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Concurrent 2,4-D and triclopyr biomonitoring of backpack applicators, mixer/loader and field supervisor in forestry

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Two herbicides, 2,4-D and triclopyr esters (application ratio 1.6:1 acid equivalents) were applied as a tank mix by a crew of 8 backpack sprayer applicators, a mixer/loader, and a field supervisor. The crew was employed in a conifer release program in northern California during the summer of 2002. Biomonitoring (urine, 24 h) utilized 2,4-D and triclopyr (a.e.) as rapidly excreted exposure biomarkers. The absorbed dosages of 2,4-D and triclopyr were calculated based upon cotton whole body suits and biomonitoring. Dosages based upon accumulation of the herbicides on body suits averaged 42.6 μg (a.e.) 2,4-D/kg-d and 8.0 μg (a.e.) triclopyr/kg-d. Six consecutive days of concurrent urine collections showed that backpack applicators excreted an average of 11.0 μg (a.e.) 2,4-D/kg-d and 18.9 μg (a.e.) triclopyr/kg-d. Estimates based upon curve fitting were 17.1 and 29.3 μg (a.e.)/kg-d, respectively. Results suggest that passive dosimetry for 2,4-D consistently overestimated the dosage measured using biomonitoring by a factor of 2-3 fold, while for triclopyr, passive dosimetry underestimated the absorbed dose based on biomonitoring by a factor of 2-4 fold.

Keywords: Exposure assessment; forestry; triclopyr; 2,4-D; biomonitoring; backpack.

Introduction

Herbicides are used in forestry for the control of broad leaf weeds and brush for site preparation after timber harvest, conifer release in young regenerating forests, thinning to promote growth of remaining trees, and fuel reduction. About 27,000 lbs of 2,4-D and 5,900 lbs of triclopyr were used by the U.S. Forest Service in 2002.[1]Usually ground/aerial broadcast, high/low volume foliar, and backpack sprayers control weeds by applying a mixture of herbicides. Herbicide sprays may be directly applied by backpack sprayer to broadleaf foliage in conifer release treatments. Applicators may contact the chemicals by inhalation, ingestion, and dermal absorption. However, the first two routes are of minor importance compared to dermal exposure, which is the most important route of entry.[2,3]

Due to their high efficiency and low mammalian toxicity, pyridine and phenoxy herbicides are essential for many agricultural uses. Triclopyr (Garlon *4™; 3,5,6-trichloro-2-pyridinyloxyacetic acid) and 2,4-D (2,4-dichlorophenoxyacetic acid), two broad-spectrum broadleaf herbicides which act as auxin agonists in woody plants, and annual and perennial weeds are formulated as esters (Fig.1). In the human body the esters are hydrolyzed to release the acids, which are rapidly excreted almost completely in urine[4] with a first order excretion half-life of 16.8 h (dermal) for triclopyr[5] and 17.7 h (oral) for 2,4-D.[6] Over 80 % of triclopyr[5] and 95.1 % for 2,4-D[6] are eliminated as acids, thereby facilitating urine biomonitoring.[7]

This study gives occupational exposure estimates for 2,4-D and triclopyr resulting from backpack sprayer use in forestry. The exposures of a hand sprayer crew were determined by whole body dosimetry and biomonitoring during a 6-day reforestation project in northern California.

Materials and methods

Participants

A crew of 10 contract workers (Great Tree Tenders, Redwood Valley, CA) consisted of 8 backpack sprayer applicators, a mixer/loader, and a field supervisor (Table 1).

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Table 1. Characteristics of study participants.

Participant Number	Body Weight (kg)	Height (m)	Age (yr)	Body Mass Index[a] (kg/m²)	Work Task	Group[b]
1022	68.6	1.64	22	25.5	Applicator	A
1234	82.6	1.74	25	27.3	Applicator	A
1970	53.6	1.54	34	22.6	Applicator	A
1978	79.9	1.74	24	26.4	Applicator	A
3525	74.5	1.68	32	26.4	Applicator	A
2123	73.1	1.74	21	24.1	Applicator	B
3335	64.0	1.64	21	23.8	Applicator	B
6670	76.3	1.73	49	25.5	Applicator	B
1272	79.5	1.70	28	27.5	Mixer/loader	B
7227	69.9	1.56	28	28.7	Field Supervisor	B
Mean ± SD	72.2 ± 8.7	1.67 ± 0.07	28.4 ± 8.5	25.8 ± 1.9		

[a] Body Mass Index (BMI) = Body weight (kg) ÷ Height ^{2} (m)

[b] All workers wore coveralls. Group A used cotton union suits as inner whole body dosimeters. Group B wore standard worker protection clothing beneath coveralls. All workers provided 24 h urine specimens following a 12 h control collection.

The crews lived in an encampment near the work sites and maintained normal work schedules and practices during the study period. Their herbicide exposures were monitored from August 5 to August 10, 2002, in the Klamath National Forest near Weed, CA. During a 6-day period, Garlon* 4™ and 2,4-D LV 6™ tank mix were applied to brush for release treatment in young, regenerating conifer forests.

The protocol was approved by the Institutional Review Board, University of California, Riverside. Each worker gave written consent prior to participation. Age, body weight, and work experience were recorded (Table 1).

Conifer forests

Three application units were located about 20 air miles east of Weed, CA. The elevation was about 1830 m. Fifty-five acres, representing units of 35, 5, and 15 acres, were treated. The conifers were 8 year-old California red fir *Abies magnifica* and ponderosa pine *Pinus ponderosa* that were about 0.7 to 3 m in height. The rugged terrain was uneven and slopes ranged from 10 % to 50 %. Fifty percent or more of the ground was covered by brush species (0.3 to 2 m) including greenleaf manzanita *Arctostaphylus patula*, snowbrush *Ceanothus velutinus*, and red flowering current *Ribes sanguineum*. Weather Information Management System of
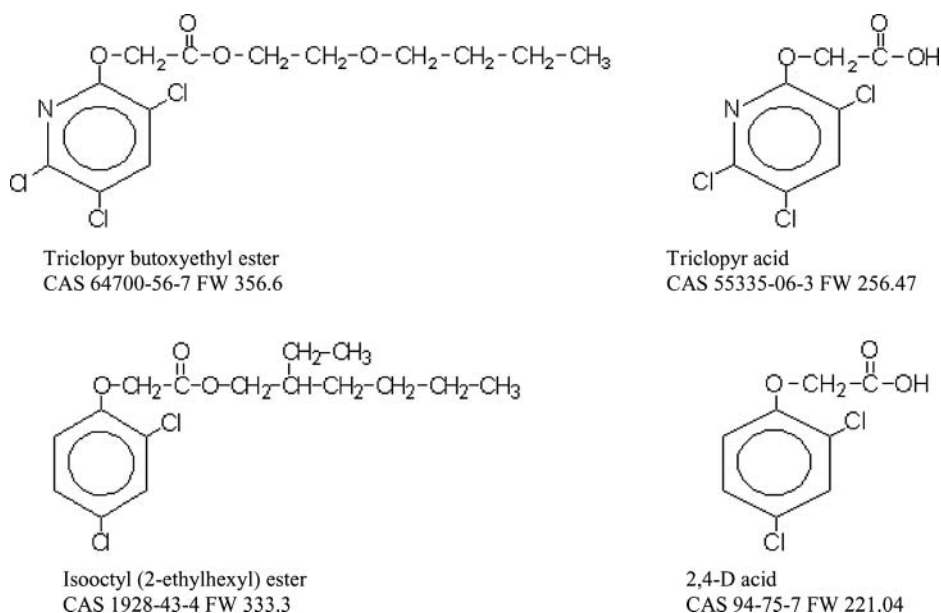

Triclopyr butoxyethyl ester
CAS 64700-56-7 FW 356.6

Triclopyr acid
CAS 55335-06-3 FW 256.47

Isooctyl (2-ethylhexyl) ester
CAS 1928-43-4 FW 333.3

2,4-D acid
CAS 94-75-7 FW 221.04

Fig. 1. Structures of Esters and Acids for Triclopyr and 2,4-D.

