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Conifer Seedling Nursery Worker Exposure to Glyphosate

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Abstract. This study addresses the measurements of glyphosate exposure received by 14 workers employed at two tree nurseries. The applicators, weeders, and scouts monitored all wore normal work clothing, which for applicators was a protective suit, rubber gloves and boots. Measurements were made of the glyphosate that was dislodged from conifer seedlings during water rinses taken twice weekly from May through August. Only 1 of these 78 dislodgeable residue samples were positive for glyphosate. Nine cotton gauze patches were attached to the clothing of each worker one day per week during this same period. Hand washes were taken on the same day that patches were worn. Most patches and hand washes from applicators and weeders contained measurable amounts of glyphosate. Analyses of individual patches showed that the body portions receiving the highest exposure were ankles and thighs. For scouts only 1 of 23 hand washes contained glyphosate. To provide a measure of the exposure occurring via all exposure routes (dermal, ingestion, and inhalation) an analysis was made of the total urine excreted. For most workers a daily total urine collection was made for 12 consecutive weeks. Urine analysis, the biological monitoring tool used to assess the total amount absorbed via all avenues, did not reveal any positive samples. The lower limit of method validation for glyphosate in the urine samples was 0.01 μg/ml.

High rainfall, or irrigation as needed, in conjunction with normal field dissipation avenues and worker training were cited as contributing factors for the low amounts of glyphosate exposure found. None of the exposure parameters indicated that glyphosate exposure poses a threat to human health when used under normal nursery conditions.

The herbicide glyphosate [isopropylamine salt of *N*-(phosphonomethyl)glycine] is widely used by many in agricultural production and lawn care. Its broad spectrum control of green plants, its lack of phytotoxicity after contacting soil, and its low mammalian toxicity have added to its popularity and use for weed control. Despite the many positive aspects of glyphosate use, there is a public concern with pesticides in general that the use of and exposure to these materials will be detrimental to the environment and to humans.

Other researchers have studied the dissipation of glyphosate, addressing persistence in soil, herbicidal efficacy, and potential adverse effects on the environment (Goldsborough *et al.* 1989; Newton *et al.* 1984; Roy *et al.* 1989; Salazar *et al.* 1982).

In an attempt to assess the potential of glyphosate to cause adverse human health effects, the USDA Forest Service sought to measure the extent of occupational exposure to individuals using the compound and to assess the margin of safety associated with its use. Glyphosate is used for weed control at several of the 15 USDA Forest Service Nurseries which produce in excess of 50 million conifer seedlings each year. Thus, this study was designed to assess the exposure occurring to humans applying glyphosate and those working in or near areas where this herbicide has been applied to vegetation in conifer seedling nurseries. For many workers, it is common to spend 7 to 10 months each year working with seedlings grown near areas treated with glyphosate. Depending on their duties, the number of applications made, and the persistence of this compound, some workers may have the potential for glyphosate exposure up to 10 months per year. Results from this study should represent a realistic measure of the season-long exposure occurring to those whose duties require them to apply or work in the vicinity of glyphosate-treated vegetation.

The primary objective of this study was to monitor workers potentially exposed to glyphosate and to quantitate the absorbed dose received by those workers over a 10-month period. Assessing the absorbed dose was deemed to be essential by the funding agency, the USDA Forest Service, since they reasoned that any potential adverse human health effects would depend on the amount of body dose. No previous human exposure studies involving biological monitoring of workers exposed to glyphosate have been published. In earlier studies, evaluating worker exposure to several other herbicides used under forestry work conditions, we have shown that a significant portion of the active ingredients of those herbicides was absorbed by the workers and excreted intact as the parent molecule in their urine (Lavy *et al.* 1980, 1982, 1987). Urine analysis as a biological monitoring tool for measuring the absorbed dose of pesticide has also been used by other researchers (Grover *et al.* 1986; Libich *et al.* 1984; Murphy *et al.* 1983; Nash and Kearney 1982; Winterlin *et al.* 1984).

