

EXHIBIT 86

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Glyphosate Skin Binding, Absorption, Residual Tissue Distribution, and Skin Decontamination

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Glyphosate Skin Binding, Absorption, Residual Tissue Distribution, and Skin Decontamination. WESTER, R. C., MELENDRES, J., SARASON, R., MCMASTER, J., AND MAIBACH, H. I. (1991). *Fundam. Appl. Toxicol.* 16, 725-732. Glyphosate is a broad-spectrum postemergence translocated herbicide. Its interactions with skin and potential systemic availability through percutaneous absorption was studied by skin binding, skin absorption, residual tissue distribution, and skin decontamination. Glyphosate in a final formulation (Roundup) undiluted and diluted with water 1:20 and 1:32, would not partition into powdered human stratum corneum (<1%). *In vitro* percutaneous absorption through human skin into human plasma as receptor fluid was no more than 2% over a concentration range of 0.5-154 µg/cm² and a topical volume range of 0.014-0.14 ml/cm². Disposition of glyphosate following iv administration of 93 and 9 µg doses to rhesus monkeys was mainly through urine excretion, 95 ± 8 and 99 ± 4% in 7 days, respectively. Percutaneous absorption *in vivo* in rhesus monkey was 0.8 ± 0.6% for the low dose (25 µg/cm²) and 2.2 ± 0.8% for the high dose (270 µg/cm²). No residual ¹⁴C was found in organs of the monkeys euthanized 7 days after the topical application. Washing the skin application site with soap and water removed 90 ± 4% of applied dose, and washing with water only removed 84 ± 3% of applied dose. Both soap and water and water only were equal in ability to remove glyphosate from skin over a 24 hr skin application period. About 50% of the initially applied dose could be recovered after 24 hr. Glyphosate is very soluble in water and insoluble in most organics (octanol/water log P = -1.70) and therefore not compatible with the lipid-laden stratum corneum. This is consistent with the low skin binding and skin absorption and also consistent with the efficient removal from skin with soap and water or water-only wash. © 1991 Society of Toxicology.

Percutaneous absorption is the process whereby a chemical in contact with skin becomes systemically available through a series of biochemical events (Wester and Maibach, 1983). A herbicide, though distributed into the environment for plant growth control, may come in contact with human skin through manufacturing, distribution, application, and residual presence in the environment. The potential human health hazard of that herbicide should include an estimate for percutaneous absorption.

Glyphosate is a broad-spectrum postemergence translocated herbicide. Through production and general use it may come in contact with human skin as indicated above. The extent that glyphosate will wash off skin, might bind to skin, or become systemically available through percutaneous absorption was studied in a series of related experiments. Potential skin binding was examined by the ability of glyphosate to partition out of its vehicle to powdered human stratum corneum. Percutaneous absorption relative to vehicle dilution

and vehicle volume was studied *in vitro* in human skin. Percutaneous absorption and residual tissue distribution were determined *in vivo* in the rhesus monkey, an animal model relative to man for percutaneous absorption (Wester and Maibach, 1975). The ability of glyphosate to be washed off skin was also determined *in vivo* in the rhesus monkey.

MATERIALS AND METHODS

In vitro percutaneous absorption. *In vitro* percutaneous absorption of [^{14}C]glyphosate (*N*-phosphonomethyl glycine) through human skin was determined using flow-through design glass penetration cells (LG-1084-LPC, Laboratory Glass Apparatus, Inc., Berkeley, CA) and radiotracer methodology. Undiluted and two dilutions of glyphosate in a formulation (Roundup, Monsanto Co, St. Louis, MO) with distilled water were utilized: undiluted, 1:20 (v/v) and 1:32 (v/v), corresponding to glyphosate concentrations of 1.1, 0.059, and 0.037 mg glyphosate per milliliter, respectively. Normal spray solutions used were in the range of 1–5%. These solutions were formulated with ^{14}C -labeled glyphosate (Monsanto Co, St. Louis, MO). The resulting specific activities of the undiluted 1:20 and 1:32 preparations were 1.8, 2.1, and 2.2 $\mu\text{Ci/ml}$ respectively.

