3039 or at rpuskas@foleyhoag.com if you have any questions with respect to this request. In addition, please forward responsive documents to me as they become available, rather than waiting for all responsive documents to be identified.

This letter authorizes the expenditure of up to $1,000 in costs; please contact me if the amount will be greater.

Sincerely,

Rebecca L. Puskas

[15] The EPA 2002 Analysis states that: "[b]ased on video of spraying operations with multiple aircraft, the number of spray lines used in modeling was 4." EPA 2002 Analysis, Section 4, Table 1.

[16] The EPA 2002 Analysis suggests that these labels were evaluated by the Agency: "[t]here is some inconsistency in the description of Cosmo-Flux in the two available labels, in Spanish and English." EPA 2002 Analysis, Section 4.

[17] The EPA 2003 Analysis states that: "During April 18 briefing, the Department of State agreed to supply the Agency with a full battery of the six acute toxicity tests on the tank mix used in the coca aerial eradication program. That information has been received and reviewed. " EPA 2003 Analysis, p. 9.

[18] This document was reviewed by the Agency for the EPA 2004 Analysis.

[19] The EPA 2004 Analysis states that: "Using runoff simulations from Agency exposure models PRZM and EXAMS, the concentration that may result from direct application of 3.75 lb acid eq/acre of glyphosate to a 1-acre, 6-foot deep pond is 230 ppb...."

Annex 58

Embassy of the United States of America, List of Aerial Eradication Verification Mission since 1997

Appendix: *Implementation of the verification protocol January – July 1998*, carried out October 18-23, 1998

(United States Embassy in Bogotá, 2011)

Embassy of the United States of America

AERIAL ERADICATION MISSION REPORTS SINCE 1997

1. October 27-November 6, 1997; November 16-21, 1997: "Trip Report – Colombia Coca Eradication"
2. October 19-23, 1998: "Implementation of the Verification Protocol: January – July, 1998"
3. October 19-23, 1999: "First Implementation of the Verification Protocol for the Period January – July 1999"
4. December 3-8, 1999: "Second Implementation of the Verification Protocol for the Period July – September 1999"
5. September 11-22, 2000: "Verification Report, Colombia, September 2000"
6. December 9-20, 2002: "2002 Colombia Coca Eradication Report"
7. 2003 – 10th National Coca Crop Verification Mission, 2003
8. 2004 – 11th Verification Mission for Efficiency and other Comments on Illicit Coca Crop Spraying Operations during 2004
9. 2005 – 12th Mission for Efficiency Verification and other Comments on Illicit Coca Crop Spraying Operations during First Semester of 2005
10. 2006 – 13th Mission for Verification of Efficacy and Other Observations on Illicit Coca Crop Spraying Operations (October 2005 to February 2006)
11. 2006 – 14th Mission for Verification of Efficacy and Other Observations on Illicit Coca Crop Spraying Operations (March – August 2006)
12. 2007 – 15th Verification Mission on Efficacy and Other Observations on Illicit Coca Crop Spraying Operations (September 2006 – February 2007)
13. 2007 – 16th Verification Mission on Efficacy and Other Observations on Illicit Coca Crop Spraying Operations (March –August 2007)
14. 2008 – Technical Report – 17th Spraying Operation Verification Mission September 2007 – February 2008
15. 2008 – Technical Report – 18th Verification Mission of Spraying Operations done Between March and August 2008
16. 2009 - Technical Report – 19th Verification Mission of Spraying Operations done Between September 2008 and February 2009
17. 2009 - Technical Report – 20th Spraying Operation Verification Mission March –August 2009
18. 2010 - Technical Report – 21th Spraying Operation Effectiveness Verification Mission (Period: September 2009 – February 2010)

Santafé de Bogotá, D.C. November 13, 1998 Sprayed: Jan – July 1998

Mr. Luis Moreno
Director N.A.S.
US Embassy

Dear Mr. Moreno

Please find enclosed the report "Implementation of the Verification Protocol: January – July, 1998", carried out October 19 – 23, 1998 for coca plantations.

Thank you for your attention

Cordially,

Luis Eduardo Parra Rodriguez
Environmental Auditor – Illegal Crop Eradication

Enclosures

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⇒ GUAVIARE – META NUCLEUS
⇒ CAQUETÁ – PUTUMAYO NUCLEUS

"SATLOC" FLIGHT RECORDS FOR THE INITIAL SAMPLE. JANUARY – JULY 1998

APPENDIX 3: ON-SITE VERIFICATION OF SELECTED AND SPRAYED COCA SITES.

INTRODUCTION

The document being submitted to the National Narcotics Directorate – NND (Dirección Nacional de Estupefacientes – D.N.E), Narcotics Police Directorate – N.P.D. (Dirección Policía Antinarcóticos – DIRAN) and the Narcotics Affairs Section – N.A.S. of the US Embassy, represents the implementation of the verification protocol[1] signed by the governments of Colombia and the United States on the efficacy of area spraying using Glyfosate (fumigation) and the respective percentage of effective eradication of illegal coca plantations in the departments of Meta, Guaviare, Caquetá and Putumayo.

This report is based on a random sampling of the sites and/or lots sprayed between January and July 1998 as well as the respective aerial and in situ inspections. This report includes a technical memoir and a detailed photographic record to serve as illustration and evidence, as well as other attachments.

In general terms and according to the in situ verifications carried out by the three observers[2] the efficacy of the spraying program and, therefore, of the effective coca eradication for the above mentioned period is 91.23% ±12.64. This figure is obtained after daily processing and comparing the observations by the evaluation committee in the presence of all evaluators. Based on this effective eradication index it is possible to say that out of the 49,527.47 hectares that were sprayed, close to 45,184 hectares of coca plantations are completely dead and out of the production of cocaine hydrochlorate. Of the other 4,343.56 ha, most are abandoned and others are being cared for in small remaining lots or areas by some people (relatives or groups of people?) but with no indication of intent to continue with large areas.
It's important to note that only on two occasions was there an area with overspray detected. These can be considered isolated events that do not in any way affect the effective eradication percentage. It is also possible to adjust the final eradication figure for 1998 after analysis of the doublespray that might take place, including the last verification for August – December 1998. This will likely take place during the first half of March 1999.

Plante and the Ministry of the Environment were also invited to this process but these institutions were not able to attend for various reasons. The verification committee included interinstitutional and international participation from the following people and organizations.

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[1] This protocol was signed by the Ministry of Defense and the United States Embassy on November 19, 1995 based on the document *Joint Verification Procedures for Illegal Coca Plantations* prepared in October, 1996 and agreed to by DNE, DIRAN and N.A.S..

[2] The three (3) evaluators were Drs. Ch, Helling and R. Collins for N.A.S.-USA and Dr. Luis Eduardo Parra R. for Colombia. The other participants from various institutions acted as observers.

NAME	POSITION	ORGANIZATION
Doctor Fernando Puerta	Consultant – Director	D.N.E.
Major Luis E. Salamanca M.	Director – Illegal crop eradication division	DIRAN
Major Leonidas Molina T.	Narcotics Director – Eastern area	DIRAN
Lieutenant James Roa	Reconnaissance Director	DIRAN
Major Gustavo Ramirez	Lead Pilot – Helicopter Squad	DIRAN
Mr. David Becker	Assistant Director	N.A.S.
Mr. Mike Kenna	Aviation Consultant	N.A.S.
Mr. Lowell Neese	Aviation Consultant	DYNCORP – N.A.S.
Mr. Nathaniel Christie	Consultant – Director	N.A.S.
Mr. Julio Dennis	Aviation Consultant	DYNCORP – N.A.S.
Dr. Charles Helling	Scientific Weed Lab Director	USDA - ARS[3]
Doctor Ron Collins	Herbicide Scientist	USDA – ARS
Dr. Jayson Page	Interpreter – Analyst	CNC - Washington[4]
Dr. Anne Mogloon	Interpreter – Analyst	CNC –Washington
Dr. Luis E. Parra R.	Director	Environmental Auditor – Illegal crop Eradication

Finally, the invaluable aid provided by Colonel Jose Leonardo Gallego, Director of the Narcotics Police must be praised. He was always ready to ensure the aerial, logistic and detection resources for the proper performance of this important part of the Program. Also the efforts of the Air Service and DIRAN's operations group, and the international cooperation represented by N.A.S. and INL under the direction of Mr. Luis Moreno.

[3] USDA – ARS = U. S. Department of Agriculture Assets and Resources
[4] CNC = Crime and Narcotics Center, office that specializes in image and photographic analysis and interpretation.

2. BACKGROUND AND JUSTIFICATION

From the onset, the program has had its own verifications practices an procedures. These have been improved and complemented over time to arrive at the current procedure. Past experience and efforts are very important because they made it possible to build the current model and practices.

Verification has been aimed at general and detailed air reconnaissance, and on site reconnaissance activities. This procedure is a continuation of similar activities carried out from 1995 through 1997, except that now there are leading edge technological resources such as optical and radar satellite images, and differential G.P.S. and others.

The current procedure is justified by the need to have an agreed mechanism for verification and quantification of the results obtained from fumigation activities carried out in 1998 using Glyfosate spraying, bearing in mind the fact that the statistics about the efficacy of eradication in previous years (1995, 1996, 1997) showed discrepancies of varying orders and magnitudes. These were the reasons why the United States and Colombian Governments set up the Protocol mentioned above.

For this verification, Environmental Audit, in agreement with N.A.S. and DIRAN, prepared the following documents.

a. **Program for verification of Illegal crop eradication – 1998**
This document was delivered to the interested institutions, including the Ministry of the Environment, on October 6, 1998. The document established:

- Areas for reconnaissance in the Guaviare – Meta (Orinoco and Amazon River Basins) and Caquetá – Putumayo (Amazon River Basin) nuclei.
- Selection of samples for verification: size, space, representation and reliability. The sites were selected by analyzing SATLOC records, satellite images, etc.
- Criteria to estimate the effectiveness of eradication in terms of methodology, process, on site verification, overspray, etc.
- Participants in the verification process
- Evaluation of results.

b. **SPOT[5] Satellite Images**
This major technological resource was used for the first time for verification and became an important planning and implementation tool.

[5] These optical SPOT III and ERS-2 radar images are included in 87 spot views, on a 1:25,000 scale, and are an analog and digital representation of the main nuclei in Guaviare – Meta, such as San Jose, El Retorno, Calamar, Miraflores, Tomachipan and Mapiripan. These spot views totally eliminate subjectivity.

The following products were selected on the basis of these SPOT views delivered by SPOT Image to DIRAN.

- Spot views of existing nuclei and regions
- Cuts and work sheets for each selected lot
- SATLOC records of the sample lots and sites and
- Evaluation form to be filled out for each selected site

With these things in mind, the verification procedures is justified because this method makes it possible to determine, with a very small error margin, the efficacy of the eradication program. This is because the program makes technical and scientific use of existing technological resources including optical satellite images (SPOT and ERS-2 Radar), SATLOC records of aerial spraying, the Environmental Audit data base by region and municipality, DIRAN´s Illica records, transportation and security helicopters, SATLOC- and differential GPS-equipped airplanes.