Table 1. Summary of Roundup® used by applicators and weeders

Nursery	Worker (No.) duty	Kg applied per hectare	Hectares treated	Kg	Application time (h)
Ashe	(1) Applicator	0.11	3.64	.40	2.5
Ashe	(2) Applicator	0.11	6.48	.71	4.0
Ashe	(4) Weeder[a]	.01	[b]	.49	56
Ashe	(5) Weeder	.01	[b]	.49	56
Ashe	(6) Weeder	.01	[b]	.60	68
Ashe	(7) Weeder	.01	[b]	.56	64
Ashe	(8) Weeder	.01	[b]	.49	56
Ashe	(9) Weeder	.01	[b]	.49	56
Ashe	(10) Weeder	.01	[b]	.49	56
Ashe	(11) Weeder	.01	[b]	.49	56
Ashe	(91) Weeder	.01	[b]	.60	68
Phipps	(31) Applicator	0.11	1.2	.13	5.0

[a] Weeders at Ashe Nursery manually applied a glyphosate solution up to 7 to 8.5 days during the season
[b] Weeders applied spot treatments within seedling beds

The first goal of this series of studies was to provide a year-long quantitative measurement of the extent of human exposure occurring as full-time nursery workers perform their normal duties. A second and perhaps even more difficult part of the study was to accurately assess safety aspects of the workers who had contact with glyphosate. Since two different approaches, passive monitoring and biological monitoring, were utilized in this study, the final stages of this paper present a comparison of the risks as deduced using each of the two approaches.

Materials and Methods

At the request of the USDA Forest Service, studies to monitor the exposure of 14 nursery field workers to glyphosate were conducted at the Ashe Nursery near Brooklyn, MS, and at the Phipps Nursery near Roseburg, OR. After preparation of a 40-page protocol following suggestions of a 15-member study development team, a meeting to discuss the protocol[1] was held with US EPA officials prior to initiation of the field exposure study.

The source of potential glyphosate exposure in these studies was the Roundup® formulation which is applied for broad spectrum weed control at the two nurseries. Information in Table 1 summarizes the Roundup® (field formulation of glyphosate) spray material used and the workers involved with the application at each of the two locations.

Although Roundup® cannot be applied to conifer seedlings without phytotoxic effects, this compound was used at the Ashe Nursery to control adjacent weeds within the bed. This was accomplished by placing a 290 ml, 2.5 X 3.5 cm cylindrical metal shield (which served as a spray chamber) over the small weeds to be sprayed. The shield protected adjacent conifer seedlings in the bed while an individual weed was sprayed by manually depressing a trigger which released approximately 0.5 ml of the spray mix. Weeders at Ashe began using the herbicide in May and continued using it intermittently through August. Each weeder at Ashe Nursery applied approximately 2 gal of a 1:40 dilution of Roundup® as many as 8½ days during the season as a part of the weeding process. In addition, glyphosate was occasionally applied to nearby weedy vegetated areas by tractor drivers using conventional tractor drawn spray equipment. At both locations, individual exposure to all workers, which included applicators, weeders, and scouts, was measured as they performed their duties under normal use conditions. The scouts did not use or apply glyphosate. Age of the workers ranged from 20 to 63 years.

In an attempt to identify the various avenues and extent of exposure occurring, several different measurements were made. Both passive dosimetry and biological monitoring methods were used in these studies. The passive dosimetry methods employed were designed to provide data measurements of the potential exposure, as measured by dislodgeable residue, gauze patches, and hand rinses. Biological monitoring was used to assess the total absorbed dose of glyphosate, as determined by analyzing total urine output.

Dislodgeable Residues

Since the duties of many of the workers often require them to directly contact seedlings in or near beds where glyphosate had been used, a passive monitoring measurement was made to determine the amount of glyphosate which could be dislodged from conifer seedlings. Workers commonly contact seedlings which have been wetted by fog, dew, rainfall, or irrigation. Thus, if the workers were exposed to glyphosate which dislodged from conifer seedlings or weeds containing residual levels of glyphosate, a water rinse should provide a good vehicle for allowing quantification of the amount of glyphosate which could be dislodged. Three randomly selected samples of approximately 100 g of fresh conifer seedlings were taken twice weekly at Ashe Nursery from May through August. The amount of glyphosate removed by shaking 100 g of fresh conifer seedlings with 250 ml of water for 45 sec was deemed to provide a realistic measure of "dislodgeable residues."

Passive Monitoring

Another passive dosimetry measurement tool involved the use of cotton gauze patches. These patches were attached one day each week to the clothing worn by the workers during their regular field operations. Portions of the body identified as most likely to be exposed to glyphosate had 7.5 by 7.5 cm cotton gauze patches attached to nearby clothing being worn by the workers. Patch construction consisted of stapling eight layers of the 7.5 × 7.5 cm gauze material to a comparable dimension filter paper backing to create the patch. The location of the nine gauze patches per worker were as follows: two patches near the ankles, two on the front of the thighs, two on the lower forearms [two on the lower forearms inside the shirt for some workers], one on the upper chest, one on the upper back near the neck, and one on the hat. Patches were attached to the worker's normal clothing using two metal safety pins. When long sleeved shirts were not worn a velcro bracelet was used for attaching patches to the lower forearms. On a few pre-selected sampling dates each of the nine strategically placed patches on the workers were analyzed separately; however, for the majority of the study the nine patches were composited into one sample on location. By compositing these patches only one patch sample was generated per worker for analysis.