Human thigh skin was obtained at autopsy from various donors and dermatomed to a thickness of 0.5 mm. The dermatomed skin, stored in Eagle's minimum essential cell culture medium at 4°C, was used within 5 days. Circular pieces of skin were clamped between the two ground-glass portions of the penetration cells. Top sections of the cells were open to the environment and allowed application of each glyphosate dilution directly to the exposed skin surface area of 1.0 cm². The receptor volume of each cell was 3 ml. The water-jacketed penetration cells were maintained at 37°C using a recirculating constant temperature water bath. Human plasma (Irwin Memorial Blood Bank, San Francisco, CA) was used as the receptor fluid (Wester *et al.*, 1985). Plasma, pumped through the receptor chambers of the cells at 3 ml/hr, was collected in 5-ml aliquots directly into scintillation vials. Plasma in the receptor chambers was continually mixed by magnetic stir bars.

For each dilution, three cells were dosed with 0.014, 0.07, and 0.14 ml for the designated time periods. Exposure times were 30 min, and 4, 8, and 16 hr. The plasma receptor fluid was analyzed for amount of radiolabel by liquid scintillation counting.

Binding to powdered human stratum corneum. The binding behavior of [^{14}C]glyphosate in formulation and water dilutions of formulation to powdered stratum corneum was determined (Wester *et al.*, 1987). Powdered stratum corneum was prepared as follows: Callus (obtained from the California College of Podiatric Medicine) was

cut into fine pieces with scissors and pulverized in a mortar and pestle containing dry ice. Particles of stratum corneum that would pass through a 48-mesh sieve but were retained by an 80-mesh sieve were used (180 to 300 μm). In a plastic microcentrifuge tube, 1.0 mg of powdered stratum corneum was mixed with 1.0 ml of glyphosate solution by vortexing. The durations of contact were as follows: undiluted (30 min, 4 and 8 hr), 1:20 dilution (30 min, 4, 8, and 24 hr); and 1:32 dilution (30 min, 4, 8 and 24 hr). After a given contact time, the mixture was separated by centrifugation and the supernate removed. The stratum corneum pellet was resuspended three times in distilled water to remove material adsorbed on the surface. Four tubes were prepared for each test.

In vivo percutaneous absorption. Young adult female rhesus monkeys, 4 to 10 kg, (*Macaca mulatta*), selected from the colony at the California Primate Research Center (CPRC), were used per study group ($n = 3$ or 4). The monkeys were naive to prior radioactivity administration prior to the start of the study. The animals were placed in metabolic chairs for the first 12 hr of the study (dosing period), then housed individually in metabolic cages. A belly plate and apron were positioned on the metabolism chair under the skin-dosing site. The intravenous dose was a bolus injection of [^{14}C]glyphosate in Roundup formulation diluted with saline (93 μg in 790 μl , 4.5 μCi ; and 9 μg in 940 μl , 3.1 μCi) into the saphenous vein. Topical [^{14}C]glyphosate was Roundup-formulation diluted 1:29 with water and spread evenly over the skin surface of the abdomen (5400 $\mu\text{g}/15 \mu\text{l}/20 \text{cm}^2$, 5.46 μCi , and 500 $\mu\text{g}/40 \mu\text{l}/20 \text{cm}^2$; 7.94 μCi).

After 12 hr, the site of topical application was washed with soap and water. This procedure consists of washing the site of application with a liquid soap (Ivory):water (1:10 v/v) solution and water rinses. Each solution was applied to a cotton dental log. The application site was wiped with the solvent-laden cotton dental log. Wash samples from the skin site of application, chair wash samples, belly plate washes, and aprons were collected for analysis of the amount of ^{14}C removed from the skin surface. Urine was collected for 24 hr before dosing, then at 0–6, 6–12, and 12–24 hr the day of dosing, then daily for 7 or 8 days. Pan washes were collected at 0–6, 6–12, and 12–24 hr the day of dosing, then daily for 7 or 8 days. Pan wash ^{14}C amounts were added to ^{14}C urine totals. Contaminated solids (residual food, hair) were collected daily for 7 or 8 days. One monkey from each topical group was euthanized after the seventh day and residual ^{14}C determined for various organs.