ACTIVITIES

This verification required the following tasks:

DATE	ACTIVITY	REGION AND/OR MUNICIPALITY	COMMENTS
01-10-98/06-10-98	Preparation of the program for verification of illegal crop eradication for 1998	Meta, Guaviare, Caquetá and Putumayo	Included delivering documents to all institutions, interinstitutional discussion and adjustment. Activities carried out by Environmental Audit
07-10-98 / 17-10-98	Selection and determination of weighted sample for verification	Meta, Guaviare, Caquetá and Putumayo	Selection of spot views, preparation of image clippings and worksheets, selection of SATLOC records and delivery of documents. Activity carried out by Environmental Audit with cooperation from DIRAN and DYNCORP
16, 17-10-98	General aerial reconnaissance to major nuclei to be verified	Meta, Guaviare, Caquetá and Putumayo	Activity carried out together by DIRAN and Environmental Audit. Notes about safe routes and accessibility. Logistics.
19-10-98/23-10-98	On site verifications	Meta, Guaviare, Caquetá and Putumayo	Detailed aerial and ground (on site) verification carrie□d out by the committee

For security reasons intelligence, operations, operating groups, movements, etc. aimed at ensuring the safety of the verification committee and other participants are not included.

METHODOLOGY

Verification was carried out within the framework of the verification protocol and using as a basis the methodology proposed in the preliminary document. After the committee was established, the methodology was submitted to Environmental Audit, adjusted by all members of the committee, and defined by agreement among the parties.

4.1 SELECTION AND REPRESENTATION OF THE SAMPLE

The following criteria were used to determine this important issue.

a. Fumigation period to be verified

Verification was made of the sprayed illegal crops sprayed from January through July 1998. The area sprayed during this period in the departments of Guaviare, Meta, Vichada, Vaupés, Caquetá and Putumayo was 49,527.47 ha out of a total of 55,615 ha sprayed to October 31 1998, which represent 89.05% of the total sprayed to date.

b. Area to be verified

The verification program will be applied to the illegal coca crops sprayed between January and July 1998. In considering the logistics and geographic distribution aspects of the most important nuclei with illegal coca crops for verification, the nuclei were divided into two (2) major regions.

- Guaviare – Meta nucleus. This corresponds to the Amazon and Orinoco biomes
- Caquetá – Putumayo nucleus. This corresponds to the Amazon biome

The nuclei of illegal coca plantations located in the departments of Vichada and Vaupés were excluded from this verification because the sprayed areas are very small compared to the national total (0.59% and 0.704%). This means that the universe of sprayed areas to be verified represented actually 98.7% of the total spraying performed between January and July 1998. Table 1 shows a summary of the fumigations and their detailed participation.

c. Sample selection

Establishing a reliable and representative sample is the first step in achieving objective and credible results from verification. From the start it was decided that:

- The size of the sample should be at least 10% of the total area sprayed from January through July 1998.
- A statistical population distributed by region (municipality) would be used to select the sample, and its percentage weight compared to the total area sprayed in the country. The percentage weight for the region by month was also determined, and
- On the basis of these two (2) criteria, the days with the largest area sprayed for each month. Using these representative sample areas

TABLE 1
CONSOLIDATED NATIONAL TOTAL FOR THE ERADICATION OF ILLEGAL COCA PLANTATIONS
1998

DEPARTMENT	MUNICIPALITY	ACCUMULATED Total (ha)	JAN (ha)	FEB (ha)	MARCH (ha)	APRIL (ha)	MAY (ha)	JUNE (ha)	JULY (ha)	PERCENT OF TOTAL SPRAYED AREA
	Calamar	754.90	100.0	317.2	157.0		116.8		64.0	1.52
	El Retorno	2813.55		1073.0	600.3		481.9	83.8	574.6	5.68
GUAVIARE	Miraflores	18854.28	6196.8	4336.4	1493.1	1493.3	2844.4	855.2	1635.1	38.07
	San Jose del Guaviare	4771.06	961.5	1619.6	1541.2	311.4	193.3	49.1	94.9	9.63
	Subtotal	27193.79	7253.3	7346.1	3791.6	1804.7	3636.4	988.1	2368.5	54.91
	Mapiripan	1899.56		833.3	695.1	191.3			269.9	3.84
	Puerto Rico	2439.75		492.2	389.7		105.9	98.2		4.93
META	Vista Hermosa	425.37			100.8	216.7		107.9		0.86
	Subtotal	4764.68	1353.8	1326.4	1095.6	408.0	105.9	206.1	269.9	9.62
	Albania	424.65	260.9						163.8	0.86
	Cartagena del Chairá	4140.47	243.4			2466.3	1124.9	176.4	129.6	8.36
CAQUETÁ	Cunillo	739.89	345.5						394.4	1.49
	Milan	1966.11	713.9	66.3			533.3	235.7	416.9	3.97
	Montañita	61.0	61.0							0.12
	Puerto Rico	666.08					461.1		205.0	1.34
	Solano	2092.87	363.7	154.2		456.6	513.5	275.5	329.4	4.23
	Solita	2714.46	170.9	200.0			642.0	577.9	923.7	5.48
	Valparaiso	1381.22	428.4				336.0		616.8	2.79
	Subtotal	14186.75	2587.6	420.5	0.0	922.9	3810.7	1265.5	3179.6	287.6
PUTUMAYO	Puerto Guzmán	2736.72	126.1	0.0	0.0	219.1	746.9	1053.9	590.7	5.53
	Subtotal	2736.72	126.1	0.0	0.0	219.1	746.9	1053.9	590.7	6.5
VICHADA	Siare Guajibos	296.70		296.7						0.60
	Subtotal	296.70								
VAUPÉS	Carurú	348.83		68.9			279.9			0.70
	Subtotal	348.83		68.9			279.9	0.0	0.0	0.7
TOTAL COUNTRY		49527.47	11325.7	9457.7	4887.2	5354.7	8579.8	3513.6	6408.8	100.0
PERCENT OF MONTHLY AREA SPRAYED (%)			22.87	19.10	9.87	10.32	17.32	7.09	12.94	

Data current as of July 31 1998

Sources: Narcotics Police, SATLOC/PATHCOR activity report and daily operation support DYNCORP, Environmental audit Data Base

It was therefore decided to adopt a totally random sample of some of the lots that made up the initial sample of 10% or more, as shown in Table 2, using the following criteria:

- Final selection of the lots to be sampled was made preferentially by Dr. Helling and Collins from USDA-ARS.
- For the Guaviare nucleus it was agreed that, since SPOT satellite images were available, these would be used to superimpose the SATLOC records for the fumigation flights performed from January through July 1998.
- Each lot selected in Guaviare had a graphic record of the Lot, the clipping and the lot itself (seen Appendix 1), in addition to its SATLOC records.
- For the Meta and Caquetá-Putumayo nuclei, there being no satellite images, the sample lots were selected only on the basis of the SATLOC records from the initial sample (see Appendix 1); and
- Under these conditions, the experts agreed that the sample that was finally selected is **representative and reliable**. Therefore, the results are applicable to the totality of coca fumigation activity in terms of efficacy and effective eradication of these illegal crops during the period under consideration. As additional information, Appendix 2 shows the records for the initial sample and the respective flights or missions.

4.2 EFFICACY OF ERADICATION

Determining the area that has been effectively eradicated requires the use of agronomic observation techniques (physiological and toxicological) through objective criteria and the application of expertise in evaluating the damage that destroy or disable illegal coca plantation for the production of the drug (cocaine hydrochlorate).

Table 2

Determining the effective death of the illegal coca plantations sprayed with Glyfosate requires an evaluation of the following specific issues:

- Percentage death or control of the sprayed coca by rating or evaluation of the aircraft pass. (Pass is the effective coverage of the spray). Since the main spraying method uses parallel lines that define the pass of the aircraft, this is the best variable for rating the fumigation effort.

  This issue became key to determine the efficacy of the program and so it was agreed with the specialists from Washington, Drs. Helling, Collins and Page. This issue was rated using two (2) methods: detailed helicopter flights over the passes and over the lot itself, and using the evaluation of death of ten plants in three (3) different locations in the same lot where a landing or on site verification took place. This was done using a field questionnaire (Appendix 3).

- The SATLOC records of the flights or missions for the selected days were studied. Lots having the passes with greatest sprayed area or the nuclei with the largest number of adjoining passes were selected.

However, during the joint meeting with the American experts from USDA-ARS and CNC, it was decided that, verifying a sample of that size, even using a combined detailed aerial and ground reconnaissance (landing on some selected sites), in addition to being ambitious was not possible considering the hazardous conditions of public order (security) and the time available for the task.

TABLE. 2
SAMPLES OF COCA AREAS SPRAYED AND PERCENT SAMPLED

JANUARY - JULY 1998

DEPARTMENT	MONTH MUNICIPALITY	AREA SPRAYED (HA)							TOTAL SPRAYED (HA)	SAMPLE	
		JAN	FEB	MARCH	APRIL	MAY	JUNE	JULY		AREA (HA)	%
META	Puerto Rico	1353,8	492,2	389,7	-	105,9	98.2	-	2439,8	34,0	1,4
GUAVIARE	El Retorno	-	1073.0	600,3	-	481,9	83,8	574,6	2813,6	22,5	0.8
	Miraflores	6196,8	4336,4	1493.1	1493,3	2844,4	855,2	1635,1	18854..3	353.5	1.9
CAQUETÁ	Cartagena del Chairá	243,4	-	-	-	-	-	163.6	407.2	92.0	22.6
	Curillo	345,5	-					394.4	739,9	12,0	1,6
	Solita	170.9	200.0			842,0	577,9.	923,7	2714,5	45,0	1.7
	Valparaiso	428,4	-		-	336.0		616.8	1381,2	20.0	1,4
PUTUMAYO	Puerto Guzmán	126.1			219.1	746,9	1053.9	590.7	2736,7	70,0	2,6
	TOTAL	8864,9	6101.6	2483,1	1712,4	5357,1	2669,0	48991	2087,2	70.0	2.6

In order to make this a systematic rating[6], a table with the following characteristics or scales was used:

SCORE	EFFECTIVE DEATH RATE (%)
1	0 –50
2	50 – 75
3	75 – 90
4	>90

The issued to be rated, slightly more subjective but important nonetheless, is the lot or nucleus. One of the evaluators, Dr. Collins, preferred not to this because he considered too subjective and lacked sufficient methods for its application. Dr. Helling rated the nuclei or lots only for Caquetá and Putumayo.

Tables 3 and 4 show the lots that were ultimately sampled in the Guaviare-Meta and Caquetá-Putumayo nuclei respectively. Tables 5 and 6 show the quantitative results of evaluating the efficacy of spraying to determine the amount of coca eradicated and/or killed. In general, for January – July 1998, the national figure for the death of spayed coca plants is 91.23% ± 12.64%, not including any adjustments that could result from the double spray or overlap in the fumigation lines.