A hand wash from the workers was taken at the end of each day patches were worn by using 250 ml of 10% methanol/ 90% water solution. As a measure of the total passive exposure occurring to each worker the amount of glyphosate present in the wash from both hands was added to that found from the patch analyses. This information is important in the calculations of the risk assessment data which will be presented later.

A supplemental investigation involving analysis of individual

[1] The protocol can be found in "Pesticide Exposure Assessment of Nursery Workers-Project Completion Report," 1989. Principal investigator T. L. Lavy. USDA Forest Service, 227 pp.

patches was conducted to gain an understanding of which part of the body was most heavily exposed and to learn the extent of protection afforded to workers who wore normal work clothing. For some preselected workers, patches were attached inside the normal clothing but near an outside patch. Care was taken to prevent the outside patches from covering areas where patches were attached inside the clothing. A comparison of the amount of glyphosate present on outside patches versus the amount present on inside patches allowed calculation of the amount of glyphosate penetrating the clothing of the workers. The US Environmental Protection Agency (EPA) requested this patch and hand rinse data be collected, to provide information for their generic pesticide monitoring data base.

Biological Monitoring

Since the weeders and the scouts duties required them to routinely re-enter treated areas, continuous biological monitoring techniques were employed to allow quantification of the total season-long absorbed dose of glyphosate. To aid in this biological monitoring scheme, each worker was issued a daily urine container and instructed to collect the total urine excreted. Collections were initiated before potential exposure could have occurred and continued well into the season. The final tests were performed at least eight weeks after glyphosate had last been applied. No pharmacokinetic studies on the urinary excretion of glyphosate for humans have been published. However, monkey pharmacokinetic studies contracted by the manufacturer have demonstrated that the majority (89.1%) of glyphosate entering the body through dermal exposure was excreted in urine within 5 days (Dirks 1982). In our previous worker exposure studies involving other herbicides, a 5-day collection period was sufficient to collect over 90% of the total absorbed dose (Lavy *et al.* 1980, 1982, 1987).

In the current studies, only the applicators driving tractor drawn equipment had a definitive "exposure day." Since weeders and scouts potentially had numerous days during which exposure could have occurred as they re-entered treated areas a continuous total urine collection scheme was prescribed for all weeders and scouts for 12 consecutive weeks. After this intensive 12-week collection period, a 24-h sample was collected each Wednesday from each worker for the next 5 months. By summing the total glyphosate present in urine over this period, a total absorbed dose for each worker for the entire season could be calculated.

The successful completion of this project was dependent on the full cooperation of the field workers which included providing the research team with their total urine output for this 12-consecutive-week period. Many of the workers were enthusiastic about being a part of this comprehensive nursery worker exposure study. Nevertheless, it was decided that a monetary incentive be provided to help insure cooperation over the long collection period. Workers were aware that creatinine tests were available which could show if their total urinary output was being provided to the researchers. Provisions were made for them to report occasional partial spills, etc. A payment totalling $10 per day was provided to those workers who fully complied with the collection scheme prescribed.

Analytical

A portion of the dislodgeable residues, patches, hand rinses, and urine samples was fortified with appropriate solutions at laboratories adjoining each field location. These fortified samples were included to insure that any degradation occurring during storage and shipment could be quantitatively assessed and appropriate corrections made if necessary. The number of field fortified samples was approximately 10% of the total number of samples generated. Samples were immediately frozen after collection and stored at field nursery freezers prior to shipping to the University of Arkansas Pesticide Residue Laboratory in Fayetteville, Arkansas. At this location, a different coding system was developed prior to preparing an additional 10% of "blind spikes" which were randomly interspersed among the "unknown" field samples. All glyphosate analytical determinations were made by Monsanto Co. in St. Louis, Missouri. Specifics regarding concentrations of the fortified samples and identification of the blind spike samples were not made available to Monsanto personnel until all analytical determinations had been returned to the principal investigator. Detailed analytical procedures used for these measurements can be found in Cowell and Steinmetz (1990). For individual sample analyses and other details regarding information presented in this study including the protocol, one may refer to the 475-page USDA Completion Report (Lavy *et al.* 1989) or to the Monsanto report listed (Cowell and Steinmetz 1990).