Urine samples were analyzed in duplicate for ^{14}C . A 3-ml aliquot of each urine sample was assayed in 16 ml of scintillation cocktail (Optifluor, United Packard Technologies, IL) with a Packard 4640 liquid scintillation spectrophotometer. Fecal samples and contaminated solids were homogenized in distilled water using a Waring commercial blender. A 500- μl aliquot of each fecal homogenate or contaminated solid homogenate was combusted (Packard Tri-Carb Oxidizer Model 306) and assayed with a liquid

GLYPHOSATE PERCUTANEOUS ABSORPTION

scintillation spectrophotometer (Packard 4640). Pan wash samples were analyzed in duplicate for ¹⁴C. A 3-ml aliquot of each pan wash sample was assayed in 16 ml of scintillation cocktail with the liquid scintillation spectrophotometer. The wash samples were analyzed for ¹⁴C. The cotton dental log from the washing of the site of application, and gauze from the chair washing, were individually counted in 16 ml of scintillation cocktail with the liquid scintillation spectrophotometer. The belly aprons were analyzed for ¹⁴C. Each belly apron was extracted three times with 100 ml of 0.05 M ammonium bicarbonate. A 3-ml aliquot of each extract was counted in 16 ml of scintillation cocktail with the liquid scintillation spectrophotometer. Whole blood samples were assayed by direct liquid scintillation counting or by combustion first followed by scintillation counting.

In vivo skin decontamination. Four rhesus monkeys (4) were isolated in metabolic chairs. The abdominal skin was marked with a single site or a series of 1-cm² areas. [¹⁴C]glyphosate in formulation diluted 1:20 with water was applied to each marked area. Dosing was 7 μl/cm² skin area containing 0.4 μg glyphosate/cm². The single site decontamination was done by washing with a cotton ball laden with water only or 50% soap (Ivory liquid) and water (v/v). The initial washing was followed with two water-only rinses. This was done within a 5-min time period following skin application. With the grid method (Fig. 1), all of the 1-cm² skin areas were dosed with the same glyphosate formulation. At the designated time period (where 0 hr is within the first 5 min), the skin was washed with a cotton applicator (Q-tip, Chesebrough-Pond, Inc., Greenwich, CT) laden with water only or 50% soap and water. With the grid method, the cotton applicator was better confined to each 1-cm² skin area and did not touch any other area.

Scintillation counting. Background control samples and test samples were counted in a Packard Model 4640 or Model 1500 counter (Packard Instruments). Control and test sample counts were transferred to a computer program (Appleworks/Apple IIE computer; Apple Computer Co., Mountain View, CA) which subtracted 1X background control samples and generated a spreadsheet with the data reported under Results. The counting process and computer program have been verified to be accurate by a quality assurance officer (Wester *et al.*, 1990).

RESULTS

Table 1 gives the *in vitro* percutaneous absorption of [¹⁴C]glyphosate through human skin. Percentage dose absorbed through skin for time periods of 30 min and 4, 8, and 16 hr is given for undiluted glyphosate in formulation and that diluted 1:20 and 1:32 with water. Volumes of test material ranged 10-fold

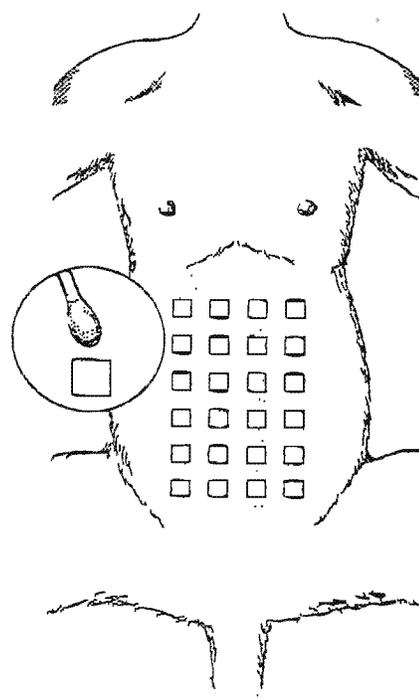


FIG. 1. Illustration of grid method where multiple single doses are marked on rhesus monkey abdomen for skin decontamination with time using a cotton-tip applicator laden with appropriate solvent.

from 0.014 to 0.14 ml/cm² skin surface area. In all cases *in vitro* percutaneous absorption was low, 2.2% or less of applied dose.