Table 2. Daily acres treated and tank mix applied during conifer release study.

				Triclopyr		2,4-D	
Day	Acres Treated	Hours Worked	Gallons Tank Mix	Garlon[a] (gallons)	Triclopyr acid (lb)	2,4-D LV 6[b] (gallons)	2,4-D acid (lb)
1	7.5	7	200	3	12	3	16.5
2	11	8	300	4.5	18	4.5	24.75
3	10	8.5	300	4.5	18	4.5	24.75
4	9	8	300	4.5	18	4.5	24.75
5	10	8.5	300	4.5	18	4.5	24.75
6	7.5	7	200	3	12	3	16.5
Total	55	47	1600	24	96	24	132

[a]Garlon *4 contains 4 lb/gal triclopyr acid.
[b]2,4-D LV 6 ester contains 5.5 lb/gal 2,4-dichlorophenoxyacetic acid.
Application ratio as acid equivalents: 132/221: 96/256 = 1.6:1

National Fire and Aviation Management (Kansas City, MO) provided weather data. No rain occurred and daytime temperatures ranged from 5°C to 33°C, relative humidity from 9 % to 75 %, and wind speed from 0 to 16 km/hour during the time of application.

Spray mix

Garlon* 4™ (EPA Reg. No. 62719-40; Dow AgroSciences, Indianapolis, IN) and 2,4-D LV 6™ (EPA Reg. No. 228-95; Riverdale Chemical Company, Glenwood, IL) were purchased from the normal channels of trade. Garlon* 4™ was formulated as 3,5,6-tricloro-2-pyridinyloxyacetic acid butoxyethyl ester, equivalent to 44.3 % (w/v) triclopyr. 2,4-D LV 6™ was 2,4-dichlorophenoxyacetic acid 2-ethylhexyl ester, equivalent to 57.9 % (w/v) 2,4-D (Figure 1). The aqueous tank mix also contained Phase™, a blend of methylated esters of fatty acids and organosilicon surfactant (EPA CA Reg. No. 36208-50031; Loveland Industries, Inc., Greeley, CO). Spray Tracer Purple™ (Becker Underwood, Inc., Ames, IA), was also added to visualize the spray. All the chemicals were stored on a flatbed utility truck in the field under ambient conditions.

Tank mix was prepared daily by a mixer/loader who was a California-certified Pest Control Operator. He also filled individual 4-gallon backpack sprayers (Shindaiwa, Inc., Tualatin, OR) and assisted with minor maintenance during the work day. A 500-gallon, custom mixing unit on a utility truck was filled with water from a local creek. Garlon* 4™, 2,4-D LV 6™ and Phase™ were mixed at 1:1:2 (v/v). The 2,4-D:triclopyr mole ratio was 1.6:1 (a.e.). The mix was applied to foliage at 30 gallons/acre (2 quarts a.i./acre) using handwands (Table 2). During the spray operation workers often used their lower legs and feet to clear conifers away from brush, creating opportunity for leg contact with sprayed foliage. The applicators began spraying and returned to refill their hand pump sprayers at about 30 min intervals. The crew followed virtually the same work schedule each day.

Study design

Backpack sprayer applicators, the mixer/loader, and the field supervisor provided a pre-work and complete 24-h urine specimens during a 6-day spray program. Each worker wore either Worker Protection Standard (WPS)

Table 3. Tank mix analyses.

		Triclopyr (mg/L)				2,4-D (mg/L)			
Tank Mix	Time	Ester	Acid	Total ester equivalent[a]	Percent recovered[c] (%)	Ester	Acid	Total ester equivalents[b]	Percent
Tank Mix Sub1	Day 2	9,746	0	9,746	105	3,798	4,360	10,372	81
Tank Mix Sub2	Day 2	10,364	0	10,364	112	4,315	3,780	10,014	79
Tank Mix Sub10	Day 2	586	5,600	8,373	91	935	7,180	11,761	92
Tank Mix Sub11	Day 2	545	5,650	8,401	91	906	7,150	11,687	92
Tank Mix Sub20	Day 4	591	5,712	8,534	92	909	7,580	12,338	97
Tank Mix Sub21	Day 4	965	5,100	8,057	87	1,572	6,590	11,508	90
Mean ± SD				8,913 ± 920	96 ± 10			11,280 ± 894	88 ± 7

[a]Total triclopyr ester equivalents(mg/L) = triclopyr ester (mg/L) + triclopyr acid (mg/L) × (FW triclopyr ester ÷ FW triclopyr acid).
[b]Total 2,4-D ester equivalents(mg/L) = 2,4-D ester (mg/L) + 2,4-D acid (mg/L) × FW 2,4-D ÷ FW 2,4-D acid.
[c]Percent recovered (%) = Concentration recovered ÷ Nominal concentration × 100%. The nominal concentration for triclopyr ester was 9,240 mg/L and 2,4-D ester was 12,750 mg/L (2,4-D/221:Triclopyr/256 (a.e.) = 1.6).

clothing consisting of their personal long sleeved shirt and long pants or a cotton union suit (Sears®) beneath long-sleeved cotton coveralls. Serving as passive dosimeters, the union suits (whole body suit) were changed daily and analyzed for herbicide residues that were considered potential dermal exposure (DE). Additionally, a plastic safety helmet, impervious nitrile work gloves (14 mil gauntlet type), and knee-high, corked rubber boots were worn. Five spraymen (Group A) were randomly selected by draw to wear whole body, cotton suits. All workers were supplied daily with cotton socks and light cotton gloves that were worn beneath their work gloves. The mixer/loader and field supervisor wore their usual WPS clothing beneath their cotton coveralls.

Spraymen in Group A wore whole body suits under long sleeved cotton coveralls. The body suits served as passive whole body dosimeters. In addition, the spraymen provided 24 h urine specimens. Those in Group B wore their normal work clothes and provided only 24 h urine collections each day. Clean cotton coveralls were supplied to both groups each day.

Urine

Urine collected from university volunteers without known herbicide exposure was used for fortification following the loss of the primary control urine samples during transport from the field laboratory to the university. Aliquots (24 mL)

Table 4. Recovery rates for spiked dosimeter samples.

Sample	Scheme [a]	Recovery (%) Triclopyr	2,4-D
Body suit	lab spike	94 ± 36	50 ± 23
	travel spike	79 ± 12	29 ± 8^{b}
	travel spike second hydrolysis	87 ± 13	72 ± 7
	field spike	114 ± 35	62 ± 31
	matrix blank	100 ± 15	77 ± 8
Urine	lab spike at high level of acids [c]	112 ± 15	109 ± 10

[a]The spike scheme is as follows: Washed, unused body suits (control) containing no triclopyr or 2,4-D were analyzed with spiked samples. Matrix blank: spiked samples in the analytical lab with the analysis of real samples; lab spike: spikes before the field study; travel spike: spikes right before the trip to the study site; field spike: spiked in the field. Recovery is per cent of nominal triclopyr (15.4 mg) and 2,4-D (21.8 mg) ester added as an acetone spike of body suit.

[b]This recovery rate was low and a second hydrolysis for the samples was performed. An improved recovery was achieved as shown in the rehydrolysis result.

[c]Blank urine was spiked at UC Riverside with triclopyr and 2,4-D acids. But the spiked urine samples for lab, travel, and field were lost during transport. New lab spiked urine samples were prepared after the field study and only high level spikes within the working range of the study were used. Day 0 urine specimens of study participants (Table 6) contained some triclopyr and 2,4-D perhaps as a result of handling materials and equipment in preparation for the spray program.

containing triclopyr and 2,4-D at 0.25 ppm and 25 ppm were prepared 4 days before the study and on Days 1 and 3.