The recovery of glyphosate from fortified urine was 90.7% with a standard deviation of $\pm$ 7.28 for 132 samples fortified from 0.01 to 0.1 ppm (μg/g). The limit of detection was 0.002 ppm while the limit of quantitation was 0.00356 ppm. For urine samples the lower limit of method validation (LLOMV) defined as the lowest fortification level with recovery >70%, was 0.01 ppm. The LLOMV for dislodgeable residues was 0.02 μg/ml. The LLOMV for glyphosate on nine composited patches per worker was 12.5 micrograms while individual patches had a LLOMV of one microgram. The LLOMV for the hand rinse samples was 5 μg or 0.02 μg/ml.

Results and Discussion

Dislodgeable Residues

At Phipps Nursery, no dislodgeable residue samples were collected from the conifer seedlings since no glyphosate was used in the beds. At Ashe Nursery, glyphosate was used for weeding within the beds approximately nine times over an 88 day period beginning on May 9. Samples were not taken from June 4 to July 17, since no glyphosate was applied at that time. This sampling scheme resulted in 78 samples which were analyzed in the 21 sampling periods between May 9 and August 5; in only one of these dislodgeable residue samples was glyphosate detected. The one positive dislodgeable residue sample, collected on May 9, contained 138.5 micrograms glyphosate. The low frequency of detection for glyphosate in these dislodgeable residue samples from the conifer seedlings suggest that one or more of the following reasons were important: (1) irrigation and rainfall removed the glyphosate prior to sample collection; (2) the spray shield apparatus used by those manually applying glyphosate was highly effective in preventing this compound from contacting the seedlings; (3) the water rinse did not efficiently dislodge glyphosate after it dried on conifer seedlings; or (4) other environmental factors were effective in degrading glyphosate to levels below the detection limit.

These findings of essentially no glyphosate on the seedlings should not be surprising since care was taken to keep the phytotoxic glyphosate off the conifer seedlings. In addition, the 72 cm rainfall and irrigation at the Ashe Nursery may have washed some glyphosate off the vegetation prior to sample collection. The lack of significant findings of water extractable dislodgeable glyphosate on the seedlings indicates that the presence of dislodgeable residues as a source

Table 2. Individual patch analysis

Worker	Date	Ankles	Thighs	Forearms Outside (μg)	Forearms Inside	Chest	Back	Head	Total per worker
1 A[a]	7/22	.89	34.50	.29	.11	.29	.02	.12	36.22[b]
2 A	7/29	.42	.12	.28	.06	.04	.04	.02	.98
4 W	6/10	4.46	6.79	.01	.00	.00	.00	.01	11.27
5 W	6/10	11.10	.07	.03	.00	.00	.00	.03	11.23
5 W	6/13	8.93	—.[c]	.03	.02	.01	.00	.01	9.00
31 A	8/28	.01	.02	.00	.00	.00	.00	.00	0.03
		24.92	7.00	.35	.08	.05	.04	.07	
% of total		76.7	21.5	1.1	.3	.1	.1	.2	

[a] A = applicator; W = weeder
[b] Although all workers received the same instructions, worker number 1, a tractor-driver applicator indicated that he thought he was supposed to become highly exposed. His patches indicated that he received 36 times more exposure than his spray partner who used more material and sprayed more hectares. His data are recorded in Table 2 but do not appear in the means
[c] Sample missing

of exposure to personnel working in, around, or re-entering the seedling beds is minimal.

Patches and Hand Washes

Gauze patches attached to normal work clothing were worn one or two days each week from April to June by weeders and scouts at both nurseries. Some were also worn later in the year. Weeders at Ashe Nursery, who applied glyphosate manually as a part of the weeding procedure, wore patches each day that glyphosate applications were made. Although scouts did not apply glyphosate, their duties often required them to measure and count seedlings in the beds or diagnose for potential pest problems. Tractor drivers who applied glyphosate to nearby fields or between beds used conventional ground application spray equipment. These tractor drivers, numbers 1, 2, and 31 (Table 1) wore protective coveralls and rubber gloves. Patches were attached to their protective clothing prior to them mixing or spraying glyphosate.

In an attempt to best identify the body portions most likely to be exposed, analyses of individual patches were made for randomly selected workers. Results of these analyses showed that ankles and thighs received the largest majority of the glyphosate exposure which occurred (Table 2).