Table 2 gives the partitioning of [¹⁴C]-glyphosate from vehicles to powdered human stratum corneum. The partitioning is from undiluted formulation vehicle and formulation diluted 1:20 and 1:32 with water. Exposure times were for 30 min and 4, 8, and 24 hr. The water rinse given is the first of three rinses (numbers 2 and 3 contained no [¹⁴C]-glyphosate), and SC is the stratum corneum pellet after the water rinses. In all cases better than 90% of the glyphosate stayed in the vehicle and did not partition into powdered human stratum corneum. In all cases the amount remaining with the powdered human stratum corneum pellet after the water rinses was less than or equal to 0.05%.

Table 3 gives the *in vivo* disposition in rhesus monkeys following intravenous administration of two glyphosate doses of 93 μg (dose

TABLE I
IN VITRO PERCUTANEOUS ABSORPTION OF GLYPHOSATE

Dilution	Concentration (µg/cm ²)	Volume (ml/cm ²)	Percentage of dose absorbed ^a			
			30 min	4 hr	8 hr	16 hr
Undiluted	15.4	0.014	0.02 ± 0.02	0.3 ± 0.0	0.06 ± 0.1	— ^b
	77.0	0.07	0 ± 0	0.03 ± 0.06	0 ± 0	—
	154.0	0.14	0.02 ± 0.02	0.0 ± 0.0	0.4 ± 0.2	—
Diluted 1:20	4.1	0.07	0 ± 0	1.4 ± 0.7	0.8 ± 1.3	0.2 ± 0.1
	8.3	0.14	0 ± 0	0.9 ± 1.3	0.1 ± 0.1	1.6 ± 2.3
Diluted 1:32	2.6	0.07	0.1 ± 0.07	0.1 ± 0.01	2.2 ± 0.5	0.3 ± 0.2
	5.2	0.14	0.7 ± 1.2	0.3 ± 0.5	0.9 ± 1.5	0.5 ± 0.6

^a Data expressed as means ± SD.

^b Not done.

A) and 9 µg (dose B). Almost all of each dose was excreted into urine within the 7-day urine collection period. The majority of the ¹⁴C urinary excretion was in the first 24 hr (Fig. 2). Overall accountability was greater than 95% of administered doses.

Table 4 gives the data for *in vivo* disposition in rhesus monkeys of topical administration

of two doses of [¹⁴C]glyphosate with a 10-fold difference in amount (dose C, 5400 µg/200 cm²; dose D, 500 µg/20 cm²). Only 2.2 and 0.8% of dose C and dose D, respectively, were excreted in urine during the 7-day excretion period, the highest amount in the first 24 hr (Fig. 3). Since all of the iv-administered doses (Table 3) were excreted in urine, the percu-

TABLE 2
GLYPHOSATE PARTITION FROM ROUNDUP VEHICLE TO POWDERED HUMAN STRATUM CORNEUM

Parameters	Percentage dose ^a		
	Undiluted	Diluted 1:20	Diluted 1:32
30 min exposure			
Vehicle	96.2 ± 0.9	101.6 ± 1.2	96.4 ± 1.6
Water rinse	2.4 ± 0.2	1.2 ± 0.07	1.1 ± 0.2
SC	0.04 ± 0.04	0.03 ± 0.03	0.02 ± 0.01
4 hr exposure			
Vehicle	96.2 ± 2.4	104.2 ± 1.3	91.3 ± 2.9
Water rinse	3.3 ± 0.8	1.2 ± 0.2	5.0 ± 2.2
SC	0.01 ± 0.01	0.02 ± 0.03	0.05 ± 0.04
8-hr exposure			
Vehicle	96.0 ± 0.4	98.0 ± 6.6	94.5 ± 1.4
Water rinse	3.0 ± 0.2	2.2 ± 0.5	2.3 ± 1.2
SC	0.02 ± 0.0	0.01 ± 0.01	0.02 ± 0.01
24-hr exposure			
Vehicle	—	103.4 ± 3.1	95.8 ± 1.2
Water rinse	—	2.4 ± 1.5	2.7 ± 1.4
SC	—	0.01 ± 0.0	0.01 ± 0.01

^a Undiluted, 1.1 mg Gly/ml/mg SC; 1:20, 0.059 mg; 1:32, 0.037 mg. Data expressed as mean ± SD.