Quality assurance

An independent QA professional observed field operations during a one-day visit to the study site. Field operations of the lab personnel, data recording, sample storage and sample labeling and processing were also reviewed.

Samples and analysis

Tank mix

About 20-30 mL tank mix was collected from each of the 6 batches of spray mix on Days 2 and 4. Esters and acids of triclopyr and 2,4-D in the tank mix were analyzed at Carbon Dynamics Institute, LLC, Springfield, IL 62702 (Table 3).

Cotton body suits

Collected body suits were stored frozen on dry ice and shipped to McKenzie/Wright Laboratories, College Station, TX, for triclopyr ester and 2,4-D ester analysis. Analysis for both esters was performed by extraction of all ester and corresponding free acid residues from the body suits, reduction of all extracted esters to their corresponding free acid and quantification of the extracted and reduced free acid totals by LC/MS/MS. The quantified 2,4-D and triclopyr acid residues were reported as their corresponding ester by applying an appropriate molecular weight conversion calculation. The suits were extracted in 2.5 L of pH 7 water:acetone (60:40 v/v) solution and shaken for 30 min. A portion of this extract was basified to pH 12 using NaOH and placed in a 60°C water bath for 2 hours. After the sample was chilled, the pH was adjusted to 2 with H_2SO_4. The sample was then transferred to pre-conditioned CarboPrep 200 SPE cartridge (Restek, Bellefonte, PA) for clean-up. Quantification of triclopyr and 2,4-D was performed using a Thermo Finnigan Surveyor LC/MS/MS system configured with a C_{18} Phenyl LC column (2.1 mm I.D. × 100 mm; 3.5 μm particle size). The mobile phase was MeOH:H_2O (60:40 v/v) at a flow rate of 300 μL/min. The limit of quantification for the method was 150 μg/suit for triclopyr ester and 3000 μg/suit for 2,4-D ester (Table 4).

Urine specimens

Urine was collected in 8 h and 16 h portions each day that were combined in the field to form a 24 h specimen. Urine volume was determined gravimetrically and a 25 g portion was collected and frozen for shipment to Pacific Toxicology Laboratories, Chatsworth, CA, for analysis of triclopyr and 2,4-D acids. Frozen specimens were thawed, and a 4 g aliquot was transferred to an 8 mL vial. $^{13}C_6$-2,4-D was added as an internal standard. The sample was treated with

1 mL of concentrated (12N) HCl and heated in a 90°C water bath for 60 minutes. Dichloromethane was added, mixed and the organic layer removed. The sample was re-extracted with dichloromethane. The combined extracts were concentrated and derivatized with diazomethane to produce methyl esters of 2,4-D, triclopyr, and the internal standard. The sample was analyzed by GC/MS equipped with a J&W Scientific, Folsom, CA, fused silica capillary column (30 m × 0.18 mm i.d. and 0.3 μm film thickness). Following 1 μL sample injection, the oven was held at an initial temperature of 80°C for 1 min. Temperature was increased 10°C/min to 180°C and then temperature was increased 20°C/min to reach final temperature of 280°C that was held for 2 min. The limit of quantification was 5 μg/L for triclopyr and 2 μg/L for 2,4-D.

Concurrent analysis of worker urine samples for creatinine (Cn) using the Jaffé method was performed to evaluate the completeness of the 24 h urine specimens. The change in absorbance at 520 nm for 30 μL urine was measured after adding an alkaline picrate solution. The test was performed on a Beckman CX7 analyzer using Beckman Coulter Synchron C® Systems Creatinine Reagent Kit (Beckman Coulter, Inc. Fullerton, CA). The limit of quantification was 0.14 μg/L.

Data analysis

Residues recovered from body suits and biomonitoring data are reported as acid equivalents. Dermal exposure (DE), absorbed dosage (AD) and absorbed daily dosage (ADD) are defined as follows:

DE = herbicide residue retained on the body suit (mg a.e./d; Table 5).

AD = DE × default dermal absorption of herbicide (%/24h).

ADD = AD/body weight (μg/kg-d; Table 5)

DE represents the herbicide dose that penetrated the cotton coverall to the whole body suit. The default dermal absorption percentage was 5.8 % for 2,4-D[4] and 1.65 % for triclopyr.[5] The results are presented in Table 5.

Urine biomonitoring: 2,4-D and triclopyr acids

Individual daily excreted amounts of 2,4-D and triclopyr acids (mean ± s.d.) are reported in Table 6. These values represent cumulative 24 h urine collections for each of 6 successive work days by each study participant including the mixer/loader and the field supervisor. The excreted levels of biomarkers were not adjusted for partial excretion via the urine pathway or for half-life derived from bolus oral dosing studies.

Urine excretion curve fitting

To approximate the steady-state absorbed dose levels for 2,4-D and triclopyr, the mean daily dosage values for each cohort were plotted (*x* values represent Day, i.e., 0 to 6 and *y* values represent ADD) using TableCurve 2D, version 5.01 for Windows© (2002, SYSTAT Software Inc.). TableCurve 2D provides curve fitting and associated best fit statistical analyses (e.g., r^2 values) for linear and non-linear functions for a given set of data (see http://www.systat.com/products/tablecurve2d/ for more details). TableCurve 2D uses four common goodness-of-fit statistics [using the following descriptors: SSM - the sum of squares about the mean; SSE – the sum of squared errors (residuals); n - the total number of data values, and m - the

Table 5. Estimated triclopyr and 2,4-D absorbed dosages of backpack applicators based upon cotton whole body suit dosimeters (WBD) worn beneath coveralls.

Applicator Number	Body Weight (kg)	WBD Dose (mg a.e.)[a]		Estimated Absorbed Dose (mg a.e.)[b]	
		2,4-D	Triclopyr	2,4-D	Triclopyr
1022	68.6	19 ± 13	37 ± 29	1.90	0.39
1234	82.6	42 ± 23	104 ± 80	4.20	1.09
1970	53.6	7 ± 5	13 ± 12	0.70	0.14
1978	79.9	15 ± 13	32 ± 38	1.50	0.33
3525	74.5	70 ± 68	89 ± 59	7.00	0.93
Average WBD Dose (mg)[b]		31 ± 24	55 ± 44	3.1	0.57
Avg.WBD Dosage (μg/kg)[c]				42.6	8.0
BM Dosage (μg/kg)[d]				11.0	18.9
Ratio WBD:BM[e]				3.87	0.423

[a] Mean ± s.d. herbicide a.e. recovered from WBD
[b] Mean dose derived from WBD/travel spike recovery (Table 4) × 0.5 clothing penetration × fraction dermal absorption. Dermal absorption$_{24h}$ estimates of 1.65 % for triclopyr[5] and 5.8 % for 2,4-D.[4]
[c] Absorbed dose WBD normalized to average body weight of 71.8 kg
[d] Biomonitoring dosage from Table 7
[e] Ratio of absorbed dosages, WBD (passive dosimetry) to BM (biomonitoring)

Table 6. Urine biomonitoring of backpack applicator crew, mixer/loader, and field supervisor.