All patches on applicators, with the exception of the tractor spray applicator at Phipps Nursery, had measurable levels of glyphosate. Data in this table reveal that over 76% of the exposure, as measured by individual patch analyses, occurred on the ankles, while the thigh area received over 21% of the total. Since 98% of the exposure occurs at or below the thigh area, any changes in protection should emphasize protecting the lower parts of the body.

Even though tractor drivers wore special clothing, analyses of patches and hand washes revealed that they received more exposure than did the scouts and weeders (Table 3). Hand weeder number 4 at Ashe Nursery, the worker who handled the concentrate by diluting and filling the manual spray applicators for the other weeders, received much higher exposure than the eight other workers except for weeder number 5. There was an 18-fold difference in the hand wash levels between worker 5 and worker 6 who had similar job assignments. This wide range in exposure is not unlike some of the differences reported in our earlier studies which evaluated 2, 4, 5-T exposure by backpack applicators (Lavy *et al.* 1980). Thus, data continue to indicate that it is common for workers engaged in similar duties to receive a wide range of exposure. Much of this variation appears to be associated with worker habits and chemical hygiene. Others have observed widespread variation in worker exposure to pesticides (Nash and Kearney 1982; Libich *et al.* 1984).

Evaluation of hand washes from scouts showed that only 1 of 23 was positive for glyphosate (this lone positive sample contained 7.3 micrograms glyphosate). However, for applicators and weeders, 85 of 89 hand washes contained glyphosate above the 0.2 μg/ml level. Although much of the hand wash data show some worker exposure, in general the amount present was considerably less than the amounts found on the patches.

Essentially all of the applicators and weeders wore rubber gloves. Worker number 6 who wore a rubberized cotton glove recorded average to low exposure levels. Since all workers, except the scouts, had positive hand washes and yet all wore gloves, it is apparent that total protection was not achieved or that hands became contaminated while removing the gloves at the end of the day. To decrease this exposure it is suggested that the outside of the rubber gloves be washed off before removing them. Theoretically, one would expect that the glyphosate present on the hands would constitute a substantial exposure since no protective clothing separates this deposit from the potentially absorbent dermal surface.

Of the 34 individual and composited patches attached to clothing of scouts at this location, only one had a patch positive for glyphosate. Thus, scouts had little exposure. However, of the 95 composited nine-patch samples for the weeders at Ashe Nursery who applied glyphosate using individual manual applicators, 80 were positive for glyphosate. An evaluation of the findings from patch analysis and hand washes of the applicators and weeders at Ashe Nursery re-

Table 3. Passive monitoring data used for estimating absorbed dose of glyphosate

Worker				Micrograms glyphosate					Normalized	
no.	duty	Body wt.	No. observ. per.	Hand wash[a]	Exposed skin[b]	Covered skin[c]	Total	Est. dose[d]	Per hour[e] applied	Per kg[f] applied
				A	B	C	D	E	F	G
		(kg)		Daily Average (μg)					mg/kg/hr	mg/kg/kg
2	A	97.5	1	1080	619	5928	7627	137.3	3.5×10^{-4}	2.0×10^{-3}
31	A	99.8	2	60	769	2,044	2,873	51.7	1.0×10^{-4}	4.0×10^{-3}
4	A/W	63.5	8	108	13,635	34,876	48,619	875	1.7×10^{-3}	2.8×10^{-2}
5	W	79.4	7	675	3,780	9,651	14,106	254	4.0×10^{-4}	6.5×10^{-3}
6	W	50.8	15	38	1,457	3,726	5,221	94.0	2.3×10^{-4}	3.1×10^{-3}
7	W	65.8	10	95	855	2,187	3,137	56.5	1.1×10^{-4}	1.5×10^{-3}
8	W	70.8	7	54	727	1,859	2,640	47.5	8.5×10^{-5}	1.4×10^{-3}
9	W	74.4	7	306	893	2,279	3,478	62.6	1.1×10^{-4}	1.7×10^{-3}
10	W	70.8	7	139	3,917	10,032	14,088	254	4.5×10^{-4}	7.3×10^{-3}
11	W	81.7	7	71	344	878	1,293	23.3	3.6×10^{-5}	5.8×10^{-4}
91	W	69.9	14	103	1,340	3,422	4,865	87.6	1.6×10^{-4}	2.1×10^{-3}
12	S[g]	64.9	9	0.0	0.0	0.0	0.0	0.0	0.0	[h]
13	S[g]	64.9	11	0.7	24.4	62.3	87.4	1.6	3.1×10^{-6}	[h]