GLYPHOSATE PERCUTANEOUS ABSORPTION

TABLE 3

DISPOSITION OF GLYPHOSATE FOLLOWING INTRAVENOUS ADMINISTRATION TO RHESUS MONKEYS

Disposition site	Percentage administered dose ^a	
	Dose A	Dose B
Urine	94.9 ± 8.6	98.8 ± 3.8
Feces	1.3 ± 0.5	0.8 ± 0.4
Contaminated solids	0.2 ± 0.1	0.2 ± 0.3
Total	96.4 ± 8.2	99.8 ± 4.1

^a Means ± SD for three monkeys. Dose A, 93 µg; Dose B, 9 µg.

TABLE 4

DISPOSITION OF GLYPHOSATE FOLLOWING TOPICAL ADMINISTRATION TO RHESUS MONKEYS

Disposition site	Percentage applied dose ^a	
	Dose C	Dose D
Urine	2.2 ± 1.5	0.8 ± 0.6
Feces	0.7 ± 0.5	3.6 ± 1.6
Surface washes ^b	73.5 ± 6.0	77.1 ± 9.2
Contaminated solids	0.05 ± 0.1	0.3 ± 0.1
Total	76.5 ± 6.7	81.8 ± 6.9

^a Means ± SD for four monkeys. Dose C, 5400 µg/20 cm²; Dose D, 500 µg/20 cm².

^b Surface washes: skin application site, chair, belly plate, saliva, apron, final body wash.

taneous absorption of glyphosate is estimated to be 0.8–2.2% of applied dose. The majority of the applied dose (approximately 75%) was recovered in the surface washes (most of which was the skin surface wash). Accountability was 75–80% of administered doses.

Table 5 gives the glyphosate ¹⁴C equivalents blood concentrations following the two iv (A and B) and two topically administered doses (C and D). ¹⁴C blood levels were detectable after iv administration, but were near or at background level following topical administration.

Two monkeys from each topical dose level (a total of four monkeys) were euthanized after the 7-day excretion period and tissues were assayed for ¹⁴C content. No radioactivity was detected in spleen, ovaries, kidney, brain, liver, abdominal fat, bone marrow, upper spinal column, or central nervous system fluid. Skin that contained the applied dose for 12 hr and then was washed with soap and water contained 0.006 ± 0.0007% applied dose;

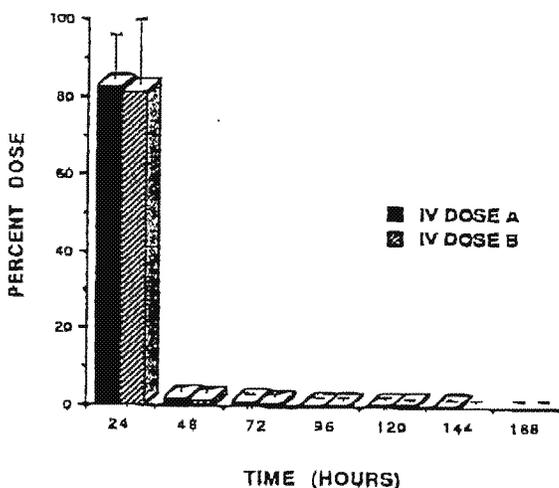


FIG. 2. Daily urinary excretion of ¹⁴C following iv administration of two doses (A), 93 µg; (B), 9 µg of [¹⁴C]glyphosate. Data are expressed as means ± SD.