| Absorbed Dose and Dosage | Study day | Group A (n = 5) | | Group B | | | | | |
| | | | | Applicators (n = 3) | | Mixer/Loader | | Field Supervisor | |
		Triclopyr	2,4-D	Triclopyr	2,4-D	Triclopyr	2,4-D	Triclopyr	2,4-D
AD (μg a.e./day)	Day 0	4.3 ± 7.2	11 ± 12	2.2 ± 1.4	23.2 ± 23	15.8	9.3	2.2	17.2
	Day 1	393 ± 291	186 ± 61	206 ± 123	204 ± 143	64.7	67.6	114	78.2
	Day 2	783 ± 90.6	481 ± 170	1267 ± 918	961 ± 848	137	167	221	188
	Day 3	1107 ± 378	711 ± 242	1058 ± 645	908 ± 668	186	217	304	293
	Day 4	1557 ± 537	1069 ± 442	1071 ± 748	773 ± 535	319	212	311	334
	Day 5	2118 ± 765	962 ± 283	1203 ± 967	981 ± 820	400	256	360	237
	Day 6	2181 ± 748	1203 ± 306	2877 ± 3143	1883 ± 2399	357	381	551	413
	Mean ± SD (Days 1 to 6)	1349 ± 831[a]	768 ± 438[b]	1281 ± 1470[a]	951 ± 1089[b]	244 ± 134	217 ± 103	310 ± 146	257 ± 117
ADD (μg a.e./kg-d)	Day 0	0.1 ± 0.1	0.1 ± 0.1	0.04 ± 0.01	0.3 ± 0.3	0.2	0.1	0.03	0.3
	Day 1	5.6 ± 4.3	2.7 ± 0.9	2.8 ± 1.5	2.8 ± 1.8	0.8	0.9	1.7	1.1
	Day 2	10.5 ± 2.2	6.6 ± 1.6	17.5 ± 11.6	13.1 ± 10.7	1.7	2.1	3.2	2.7
	Day 3	15.2 ± 4.5	9.7 ± 3.0	15.0 ± 9.2	12.6 ± 8.6	2.4	2.7	4.3	4.2
	Day 4	21.6 ± 6.4	14.7 ± 5.0	14.8 ± 9.4	10.7 ± 6.7	4	2.7	4.5	4.8
	Day 5	29.6 ± 9.8	13.5 ± 3.7	16.5 ± 12.2	13.5 ± 10.3	5	3.2	5.2	3.4
	Day 6	30.8 ± 10.4	16.6 ± 2.8	39.3 ± 40.4	25.3 ± 31	4.5	4.8	7.9	5.9
	Mean ± SD (Days 1 to 6)	18.9 ± 11.4[c]	11 ± 5.7[d]	17.7 ± 19.3[c]	13.0 ± 14.1[d]	3.1 ± 1.7	2.7 ± 1.3	4.5 ± 2.1	3.6 ± 1.7

Numbers with same letters are not significantly different.

number of coefficients in the model. DOF, the degree of freedom, is n – m].

1. Coefficient of Determination (r squared); $r^2 = 1 - SSE/SSM$
2. Degree of Freedom Adjusted Coefficient of Determination; $DOF\ r^2 = (1 - SSE*(n - 1))/(SSM*(DOF-1))$
3. Fit Standard Error (Root MSE); $StdErr = sqrt(SSE/DOF)$
4. F-statistic; $F\text{-stat} = ((SSM - SSE)/(m - 1))/(SSE/DOF)$

As a fit becomes more ideal, the r^2 values approach 1.0 (0 represents a complete lack of fit), the standard error decreases toward zero, and the F-statistic goes toward infinity. Optimally fitting functions (or equations) can therefore be readily identified using these statistical criteria.

Completeness of daily urine collections

Urine Cn (g/L) was used to evaluate the completeness of 24 h urine collections. Excretion in the range of normal Cn excretion for adult males (1.7 g/day) was regarded as complete.

Additional statistical analysis

Descriptive statistics, i.e. means and standard deviations, were calculated. Statistical comparisons of exposures were made between groups and among workdays using either two sample t-test or ANOVA. The difference was regarded as significant when $p < 0.05$.

Results

Participant data

The average body weight (mean ± s.d.) was 72.2 ± 8.7 kg and the average applicator age was 28.4 ± 8.5 yr (Table 1). The crew was regularly employed in landscape maintenance. They were normal to overweight with an average Body Mass Index of 25.8 ± 1.9. Each of them demonstrated good physical conditioning by uninterrupted hard work in rugged terrain during the spray operations each day. The workers wore WPS clothing or a cotton body suit beneath cotton coveralls. The crew sprayed 55 acres of forest land with 24 gallons of Garlon*4™ and 24 gallons of 2,4-D LV6™ in 1600 gallons of tank mix during the 6-day monitoring period (Table 2).

During the conifer release program, all workers provided complete urine samples. No adverse health effects were reported or observed.

Tank mix samples

Tank mix containing Garlon*4™ and 2,4-D LV 6™ was sampled on Days 2 and 4. The nominal concentrations were 9,240 mg/L triclopyr butoxyethyl ester and 12,750 mg/L 2,4-D 2-ethylhexyl ester. The levels measured in tank mix were 8,913 ± 920 mg ester equivalents triclopyr/L and 11,280 ± 894 mg ester equivalents 2,4-D/L representing 96 ± 10 % and 88 ± 7 % of the nominal herbicide concentrations (Table 3). The nominal ratio of 2,4-D:triclopyr (a.e.)

was 1.6:1, while the measured ratio in tank mix was 1.5:1 (Table 3). However, the ratio of ester to acid for each active ingredient varied both within days (e.g., day 2) and between days.

Whole body suits

The herbicides were stable on the body suits during monitoring, transport, and analytical phases of the work. Recovery of esters from field-fortified cotton body suits ranged from 62-114 % (Table 4). As shown in Table 4, triclopyr ester recoveries were consistently greater than recoveries of 2,4-D from similarly treated body suits even though the application ratio of 2,4-D:triclopyr (a.e.) was 1.5:1. Five participants (Group A) wore body suits each day. The resulting herbicide levels as acid equivalents are listed in Table 5. The apparent Dermal Exposure (DE; mean ±s.d.) based upon the body suit level for 2,4-D was 31 ± 24 mg/d and the mean for triclopyr was 55 ± 44 mg/d, consistent with the analytical results of known spikes in Table 4. Results were not adjusted for recovery, except where noted for estimating absorbed dose.

Estimating ADD using WBD data

In order to account for incomplete recovery of the analyte (2,4-D or triclopyr) from the WBD (whole body dosimeter), the amount measured on clothing was corrected for recoveries from Table 4. The most appropriate estimate of recovery was judged to be 29 % and 79 % for 2,4-D and triclopyr, respectively. The fortified travel spike was used in preference to the field fortification because conditions for spiking were more controlled, and at the same time, the travel spike included exposure to the environmental conditions in the field. Workers wore WBD under their normal work clothing. The dose measured on the WBD represents potential dermal dose, i.e., dose that would reach the skin if there were no second layer present. However, to account for the effect of a second layer, clothing penetration was estimated to calculate absorbed dose for comparison to the concurrent biomonitoring. A measured clothing penetration factor has been determined from the Pesticide Handlers Exposure Database.[8] Clothing penetration increases as dose decreases, and the equation relating penetration to deposition is shown in equation [1] below adapted from Driver et al.[8]

$$\text{Dose at skin} = 10 \, (0.6731 * LOG(WBD) - 1.521) \quad (1)$$

In the range of potential dermal dose deposition on the WBD (assuming uniform distribution), the clothing penetration factor ranges from 40-60 %. Thus, a fixed 50 % penetration was assumed for estimating the amount of potential dermal dose on the WBD that actually penetrated through the WBD to the worker's skin.

Biomonitoring

Mean AD and ADD expressed as acid equivalents were calculated from daily biomarker concentrations in 24 h urine specimens. Group A applicators (n = 5) excreted a mean daily 768 ± 438 μg (a.e.) and Group B Applicators (n=3) excreted 951 ± 1,089 μg (a.e.) 2,4-D during the monitoring period (Table 6). Group A and Group B excreted relatively similar amounts of triclopyr biomarker. For neither herbicide were the differences statistically different between groups. The activities of the mixer/loader were very limited to preparation of tank mix and frequent refilling of sprayers. The field supervisor had continual contact with sprayed foliage as he actively guided applicators to assure full brush coverage during the conifer release program. Based upon the nature of their respective work tasks, the field supervisor had higher exposure than the mixer/loader but lower levels than the applicators, and this is reflected in the biomonitoring.