[a] Average glyphosate measured in hand rinse on the day patches were worn
[b] Amount deposited on exposed skin = μg glyphosate/cm^2 on exposed patches multiplied by the area of exposed skin (1560 cm^2)
[c] Glyphosate penetrating clothing = covered skin area × μg/cm^2 glyphoate on patches × 0.229 (clothing penetration factor)
[d] Estimated dose = total glyphosate contacting hands, exposed skin and covered skin × .018 dermal absorption value (Dirks 1982)
[e] mg/kg/hr = estimated body dose (converted to mg)/kg body weight/hour exposed. The exposure time for weeders and scouts was 8 hours and for applicators #2 and #31 it was 4 and 5 hours, respectively
[f] Weeders applied 0.068 kg except weeder #4 who applied 0.54 kg, an estimation arrived at by multiplying 0.068 by 8 (the number of weeders serviced by #4 who mixed and poured the solutions for all of the other weeders). Applicator #31 treated 1.2 hectares using 3.8 L Roundup®/935 L water/ha. There is 1.4 kg glyphosate (acid equiv.)/gal Roundup®. The total kg glyphosate applied by #31 = 4.1 kg
[g] Of the 57 samples from scouts analyzed for glyphosate, worker #13 had one positive hand wash (7.3 μg) and one positive patch sample (19.4 μg)
[h] Scouts walked through the area treated by weeders. Because glyphosate was not applied by scouts, mg/kg/kg calculations were not made

vealed that for weeder-applicators, a high percentage of these samples were positive for glyphosate. Thus, the nursery workers most likely to receive an absorbed dose of glyphosate were the applicators and weeders at Ashe Nursery.

To gain some insight regarding the extent of protection afforded these workers by the clothing they were wearing, a comparison of the glyphosate present on the paired patches worn inside and outside the forearm clothing was made (Table 2). Dividing the amount of glyphosate found on the inside patch by the total amount found on both patches and multiplying by 100 gives the percentage of glyphosate that penetrated the workers' clothing. Since only a limited number of inside and outside patch comparisons were made and a relatively high error is involved in making comparisons of trace levels of glyphosate, these values are an estimate of the actual percentage of glyphosate penetrating the clothing. Nevertheless, average values for all paired patches showed an average of 22.9% of the glyphosate deposited on worker clothing might be expected to penetrate through it.

Some patches were attached to the workers' clothing which had been strategically located near exposed, noncovered skin areas. By multiplying the amount of glyphosate on the patch on a μg/cm^2 basis by the area of this uncovered skin (cm^2), a value can be calculated which represents the total glyphosate (μg) conceivably deposited on the uncovered area, assuming comparable deposition on all surfaces. In addition, an estimation of the amount of glyphosate deposition resulting from penetration through clothing on covered skin areas can be calculated by multiplying the amount of glyphosate per cm^2 on the nearest patch, the area of the covered portion and 0.229 (due to the 22.9% penetration value discussed earlier). Summing the values from all body parts provides an estimate of glyphosate deposited on skin under clothed portions of the body. The 22.9% clothing penetration value used may be somewhat higher than actual values since some body parts are covered by more than one layer of clothing.

The amount of glyphosate present in the hand wash solutions provides an estimate of the glyphosate contacting the hands. Adding the amount found on the hands to that deposited on other uncovered body parts and that portion penetrating clothing provides a value which represents the total glyphosate contacting the body of each worker. Individual total dermal deposition values are found in column D of Table 3.

Monkey pharmacokinetic studies (Dirks 1982) show that 1.8% of the glyphosate contacting dermal surfaces was absorbed through the skin. Using the hand rinse and gauze patch data and accepting the 1.8% absorption value as the best number for estimating absorption through human skin, allows calculation of the amount of glyphosate which could conceivably penetrate the skin of exposed nursery workers in our studies. Using the data collected from the passive dosimetry studies and assuming that the 1.8% value is valid for humans, an estimate of absorbed dose as measured by the passive dosimetry studies is presented in column E of Table 3. When the values are normalized for body weight and exposure period, the data show that applicators and the weeder who mixed and loaded the spray bottles at the Ashe Nursery received the most exposure. Average values for these groups were 7.2×10^{-4}, and 2.0×10^{-4}, and 1.6×10^{-6} mg/kg/h for applicators, weeders, and scouts, respectively. Thus, based on extrapolations from the passive dosimetry data, applicators would have received 3.6 times more exposure than weeders and 450 times more exposure than scouts.

In summary, these passive monitoring findings suggest that use of glyphosate by applicators, tractor sprayers or individual applications by weeders, provides potential for glyphosate exposure. The perceived significance of this potential exposure will be addressed later in the paper.

