Caswell file

Glyphosate / Tox

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

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RELEASABLE

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MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: EPA Reg. # 524-308; Roundup; Glyphosate; Miscellaneous Data; Dermal ~~Sensitization~~ and Dermal Absorption
Caswell #: 661A
Accession #: 252142

TO: Robert Taylor
Product Manager (25)
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THRU: Robert P. Zendzian, ~~Ph.D.~~
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3/26/85

FROM: William Dykstra, Ph.D.
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William Dykstra 3/21/85

Conclusion:

1. All but one of the submitted studies are acceptable. The results show that Roundup and/or technical glyposate are not skin sensitizers in the guinea pig. Additionally, formulations of glyphosate did not significantly penetrate in vitro human skin samples. C^{14}-glyphosate was rapidly excreted in the urine of Rhesus monkeys following intramuscular injection. C^{14}-glyphosate was absorbed through the skin of Rhesus monkeys following dermal application but the absorption could not be quantitated. This study is unacceptable, since the majority of the dose could not be accounted for.

REVIEW: .

1. The dermal sensitization study in guinea pigs (Biodynamics Project # 4234-83, October 7, 1983).

Test Material: Roundup formulation; yellow liquid; Lot # LDRP 01-006.

Positive Control: 1-chloro-2,4-dinitrobenzene (DNCB); yellow granules, Lt # A1B

Negative Control: 0.85% saline; acetone.

a. Range-finding Study

A group of six Hartley albino guinea pigs were used to determine a slightly irritating concentration for topical induction and non-irritating concentration for the challenge application for the main study.

Six guinea pigs (3 male and 3 female) were treated topically with 100% test material (in 80% ethanol), 50%, 25% and 10% V/V (4 patches per animal) on the shaved skin and held in place under an impervious cuff for 6 hours. Following exposure, the test sites were washed and evaluted for irritation at 24 and 48 hours.

Results: As presented in Appendix A, no dermal irritation occurred in any test site.

Based on these study results, a 100% concentration was found to be non-irritating and was selected for both induction and challenge administration.

b. Main Study

In the sensitizations study, three groups of 5 male and 5 female Hartley albino guinea pigs were used. Group 1A was saline (negative control), II-A was DNCB (positive control) and III-A was Roundup formulation. In order to differentiate dermal reactions producted by irritation from those produced by sensitization, three groups of 3 male and 3 female untreated guinea pigs were subjected to the same challenge procedures as the animals which received the nine induction exposures of either the saline, DCNB or test material, (groups I-B, II-B, and III-B, respectively).

In the induction phase of the study, one induction dose (0.2 ml) was applied for 6 hours a day for 3 days per week for 2 weeks (total 9 exposures per animals). The induction material was applied to the right side of the midline of the shaved skin of the rabbit. Due to signs of severe irritation and tissue damage seen in some animals treated with 0.3% DNCB after the fourth, sixth, and eighth induction exposures, the location of the dosing site was adjusted so that patches were not placed over damaged skin. Animals were reclipped as needed. The evalution of demal response for the induction phase was 24 and 48 hours after each dose.

In the challenge phase, fourtheen days after last induction dose, animals were clipped free of hair and the test or control substance was administered in the same manner as in the induction phase but at a second site on the left side of the midline.

After 6 hours of exposure, the patches were removed and the skin wiped free of excess material. The evaluation of dermal response for the challenge phase was at 24 and 48 hours after dosing.

No statistical analysis of the data was performed.

Results

These were no deaths in the study. Body weight gain was comparable between control and treated animals.

The incidence of dermal response at challenge is shown in the table below taken from the report.

INCIDENCE OF DERMAL RESPONSE AT CHALLENGE

Group	Material	Interval Hr.	Dermal Scores[a] 0	+	1	2	3	Ed	N	E	Total # of Animals
1 A	Vehicle (Acetone)	24	10	0	0	0	0	0	0	0	10
		48	10	0	0	0	0	0	0	0	10
1 B	Vehicle (Acetone) (Irritation Control)[b]	24	6	0	0	0	0	0	0	0	6
		48	6	0	0	0	0	0	0	0	6
IIA	DNCB (0.3%)	24	0	0	1	7	2	8	0	0	10
		48	0	1	1	7	1	9	0	0	10
1IB	DNCBc(0.3%) (Irritation Control)[b]	24	6	0	0	0	0	0	0	0	6
		48	6	0	0	0	0	0	0	0	6
IIIA	Roundup Formulation (Challenge)	24	10	0	0	0	0	0	0	0	10
		48	10	0	0	0	0	0	0	0	10
IIIB	Roundup Formulation (Challenge Irritation Control)[b]	24	6	0	0	0	0	0	0	0	6
		48	6	0	0	0	0	0	0	0	6

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[a] Scored using the scale presented in Appendix B.

[b] Irritation control groups were treated at challenge only. The same six animals were used for Groups IB and IIB.