[6] This table was applied as of the second day of verification, i.e., in Miraflores, Caquetá and Putumayo and produced good results

TABLE 3
PLOTS FINALLY SELECTED FOR VERIFICATION
JANUARY - JULY 1998
GUAVIARE –META NUCLEUS

DATE SPRAYED	VERIFICATION DATE	DAYS SPRAYED	LOT NO.	PLOT	CUT	COORDINATES "SPOT" (ILLICO)		LOT AREA (ha)	LOCATION
						N	W		
26/01/98	20/10/96	266	1(10)	112	-	03°00.0583'	73°24.2483'	20.00	Puerto Rico – Meta
26/0198	20/10/98	266	2(11)	112	-	03°00.3619'	73°23.2855'	14.00	Puerto Rico - Meta
05/07/98	20/10/98	108	3(3)	190	D2	02°11.8166'	72°27.1500'	14.30	El Retorno - Guaviare
13/07/98		100							
05/07/98	20/10/98	108	4(4)	190	D2	02°11.1667'	72°26.6666'	8.21	El Retorno - Guaviare
13/07/98		100							
08/02/98	20/10/98	255	5(1)	264	A3	01°38.2500'	72°03.3334'	39.50	Miraflores - Guaviare
11/02/98		252							
27/04/98		177							
09/02/98	20/10/98	254	6(2)	264	C3	01°33.1000'	72°02.7501'	16.20	Miraflores - Guaviare
27/04/98		177							
05/01/98	20/10/98	289	7(3)	264	C4	01°33.2667'	72°00.3834'	31.00	Miraflores - Guaviare
09/01/98	20/10/98	285	8(4)	265	B1	01°35.1166'	71°59.7835'	12.74	Miraflores - Guaviare
09/01/98	20/10/98	285	9(5)	265	C2	01°33.3000'	71°56.6166'	53.87	Miraflores - Guaviare
17/02/98	20/10/98	246	10(6)	266	D3	01°32.2500	71°53.0000'	13.25	Miraflores - Guaviare
04/06/98	20/10/98	139	11(7)	283	A4	01°29.3500'	71°52.8501'	15.00	Miraflores - Guaviare
27/04/98	20/10/98	177	12(8)	283	B4	01°27.2334'	71°50.8501'	16.04	Miraflores - Guaviare
10/06/98	20/10/98	133	13(9)	283	C4	01°23.2000'	71°51.7501'	5.39	Miraflores - Guaviare
08/07/98		105							
20/03/98	20/10/98	215	14(10)	283	B2	01°26.5334'	71°56.2167'	15.49	Miraflores - Guaviare
19/02/98	21/10/98	245	15(11)	300	B3	01°17.3834'	72°04.0166'	15.66	Miraflores - Guaviare
19/02/98	21/10/98	245	16(12)	300	B3	01°16.7501	72°03.6668'	12.30	Miraflores - Guaviare
22/02/98		242							
19/02/98	21/10/98	245	17(13)	300	B2	01°15.1333'	72°05.5834'	8.50	Miraflores - Guaviare
22/02/98		242							
26/01/98	21/10/98	269	18(14)	300	C4	01°14.4667'	72°01.9166'	40.22	Miraflores - Guaviare
26/05/98		154							
18/03/98	21/10/98	218	19(15)	301	A1	01°10.8335'	71°59.7335'	6.83	Miraflores - Guaviare
18/03/98	21/10/98	218	20(16)	301	D1	01°10.8335'	71°59.7335'	51.57	Miraflores - Guaviare
TOTAL SAMPLE AREA								410.09	

Notes:

1. Plot number is the sequence established in the program. Cut is a square on the plot
2. There are no SPOT images for lots 1 and 2 in plot 112 so the coordinates were obtained from SATLOC.
3 Lot 2 could not be verified due to hostile fire from armed groups on two different occasions
4 Coordinates for lots 3 through 20 are for the center of the lot converted to SATLOC system for greater precision in the field. However. Illico presents them in the sexagesimal system and the
5. Lot are is precisely measured on the SPOT image. The number in parenthesis is the order assigned to the lot by the American inspectors in the field.

TABLE 4

LOTS FINALLY SELECTED FOR VERIFICATION
JANUARY – JULY 1998
CAQUETÁ – PUTUMAYO NUCLEUS

DATE SPRAYED	DATE VERIFIED	DAYS SPRAYED	LOT NO.	PLOT	SATLOC COORDINATES		LOT AREA (ha)	LOCATION
26/01/98	23/10/98	271	1(1)	61	01°03.9092'	76° 03,3823'	12	Curilla – Caquetá
05/06/98	23/10/98	141	2(2)	73	00° 53,8200'	75° 58,8000	10	Puerto Guzmán - Putumayo
05/06/98	23/10/98	141	3(3)	73	00° 56,1922'	75° 56,1678'	15	Puerto Guzmán - Putumayo
29/01/98	23/10/98	268	4(4)	73	00°55.1109'	75°57.5903	20	Puerto Guzmán - Putumayo
23/05/98	23/10/98	154	5(5)	73	00°54.2000'	75°54.3200'	25	Puerto Guzmán - Putumayo
02/05/98	23/10/98	175	6(6)	62	010°00.7136'	75°48.5959'	10	Solita – Caquetá
02/07/98	23/10/98	114	7(7)	62	01° 03,4626'	75°44,5799'	20	Valparaiso – Caquetá
02/05/98	23/10/98	175	8(8)	74	00°56,7700'	75°43,7072'	35	Solita – Caquetá
27/04/98	23/10/98	180	9(13)	137	00°29.6424'	74°14.3274'	30	Cartagena del Chairá – Caquetá
24/04/98	23/10/98	183	10(14)	119	00° 34,6866'	74°15,4479'	12	Cartagena del Chairá – Caquetá
18/04/98	23/10/98	191	11(15)	116	00°37,0179'	74°21,2587'	10	Cartagena del Chairá – Caquetá
15/04/98	21/10/98	192	12(16)	118	00°39,1007'	74°27.9887'	40	Cartagena del Chairá – Caquetá
TOTAL AREA OF SAMPLE							239.	

Notes:

1. Number in parenthesis represents lot order assigned by the American inspectors
2. Drs. Collins, Helling and Page selected a sample of 20 lots from the initial sample taken from the SATLOC records for the southwest (SW) and southeast (SE) regions in the Larandia base and a total of 12 lots were checked
3. Lot area was estimated o within ± 2 ha

TABLE 5

EVALUATION OF THE EFFICACY OF AERIAL SPRAYING IN ILLEGAL COCA PLANTATIONS
GUAVIARE - META NUCLEUS

INSPECTOR	SITE 10		PLOT 198				PLOT 254						PLOT 265									
	STEP		R-D2		R-D2		R-A3		R-C3		R-C4		RB-1		R-C2		R-D3		R-A4		R-B4	R-
	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%	NO	%	NO	%	NO	%	NO	%	NO	%
CHARLES HELLING	-	64	-	90	-	90	4	95	4	95	2	50	4	95	3	82.5	3	82.5	4	95	3	82
RON COLLINS	-	35	-	95	-	95	3	82.5	4	95	4	95	4	95	4	95	3	82.5	3	82.5	4	96
LUIS E. PARRA		90	-	100	-	100	4	95	4	100	4	100	4	95	4	100	4	100	3	95	4	96
ARITHMETIC MEAN		63.0	-	95.0	-	95.0	-	90.8	-	96.7	-	81.7	-	85	-	92.5	-	88.3	-	90.6	-	90
STANDARD DEVIATION		27.5	-	5.0	-	5.0	-	7.2	-	2.9	-	27.5	-	0.0	-	9.0	-	10.1	-	7.2	-	7.2
			-																			

Notes:

1. No. value given to the evaluation of the aircraft pass, expressed according to the following table - % effective death (%) (Scale of values)

SCORE	% EFFECTIVE DEATH
1	0-50
2	50-75
3	75-90
4	>90

2. When the inspector rated using only a numeric value (No.) the percent rating (%) was taken as the average value.....Drs. Helling and Collins gave their % rating for aircraft pass in plot 190. Lots 3 and 4 were not rated numerically because their was on-site verification
3. When the inspector rates spraying efficacy at 100% it means that the coca plants are completely dead and the lot.....
4. Plot: A 10 X 10 mile square (10 X 10 geographic minutes)
5. R-A1, R-C3, C-Cn. Cut: squares that make up a plot, equivalent to 1/16 of the plot
6. Table 1 also shows the number, location of the lots or site, according to plot and cut number

TABLE 6

EVALUATION OF EFFICACY OF AERIAL SPRAYING IN ILLEGAL COCA PLANTATIONS
CAQUETÁ – PUTUMAYO NUCLEUS

EVALUATION	SITE 1		SITE 2		SITE 3		SITE 4		SITE 5		SITE 6		SITE 7		SITE 8		SITE 13	
	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%	NO	%
CHARLES HELLING	4	95	3	80	4	90	4	90	4	90	1	30	2	50	4	100	4	100
RON COLLINS	4	95	3	82.5	4	95	4	95	4	95	4	95	4	95	4	95	3	82.5
LUIS E. PARRA	4	95	4	95	4	100	4	100	4	95	3	85	4	95	4	100	4	100
ARITHMETIC MEAN	-	95.0	-	85.8	-	95.0	-	95.0	-	93.3	-	70.0	-	80.0	-	98.3	-	94.2
STANDARD DEVIATION	-	0.0	-	8.0	-	5.0	-	5.0	-	2.9	-	35.0	-	26.0	-	2.	-	10.1

Notes:

1. No. value given to the evaluation of the aircraft pass, expressed according to the following table - % effective death (%) (Scale of values)

SCORE	% EFFECTIVE DEATH
1	0-50
2	50-75
3	75-90
4	>90

2. When the inspector rates using only a numeric value (No.) the percent rating (%) was taken as the average value.....
3. When the inspector rates spraying efficacy at 100% it means that the coca plants are completely ...
   In this case, Drs. Helling and Parra expressed the percent eradication for the pass and the lot.
4. SL: Site not identified by Dr. Ron Collins. This evaluation was not taken into consideration in finding the averages for Site 14
5. Table 2 shows identification, location and geographic coordinate for the sites that were evaluated

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 ERADICATION OF COCA PLANTATIONS

- The representation and reliability criteria established in the verification procedure and agreed with international cooperation (USA) were met with full scientific rigor.
- Therefore, the national average for effective eradication through fumigation or the death of coca plants is 91.23% with a standard deviation of ± 12.64%. The percent of eradication for the Meta-Guaviare nucleus is 91.12% ± 11.79% and for the Caquetá-Putumayo nucleus it is91.4% ± 14.1%.