However, the use of daily mean values did not reflect the incomplete daily excretion of the absorbed dose from the first days of continuing exposure. As a result ADDs were lower when based on means than when estimated from pseudo-steady state excretion data in cases where exposure continued day-to-day (Table 7).

Estimate of steady state biomarker excretion by curve fitting

Excretion data from several worker cohorts were also analyzed. Table 6 presents estimates of mean ADD for 2,4-D and triclopyr for each worker cohort (applicators, mixer/loader, and the field supervisor), by consecutive day of urine monitoring. Figure 2 presents a simple plot of ADD for each worker cohort for Days 1 to 6. Given that the urinary elimination half-lives of both 2,4-D and triclopyr are less than 24 hours (i.e., 17.7 hrs in the case of 2,4-D[6]) and occur by an apparent first-order rate process,

Table 7. Summary of absorbed daily dosages estimated by curve fitting of urine biomonitoring data.

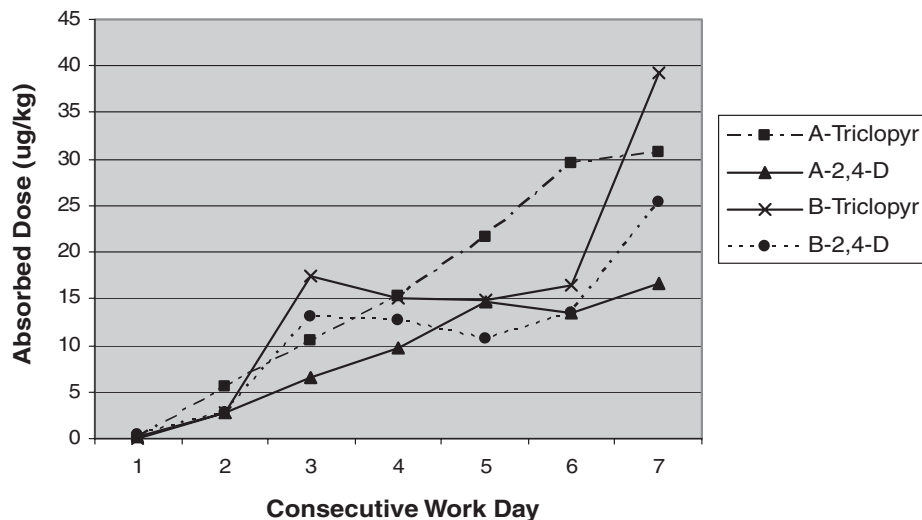| | Absorbed Daily Dosage (μg a.e./kg-d) | | | |
| | 2,4-D | | Triclopyr | |
Work Tasks	Daily Mean	Curve Fitting	Daily Mean	Curve Fitting
Group A				
Applicators (n=5)	11.0	15.3	18.9	28.1
Group B				
Applicators (n=3)	13.0	20.2	17.7	31.3
Mixer/Loader	2.7	3.9	3.1	4.5
Field Supervisor	3.6	5.1	4.5	6.4
Weighted mean for all applicators (n=8)	11.8	17.1	18.5	29.3

Fig. 2a. Triclopyr and 2,4-D Backpack M/L/A Exposure- Absorbed Dose as a Function of Work Day. On Work Day 1 the prespray urine was collected (Table 6). Day 6 was the last spray day and the urine specimens were collected the following morning (Day 7). Contact with contaminated equipment may have occurred on Day 6 (see also Field Supervisor).

it would be expected that following four to five half-lives (or after approximately 3 to 4 days of repeat exposure in the case of 2,4-D), a steady-state would be established and reflected as a "plateau" daily absorbed dose level in the 2,4-D and triclopyr worker cohorts.

Figures and associated tables generated using Table-Curve 2D for each of the six worker cohorts (2,4-D Applicators or A1-2,4-D, Field Supervisor-2,4-D, and Mixer/Loader or ML-2,4-D, and similarly designated cohorts for triclopyr) are provided below (Figures 2–4). The equations selected and their associated best fit statistics are indicated at the top of each figure. These figures depict a plateau of 2,4-D and triclopyr ADDs achieved by days 4 to 6. These data can be used to conservatively estimate equa-

tions provided for each chemical and cohort, based on use of an x-value of "6," i.e., estimation of plateau daily absorbed dosage at day 6. The measured urine levels on day 6 were very similar to the levels predicted by curve fitting. The tabular results (Table 7) associated with each figure provide Day 6 predicted values and can be compared to the mean excretion from days 1-6. The mean estimated daily excretion values are consistently lower than the day 6 value predicted from curve fitting due to incomplete excretion of herbicide during the first two days of collection.

The exposures of the mixer/loader and the field supervisor demonstrate the significance of work task as a determinant of exposure. The ADDs of the mixer/loader who seemed to have the least direct herbicide contact were
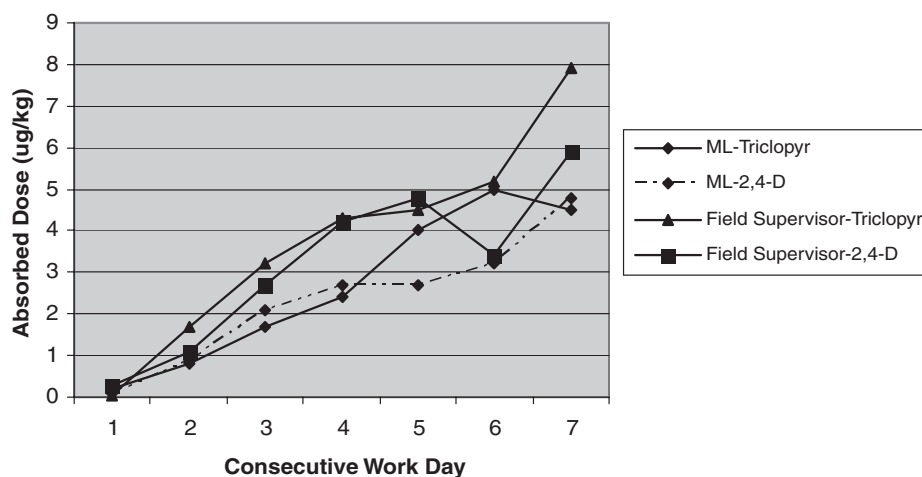
Fig. 2b. Triclopyr and 2,4-D Backpack Mixer/Loader (ML) and Field Supervisor Exposure (Absorbed Dose) as a Function of Work Day.
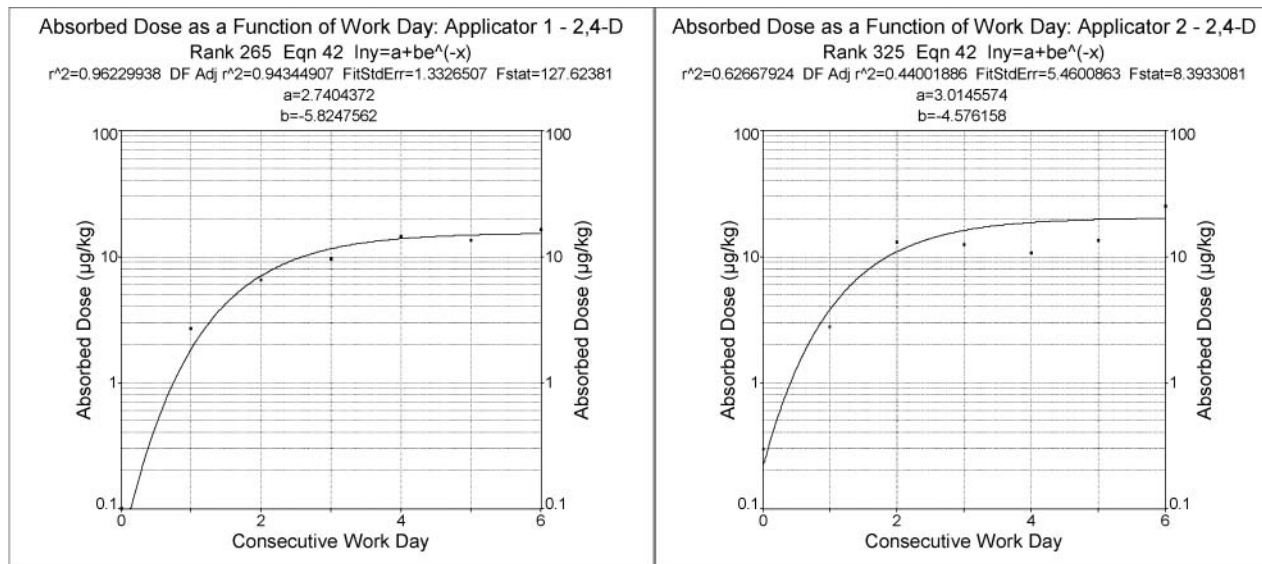
Figure 3 Group A 2,4-D Applicators (n=5)
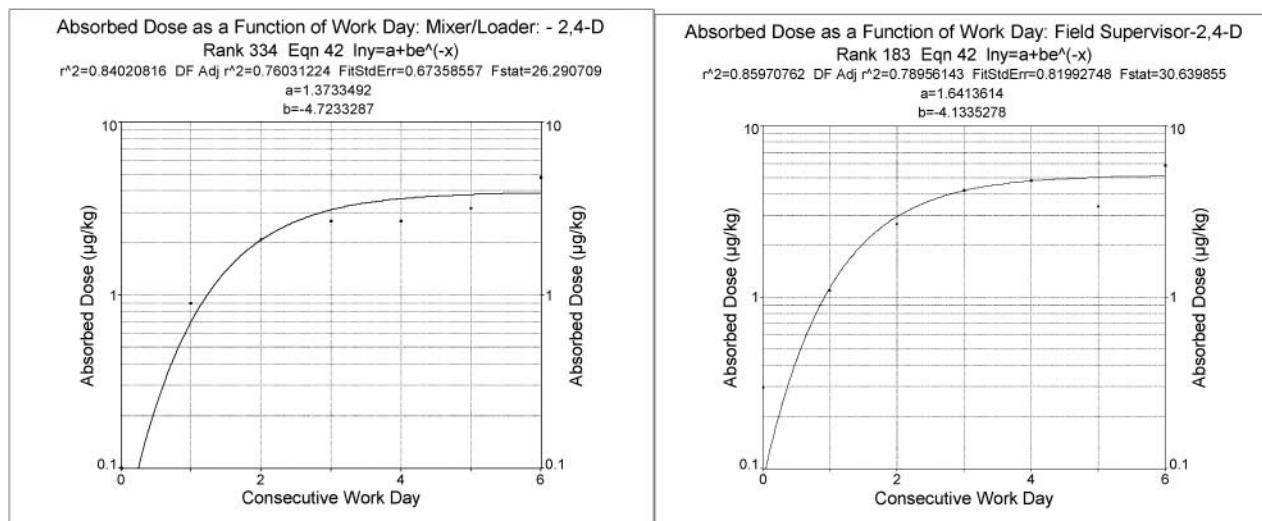

