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Irritation, sensitization, photoirritation and photosensitization assays with a glyphosate herbicide

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Glyphosate, a widely utilized herbicide, was evaluated for acute irritation, cumulative irritation, photoirritation and allergic and photoallergic contact potential in 346 volunteers. The herbicide was less irritant than a standard liquid dishwashing detergent and a general all purpose cleaner. There was no evidence for the induction of photoirritation, allergic or photoallergic contact dermatitis. 10% glyphosate in water is proposed as a diagnostic patch test concentration.

Key words: Glyphosate – irritation – cumulative irritation – phototoxicity – photoallergic contact dermatitis – patch test concentration – herbicide.

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Glyphosate is a well-known herbicide which is applied as a foliage spray for the control or destruction of most herbaceous plants. First sold for non-crop industrial uses in 1974, its authorized uses have expanded to agricultural, recreational and public area and road side applications as well as home use. Methods of application vary from hand-held sprayers to specialized large scale spray equipment.

Because in vivo techniques for evaluating a compound's potential to initiate dermatitis have become increasingly predictive due to experimental sophistication and accuracy, further evaluation of its potential for causing dermatitis was desirable.

This study was initiated to evaluate a glyphosate herbicide's potential to cause cumulative irritation, allergic contact dermatitis, photoirritation and photoallergic reaction, and to provide data permitting estimation of an appropriate diagnostic patch test concentration. In addition to the herbicide, 3 well-known household compounds were used as comparative controls.

Material and Methods

Test compounds

(i) Glyphosate herbicide, (Roundup®, Monsanto Company, St. Louis, Missouri 63167) (see Fig. 1). Glyphosate is N-(phosphonomethyl) glycine; the formation utilized contained 41% glyphosate. The test compound herbicide is 98.4% pure and contains the isopropylamine salt of glyphosate, water and surfactant.

(ii) General all purpose cleaner (Pinesol Liquid®, American Cynamid, Wayne, New Jersey 07470).

(iii) Shampoo (Johnson Baby Shampoo®, Johnson & Johnson, New Brunswick, New Jersey 08933).

(iv) Liquid dishwashing detergent (Ivory Liquid®, Proctor & Gamble, Cincinnati, Ohio 45201).

Test compounds were used at full strength and diluted (10% v/v) in distilled water. Distilled water also served as the negative control vehicle.

Fig. 1. Formula of glyphosate.

Test population
A total of 346 normal volunteers were studied. All subjects, male and female, were between the ages of 18 and 80 years. None of the test subjects had active skin pathology or disease which would adversely affect the study.

Path occlusion material
Skin test sites were covered with non-woven fiber patches (Webril®, Curity Inc., New York, New York). Patches were fixed in place with hypoallergenic plastic tape (Blenderm®, 3M Co., St. Paul, Minnesota 55106).

Test scoring scale
Skin test sites were evaluated for evidence of inflammation and graded as follows: negative reaction [0]; equivocal reaction [+/−]; erythema [1]; erythema and induration [2]; erythema, induration and vesicle [3]; and erythema, induration and bullae [4]. In addition, the subjects were questioned about the presence or absence of burning, stinging or itching. To ensure consistency, test site reactivity was evaluated by the same individual for each study. To prevent experimental bias, all studies were performed on a double-blind basis.

Data analysis
At the conclusion of each study, the skin test scores for each compound were summed. The mean and standard deviations for each compound were determined.

Single application irritancy assays
Full strength test compounds (0.1 ml) were applied to intact and Draize-type abraded skin on the backs of 24 normal volunteers (1). Patches were removed at 24 h. Skin test sites were evaluated at 24 and 48 h after application of the compounds.

21-day cumulative irritancy assays
The method used was that described by Phillips et al. (2). Using 23 normal volunteers, the test materials were applied 5 days weekly for 21 days to the same site. Patches were not reapplied on weekends; they remained in place for these periods. Test site readings were made at each removal of the patch.

Modified Draize skin sensitization study
To further evaluate the herbicide for irritation and sensitization by repeat insult patch testing, a modified Draize skin sensitization study was performed on 204 adult volunteers (3). For this investigation, test patches moistened with approximately 0.2 ml (0.2 mg) of the full strength test solutions were applied to the subjects' upper arms or back. During the 3-week induction period, each volunteer was patched 3 times per week, and the patches left in place for 48-72 hours. After induction, the subjects received no patching for 2 weeks. Then, after the 2-week rest period, each volunteer received a challenge patch containing the compounds for a 48-h application. The challenge site was examined and graded upon patch removal, and again 48 h later.

Photoirritation [phototoxicity] study
The study was performed by a modification of the procedure outlined by Marzulli et al. (4). Skin on the upper arms or backs of 15 subjects was stripped to glistening with cellophane tape to remove most of the stratum corneum. Full strength test compounds were applied to the test sites for 24 h. The test sites received irradiation with ultraviolet A light (UVA) from a Hanovia Inspectalight for 45 min at 9 inches distance. After UVA irradiation, the sites were exposed to 2/3 MED of UVB irradiation from an air-cooled Kromayer light. Each site was evaluated at 24 h after UVA irradiation. Non-irradiated cellophane-tape-stripped application sites provided internal control for irradiation.