5.1.1 Guaviare-Meta Nucleus

- Nineteen lots were verified in this nucleus, as follows: Two in Meta of which only in one was it possible to land and make an on site verification; in the other, verification was made quickly from the air because the helicopters were harassed by gunfire. These sites are located in Puerto Toledo, jurisdiction of Puerto Rico. This nucleus still contains 300-500 ha of coca plants in lots of more than 10 ha each that are being farmed intensively.
- In El Retorno, in the Guaviare nucleus, on site verification was made in two lots. The findings were 95% ± 4.47% effective control or eradication within the airplane path and on the lot itself. These lots were prepared and selected using SPOT images from December '97 and January '98 which were available at DIRAN. In the other nuclei in the municipalities of El Retorno, Calamar and San Jose del Guaviare, regional control and eradication of coca plantations is greater than 90% of the area that existed in 1994/1998. Today, there are no more than 500-600 ha in those municipalities, and the trend is toward smaller plantations (less than 2-3 ha), interspersed with tree cover (forest farming system) whose overhead cover is made up of *yarumos,* balsa wood and other widely distributed halophytic pioneer species.
- In Miraflores, in the Guaviare nucleus, 16 lots were verified using detailed aerial reconnaissance in slow circular overflights at ground level for each lot. A 92.39% ± 8.79% effective eradication was measured.
- In summary, effective eradication from spraying in the Guaviare/Meta nucleus, including doublespray[7], is 91.12% with ± 11.79 standard deviation. These results indicate

1. Actual decrease or effective reduction of planted areas by over 90% in January 1998.
2. This means that the El Retorno, Calamar and San Jose del Guaviare with their respective areas, do not exceed, taken together, 500-600 ha. The individual plantations are small – 4 or 5 ha each, and are located in marginal rural consolidated settled areas.
3. The Puerto Rico-Meta, as was said before, still includes around 300-500 ha that are located in an area that historically has been high risk due to attacks with firearms against spay planes and security helicopters. However, under current

[7] Evaluation of doublespray must be performed using a random sample from the SATLOC registry. As a very quick estimate, the figure would not exceed 5%. In this kind of spraying over illegal plantations, where it is not possible to foresee obstacles or high risk situations, overspray should be considered negligible. This is not commercial spraying. The efficacy of the spraying is more closely tied to other technical and environmental parameters

conditions, some 10 or 12 OV-10 or T-65 highly controlled precision missions would be enough to destroy the existing illegal plantations.

4. The Miraflores nucleus includes two (2) different sectors. The north sector located to the west, north and east of the runway which is over 85% controlled and covers an area no greater than 500-700 ha. And the south sector, located south of Lagos del Dorado, which is 60% controlled and whose remaining area is perhaps 1000-1500 ha.
5. The difference between the theoretical area after spraying and the estimated remaining areas can be explained by re-planting of small areas which, by itself, is very little. Very likely, if the eradication process continues, the country is approaching the possible scenario of a Guaviare without large coca plantations and small remaining areas that can be managed within the framework of an integrated strategy of eradication and alternate development.

5.1.2 Caquetá-Putumayo Nucleus

- This nucleus was divided into two (2) major sectors or sub-regions: the southeast is located in Caquetá and covers mainly the municipality of Cartagena del Cairá (Lower and Upper Caguán), and the southeast which is located in the departments of Caquetá and northwest of Putumayo. This sector includes the municipalities of Albania, Valparaiso, Milan, Solano, Solita and Curillo, among others.
- Verification in this nucleus consisted of twelve (12) lots or sites. In the southwest, including Puerto Guzmán, Putumayo, eight (8) lots were checked and the efficacy was found to be 89.06% ± 16.23. Four (4) sites were verified in the southeast where the efficacy of aerial spraying was found to be 96.59% ± 5.27. In general, effective eradication or death of coca plants in the Caquetá-Putumayo nucleus is 91.42% ± 14.10%.
- Based on the above, a similar trend can be seen as far as the efficacy of spraying in the Guaviare-Meta nucleus. However, the remaining area of illegal coca plantations in the department of Caquetá is greater than in Guaviare. Of the 16,923.5 ha sprayed, around 15,485.0 ha have been effectively eradicated.
- The lot size in the southwest is small to medium (2 – 8 ha) and a tendency towards forming larger groups or nuclei. In the southeast lots are mainly large (greeter than 10 ha) and sometimes 60 – 100 ha forming groups with considerable areas.
- The southeast, in view of the grouping pattern and large lots, is an area that con be conveniently sprayed using OV-10 –type platforms. This sector covers the area of Billar, Varadero, Cuba, Cubita, Lower Sunsiya River and the mouth where the Caguán river flows into the Caquetá.
- In this Caquetá-Putumayo nucleus intensive work only began in January 1998 with some interruptions in February and March of the same year. Although it is not yet possible to see a major impact or a significant decrease in the cultivated areas, there are already areas in this region where control is greater than 90% (such as Montañita and Albania). This is a positive trend and, if it continues, within one year the region could reach Guaviare's current level, i.e., that of a controllable scenario.

5.2 ABOUT ILLEGAL COCA PLANTATIONS

- In most of the lots that were inspected —either through aerial reconnaissance or on-site verification— it is possible to see residual areas that were not sprayed. Growers keep these small areas with good vegetable cover, ready for production, even though the crops would be smaller. (See Photographs VC 22-RVC 01/98 and VC 5A-RVC 02/98).
- These residual areas or "conejos" as they are commonly known, remain because they are very close to the jungle or to very tall trees that make it difficult to spray them properly, or because they are located between non-overlapping passes of the aircraft (See photograph no. VC 32-RVC 01/98).
- Elsewhere, small coca spots can bee seen (less than 1 ha) with plant in very poor condition, such as necrotic and twisted leaves, sparse foliage, stunted re-growth, descending necrosis, etc. These remains can be considered out of production (see photographs VC 13-RVC 01/98 and VC 24A-RVC 02/98) because the plants exhibit severe physiological damage.
- In Meta-Guaviare there has been a considerable decrease in deforestation of the Amazon and Orinoco jungles. This indicates that there has been no translation or metastasis from the coca plantations. In this nucleus it is not true that dead crops are replaced.
- In Caquetá, especially in the southeast sector (lower and middle Caguán) there is a very significant nucleus or large-scale illegal coca plantations. In this nucleus the impact of spraying has not discouraged cultivation. However, the balance in July 1998 is very encouraging because some nuclei with an excellent degree of control can be seen already (e.g., southeast Caquetá).
- Although Caquetá shows some areas in the process of deforestation, they do not have the same rate and level as in 1997. This is also an indication that illegal coca plantations have decreased in this region.
- In conclusion, the Meta-Guaviare nucleus shows a significant decrease in cultivated areas —close to 90%— by January 1998. However, it will be necessary to maintain control over this area to prevent the growth of existing illegal coca plantations and the creation of new nuclei In Caquetá there is still a large area planted with coca that could easily exceed 20,000-25,000 ha.

6. QUALITATIVE ENVIRONMENTAL ASSESSMENT OF ILLEGAL CROPS AND SPRAYING

The environmental disaster produce by illegal coca plantations from their start all the way through production (agricultural work) until the coca leaf is processed into cocaine base and cocaine hydrochlorate are very visible and undeniable. For the specific situation of this verification it is necessary to divide the major environmental effects resulting from these activities.

6.1 ENVIRONMENTAL IMPACT OF AERIAL SPRAYING

- Only twice in the whole sample was there evidence of overspraying or drift as a consequence of runoff over an area less than 500 m^2 (0,05 ha(in each case. These isolated events are the result of obstacles at the end of the fumigation run (large trees over 40 m tall) that force the pilot to perform a very risky maneuver and close the bypass very tight. (See Photograph VC 34A-RVC 02/98).
- In situations such as these it is recommended to close the bypass 20 m before the last obstacle in the spray line. This could lead to lots being left with more small areas that have not been sprayed. In conclusion, the sampled areas do not show any significant damage in terms of size or duration, from lateral drift or poor applications. On close inspection, the natural vegetation around or next to the sprayed lots continues its natural succession with the flora in the abundance and composition that are typical for this type of ecosystem (see photograph 17A-RVC 02/98).
- Shrubs and small tress of the *Yarumo, Balso and Manchador* species found within the coca lots die after being sprayed as do the few grain and herbaceous species as well as the few plantain or yucca plants associated with illegal coca plantations. (See photographs VC6-RVC and VC 23A-RVC 02/98).
- The effect on the interspersed vegetation is very localized and low magnitude compared to the destruction of the tree cover produced by deforestation and fires set to set up illegal coca plantations. (See photographs VC 30A-RVC 02/98 and VC 8-RVC 03/98.)
- There were no instances where the on site inspections showed damage to the insect, bird or reptile population. No spraying over bodies of water was observed, whether lakes, lagoons, streams or rivers.

6.2 ENVIRONMENTAL IMPACT OF ILLEGAL CROPS

- There is no denying that the greatest environmental impact caused by illegal coca plantations is deforestation and its consequences such as loss of biodiversity, destruction of priceless and unknown bio-genetic resources, exhaustion of significant germ plasm banks, potential sources for future wealth and well-being for mankind, damage to the soil from fires that lead to erosion and a decrease in edaphic, aquatic, terrestrial and aerial fauna.

  This large scale impact, in the case of Guaviare and Meta, has had a significant decrease in size. This means that the crops that have been sprayed and eradicated have not been replaced at the same rate of one for one, and they have not moved

deeper into the jungle, either The few crops that have been replaced have been re-planted or weeded out in the same fields that have been sprayed, with 10% maximum growing potential, or in areas that have secondary forest cover (*barbecho*). (See photograph VC 28A-RVC 02/98.)

- The cumulative environmental impact of deforestation (fires and fellings) in Guaviare and Meta during 1970 – 1996 caused a real forest massacre that became the first step towards turning the Colombian Amazon and Orinoco areas into pastures through the creation of extensive cattle ranches.

  In the specific case of the department of Meta the loss of tree cover caused by illegal coca plantation had a growing and devastating impact on the gallery forest ecosystems or "mountain plants" which are the only tree and shrub vegetation in the biome of the Orinoco plains. (See photograph VC 15-RVC 01/98). These forests are the places where all the ecological interactions vital for the biome in question take place. And these forests are also the vegetable mass that produce and regulate countless rivers and rivulets that have their origins here with their inherent water supply.

- In Caquetá, the new clearings or deforestation are greater than in Guaviare and are a major concern. In Putumayo the situation is very different It is characterized by large areas of highly technified illegal coca plantations, massive deforestation and uncontrolled fires. It would seem as is the big backlash of illegal plantations in the Orinoco and Amazon areas were taking place in Putumayo.
- In all lots verified from the air or on the ground, an aggressive natural regeneration process can be observed, with a large number of species. (See photograph VC 16-RVC 03/98). In general, it has been more than 180 days since these lots were sprayed. The excellent biological activity in the soils, both micro-fauna and arthropods, is also worth noting. This means that a good number of insects can be found mainly representatives of the annelids, termites, ants and spiders.
- The main species in natural regeneration present in the vegetable succession that are abundant and frequent in the Orinoco biome after spraying are listed below. Structurally, this succession is dominated by a thick herbaceous cover in grasses of the *Panicum sp.* and *Paspallum sp.*, among others.