Figure 3 Group B 2,4-D Applicators (n=3)

Figure 3 Group B 2,4-D Mixer/Loader

Figure 3 Group B 2,4-D Field Supervisor

Fig. 3. Summary of 2,4-D Curve Fitting Urine Biomonitoring Data of Backpack Spray Crew.

3.9 μg 2,4-D/kg-d and 4.5 μg triclopyr/kg-d. The field supervisor's exposure was acquired by intermittent contact with sprayed brush and weeds as he continually moved over the work site. ADDs of the supervisor for 2,4-D and triclopyr were 5.1 and 6.4 μg/kg-d, respectively, for the 6-day monitoring period (Figure 3 and 4; Table 7).

The completeness of urine collection was used as an indicator of worker compliance with the study protocol. The average of daily Cn excretion for all workers was 1.6 ± 0.4 g/day. The daily Cn excretion is in the range of the reported norm,[9] which is 1.7 g Cn/day for adult males (one sample t–test, $p > 0.05$).

ADD per pound herbicide applied

Perspective on the extent of exposure during this backpack application can be obtained by calculation of the ADD (weighted means from curve fitting, Table 7) per pound herbicide applied. In the case of triclopyr at 2 pounds a.e. per day, the mean μg triclopyr/kg-d/lb applied was 14.7.
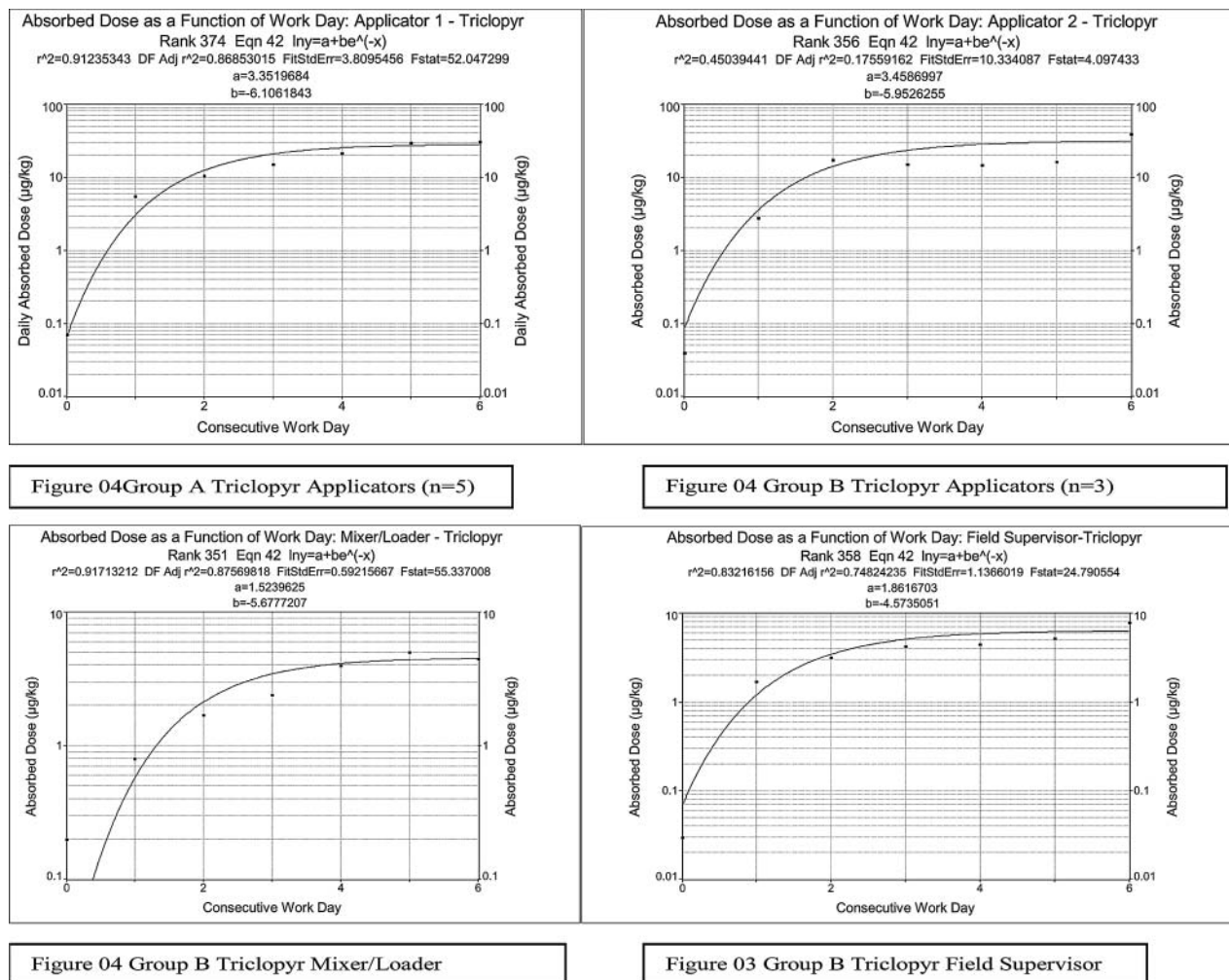
Absorbed Dose as a Function of Work Day: Applicator 1 - Triclopyr
Rank 374 Eqn 42 lny=a+be^(-x)
r^2=0.91235343 DF Adj r^2=0.86853015 FitStdErr=3.8095456 Fstat=52.047299
a=3.3519684
b=-6.1061843