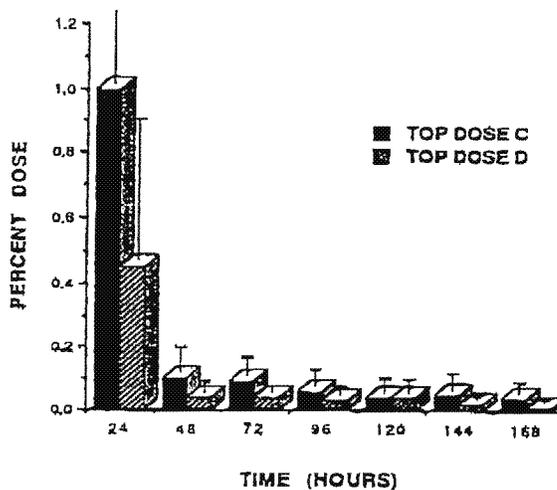


FIG. 3. Daily urinary excretion of ¹⁴C following topical administration of two doses (C), 5400 µg/20 cm²; (D), 500 µg/20 cm² of [¹⁴C]glyphosate. Data are expressed as mean ± SD.

TABLE 5
GLYPHOSATE ¹⁴C BLOOD CONCENTRATIONS (μg eq/ml)

Time (hr)	Group			
	Intravenous A	Intravenous B	Topical C	Topical D
-24	0.00	0.00	0.00	0.00
1	0.015 ± 0.004	0.003 ± 0.002	0.0001 ± 0.0001	0.00
6	0.005 ± 0.004	0.002 ± 0.001	0.00	0.00
24	0.004 ± 0.004	0.002 ± 0.001	0.00	0.00
AUC ^a	0.13 ± 0.096	0.044 ± 0.032	0.001 ± 0.002	0.0006 ± 0.0009

^a AUC, area under blood concentration versus time curve. Data expressed as means ± SD.

untreated skin contained levels of 0.0012 ± 0.0002%. Therefore, there was no residual dose in tissues or in skin. Thus, the 75–80% accountability for topical application (Table 4) and no residual compound in tissues or skin suggest that the “missing” 20–25% dose was lost during procedure. Such a loss of 20–25% of the topically applied dose is not unusual. Similar losses occurred in previous studies (Longacre *et al.*, 1989; Wester *et al.*, 1989). *In vivo* skin undergoes exfoliation, a continual shedding of the top layer of the stratum corneum. This process will scatter microscopic tissue and bound chemical to the atmosphere, making total accountability impossible to achieve.

Table 6 compares skin decontamination of glyphosate from a single site 5 min after ap-

plication versus sequential decontamination from skin grid site. Data are given for both soap and water decontamination and water-only decontamination. A soap and water wash followed by two water rinses of a single skin application site removed 89.6 ± 3.5% of the applied dose. The equivalent procedure with the grid system removed less (but not statistically different) glyphosate (71.4 ± 12.9%). With water as the decontamination solvent, 83.6 ± 3.3% of the applied dose was removed from the single site and a lesser amount (*p* = 0.03) of 76.9 ± 3.6% was removed by the grid system.

Using the grid system, skin decontamination of glyphosate from monkeys *in vivo* was done over a 24-hr period with soap and water and with water only (Table 7). The recovery

TABLE 6
SKIN DECONTAMINATION OF GLYPHOSATE SINGLE SITE VERSUS SKIN GRID APPLICATION

Wash number	Percentage applied dose			
	Soap and water		Water-only	
	Single site	Grid	Single site	Grid
1	85.1 ± 3.1	68.7 ± 11.7	78.9 ± 3.9	72.2 ± 5.4
2	3.9 ± 2.2	2.4 ± 1.5	3.9 ± 1.8	4.2 ± 2.4
3	0.5 ± 0.06	0.6 ± 0.6	0.8 ± 0.3	0.5 ± 0.3
Total	89.6 ± 3.5	71.7 ± 12.9	83.6 ± 3.3 ^a	76.9 ± 3.6 ^a

^a Statistically different (*p* = 0.03). Data expressed as means ± SD.