Biological Monitoring Data

Analyses of 355 urine samples monitored over 1,138 worker-days of total urine collection from the 14 workers in these studies did not reveal the presence of glyphosate. At first glance, these findings may be somewhat surprising since significant levels of glyphosate were present on patches and hand rinses. Also, previous studies (Dirks 1982) have shown that 1.8% of the glyphosate deposited onto monkey skin was excreted in the urine of the treated monkeys. The fact that no glyphosate was found in the urine of the workers in our study may be interpreted in several ways.

1. Human skin is less penetrable by glyphosate than monkey skin (*i.e.*, no glyphosate penetrated the skin of these exposed workers)
2. Glyphosate deposited on skin in a real-world worker situation does not penetrate at the same rate as the laboratory studies due to other parameters such as perspiration, runoff, etc.
3. Glyphosate was metabolized to a non-detectable metabolite. This is not probable since in the Dirks (1982) monkey study using ^{14}C-labeled glyphosate more than 89% of an applied dermal dose was accounted for in the parent or metabolites being monitored.

In addition to the 355 urine samples analyzed from the field workers, 74 fortified control urine samples were collected from on-site office employees and were shipped and stored with the actual samples as a part of the quality control program. Fortification levels ranged from 0.10 to 0.58 μg glyphosate/ ml urine. Percentage recoveries for these fortified samples ranged from 57.7 to 168 with an average of 100. All field fortified samples contained quantifiable amounts at the time of analysis.

Data Assessments

At the initiation of this nursery-worker exposure study, it was thought that the amount of dislodgeable glyphosate on the surfaces of the vegetation could represent a significant source of exposure. Thus, a measure of this potential exposure source was deemed important. If a high correlation could be shown between the absorbed dose and the amount of glyphosate present as a dislodgeable residue or the amount present on gauze patches or hand rinse, future worker exposure studies could be markedly simplified. Dislodgeable residue, as well as patch or hand rinse samples are simpler to collect, quantify and analyze than are total urine samples. Thus, finding a good correlation between the amount determined by passive dosimetry and the amount excreted in urine could also provide a significant savings in time, effort and dollars. However, since no glyphosate was found in urine no definitive correlation could be made between the actual amount deposited on the body or foliage and the amount in urine.

With respect to reporting and interpreting data from human exposure to pesticide studies, a new concept has been adopted by the US EPA, the specifics of which are presented in *Pesticide Assessment Guidelines, Subdivision U, Applicator Exposure Monitoring*. The new concept resulted from the rapid developments in the area of analytical chemistry methodology which allow for the detection of lower and lower levels of pesticides. Therefore values which in the past were reported as 0 or ND (non-detected), may today have a definitive quantitative value. Correspondingly, 0 or ND values today may also in the future be found to have a definitive value. Thus, the EPA has developed guidelines which, in their opinion, more accurately report (or estimate) trace amounts of materials. These guidelines indicate that for all samples found to be below the detection limit, a value equivalent to ½ the detection limit will be assumed to be present in the sample.

In this study, we used the lower limit of method validation (LLOMV), defined as the lowest glyphosate level that was fortified to a check urine sample and found to be accurately (>70%) and reproducibly (<25% CV) recovered, instead of the limit of detection. (The definition is generic but the values are specific for urine samples in this study.)

Although this concept will help insure that samples with trace levels will not go unreported, it suffers from the major obstacle that samples which are truly zero will no longer be accurately reported.

It is essential that the reader be able to differentiate between those values which have arisen from true analytically positive detections and those which have arisen based solely from data emanating from calculations of ½ the LLOMV. Hereinafter in this paper data values calculated using ½ the LLOMV will be referred to as "postulated data". All risk assessment information generated from biological monitoring data in this study is classified as postulated data since none of these biologically monitored urine samples were positive for glyphosate or its metabolites.

Thus, in order to provide an estimate of any exposure occurring which is below our analytical detection limits for the urine samples, a value of ½ the LLOMV per ml of urine excreted is being used to calculate an assumed absorbed dose. Since the LLOMV value for glyphosate and glyphosate metabolites in human urine was 0.010 μg/ml, a concentration of 0.005 μg glyphosate per milliliter of urine was assumed to be present in all urine samples (although no urine samples were positive for glyphosate or its metabolites). The product of this concentration and the total volume of urine excreted provides a value which will be used to approximate

Table 4. Mean comparison of biological monitoring data using "postulated data" and passive total body dose estimates for applicators, weeders and scouts