Ed=Edema; N=Necrosis; E=Eschar

Very slight to moderate dermal irritation was observed in all animals treated with Roundup beginning at the third induction dose. Since results at challenge indicated no sensitization had occurred, the dermal irritation was considered due to cumulative irritation.

Conclusion: Roundup formulation is not considered a skin sensitizing agent.

Classification: Core Minimum Data.

2. A dermal sensitization s;tudy in guinea pigs with glyphosate (Biodynamics Study # BD-83-008; 7/22/83).

Test Material: Technical glyphosate; Lot # NBP 1782608; 99.7% purity; white powder.

Positive control Material: 2,4-dinitrochlorobenzene (DNCB); yellow granular solid.

Vehicles: 80% ethanol, acetone.

Negative Control Material: Saline

a. Range-finding Study

A group of six Hartley albino guinea pigs were used to determine a slightly irritating concentration for topical induction and non-irritating concentration for the challenge application.

Six guinea pigs (3 male and 3 female) were treated topically with 100% test material (in 80% ethanol), 50%, 25% and 10% (V/V) (4 patches per animal) on the shaved skin and held in place under an impervious cuff for 6 hours. Following exposure, the test sites were washed and evaluated for irritation at 24 and 48 hours.

Results:

Based on the result of this study in Appendix A, a 100% concentration was found to be non-irritating and was selected for both induction and challenge administration. No dermal irritation occurred in any test site.

b. Main Study

In the sensitization study, three groups of 5 male and 5 female Hartley albino guinea pigs were used. Group I-A was treated with saline (negative control), II-A was treated with DCNB (positive control) and III-A was treated with 100% glyphosate. In order to differentiate dermal reactions produced by irritation from those produced by sensitization, three groups of 3 male and 3 female untreated guinea pigs were subjected to the same challenge procedures as the animals which received the nine induction exposures of either saline (IB), DCNB (group II-B) or 100% glyphosate (III-B).

In the induction phase of the study, one induction dose (0.2 ml) was applied for 6 hours a day for 3 days a week for 3 weeks (total of 9 exposures per animal). The induction material was applied to the right side of the midline of the shaved skin of the rabbit.

Due to signs of severe irritation and tissue damage seen in some animals treated with 0.3% DCNB after the fourth, sixth and eighth induction exposures, the location of the dosing site was adjusted so that patches were not placed over damaged skin. Animals were reclipped as needed. The evaluation of dermal response for the induction phase was 24 and 48 hours after each dose.

In the challenge phase, fourteen days after the last induction dose, animals were clipped free of hair and the test or control substance was administered in the same manner as in the induction phase, but at a second site on the left side of the midline.

After 6 hours of exposure, the patches were removed and the skin wiped free of excess material.

The evaluation of dermal response for the challenge phase was at 24 and 48 hours after dosing.

No statistical analysis of the data was performed.

Results

There were no deaths. Bodyweight gain was comparable among all groups.

The incidence of dermal response at achallenge is shown in the table below taken form the report.

INCIDENCE OF DERMAL RESPONSE AT CHALLENGE

Group	Material	Interval Hr.	Dermal Scores[a] 0	+	1	2	3	Ed	N	E	# Total of Animals
1 A	Saline	24	10	0	0	0	0	0	0	0	10
		48	10	0	0	0	0	0	0	0	10
1 B	Saline (Irritation Control)[b]	24	6	0	0	0	0	0	0	0	6
		48	6	0	0	0	0	0	0	0	6
IIA	DNCB (0.3%)	24	0	0	2	8	0	7	0	0	10
		48	0	0	7	3	1	6	0	0	10

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INCIDENCE OF DERMAL RESPONSE AT CHALLENGE (Cont'd.)

IIB	DNCB (0.3%) (Irritation Control)[b]	24	4	2	0	0	0	0	0	0	6
		48	6	0	0	0	0	0	0	0	6
IIIA	Glyphosate	24	10	0	0	0	0	0	0	0	10
		48	10	0	0	0	0	0	0	0	10
IIIB	GLYPHOSATE (Irritation Control) [b]	24	6	0	0	0	0	0	0	0	6
		48	6	0	0	0	0	0	0	0	6

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[a] Scored using the scale presented in Appendix B.

[b] Irritation control groups were treated at challenge only. The same six animals were used for Groups IB and IIB.

Ed=Edema; N=Necrosis; E=Eschar

Slight to severe irritation was observed in all animals treated with glyphosate beginning with sixth induction dose. Since results at challenge indicated no sensitization had occurred, the dermal irritation was considered due to cumulative irritation.

Conclusion: Glyphosate technical was not considered a skin sensitizing agent.

Classification: Core Minimum Data.

3. (a) Elimination of C^{14}-Glyphosate in Rhesus monkeys following a single dose.

