



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

PESTICIDES
SUBSTANCES

OFFICE OF
PREVENTION,
AND
TOXIC

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 LD₅₀ (rat; MRID 45929403), acute dermal LD₅₀ (rat; MRID 45929402), acute inhalation LC₅₀ (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:

<u>Study Type</u>	<u>Tox. Cat.</u>	<u>Classification & MRID #</u>
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
MRID No.: 45929403

Reviewer: Byron T. Backus, Ph.D.

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
MRID No.: 45929402

Reviewer: Byron T. Backus, Ph.D.

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
MRID No.: 45929404

Reviewer: Byron T. Backus, Ph.D.

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 $\leq 4 \mu\text{m}$. The MMAD was $2.9 \mu\text{m}$, 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
MRID No.: 45929405

Reviewer: Byron T. Backus, Ph.D.

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

^aScore of 2 or more considered positive.

^bFluorescein 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
MRID No.: 45929406

Reviewer: Byron T. Backus, Ph.D.

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
MRID No.: 45929407

Reviewer: Byron T. Backus, Ph.D.

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 \pm (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 ≤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-Sensitizer	A

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