		Biological monitoring mg/kg/hr	Passive dosimetry mg/kg/hr
Applicators[a]	x̄	6.5×10^{-5}	7.2×10^{-4}
	S.D.	1.1×10^{-5}	8.6×10^{-4}
Weeders	x̄	3.9×10^{-5}	2.0×10^{-4}
	S.D.	1.8×10^{-5}	1.5×10^{-4}
Scouts	x̄	8.0×10^{-5}	1.6×10^{-6}
	S.D.	5.7×10^{-5}	2.2×10^{-6}

[a] Workers 2, 4 and 31 were included as applicators

Table 5. Margin of safety[a] calculations based on biological monitoring "postulated data" and passive dosimetry for nursery worker duties

	Biological monitoring	Passive dosimetry
	(MOS)	(MOS)
Applicators	6,410	579
Weeders	10,684	2,083
Scouts	5,208	260,400

[a] Based on 10 mg/kg/day rat feeding studies

a measure of the absorbed dose. In monkey pharmacokinetic studies (Dirks 1982), 84% of the total glyphosate entering the body was excreted within a 72 h period. For several of the weeder-applicators daily, total urine collections were made over a consecutive 84-day period during which they made as many as eight glyphosate applications. Based on finding no glyphosate in the urine but using the ½ LLOMV value (0.005 μg/ml), the calculated absorbed dose for each worker will be directly proportional to the volume of urine excreted. Use of guidelines which result in the highest exposure estimates for those excreting the highest quantity of urine appears to lack scientific validity.

Although biological monitoring of urine failed to show any worker's urine to be positive for glyphosate, data in Table 4 provides a comparison of passive dosimetry and biological monitoring exposure estimates based on assumptions made for each measurement scheme. Data calculations in Table 4 show higher passive dosimetry readings than biological monitoring readings for both applicators and weeders. It should be pointed out that urinary excretion volumes were fairly uniform for all workers, but scouts excreted somewhat larger volumes. Since no glyphosate was found in any sample, the calculated amount listed in Table 4 reflects the volume of urine excreted multiplied by ½ the LLOMV value (i.e. 0.005 μg/ml). Passive dosimetry values for scouts were quite small since almost all patches and hand wash samples contained no detectable glyphosate.

Multiplying the data in Table 4 by 24 h, calculating margins of safety based on a 10 mg/kg/day no observable effect level (NOEL) (Federal Register, 1990) and assuming that exposure occurs each day provides the information listed in Table 5. Margins of safety (MOS), derived by dividing calculated dose levels from the passive monitoring study into a 10 mg/kg/day rat NOEL, ranged from 579 (applicators) to 260,400 (scouts). For the biological monitoring data using "postulated data", a much closer range was found. These biological monitoring data reveal that relatively high MOS values are present, even after assuming that an unmeasurable amount of glyphosate was present.

The 10 mg/kg/day NOEL was obtained from trials in which rats were continuously fed 10 mg of glyphosate per kilogram body weight each day. For applicators and weeders, patches and hand washes were used as monitors only on the day that glyphosate was applied. If average daily values obtained in our passive and biological monitoring schemes are divided into the 10 mg/kg/day NOEL, this results in MOS values that assume every day is an application day. Applications were made as many as 1 to 8½ days per year by applicators and weeders. Thus, the MOS values presented here represent worst case calculation of our data. Larger margins of safety would have resulted if the actual exposure days had been used in the calculations. Nevertheless, high MOS are present even when this conservative approach is taken.

If we had ignored the new EPA Guidelines and chosen not to evaluate our data following this new concept, the passive monitoring data would have been accepted as the best estimate of exposure. Our calculations indicate that passive monitoring may have over-estimated exposure assessed by biological monitoring by at least 11 fold for the most highly exposed workers. This conclusion is based on assuming that 1.8% of dermally deposited glyphosate penetrates human skin and that at least 89% of the internal glyphosate is excreted in urine (Dirks 1982).

Use of the ½ of the LLOMV value for biological monitoring data likely over-estimates the actual exposure (and absorbed dose) occurring; nevertheless, as analytical sensitivity improves, this approach can be used to assist in evaluating risks for humans contacting pesticides. Ultimately the goal should be to perfect the methodology and understanding of absorbed dose to the point where the appropriate biologically monitored component can be quantified to accurately assess the extent of risk associated with exposure to any real or potential toxin. As limits of detection continue to decrease, valid MOS will increase if samples recorded as 0 are true 0's.

Results from these year-long studies suggest that glyphosate exposure via dislodgeable residue, direct contact during glyphosate application or season-long exposure to this herbicide should not pose a threat to human health when used under normal nursery conditions.

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