GLYPHOSATE PERCUTANEOUS ABSORPTION

TABLE 7
SKIN DECONTAMINATION OF GLYPHOSATE

Time (hr)	Percentage applied dose ^a	
	Soap and water	Water only
0	71.7 ± 12.9	76.9 ± 3.6
0.5	70.9 ± 7.2	74.9 ± 11.6
3	47.7 ± 23.0	68.0 ± 18.0
6	60.6 ± 19.2	67.7 ± 11.4
24	48.5 ± 17.5	50.6 ± 19.2

^a Data expressed as means ± SD.

with soap and water was 71.7 ± 12.9% at 0 hr (same value as in Table 6) and decreased with time to a recovery of 48.5 ± 17.5% after a 24 hr skin residence time. Equivalent numbers were found using water only. There was no difference whether skin was washed with soap and water or water only (Fig. 4).

DISCUSSION

Glyphosate solubility in water at 25°C is 12 g/liter and it is insoluble in most organic solvents. The formulation utilized is the mono isopropylamine salt of glyphosate and it is very soluble in water. The octanol/water log P of glyphosate is -1.70 (Martin and Edgington, 1981). The outer layer of the skin that is in contact with the environment is the stratum corneum. The biochemistry of the stratum corneum is that of a lipid-laden tissue. Given the above simple biochemistry it appears that the water soluble/organic insoluble glyphosate would not react with the stratum corneum, and this is reflected in the data. Glyphosate stayed in the formulation, whether undiluted or diluted with water, rather than partition into the powdered human stratum corneum. This result is consistent with that reported by Wester *et al.* (1987). Given that little glyphosate would partition from a water-based vehicle to skin, then skin absorption would be expected to be slight. This is reflected in the *in vitro* absorption where no more than 2% was ab-

sorbed into human skin for up to 16 hr exposure with changes in concentration, dilution, and applied volume. The *in vivo* absorption in the rhesus monkey was consistent in that only 1.5% glyphosate was absorbed during 12 hr skin exposure. And, since little was absorbed into skin, residual dose in all systemic organs after topical application was nil. Glyphosate on skin could be readily washed off with soap and water or water only. The best system (single site) removed 90% of applied dose. The above data show that glyphosate had adversion to the skin and to transit through the skin into the body.

Glyphosate has an oral LD50 of 5600 mg/kg in rats. Absorption across the gastrointestinal membranes is thought to be minimal since the majority of an oral dose is rapidly eliminated unchanged in feces. Less than 2% of a dose remains in the tissues after 24 hr (Duarson and Sipes, 1987). It was confirmed that oral absorption in the rat was incomplete; a similar conclusion was reached for the rabbit (FAO, 1987). The above would postulate a correlation between low oral toxicity and incomplete oral absorption of glyphosate. There

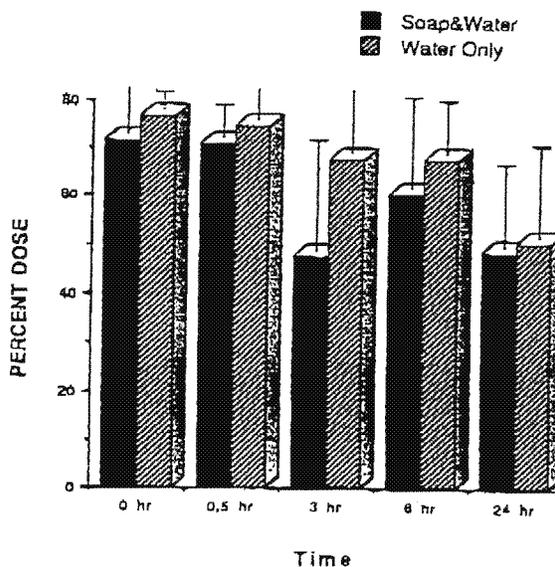


FIG. 4. Glyphosate removal from rhesus monkey skin using soap and water or water only over a 24-hr time period. Data are expressed as means ± SD.

are no dermal toxicology data to compare with the oral toxicology data. However, the percutaneous absorption of glyphosate in the rhesus monkey is low (0.8–2.2%). Since the rhesus monkey is a good animal model for percutaneous absorption relevant to humans (Wester and Maibach, 1975), we can assume that the potential for glyphosate dermal toxicity in humans is also low.

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