(b) Percutaneous absorption of C^{14} Glyphosate in Roundup® formulation in Rhesus monkeys following a single topical dose. (Howard Mibach, M.D., U. California School of Medicine; Study # MA-81-349; 4/1/83)
Study (a). Four adult male Rhesus monkeys each received a single one ml. dose of C^{14}-glyphosate (specific activity of 84.6 micro curies/millimole, mulecular weight of 169.1) by intramuscular injection into the thigh.

Urine samples were collected at 4, 8 and 12 hours the first day, then every 24 hours for seven days. A 5 ml. aliquot of each urine sample was assayed by liquid scintillation spectrophotometry.

Results: Peak excretion was 0-4 hours. The urine contained an average total of 89.9% of the C^{14} labeled glyphosate. The C^{14}-labelled gylyphosate had an average elimination half-life of 19.7 hours; however, two-phases of excretion were noted. The first phase had a T-1/2 of 6.9 hours and the second phase had a T-1/2 of 35.1 hours.

Conclusions: The C^{14}-glyphosate was rapidly excreted in the urine following intramuscular injection.

Classification: Acceptable.

Study (b). Six male Rehesus monkeys each received a single dose of 0.80 microcuries of C^{14}-labeled glyphosate (specific activity of 9.4 microcuries/millimole, (M.W. of 169.1). Twenty-five microliters of the glyphosate preparation was applied over the shaved skin of 7.9 square centimeters of abdomen. After 24 hours, the site of application was washed two times with distilled water, two times with acetone, then two times with distilled water. Washes were measured by Liquid scintillation. Urine samples were collected at 4, 8, and 12 hours the second day, then every 24 hours for 7 days. Urine content of C^{14} was measured by liquid scintillation.

Results: The washing procedure removed 14.2% average of the applied C^{14} glyphosate. The urine contained an average corrected total value of 1.8%. Peak excretion occurred between 8-36 hours.

The total percent recovery (percent label removed by washing plus percent label contained in urine) was low, e.g., 16%. The C^{14}-label on the glyphosate

had an average elimination T-1/2 of 59 hours.

All calculated excretion values were corrected for incomplete urinary excretion with a parenteral excretion factor of 89.9%.

Conclusion:

The low total recovery make it impossible to quantitate the dermal absorption of C^{14} glyphosate in this study. The author of the report writes. "Although a definitive explaination can not be offered for the low recovery, previous experience suggests that much of the test material may in some way bind to or in the skin and can not be removed by washing. This bound material is not apparently available for systemic absorption." This supposition cannot be distinguished from the possibility that the unrecovered material was lost from the skin.

If the first possibility, binding to the skin, is true one must assume that ultimately this material will be absorbed and the total absorption over time will be in the order of 85%. If the second possibility, loss of material from the skin, is true one must assume that the acutal skin dose is only in the order of 16% of the intended dose and the absorption is in the order of 13%.

Classification: Unacceptable. The majority of the dose could not be accounted for.

4. Evaluation of the percutaneous absorption of Roundup formulations in man using an in vitro technique (Thomas J. Franz, M.D., School of Medicine, University of Washington: Study # UW-81-346; 8/30/83).

Test Material: C^{14} labeled glyphosate from three formulations

a) Roundup formulation; Lot #s: 2313601, 2313920-B

b) Mon 0139; Lot #s: 2313602, 2440952-E

c) Roundup Spray Solution; Lot #s: 2313601, 2313920-C.

Human cadaver skin taken from the abdomen was used to determine the absorption of each of the three glyphosate formulations which were labeled with C^{14}-glyphosate.

The method consisted of mounting skin between two specially constructed glass chambers. The dermis was bathed by isotonic saline at pH 7.4 and 37°C and the epidermis was exposed to air. A 10 microliter sample of each formulation was applied to the epidermis and absorption was determined by removing the dermal solution and analyzing it for radioactive content. Sampling was done a 1, 2, 4, 6, 8, 12, 24, 36 and 48 hours after application.

Results: By using tritiated water, it was determined that water penetration ranged form 0.06 to 0.49% of the applied dose. These data demonstrated that the integrity and viability of the skin samples were sufficient for conduct of the test.

Glyphosate recovery form the dermal bathing solution and from the dermis were low.

Total recovery of the applied dose form the epidermis and washes was approximately 100% as shown in the following table taken form the report.

	Percent of Dose (Mean ± SD)		
	MON 0139	Roundup Formulation	Roundup Spray Mix
Skin Washes	98.93 ± 10.80	100.85 ± 5.66	92.09 ± 12.97
Epidermis	0.04 ± .05	0.14 ± .28	4.02 ± 4.05
Total Absorbed	0.028 ± .019	0.063 ± .074	0.152 ± .101
Total Recovery	99.00 ± 10.80	101.05 ± 5.67	96.26 ± 13.59

Conclusions: The three glyphosate formulations were poorly absorbed through human cadaver skin in vitro. The epidermis provided the most effective barrier to absorption and approximately 97% of the applied dose of glyphosate could be removed by water rinses.

Classification: Acceptable.