Absorbed Dose as a Function of Work Day: Applicator 2 - Triclopyr
Rank 356 Eqn 42 lny=a+be^(-x)
r^2=0.45039441 DF Adj r^2=0.17559162 FitStdErr=10.334087 Fstat=4.097433
a=3.4586997
b=-5.9526255

Figure 04 Group A Triclopyr Applicators (n=5)

Figure 04 Group B Triclopyr Applicators (n=3)

Absorbed Dose as a Function of Work Day: Mixer/Loader - Triclopyr
Rank 351 Eqn 42 lny=a+be^(-x)
r^2=0.91713212 DF Adj r^2=0.87569818 FitStdErr=0.59215667 Fstat=55.337008
a=1.5239625
b=-5.6777207

Absorbed Dose as a Function of Work Day: Field Supervisor-Triclopyr
Rank 358 Eqn 42 lny=a+be^(-x)
r^2=0.83216156 DF Adj r^2=0.74824235 FitStdErr=1.1366019 Fstat=24.790554
a=1.8616703
b=-4.5735051

Figure 04 Group B Triclopyr Mixer/Loader

Figure 03 Group B Triclopyr Field Supervisor

Fig. 4. Summary of Curve Fitting Triclopyr Urine Biomonitoring Data of Backpack Spray Crew.

The corresponding measure for 2,4-D at 2.75 pounds a.e. per day was 6.2 μg 2,4-D/kg-d/lb applied.

Pesticide handler exposure database (PHED)[10] default exposure estimate

The generic PHED[10] exposure database is a useful tool to inform initial estimates of pesticide handler dermal exposure. Its use for exposure assessments is limited by default assumptions that must be used to represent specific work tasks including backpack sprayers in forestry. Using PHED[10] for the present case, an estimate of ADD was obtained (Table 8). Hand protection was assumed to be complete based upon the use of impervious nitrile gloves worn over light cotton gloves that retained insignificant (1-2 μg/pair-d) amounts of herbicide residue. The total dermal exposure was reduced to 202 mg/lb applied by adjusting dermal exposure by 50 % based upon clothing penetration for a second layer.[8] Head and neck exposure were not considered in this estimate since workers wore impervious helmets. Collars on coveralls provided additional protec-tion of the neck. The average rates of use of 2,4-D and triclopyr were 2.75 lb/d and 2 lb/d (a.e.) respectively, the dermal dose for 2,4-D was 369 mg and 290 mg for triclopyr. The resulting absorbed doses were 21.4 mg 2,4-D and 4.8 mg triclopyr. When factored by mean weight of the 8 applicators (71.6 kg) the estimated ADDs were 299 μg 2,4-D/kg-d and 67 μg triclopyr/kg-d (Table 8).

Discussion

The backpack spray crew members applied a 2,4-D and triclopyr tank mix during a 6-day period in very rugged terrain in northern California. The workers lived in tents and cooked their meals at a base camp about a mile from the primary work area. Most of the crew had backpack sprayer experience during the previous season. They were in excellent physical condition based upon their steady day-to-day performance. The workers' average body weight was 72.2 kg with a BMI of < 26, objective evidence of their fitness.

Table 8. Estimates of 2,4-D and triclopyr absorbed daily dosages from the pesticide handler exposure database, Version 1.1, Scenario 20 (backpack applicator).[10]

(1) TOTAL Dermal Exposure (mg/lb a.i. handled-d)	680
(2) Full hand protection (nitrile gloves over light cotton gloves)	276
(3) Adjusted potential dermal exposure (1) - (2)	404
(4) Dermal exposure @ 50% clothing penetration 0.5 × (3)[8]	202
(5) Active ingredient handled (5a) 2,4-D ester 2.75 lb/d (5b) Triclopyr ester 2 lb/d	
(6) Dose (4) × (5)	
(6a) 2,4-D as acid eq (4) × (2.75 × 221/333)	369
(6b) Triclopyr (mg) as acid eq (4) × (2 × 256/357)	290
(7) Absorbed Dose (%/d; mg a.e./d)	
(7a) 2,4-D 5.8%[4]	21.4
(7b) Triclopyr 1.65%[5]	4.8
(8) Absorbed Dosage (Absorbed Dose/applicator body weight 71.6 kg, n=8)	
(8a) 2,4-D, μg a.e./kg-d	299
(8b) Triclopyr	67
(9) Mole ratio	
299/221:67/256	5.2

Inhalation exposure was not monitored due to the small contribution of the air route when semi-volatile pesticides are handled under field conditions.[10] Vegetation would also cause potential interference of breathing zone monitoring devices during normal work tasks, making inhalation monitoring very difficult, if not impossible. The vapor pressures at 25°C of triclopyr and 2,4-D are 1.50×10^{-6} mm Hg[11] and 1.42×10^{-7} mm Hg[12] respectively. Airborne exposure was further reduced relative to dermal exposure by the routine work practice of directing spray downward and away from the breathing zone. Large diameter spray droplets (generally greater than 300 μm VMD) also minimize drift from the backpack sprayers,[3] and further reduce the inhalation contribution to the AD.[2, 3]

In this study dermal exposure monitoring was limited to whole body suits worn by Group A applicators (n = 5) under coveralls. Their counterparts in Group B (n=3) wore WPS clothing under coveralls. We were prepared to use cotton patch dosimeters for passive monitoring of external exposure; however, dosimeters were torn from their cardstock holders after short work periods under the prevailing brushy work conditions when tested in pilot trials. Knee-length work boots and gloves prevented herbicide contact with cotton socks and gloves so those body regions made a negligible contribution to Potential Dermal Exposure (PDE) (data not shown). As a result only whole body suits represented DE as passive dosimeters (Table 5). DE was 31 ± 24 mg/d 2,4-D (a.e.) and 55 ± 44 mg/d triclopyr (a.e.) for Group A applicators who wore whole body suits beneath coveralls in lieu of WPS clothing. The greater estimated 2,4-D absorbed dose (3.1 mg 2,4-D vs 0.57 mg triclopyr; Table 5) results from application of the larger default dermal absorption estimate of 5.8 % for 2,4-D[4,13,14] versus 1.65 % for triclopyr[5] and the lower spike recovery of 2,4-D from the WBDs (Table 4).

The similarity of the urine biomonitoring data from Group A applicators (n = 5) and Group B applicators (n = 3) is of interest and of potential importance in future studies. Biomonitoring can yield a direct estimate of aggregate exposure. Biomonitoring furthermore does not require use of monitoring ensembles that may interfere with normal work practices or produce heat stress. The whole body suits were well tolerated and occasionally showed evidence of minor break through of blue-dyed spray. Previously, indoor studies showed that passive dosimeters (whole body suits) retained chlorpyrifos residues that were available for dermal absorption.[15] In the present study, the ADs of the applicators in Groups A (whole body suits) and B (WPS clothing) were not significantly different, supporting our conclusion that the passive dosimeters and the WPS clothes behaved similarly during the day-long monitoring periods.