COMMON NAME	SCIENTIFIC NAME	STRATUM
Guaramo – Yarumo	*Crecropia sp*	Tree
Tabaquilla	*Aegiphila sp.*	Tree
Tórtolo	*Schefiera morototoni*	*Tree*
Balso	*Ochroma piramidale*	Tree
Gualanday	*Jacaranda lassiogime.*	Tree
Tuno peludo	*Clidemia sp.*	Shrub
Cadillo	*Triumfetta sp.*	Shrub
Punta lanza	*Vismia laurifalia*	Shrub
Limoncillo	*Siparune sp,*	Shrub
Cucharo	*Myrsine sp.*	Shrub
Lechero	*Euphorbia sp-*	Herbaceous
Bledo	*Achyranthus sp.*	Herbaceous
Violeta montañera	*Sauvagosia sp,*	Herbaceous
Cucubo	*Solanum sp.*	Herbaceous
Trepador	*Stigmaphylum sp.*	Vine
Enredador	*Hippocratea sp.*	Vine
Rabo de zorro	*Andropugurn bicornis*	Herbaceous (grasses)

The main species in the pioneer succession of the Amazon biome are characterized by the fact that they belong to an abundant community of grasses and a large variety of shrubs and trees. Structurally, individuals belong to juvenile heliophitic species of large trees with a rich and varied substratum of very active arthropod fauna. Some of these species are:

COMMON NAME	SCIENTIFIC NAME	STRATUM
Guayabo	*Psidium guaiaba*	Tree
Mendrillo	*Clavija sp..*	Tree
Guarumo rosado	*Cecropia sp*	Tree
Dormilón	*Stryphnodendron sp.*	Tree
Chocho	*Ormosia sp.*	Tree
Cordoncillo	*Piper sp.*	Shrub
Frijolillo	*Clitoria sp.*	Shrub
Venadillo	*Conyza nonariensis*	Shrub
Mispero	*Bellucia sp.*	Shrub
Batatilla	*ipornea sp.*	Vine
Agraz	*Cissus sp,*	Vine
Granadilla de montaña	*Passiflora sp.*	Vine
Platanillo	*Calathea sp.*	Herbaceous
Cucubo	*Solanum sp,*	Herbaceous
Helecho	*Pitysograma sp.*	Herbaceous
Grama	*Paspallum sp.*	Herbaceous(grasses)
Palma	*Bactris sp*	Palm

Lastly, there were very few agricultural crops next or close to the illegal coca plantations. Some corn, plantain and yucca could be seen in small lots can bee seen, sometimes interspersed with coca lots. However, the cultivation patter is characterized by large patches surrounding primary forest, illegal plantations in different sizes in the middle of the jungle, medium and small illegal coca plantations in marginal areas, either rural or with consolidated settlements, and natural pastures or natural successions with extensive cattle raising. Non of the lots verified showed any damage from Glyfosate spraying.

PHOTOGRAPHIC RECORD

GUAVIARE – META NUCLEUS
Municipality of Puerto Rico – Meta
Municipality of El Retorno – Guaviare
Municipality of Miraflores – Guaviare

CAQUETÁ – PUTUMAYO NUCLEUS
Municipality of Cartagena del Chairá – Caquetá
Municipality of Solita – Caquetá
Municipality of Valparaiso – Caquetá
Municipality of Puerto Guzmán – Putumayo

NOTE: Contains also field report forms for on-site verification of sample lots.

ON-SITE INSPECTION OF COCA LOTS SPRAYED AND SELECTED

INSPECTOR'S NAME: LUIS EDUARDO PARRA SITE No: 10 DATE 20/10/98 TIME :3:15 PM

SATLOC COORDINATES	FIELD COORDINATES (HAND-TRIMBLE-GPS)	FLIGHT CODE	PLANT NO.	% CONTROL SECTOR A	CONTROL SECTOR C	% CONTROL SECTOR C	%TOTAL CONTROL
N03° 00.0583'	N 03° 00' 17.04"	A268DCAC	1	80	100	100	93,3
W 73° 24,2483'	W 73° 24' 322"		2	80	90	100	90.0
			3	85	100	85	90.0
			4	80	100	100	93,3
			5	80	100	90	90,0
	SPOT IMAGE INFORMATION		6	80	90	100	90,0
PLOT No.	CUT No.	LOT No.	7	85	85	190	86,7
			8	80	100	85	88,3
112			9	80	100	85	88,3
			10	65	85	100	90.0
ARITHMETIC MEAN				81,5	95	93.5	90.0
STANDARD DEVIATION				2.3	6.3	6.7	2.0
TYPICAL MEAN ERROR							

A. SPRAYING OUTSIDE THE TARGET OR ON OTHER PLANTS

None. Lot very overgrown with weeds. Plants are affected only by fumigation. Remaining plants exhibit curled leaves, without terminal buds and poorly formed re-growth.

B. DRIFT AND DAMAGE

No damage was detected in the surrounding trees, grasses. No drift was observed. Ninety per cent of the area is under control

C. CONDITIONS OF THE COCA PLANT AND OTHER COMMENTS

More than 90% of the lot is completely abandoned. Houses and labs no longer exist. (Photographs 28 and 29(. The initial lot was over 20 ha. In one part of the lot (± 2 ha) coca leaves were being picked from a sector that must not have been sprayed for any number of reasons.

ON-SITE INSPECTION OF COCA LOTS SPRAYED AND SELECTED

INSPECTOR'S NAME: LUIS EDUARDO PARRA SITE No: 3 DATE:: 20/10/98 1TIME: 12:15 PM

SATLOC COORDINATES	FIELD COORDINATES (Hand-Trimble-GPS)	FLIGHT CODE	PLANT No.	% CONTROL SECTOR A	%CONTROL SECTOR C	% CONTROL SECTOR C	TOTAL CONTR(%
N 02° 11.8168'	N 02° 11.531'	G058UQBC	1	100	100	100	100
W 72° 27.1500'	W 72° 27,180'	G058WOAC	2	100	100	100	100
		G138UQBC	3	100	100	100	100
			4	100	100	100	100
SPOT IMAGE INFORMATION			5	95	100	100	98,3
	A EN SW		6	100	100	100	100
PLOT No.	CUT No.-	LOT NO.	7	90	100	100	96.7
			8	100	100	100	100
190	D2	2396	9	100	100	100	100
			10	100	100	100	100
ARITHMETIC MEAN				98,5	100	100	99,6
STANDARD DEVIATION				3,2	0	0	1.1
TYPICAL MEAN ERROR							

A. SPRAYING OUTSIDE THE TARGET OR ON OTHER PLANTS

None. Perfect SPOT image. Lot size and shape can be seen clearly

B. DRIFT AND DAMAGE

Young *yaurmo* trees that covered the coca plants as agricultural crop died. No drift.

C. CONDITIONS OF THE COCA PLANT AND OTHER COMMENTS

Photographs 4, 5, 6 and 7, last aerial shot from the NW. Natural regeneration and succession process is exuberant.

ON-SITE INSPECTION OF COCA LOTS SPRAYED AND SELECTED

INSPECTOR'S NAME: LUIS EDUARDO PARRA; SITE No: 4 DATE 20/10/98 TIME :3:15 PM

SATLOC COORDINATES	FIELD COORDINATES	FLIGHT CODE	PLANT NO.	% CONTROL SECTOR A	% CONTROL .SECTOR C	% CONTROL SECTOR C	TOTAL CONTROL %
N 02° 11.1667'	N 02° 11.217'	G058UQBC	1	100	100	100	100
W 72° 26.6668'	W 72° 26.496'	G058WOAC	2	100	100	100	100
		G138UQBC	3	100	100	100	100
			4	100	100	1130	100
			5	100	100	100	100
	SPOT IMAGE INFORMATION		6	100	100	97	99.0
PLOT No.	CUT No.	LOT No.	7	100	100	98	99,3
			8	100	90	100	96,7
190	D2		9	100	100	95	98,3
			10	100	100	96	98.7
ARITHMETIC MEAN				100	99	98.6	99.2
STANDARD DEVIATION				0	3	1.9	1.0
TYPICAL MEAN ERROR							

A. SPRAYING OUTSIDE THE TARGET OR ON OTHER PLANTS

No damage to surrounding trees or bushes. No overspray or doublespray problems detected. The SPOT image application is perfect.

B. DRIFT AND DAMAGE

None

C. CONDITIONS OF THE COCA PLANT AND OTHER COMMENTS

Natural regeneration of grains, vines, palm tress and *yarumos* is abundant. Within the lot and in the aircraft pass some dead young balsa and *yarumo* trees could be seen (Photographs 1, 2 and 3, roll 1).

ON-SITE INSPECTION OF COCA LOTS SPRAYED AND SELECTED

INSPECTOR'S NAME: LUIS EDUARDO PARRA SITE No: 16 DATE 23/10/98 TIME :3:15 PM

SATLOC COORDINATES	FIELD COORDINATES (HAND-TRIMBLE-GPS)	FLIGHT CODE	PLANT NO.	% CONTROL SECTOR A	CONTROL SECTOR C	% CONTROL SECTOR C	%TOTAL CONTROL
N 00`° 19.1007'	N 00° 38.846'	D115SHNBC	1	100	100	100	100.0
W 74° 27.9887'	W 74° 28.130'		2	100	100	100	100.0
			3	100	100	100	100.0
			4	100	100	100	100,0
	SPOT IMAGE INFORMATION		5	100	100	100	100,0
			6	100	100	100	100,0
PLOT No.	LOT: No	CUT No	7	100	100	100	100.0
			8			100	100.0
			9	1100	100		
118			10	100	100	100	400,0
				100	100	100	100.0
ARITHMETIC MEAN				100	100	100	100,0
STANDARD DEVIATION				0.0	0.0	0,0	0,0
TYPICAL MEAN ERROR							

A. SPRAYING OUTSIDE THE TARGET OR ON OTHER PLANTS

Pass control: 100%. Lot control: 100%

B. DRIFT AND DAMAGE

None

C. CONDITIONS OF THE COCA PLANT AND OTHER COMMENTS

Photographs 11, 12, 13, 14, 15, 16 and 17. Excellent natural regeneration. High development of grains, *yarumos* and herbaceous plants. Lot shows excellent control

Annex 59

United States Department of State, Bureau for International Narcotics Matters, Herbicide Selection for Coca Eradication, May 1984

(United States Department of State, Bureau for International Narcotics Matters, May 1984)

Herbicide Selection for Coca Eradication

Prepared for:
U.S. Department of State
Bureau for International Narcotics Matters

Contract No: 2071-410014

May 1984

EXECUTIVE SUMMARY

The Bureau for International Narcotics Matters (INM), U.S. Department of State, is considering conducting field studies to evaluate the effectiveness of selected aerially applied herbicides in eradicating illicit coca plants in the tropics.

This report summarizes the selection of priority herbicide candidates from approximatey 175 herbicides under consideration for use in field studies. Selection criteria were based primarily on efficacy (potential to kill coca or other perennial woody plants), practicality for use in the tropics, and general safety (including environmental and human health hazards).

Six "priority herbicide candidates" were judged as having the greatest potential for field testing: 2,4-D, dicamba (e.g. BANVEL), dichlorprop (2,4-DP), glyphosate (e.g. ROUNDUP), picloram (e.g. TORDON), and triclopyr (e.g. GARLON). Brief synopses of their efficacy, general safety, and use are given.