Modified photo Draize skin sensitization study
A modified photo Draize skin sensitization study to evaluate the herbicide for irritation and potential photosensitization was carried out on 24 adult subjects (3). Although this is a "standard" test sample in photosensitization assays, greater sensitivity would be obtained with a larger sample. Non-woven fiber patches containing 0.2 ml of the herbicide and other test compounds were affixed to the upper arm or back with hypoallergenic tape for 24 h. The procedure was performed 3 times weekly for a total of 3 weeks. After patch removal, evaluation and grading, the site was irradiated with 3 MED's of unfiltered light from an air-cooled Kromayer lamp. On the final elicitation (after induction and a 2-week rest period), a duplicate patch was irradiated with 10 MED's window glass-filtered light to remove the erythema rays. Approximately 2 weeks after the sensitization phase, challenge applications were performed. Challenge patches were applied to a previously unpatched site and were left in place for 24 h. After removal and irradiation as noted above, the subjects received examination and scoring for any reaction at 96 h following application. The volunteers also received an additional open application of 0.05 ml of the herbicide to a 2 cm area on the forearm, and were then asked about the presence or absence of burning, stinging or itching.

Table 1. Tabulation of readings for single application irritancy assay on *non-abraded skin* for glyphosate herbicide, all purpose liquid cleanser, liquid dishwashing detergent, baby shampoo and water

		Unabraded skin 24 h	48 h
glyphosate herbicide	0	24	23
	±	0	0
	1	0	1
	2	0	0
all purpose liquid cleanser	0	16	16
	±	5	4
	1	2	4
	2	1	0
liquid dishwashing detergent	0	18	16
	±	5	4
	1	2	4
	2	1	0
baby shampoo	0	23	24
	±	1	0
	1	0	0
	2	0	0
water	0	23	23
	±	1	1
	1	0	0
	2	0	0

Table 2. Tabulation of readings for single application irritancy assay on *abraded skin* for glyphosate herbicide, all purpose liquid cleanser, liquid dishwashing detergent, baby shampoo and water

		Abraded skin 24 h	48 h
glyphosate herbicide	0	10	10
	±	4	6
	1	10	8
	2	0	0
all purpose liquid cleanser	0	15	14
	±	8	7
	1	1	3
	2	0	0
liquid dishwashing detergent	0	13	10
	±	8	8
	1	3	6
	2	0	0
baby shampoo	0	21	20
	±	3	4
	1	0	0
	2	0	0
water	0	23	22
	±	1	2
	1	0	0
	2	0	0

Results

The data from single application irritancy studies on unabraded skin is shown in Table 1. The herbicide has no greater irritation potential than either the all purpose cleaner, the dishwashing liquid, or the baby shampoo. When tested on abraded skin, the herbicide had a slightly greater incidence of erythema at the 24-h reading; however, on the 48-h reading, the herbicide-induced irritation was similar to that of the all purpose cleaner and the dishwashing liquid (Table 2).

Table 3. 21-day cumulative irritation assay for glyphosate herbicide, all purpose liquid cleanser, ivory liquid dishwashing detergent and baby shampoo

Test sites	Total score	Average score	Standard deviation
glyphosate herbicide all purpose	39	1.4	3.5
liquid cleanser	343.5	12.7	8.2
liquid dishwashing detergent	372.5	13.8	8.7
baby shampoo	84	3.1	5.7

The 21-day cumulative irritancy assay was used to adequately predict the irritation potential of the test compounds. Statistical analysis of the test data showed that the herbicide and the baby shampoo were statistically less irritating than either the all purpose cleaner or the dishwashing liquid (Table 3).

In the modified Draize skin sensitization study, none of the 204 volunteers manifested significant skin irritation to any of the commercial products tested. Sensitization was not induced by any of the test compounds in any of the 204 volunteers.

Data from the studies designed to evaluate the effect of ultraviolet light on skin exposed to the test compounds demonstrated that the compounds had no potential for photoirritation or photosensitization. There were no positive skin tests induced by the 4 test compounds in the photoirritation and photosensitization studies.

In the 21-day irritancy assay, the modified photo-Draize skin sensitization study and the modified Draize skin sensitization study, the subjects were asked whether they subjectively felt any burning, stinging or itching from the test compounds. None felt any such symptoms.

Discussion

This is the first study which delineates the irritation, photoirritation, sensitization and photosensitization potential of an agricultural herbicide in man. Statistical analysis of the results indicates that the herbicide and baby shampoo have less irritant potential than either the all purpose cleaner or the dishwashing liquid. Compared to the baby shampoo, the herbicide was statistically indistinguishable in its irritant potential. The tests also show that it did not induce sensitization, photoirritation or photosensitization.

The only publication of glyphosate dermatotoxicity is that of Hindson & Diffey (5). They believed they had identified an agricultural worker with photosensitization due to glyphosate herbicide. However, after subsequent testing, they stated that the offending chemical was *not* the glyphosate but the biocide (6).

Since commercial glyphosate is not a single pure chemical, we cannot rule out the possibility that small amounts of contaminate might under unusual circumstances sensitize. The issue can be approached by testing putatively sensitized subjects with higher than usual concentrations of such materials.

Conclusion

These data, derived from utilizing normal volunteers, provide a baseline for choosing appropriate diagnostic patch test concentration. We suspect that 10% glyphosphate in water should be non-irritant, in the light of several hundred volunteers tested at full strength without significant reaction. However, without further retesting of reactive subjects, one cannot rule out the possibility of the excited skin syndrome.

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