Based on mean ADDs for 2,4-D and triclopyr calculated from daily mean urine excretion a pseudo steady state was established as illustrated in Figures 3 and 4. ADDs are presented both as daily means and as result of curve fitting that acknowledges incomplete excretion each day (Table 9). The amount of 2,4-D excreted is less than the triclopyr biomarker at each interval.

The lower amounts of 2,4-D than triclopyr biomarkers in urine are unexpected and unexplained in as much as the tank mix contained a measured 2,4-D:triclopyr mole ratio of 1.5:1. This is particularly true if the putative dermal absorption factors of 2,4-D is 5.8 %/d vs. 1.65 %/d for triclopyr are accepted. Consistently less 2,4-D than triclopyr was also recovered from the whole body suits (Table 5). This observation prompts the suggestion that under field conditions relatively more 2,4-D in the spray mix was retained by coveralls than triclopyr and therefore 2,4-D may have been less available for dermal absorption. This possibility became apparent well after the field work was completed, and it was not investigated further. However, even in this case the ratio of biomonitoring to passive dosimetry encompasses the range of 3 fold which has been suggested to indicate validity.[16]

While acknowledging the incomplete recoveries from the passive dosimetry, the results were consistent (i.e., the coefficient of variation was ~0.8 for both 2,4-D and triclopyr per Table 5). Additionally, the passive dosimetry quantitatively was similar to Spencer et al,[2] providing assurance that the currently reported data provides meaningful estimates of potential dermal exposures. More importantly, the biomonitoring data are reliable and reflect the true exposures even with two layers of clothing, because the workers normally wear two layers. Additionally, biomonitoring is generally regarded as the "gold standard" of exposure assessment.[17]

The ratio of passive dosimetry to biomonitoring may be an indication of bias introduced by the passive dosimetry

Table 9. Estimates of absorbed daily dosages of backpack applicators based upon passive dosimetry and urine biomonitoring.

Method	Absorbed Daily Dosage ug (a.e.)/kg-d		Mole Ratio 2,4-D/ Triclopyr (a.e.)
	2,4-D	Triclopyr	
Passive Dosimetry			
Group A (n=5)	42.6	8.0	6.2
Biomonitoring			
Group A (n=5)			
Daily mean	11	18.9	0.68
Curve fitting	15.3	28.1	0.63
Group B (n=3)			
Daily mean	13	17.7	0.86
Curve fitting	20.2	31.3	0.75
Applicator weighted average			
Daily mean	11.8	18.5	0.74
Curve fitting	17.1	29.3	0.69
Pesticide Handlers Exposure Database			
PHED Applicator[10]	299	67	5.2

(due to differences in affinity between dosimeter matrices and the analyte, and variations in clothing penetration).

There is considerable uncertainty associated with estimates of ADD based upon herbicide accumulation on the WBDs. Exposure due to an uncovered face and neck as well as inhalation exposure are ignored by this practice when the results are compared to ADD obtained by biomonitoring. Finally, there is uncertainty about application of the experimental dermal absorption factors to the uptake of more complex spray solution. Regardless of these uncertainties, the resulting estimates of ADD obtained by passive dosimetry are similar to those derived from biomonitoring, consistent with the general findings of Ross et al.[16]

In other forestry workers, the 2,4-D ADD ranged from 3 to 22 μg/kg-d[18] and 40 to 240 μg/kg-d.[19] In a more recent study the triclopyr ADD in forestry backpack sprayers was 58 μg/kg-d.[2] In conjunction with results from the current study, these studies demonstrate low worker exposure of herbicide backpack applicators in forestry relative to exposure estimated from the Pesticide Handlers Exposure Database.

The USEPA Registration Eligibility Decision for 2,4-D (RED)[20] identified 18 handler scenarios resulting from mixing/loading and applying 2,4-D for crop and non-crop uses including backpack sprayers in forestry. For the occupational use of 2,4-D, EPA's acceptable Margin of Exposure (MOE) is 100, which incorporates uncertainty factors

of 10x for interspecies variation and 10x for intraspecies variation.

The MOEs are the ratios of an established dose level (NOAEL) to estimated exposure. MOEs for 2, 4-D are determined by a comparison of specific exposure scenario estimates to the NOAELs for short-term and intermediate-term assessments. The appropriate NOAEL for occupational short-term dermal and inhalation exposure is 25 mg/kg-day from a rat developmental toxicity study, and the NOAEL for intermediate-term dermal and inhalation exposure is 15 mg/kg-d from a rat subchronic oral toxicity study.

Impervious nitrile gloves and hard hats as well as cotton coveralls mitigated the backpack sprayer exposures in this study. The estimated dermal exposure was 42.6 μg/kg-d 2,4-D based on the herbicide accumulated on the cotton body suit (Table 5). As a result the corresponding MOEs evaluated against either the short-term or the intermediate-term toxicity endpoints for backpack sprayers were greater than 100, i.e., the minimum MOE = 15/0.0426 = 352.

More recently Hays and Aylward[21] have used biomonitoring data in a public health assessment of a biomonitoring equivalent (BE) for risk assessment. In this case, the BE is the concentration of chemical in urine consistent with the existing health-based exposure guideline. With respect to 2,4-D the point of departure (POD) for the BE is the chronic rat dietary NOAEL of 5 mg/kg-d.[7] The interspecies uncertainty factor is applied to obtain a human equivalent POD of 0.5 mg/kg-d. The urinary level of 2,4-D associated with that exposure is 20,000 μg/L (or 30,000 μg/g Cn). Application of an additional intraspecies uncertainty factor (UF) yields an occupational BE_{RfD} of 2,000 μg/L (or 3,000 μg/g Cn). Under occupational, pseudo steady-state conditions observed here, the daily urinary excretion of backpack sprayer applicators was always less than 3,000 μg/g Cn. Aylward et al.[22] emphasize that BEs are screening values based upon existing exposure guidance and not definitive measures of risk. Thus, the backpack sprayer exposures of applicators, their mixer/loader and field supervisor are well below levels of either regulatory or public health risk thresholds.

Short-term and intermediate-term dermal and inhalation exposure assessments for triclopyr are not developed here because there are no toxicological endpoints of concern.[23] The Toxicology Endpoint Selection Committee recommended that risk assessments for short- and intermediate term exposure were not required since the NOAEL was >1000 mg/kg-d (limit dose) in a 21-day dermal toxicity study in rabbits.

When basic disposition data are available to support biomonitoring of pesticide handlers, there are compelling reasons for its use over passive dosimetry. Passive dosimetry is logistically much more complex than biomonitoring in virtually all aspects of field work. The largest component of AD is the portion of chemical retained by outer dosimeters. That sample matrix is often large and unwieldy in the field. In the laboratory there is substantial uncertainty about the

portion of the pesticide that is ultimately absorbed. When transport of dosimetry supplies, sample processing and preservation, and field storage of materials are compared to the collection and handling of urine specimens, time-effort considerations strongly favor biomonitoring. Urine biomonitoring requires minimal sample processing. Refrigeration of specimens has become a lesser concern with the availability of well-insulated containers for field use. Study participants are also less inconvenienced by biomonitoring. As a result of lesser personal inconvenience, biomonitoring may also reduce uncertainties related to assuring that the work tasks under study represent typical day-to-day conditions.

In conclusion, backpack spray applicators and their mixer/loader and field supervisor were concurrently exposed to low levels of 2,4-D and triclopyr as a tank mix in northern California. The corresponding herbicide acids were recovered as exposure biomarkers in 24 h urine specimens. The mixer/loader and the field supervisor had less contact with spray mix and treated foliage and 3- to 5-fold lower ADDs than backpack applicators. Results suggest that passive dosimetry for 2,4-D consistently overestimated the dosage measured using biomonitoring by a factor of 2-3 fold, while for triclopyr, passive dosimetry underestimated the absorbed dose based on biomonitoring by a factor of 2-4 fold. However, the dosages actually measured in this study are several-fold lower than those predicted using PHED.

Acknowledgments

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