PREFACE

This report, "Herbicide Selection for Coca Eradication", was prepared for the Bureau for International Narcotics Matters (INM), U.S. Department of State, under Contract No. 2071-410014. Mr. Robert Gifford was the contracting officer's technical representative; Mr. John McLaughlin, contract project officer, provided much of the guidance.

This report details the selection of the herbicides to be considered for the aerial eradication of coca. The six herbicides selected have been evaluated only for their use in field tests to be conducted by INM. These field tests are expected to be conducted in a way that will determine which herbicide is most effective in killing coca with the least adverse environmental and human health impacts. After these field tests have been conducted, and the results have been evaluated, INM may select a herbicide to be used in a coca eradication program.

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1.0 INTRODUCTION

The Bureau for International Narcotics Matters (INM), U.S. Department of State, is currently investigating the possible use of herbicides for the eradication of coca. In 1979 a study group for the United Nations Narcotic Laboratory examined methods for destroying illicit narcotic crops including: chemical (herbicidal), mechanical, fire, biological, and genetic (U.N.N.L. 1979). The U.N. group concluded that chemical methods for eradication are at present the best methods available. Other methods are not as practical for use in the tropics (e.g. requiring large labor forces and security) or are not adequately developed for use. INM's investigation focuses on the use of chemicals applied aerially to the plant foliage. The purpose of this report is to select and list those available herbicides which offer the greatest potential for coca eradication.

Because of the lack of research on the effectiveness of herbicides on coca, SRA project staff members approached this task by utilizing one basic assumption; herbicides that exhibit effectiveness on plants botanically similar to coca should be potentially effective on the coca plant. Therefore, herbicidal evaluations have been based on the effectiveness of the herbicide in killing perennial broadleaf weeds and woody plant species. These herbicidal evaluations have been developed through a screening process (discussed in Section 2) that eliminated herbicides unable to meet specific criteria. The screening process identified six priority herbicides for which brief synopses were developed summarizing

efficacy, general safety, and use. These six herbicides will be evaluated for use on coca in future field tests.

2.0 HERBICIDE SELECTION

All herbicides listed in the Herbicide Handbook of the Weed Science Society of America (WSSA 1983) were evaluated for use in the field test program for coca eradication. Farm Chemicals Handbook (Meister 1984) was used as a secondary reference to ensure a comprehensive review of all major herbicides.

2.1 Level 1 Selection Criteria

Approximately 175 herbicides were screened (WSSA 1983, Meister 1984) to include only those that meet the following criteria:

- Control perennial broadleaf weeds or woody plants, excluding those limited to suppressing vegetative growth and seedhead production.
- Currently registered with the U.S. EPA.
- Currently manufactured in the U.S.
- Developed for terrestrial applications, excluding those restricted to aquatic weed control.
- Developed for postemergence application.

The 55 herbicides meeting these criteria are presented in Table 1. Appendix A presents a detailed version of Table 1 that includes specific trade names of herbicidal products and their manufacturers.

2.2 Level 2 Selection Criteria

The herbicides presented in Table 1 were further screened on the basis of meeting at least one of the following criteria:

- Demonstrated positive herbicidal effects on coca.
- Demonstrated positive control of deep-rooted perennials, woody plants, or brush.
- Demonstrated use in tropical locations or on tropical food crops (e.g. sugarcane, bananas, pineapple, coffee), or use in similar conditions such as in the southern United States (i.e. Florida, Texas).
- Demonstrated use for rights-of-way or general vegetation control, field applications that are similar to the narcotics control mission.

Table 2 lists 24 herbicides that meet at least one of the above criteria.

Table 1

Level 1 Herbicide Candidates

(listed alphabetically)

- Acifluorfen - sodium
- Ametryn
- Amitrole
- AMS
- Asulam
- Atrazine
- Benazolin
- Bentazon
- Bifenox
- Borate (meta)
- Borate (octa)
- Borax
- Bromacil
- Butachlor
- Cacodylic acid
- CDAA
- Chloroxuron
- Chlorsulfuron
- Cyanazine
- 2,4-D
- 2,4-DB
- Dicamba
- 3,6-Dichloropicolinic acid
- Dichlorprop
- Diquat
- Diuron
- Endothall
- Fenac
- Fenuron
- Fenuron TCA
- Fosamine Ammonium
- Glyphosate
- Hexazinone
- Karbutilate
- MCPA
- MCPB
- Mecoprop
- Metribuzin
- Monuron TCA
- MSMA
- Nitrofen
- Oxyfluorfen
- Paraquat
- Pendimethalin
- Picloram
- Prometon
- Pronamide
- Propanil
- Simazine
- Sodium Chlorate
- TBA
- Tebuthiuron
- Terbacil
- Terbutryn
- Triclopyr

Table 2

Level 2 Herbicide Candidates

(listed alphabetically)

- Acifluorfen - sodium
- Ametryn
- AMS
- Asulam
- Atrazine
- Bromacil
- 2,4-D
- Dicamba
- Fenac
- Fenuron
- Fenuron TCA
- Fosamine Ammonium
- Glyphosate
- Hexazinone
- Karbutilate
- Oxyfluorfen
- Paraquat
- Picloram
- Sodium Chlorate
- TBA
- Tebuthiuron
- Triclopyr

2.3 Priority Herbicide Selection

The 24 herbicides in Table 2 were further evaluated for their degree of potential to eradicate perennial woody plants, their level of ability to perform in tropical environments, and their general safety.

The "Priority Herbicide Candidates" listed in Table 3 were judged to be the best candidates for field testing. Their primary advantages are that they: are primarily foliarly active, may be aerially applied, function at low application rates, may be used in combination with each other, and are not dependent on rainfall for activity.

Priority herbicides may be more effective in eradicating coca when used in combination (e.g. picloram may enhance the translocation of 2,4-D according to Kasasian, 1971). Those combinations thought to have the greatest potential are: 2,4-D and picloram (TORDON 101, TORDON RTU, AMDON 101); 2,4-D and dicamba (WEEDMASTER, BANVEL 520, BANVEL 720, BANVEL K, ACME Brush Killer 800, ACME Industrial Brushkiller); and 2,4-D and triclopyr (ESTERON BK).

Bromacil, hexazinone, and tebuthiuron would probably be effective for coca eradication; however they are primarily slow acting and are soil active. These compounds have other disadvantages such as usually requiring granular application and being dependent upon rainfall for activation.

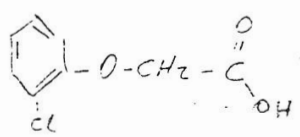
Table 3

Priority Herbicide Candidates for Coca Eradication

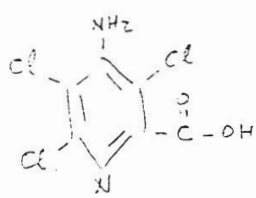
(listed alphabetically)

- 2,4-D { selectivo a gramíneas; translocable; Aplicación al follaje o suelo; toxicidad baja a dosis agudas
- Dicamba
- Dichlorprop
- Glyphosate
- Picloram ≅ 2, 4-D
- Triclopyr

PARAQUAT: No selectivo de contacto (no residual)
GLIFOSFATO: No selectivo, sistemico, translocable



2, 4-D (ácido 2, 4 - diclorofenoxiacético)



Picloram: ácido - 4 - amino - 3, 5, 6 - tricloropicolínico

Paraquat would probably be the most effective herbicide for simple defoliation or leaf dessication. Its potential to kill coca would be improved if used in combination with translocated herbicides such as 2,4-D, Dicamba, Picloram, or Triclopyr. Paraquat or other contact herbicides should not be applied at high rates when used in combination with a translocated herbicide. At excessive rates of Paraquat, leaf defoliation may occur before the other herbicide has effectively translocated from the leaf into the conductive tissues (Kasasian 1971, Johnson 1984). If a determination is made to field test paraquat, application rates will have to be adjusted to site-specific conditions.

The other herbicides in Table 2 were considered to be potentially less effective than those previously mentioned in this section, were considered impractical for use in the tropics, or required special safety considerations (e.g. TBA, Sodium Chlorate). Information for this section was primarily based on discussions with five herbicide field specialists: Curry (1984), Johnson (1984), McGlamery (1984), Palmer (1984), and Worsham (1984).

3.0 PRIORITY HERBICIDE CANDIDATES

In this section the six priority herbicides (Table 3) are discussed with respect to the following characteristics: efficacy, environmental impact, human health, logistics, and cost.

The components of these various characteristics are as follows:

- Efficacy
  - general herbicidal use relevant to coca
  - herbicidal absorption and translocation

- Environmental Impact
  - persistence in water and soil
  - toxicity to fish and wildlife

- Human Health Hazards
  - signal word*
  - outstanding hazards and precautions

- Logistics
  - method of application and carrier
  - relevant premixed trade products and mixtures

- Cost
  - actual herbicide costs

---

* The "signal word" appears on all EPA approved herbicide labels. These correspond to specific rankings into a "Toxicity Category" based on results of acute oral, dermal, and inhalation toxicity studies. In decreasing order of toxicity these signal words are: Danger, Warning, and Caution. (The signal word for some herbicide labels may be upgraded within the next 6 months by the U.S. EPA.)

3.1 Efficacy

2,4-D. 2,4-D, which controls broadleaf weeds, translocates within the phloem after foliar applications and upward in the transpiration stream after basal applications (WSSA 1983).

Studies are currently being conducted on the basal and foliar applications of 2,4-D on E. coca, but results have not been published (SSIE 1983, Gentner 1984).

Dicamba. Foliar and soil applications of dicamba will control perennial broadleaf weeds and woody brush species, including those species that are resistant to phenoxy herbicide treatment. Both leaves and roots of plants readily absorb dicamba. It readily translocates via the plant root systems or from the leaves (WSSA 1983.)

Dichlorprop. Dichlorprop controls a broad spectrum of weeds and is used for brush control in nonagricultural land. Dichlorprop translocates throughout the plant and is widely used in combination with other herbicides (WSSA 1983).

Glyphosate. Glyphosate exhibits effective control over deep-rooted perennial species, broadleaf weeds, and woody brush species. Glyphosate is absorbed through leaves and translocated throughout the plant. Translocation to underground systems of perennial species prevents regrowth and

Subterranean

results in subsequent destruction. Foliar application of glyphosate at 8.9 kg/ha in 187 l/ha defoliated coca but did not inhibit the regrowth of smaller leaves. The treatment therefore did not destroy the cambium layer and the plant was not killed (SSIE 1983).

Picloram. Picloram controls perennial broadleaf weeds, including deep-rooted herbaceous weeds and woody plants. Picloram is rapidly absorbed by leaves and roots and translocates both up and down in plants (WSSA 1983).

Triclopyr. Triclopyr controls woody plants and broadleaf weeds. Triclopyr is readily absorbed by leaves and roots and translocates both up and down in plants (WSSA 1983).

3.2 Environmental Impacts

2,4-D. When applied at recommended rates, 2,4-D persists in warm, moist soils for 1 to 4 weeks. 2,4-D ester is relatively toxic to fish and should be used with care in aquatic environments. Low volatile ester formulations are available and could be used to reduce possible off-target impacts (WSSA 1983).

Dicamba. When applied at recommended rates, dicamba is moderately persistent (Kasasian 1971). It will persist in moist soils for 3 to 12 weeks and may persist longer in soils exhibiting lower moisture levels. Under conditions of rapid metabolism, such as those found in tropical climates,

dicamba exhibits a half-life of less than 14 days. Dicamba exhibits a low order of toxicity to fish and wildlife (WSSA 1983).

Dichlorprop. Dichlorprop is chemically similar to 2,4-D and is expected to act similarly in the environment. It is toxic to fish and should not be applied when runoff is likely to occur or under conditions favoring spray drift (Union Carbide 1983, Johnson 1984).

Glyphosate. Glyphosate is a non-volatile herbicide and is relatively nonpersistent in soils when applied at recommended rates. Glyphosate exhibits a relatively low order of toxicity to fish and wildlife (WSSA 1983.)

Picloram. The persistence of picloram in soils may be considerable; depending on geographic location, climatic conditions, and rate of application (Kasasian 1971). Persistence is lower in warm, humid conditions. Picloram exhibits a low order of toxicity to fish and wildlife (WSSA 1983.)

Triclopyr. When applied at recommended rates, triclopyr exhibits moderate persistence in soils, with a half-life of 46 days depending on soils and climatic conditions (WSSA 1983, Johnson 1984).

3.3 Human Health Hazards

2,4-D. The signal word (see page 9) on 2,4-D labels is "Caution." The acid form is nearly twice as toxic as the butyl ester formulations. The oral LD50 for the acid form is 370 mg/kg in rats (Meister 1984). 2,4-D is believed to have little potential for causing human health problems (U.N.N.L. 1979).

Dicamba. The signal word for dicamba is "Caution." It is of a low order of acute toxicity (VELSICOL 1981). The oral LD50 is 1,707 to 2,900 mg/kg in rats (Meister 1984).

Dichlorprop. The signal word for dichlorprop is "Caution." It is of a low order toxicity to mice and rats. The oral LD50 is 800 mg/kg for rats (Meister 1984).

Glyphosate. The signal word for glyphosate is "Warning." Although moderately toxic, it is considered to be among the least toxic herbicides (U.N.N.L. 1979). The oral LD50 for rats is 4,300 mg/kg (Meister 1984). No cases of human poisoning have been reported (WSSA 1983).

Picloram. The signal word for picloram is "Warning." It is considered moderately toxic. The oral LD50 for rats is 8,200 mg/kg (Meister 1984). Picloram is not readily absorbed through human skin (WSSA 1983).

Triclopyr. The signal word for triclopyr is "Warning." It is categorized as slightly toxic. The oral LD50 in rats is 713 mg/kg. It is generally not a dermal irritant or absorbed through the skin (WSSA 1983).

3.4 Application

2,4-D. 2,4-D application is generally basal and foliar. Salts of 2,4-D are soluble in water. 2,4-D esters are soluble in oil and other organic solvents and thus are generally applied in the form of emulsions. In premixed trade products, 2,4-D is combined with other herbicides such as; dicamba, MCPP, dichlorprop, and MSMA. 2,4-D is often mixed with picloram, benazolin, and dicamba for herbicidal use. (WSSA 1983, Meister 1984.)

Dicamba. Dicamba is generally applied both basally and foliarly. Dicamba salts are soluble in water. In premixed trade products, dicamba is combined with 2,4-D and MCPA. Dicamba is often mixed with 2,4-D, dichlorprop, and numerous other herbicides for registered use. (WSSA 1983, Meister 1984.)

Dichlorprop. Dichlorprop is generally applied foliarly and is water soluble. In premixed trade products, dichlorprop is combined with bentazon, benazolin, 2,4-D, dicamba, and MCPA. (WSSA 1983, Meister 1984.)

Glyphosate. Glyphosate is generally applied foliarly and is water soluble. Glyphosate is not used in premixed trade products and is not generally used in mixtures. (WSSA 1983, Meister 1984.)

Picloram. Picloram is generally applied both basally and foliarly and is water soluble. In premixed trade products, picloram is combined with 2,4-D (WSSA 1983).

Triclopyr. Triclopyr is generally applied both basally and foliarly and is water soluble. In premixed trade products, triclopyr is combined with 2,4-D (Dow Chemical Co. 1983).

3.5 Cost

Cost information for the priority herbicides is presented in Table 4. The actual costs of herbicides are almost negligible when compared to the costs of aircraft acquisition, maintenance, and operation; or the use of back-pack sprayers and trained personnel. Therefore, efficacy, potential environmental impacts, and human health hazards will be used to evaluate the selection of the herbicides.

4.0 SUMMARY

This report summarizes the screening of approximately 175 herbicides under consideration for use in field studies for eradicating coca in the tropics. Selection criteria were based primarily on efficacy (potential to

Table 4
Cost Information for the Priority Herbicide Candidates

Herbicide Name	Trade Product Name (used only as an example)	% Active Ingredient (a.i.) or Acid Equivalent (a.e.)	Manufacturer's Name	Dollars/Gallon[1]	Maximum Recommended Rate/Acre[2]	Dollars/Acre at Recommended Maximum Rate	Dollars/Acre at 1/2 Recommended Maximum Rate	Dollars/Acre at 1/4 Recommended Maximum Rate
2,4-D	WEEDONE LV4	3.8 lb/gal a.e.	Union Carbide	$15 - $21	2 gallons	$30 - $42	$15 - $21	$7.50 - $10.50
Dicamba	BANVEL D	60.2% a.i.	Velsicol	$47 - $57	2 gallons	$94 - $114	$47 - $57	$23.50 - $28.50
Dichlorprop	WEEDONE 2,4-DP	3.7 lb/gal a.e.	Union Carbide	$17 - $23	2 gallons	$34 - $46	$17 - $23	$8.50 - $11.50
Glyphosate	ROUNDUP	41%	Monsanto	$65 - $75	4 gallons	$260 - $300	$130 - $150	$65 - $75
Picloram	TORDON 101M	10.2% picloram 39.6% 2,4-D	Dow Chemical	$20 - $30	6 gallons	$120 - $180	$60 - $90	$30 - $45
Triclopyr	GARLON 4	61.6% a.i.	Dow Chemical	$55 - $65	2 gallons	$110 - $130	$55 - $65	$27.50 - $32.50

1/ Ranges based on Regional Distributors' costs and should be interpreted relative to other cost ranges

2/ Maximum Recommended Rate as found on EPA-registered product labels for woody plants, broadleaf weeds or rights-of-way control.

kill coca or other perennial woody plants), practicality for use in the tropics, and general safety (including environmental and human health hazards).

Six "Priority Herbicide Candidates" were judged as having the greatest potential for field testing, these are: 2,4-D, dicamba (e.g. BANVEL), dichlorprop (2,4-DP), glyphosate (e.g. ROUNDUP), picloram (e.g. TORDON), and triclopyr (e.g. GARLON). Brief synopses of their efficacy, general safety, and logistics are given.

The priority herbicides when used in combination with one another or with other herbicides such as paraquat may be as effective as or more effective than when used singly. Therefore, combinations should be considered in the design of field tests.

APPENDIX A

APPENDIX A

Level 1 Herbicide Candidates -- Detailed Listing
(listed alphabetically)

COMMON NAME	PRODUCT NAME	MANUFACTURER'S NAME
Acifluorfen-Sodium	BLAZER 2S	Rohm and Haas
	TACKLE 2AS	Rhone-Poulenc
Ametryn	EVIK 80W	Ciba-Geigy
	CRISATRINE	
Amitrole	AMITROL-T	Union Carbide
	AMIZINE (amitrole + simazine)	Union Carbide
	AMIZOL	Union Carbide
	FENAMINE (amitrole, + fenac + atrazine)	Union Carbide
	KLEER-LOT (amitrole + linuron)	Union Carbide
	WEEDAZOL	Union Carbide
	AMINO TRIAZOLE	American Cyanamid
	CYROLAMINTROLE-T	American Cyanamid
AMS	AMMATEX-NI	Dupont
	Weed & Brush Killer	
Asulam	ASULOX	Rhone-Poulenc/May & Baker
	ACTRIL DS (asulam + ioxynil)	Rhone-Poulenc/May & Baker
	(CANDEX 70 (asulam + atrazine)	Rhone-Poulenc/May & Baker
	DIALAM (asulam + diuron)	Rhone-Poulenc/May & Baker
	TARGET (asulam + dalapon)	Rhone-Poulenc/May & Baker
	TALENT (asulam + paraquat)	Rhone-Poulenc/May & Baker
Atrazine	AATREX 80W	Ciba-Geigy
	AATREX Nine-0	Ciba-Geigy
	AATREX 4L	Ciba-Geigy
	AATREX 4LC	Ciba-Geigy

Appendix A (continued)

COMMON NAME	PRODUCT NAME	MANUFACTURER'S NAME
	ATRATOL 8P (atrazine, + sodium chlorate + sodium metaborate)	Ciba-Geigy
	ATRATOL 8P (atrazine + prometon)	Ciba-Geigy
	BICEP 4.5L (atrazine + metachlor)	Ciba-Geigy
	Atrazine 4L	Shell
	Atrazine 80W	Shell
	CO-OP Liquid Atrazine	Farmland Industries
	CO-OP Atrazine 80WP	Farmland Industries
	CO-Op ATRA-PRIL	Farmland Industries
Benazolin	LEY-CORNOX (Benazolin + 2,4-DB+ MCPA)	BFC Chemicals
	TRI-CORNOX Special (Benazolin, Dicamba + 2,4-P	BFC Chemicals
	BENAZALOX (Benazolin + 3,6-dichloropicolinic acid)	BFC Chemicals
Bentazon	BASAGRAN	BASF
Bifenox	Modown 2EC	Rhone-Polulenc
	Modown 80% WP	
	Modown4- Flowable	
Borate (Meta)	MONOBOR-CHLORATE	Occidental
	MONOBOR-CHLORATE GRANULAR	Occidental
	MONOBOR-CHLORATE GRANULAR D (+ diuron)	Occidental
	New Improved UREABOR	Occidental
Borate (Octa)	POLYBOR	U.S. Borax

Appendix A (continued)

COMMON NAME	PRODUCT NAME	MANUFACTURER'S NAME
Borax	BOROCIL (borax + boromacil)	Occidental
	UREABOR mixture (borax + monuron)	Occidental
Bromacil	HYVAR-X Weed Killer	Dupont
	HYVAR-XL Weed Killer	Dupont
	KROVAR I Weed Killer (bromacil + diuron)	Dupont
	KROVAR II Weed Killer (bromacil + diuron)	Dupont
	UREABOR	Occidental
	BOROCIL (borate + bromacil)	Occidental
	UROX B	Hopkins
	UROX HA	Hopkins
	ROUT G-8 (bromacil + diuron)	Hopkins
		Hopkins
Butachlor	MACHETE	Monsanto
Cacodylic Acid	RAD-E-CATE 25	Vineland
	PHYTAR 560	Crystal Chemical
	BOLLS-EYE	Crystal Chemical
CDAA	RANDOX	Monsanto
Chloroxuron	TENORAN 50W	Ciba-Geigy
Chlorsulfuron	"Glean" Weed Killer	Dupont
Cyanazine	BLADEX 80 WP	Shell
	BLADEX 4-WDS	Shell
	BLADEX 15G	Shell
2,4-D		
a) 2,4-D Amine	WEEDAR 64	Union Carbide
	RHODIA 2,4-D Amine No.4	Rhone-Poulenc
	DMA-4	Dow
	FORMULA 40	Dow
	AMINE 4D	Diamond Shamrock
	AMINE 6D	Diamond Shamrock
	WEED-RHAP A-4D	Vertac

Appendix A (continued)

COMMON NAME	PRODUCT NAME	MANUFACTURER'S NAME
2,4-D (cont'd)	WEED-RHAP A-6D	Vertac
	WEED-OUT AMINE	Farmland
b) 2,4-D oil Soluble Amine Salt	DECAMINE	Diamond Shamrock
	EMULSAMINE	Union Carbide
c) 2,4-D ester	WEEDONE LV-4	Union Carbide
	WEEDONE 638 (2,4-D acid + 2,4-D butoxyethyl ester)	Union Carbide
	RHODIA 2,4-D Low Volatile Ester (L.V.E.)4L	Rhone-Poulenc
	ESTERON 99 concentrate	Vertac
	ESTERON 76 BE	Vertac
	ESTERON 6E	Vertac
	WEED-RHAP LV 4D	Vertac
	WEED-RHAP LV 6D	Vertac
	LO-VOL 4D	Diamond Shamrock
	LO-VOL 6D	Diamond Shamrock
	BUTYL 4D	Diamond Shamrock
	BUTYL 6D	Diamond Shamrock
	WEED-OUT 4-L.V.E.	Farmland
	WEED-OUT 6-L.V.E.	Farmland
2,4-DB	BUTOXONE	Rhone-Poulenc
	BUTOXONE Ester	Rhone-Poulenc
	BUTYRAC 118	Union Carbide
	BUTYRAC 175	Union Carbide
	BUTYRAC Ester	Union Carbide
	LEY-CORNOX (2,4-DB, benazolin + MCPA)	BFC Chemicals
Dicamba	BANVEL	Velsicol
	BANVEL II	Velsicol
	TRI-CORNOX (dicamba, benzolin + 2,4-DP)	BFC Chemicals
	BANVEL 720 (dicamba + 2,4-D Damine salts)	Velsicol
	ACME Brush Killer (dicamba, 2,4-D + dichlorprop)	PBI/Gordon Corp.

Appendix A (continued)

COMMON NAME	PRODUCT NAME	MANUFACTURER'S NAME
Dicamba (cont'd)	ACME INDUSTRIAL BRUSH KILLER (dicamba, 2,4-D, Mecoprop)	PBI/Gordon Corp.
3,6-Dichloro-picolinic acid	LONTREL	Dow
	LONTREL 3	Dow
	LONTREL 205 (3,6-Dichloropicolinic acid + 2,4-D)	Dow
	BENAZALOX (3,6-trichloro acid + benazolin)	BFC Chemicals
Dichlorprop	WEEDONE 2,4-DP	Union Carbide
	WEEDONE 170	Union Carbide
	ENVERT 171	Union Carbide
	CORNOX RK 64	BFC Chemicals
	CORNOX RK Extra concentrate (2,4-DP + MCPA)	BFC Chemicals
	TRI-CORNOX Special (2,4-DP, benazolin + dicamba)	BFC Chemicals
	Dichlorprop (Tech)	BFC Chemicals
	Dichlorprop (Tech)	Dow
Diquat	Ortho Diquat	Chevron
	REGLONE	I.C.I.
Diuron	KARMEX Weed Killer	Dupont
	KROVAR I Weed Killer	Dupont
	KROVAR II Weed Killer	Dupont
	VELPAR K-4 Weed Killer	Dupont
	DREXEL DIURON 4L	Drexel
Endothall	ENDOTHAL (Tech.)	Pennwalt
	ACCELERATE (Endothall + ammonium sulfate)	
Fenac	FENATROL	Union Carbide
	FENATROL Industrial	Union Carbide
	FENATROL Plus (Fenac + 2,4-D)	Union Carbide

Appendix A (continued)

COMMON NAME	PRODUCT NAME	MANUFACTURER'S NAME
Fenac (cont'd)	FENAVAR (Fenac, + bromacil, amitrole)	Union Carbide
	FENAVAR Granular (fenac + bromacil)	Union Carbide
	FENAMINE (fenac, atrazine, + amitrole)	Union Carbide
Fenuron	BEET-KLEEN	Shell Chemicals
Fenuron TCA	DOZER	Hopkins Agricultural Chemical Co.
Fosamine Ammonium	KRENITE	Dupont
	KRENITE S	Dupont
Glyphosate	ROUNDUP	Monsanto
	MON-0139 (for experimental purposes only)	Monsanto
Hexazinone	VELPAR Weed Killer	Dupont
	VELPAR Gridball Brush Killer	Dupont
	VELPAR L Weed Killer	Dupont
	VELPAR K (hexazinone + diuron)	Dupont
Karbutilate	TANZENE	Ciba-Geigy
	FMC 11092	Ciba-Geigy
	NIA 11092	Ciba-Geigy
	TANZENE 80W (karbutilate + simazine)	Ciba-Geigy
	TANDEX	Ciba-Geigy
MCPA	CHIPTOX	Rhone-Poulenc
	RHOMENE	Rhone-Poulenc
	RHONOX	Rhone-Poulenc
	Bronate (MCPA + bromoxynil)	Rhone-Poulenc
	DOW MCP Amine Weed Killer	DOW
	WEEDAR Sodium MCPA	Union Carbide
	BROMINAL Plus	Union Carbide
	WEEDAR MCPA Concentrate	Union Carbide

Appendix A (continued)

COMMON NAME	PRODUCT NAME	MANUFACTURER'S NAME
MCPA (cont'd)	WEEDONE MCPA Ester	Union Carbide
	MCP AMINE 4	Diamond Shamrock
	Ley-Cornox (MCPA + Benazolin + 2,4-DB)	BFC Chemicals
MCPB	CAN-TROL	Rhone-Poulenc
	THISTROL	Union Carbide
Mecoprop	ISO-CORNOX 64	BFC Chemicals
	CHIPCO Turf Herbicide	Rhone-Poulenc
	MCPP	Rhone-Poulenc
	MCPP K-4	Diamond Shamrock
Metribuzin	SENCOR	Mobay Chemical Corp.
	LEXONE Weed Killer	Dupont
	LEXONE DF Weed Killer	Dupont
	LEXONE 4L Week Killer	Dupont
Monuron TCA	UROX	Hopkins
	UROX E Weed Killer	Hopkins
	UROX Liquid Weed Killer with 2,4-D	Hopkins
MSMA	ARSONATE Liquid	Diamond Shamrock
	BUENO	Diamond Shamrock
	BUENO 6	Diamond Shamrock
	DACONATE	Diamond Shamrock
	DACONATE 6	Diamond Shamrock
	DAL-E-RAD 70 + W	Diamond Shamrock
	DAL-E-RAD 120	Diamond Shamrock
	MESAMATE 400	Diamond Shamrock
	MESAMATE 600	Diamond Shamrock
	SUPER ARSONATE	Diamond Shamrock
	TRANS-VERT	Union Carbide
	WEED-E-RAD + W	Vineland Chemical Co.
	WEED-HOE-108	Vineland Chemical Co.
	WEED-HOE-120	Vineland Chemical Co.
	WEED-HOE-2X	Vineland Chemical Co.
	BROADSIDE (MSMA + cacodylic acid)	VERTAC Chemical Co.
	DIUMATE (MSMA + diuron)	VERTAC Chemical Co.
	MAD (MSMA + 2,4-D)	VERTAC Chemical Co.

Appendix A (continued)

COMMON NAME	PRODUCT NAME	MANUFACTURER'S NAME
Nitrofen	TOK E-25	Rohm and Haas Co.
	TOK WP-50	Rohm and Haas Co.
Oxyfluorfen	GOAL 2EC	Rohm and Haas Co.
	GOAL 25-W	Rohm and Haas Co.
	GOAL 1G	Rohm and Haas Co.
	GOAL 26	Rohm and Haas Co.
Paraquat	ORTHO PARAQUAT	Chevron Chemical Co.
	GRAMOXONE	ICI
	PATHCLEAR (Paraquat, + diquat and + simazine	ICI
	PARACOL (Paraquat + diuron)	ICI
	Terraklene (Paraquat + simazine)	ICI
Pendimethalin	PROWL	American Cyanamid Co.
	STOMP	American Cyanamid Co.
	HERBADOX	American Cyanamid Co.
	GO-GO-SAN	American Cyanamid Co.
	ACCOTAB	American Cyanamid Co.
	SIPAXOL	American Cyanamid Co.
	WAX UP	American Cyanamid Co.
Picloram	TORDON	Dow Chemical Co.
	TORDON 101 (Picloram + 2,4-D)	Dow Chemical Co.
	TORDON RTU (Picloram + 2,4D)	Dow Chemical Co.
	GRAZON	Dow Chemical Co.
	AMDON 101 (Picloram + 2,4D)	Union Carbide
Prometon	PRAMITOL 25E	Ciba-Geigy
	PRAMITOL 5Ps (Prometon, + simazine, sodium chlorate, + sodium metaborate)	Ciba-Geigy
	PRAMITOL 80WP	Ciba-Geigy
	CONQUER Liquid Vegetation Killer	Ciba-Geigy

Appendix A (continued)

COMMON NAME	PRODUCT NAME	MANUFACTURER'S NAME
Pronamide	KERB	Rohm and Haas Co.
Propanil	STAM M-4	Rohm and Haas Co.
	STAMPEDE 3E	Rohm and Haas Co.
	VERTAC Propanil 4	Vertac
	VERTAC Propanil 3	Vertac
	PROPANEX	Crystal Chemical Inter-America
	SUPERNOX	Crystal Chemical Inter-America
Simazine	PRINCEP 80W	Ciba-Geigy
	PRINCEP 4L	Ciba-Geigy
	PRINCEP 4G	Ciba-Geigy
	TANZENE 80W (simazine + karbutilate)	Ciba-Geigy
Sodium Chlorate	DEFOL	Drexel
	SODIUM CHLORATE	Pennwalt Corp.
	HARVEST AID	Kerr-McGee Chem. Corp.
	TUMBLEAF	Kerr-McGee Chem. Corp.
	UREABOR (sodium chlorate + sodium metaborate + bromacil)	J. R. Simplot Co.
	HIBOR C (sodium chlorate sodium metaborate + bromacil)	J. R. Simplot Co.
2,3,6-TBA	BENZAC	Union Carbide
Tebuthiuron	GRASLAN	Elanco Products Co.
	SPIKE	Elanco Products Co.
Terbacil	SINBAR	Dupont
Terbutryn	IGRAN 80W	Ciba-Geigy
Triclopyr	GARLON 3A	Dow Chemical Co.
	GARLON 4	Dow Chemical Co.
	ESTERON BK (Triclopyr + 2,4-D)	Dow Chemical Co.

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