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Measurement of 2,4-Dichlorophenoxyacetic Acid (2,4-D) After Occupational Exposure

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The phenoxy herbicides have been used to control broad-leafed weeds in crops, water sources, forests, pastures, rangelands, gardens, lawns, and urban and industrial sites. Because of the efficiency, economy and safety, the phenoxy herbicides, especially 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2,4-dichlorophenoxyacetic acid (2,4-D), besides other herbicides, remain essential for agricultural and other uses (DIAZ-COLON & BOVEY 1976). Use of 2,4,5-T and 2,4-D on a large scale to defoliate vegetation has resulted in controversy about the fate of the chemicals. Many of the hazardous properties ascribed to 2,4,5-T are to a lesser extent shared by 2,4-D. As a result, a number of papers dealing with the fate in the environment, toxicological effects on living organisms (DIAZ-COLON & BOVEY 1976; SAUERHOFF et al. 1977; PIPER et al. 1973) have been published. COOPER (1974) compared 2,4,5-T and 2,4-D. He has reported that commercial samples of 2,4-D in mice were teratogenic and embryotoxic. Increasing controversy about their safe use has raised doubt about their utility in achieving vegetation management objectives.

A Dow Chemical report on Forestry Applicators exposure to 2,4-D has recently been published (LEVY 1980). Nonetheless, in total, only meager information regarding human (e.g., gardeners, small farmers, etc.) exposure to 2,4-D is available. This study reports an investigation conducted under conditions characteristic of actual use pattern to determine the quantity of body surface contact of applicator with the commercial 2,4-D formulation and the quantity of the chemical measurable in serum and urine.

MATERIALS AND METHODS

Eleven healthy young male (ages 19 to 31 years) human volunteers were selected and were examined by the University physician prior to the work. Each subject was examined for blood pressure, pulse rate and body temperature on the day of work, both before and after the work period, and similar examinations were made every morning for the next three days. Sterile gauze pads (size 0.67 sq. ft.) were attached on the back and chest of each volunteer. A paper cap (area 0.44 sq. ft.) was used to cover the head.

The study was carried out either on the campus of Rutgers University or near the campus in New Brunswick, New Jersey during the period from May 1981 to August 1981. The location was covered with

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weeds and grass. Each area was roughly between one-half to one acre.

Weather conditions during this study were moderate, temperature ranging between 65° and 78° with humidity from 59 to 89%. Wind was fairly calm throughout this study except on one occasion, when it gusted to 10 miles per hour.

The spraying material consisted of DMA-4 concentrate (obtained as a gift from Dow Chemical, U.S.A.) which contained 3.8 lb acid equivalent/gal 2,4-D. The herbicide was mixed with tap water prior to use and the resulting mixture was applied at a rate of approximately 1 gal/30 min by a hand sprayer held at hip level. A DuPont air sampler was attached to the applicator and was kept running during the spraying operation. The areas were covered by spraying six gallons in about three hours as per label instruction.

To ascertain body surface exposure, gauze pads and caps were collected immediately after the work and washed in methanol. Air filter absorbents were also washed in methanol. Residue separated by methanol was evaporated to dryness and then treated with 15% BF_3 in methanol. Finally, it was extracted with n-hexane (YIP 1975).

Blood samples were drawn before the work and at the end of the day's work. Three more blood samples were drawn on the following three mornings. Overnight pooled urine samples were collected before the work and the day's collections were made during and/or after the treatment. Then the pooled urine samples were collected twice a day for the next 4 to 7 days. Blood sera and urine samples were treated, extracted and analyzed by the method of SAUERHOFF et al. (1977).

Vegetation samples and soil samples from the treated area were collected before and immediately after the treatment and then once every 24 hours for the next three days. Samples were treated and analyzed by the methods of DAVIDONIS et al. (1980) and OLSON et al. (1978).

2,4-D residue extracted in n-hexane was analyzed on a gas chromatograph (Tracor Model No. 560, equipped with electron capture detector and a six foot glass column packed with 3% OV-101 on 80/100 supelcoport).

Gas chromatographic conditions used were as follows:

Detector, Electron capture Ni 63 temperature:	350°C
Gas: Methane: Argon	(5:95)
Flow rate through the column	25 ml/min
Sample volume injected	1 µl
Column material	3% OV-101 on 80/100 Supelcoport
Length of the column	6 ft
I.D. of the column	2 mm
Column temperature	187°C
Injection port temperature	200°C

Clinical determinations on blood serum and urine were made through the automated procedures of a commercial diagnostic laboratory.

RESULTS AND DISCUSSION

Distribution of 2,4-D on human volunteers (applicators) body surface is presented in Table 1.

Table 1. Distribution of 2,4-D Residue on Body Surface

Applicator No.	Head µg/sq. ft.	Chest µg/sq. ft.	Head µg/sq. ft.
1	8.55	7.81	5.10
2	14.06	11.68	10.60
3	7.06	3.37	3.77
4	222.20	171.50	99.63
5	34.22	94.53	41.16
6	141.80	111.50	74.23
7	76.16	68.39	46.64
8	16.99	13.56	11.86
9	19.12	14.58	10.29
10	18.55	31.46	4.46
11	17.80	14.95	18.63

At the end of a day's work, the applicators had an average 2,4-D residue of 52.41 µg/sq. ft. on the head, 49.39 µg/sq. ft. on the chest and 29.67 µg/sq. ft. on the back.

2,4-D residue data on vegetation and soil surface are summarized in Tables 2 and 3, respectively.

Table 2. 2,4-D Residue on Vegetation (µg/g)

Treated Area	0	X	X+1	X+2	X+3
1	0.59	28.85	16.15	16.67	20.16
2	14.74	29.60	22.43	4.32	2.13
3	00.00	25.35	7.70	1.92	3.44

Table 3. Amount of 2,4-D on Soil Surface (µg/g)

Treated Area	0	X	X+1	X+2	X+3
1	1.24	3.70	1.90	2.80	7.30
2	3.58	4.21	0.72	1.59	7.25
3	0.00	0.13	0.15	0.27	0.80

0 = pretreatment; X = posttreatment; X+1 = 1 day after treatment; X+2 = 2 days after treatment; X+3 = 3 days after treatment.

The average value of 2,4-D residue on vegetation in the treated area was 27.93 µg/g of plant tissue at the end of the working day (i.e., first day). The residue decreased to 8.57 µg/g in plant tissue on

the fourth day. On the other hand, the average value of soil residue was 2.68 μg/g soil at the end of the first day which, however, increased to 5.12 μg/g on the fourth day.

Analysis of 2,4-D retained by the air monitor filter revealed detectable residue. The amount of air drawn through the filter was calculated to be 56.6 to 84.96 liters, and the measured 2,4-D residue was 43.1 to 60.1 parts per trillion.

Level of 2,4-D residue plus intrinsic phenolics in serum and urine was measured. The tables below present values calculated by substracting the measured base (intrinsic phenolics) from each subsequent measurement.

Table 4. Amount of 2,4-D Measured as Phenolics in Serum

Applicator No.	2,4-D Residue (ng/ml) X	X+1	X+2	X+3
1	--	714.3	198.7	0
2	60.4	116.1	196.1	48.3
3	128.4	38.0	--	396.1
4	285.4	--	--	--
5	482.0	440.6	113.6	188.8
6	--	654.6	--	466.3
7	179.9	74.8	150.2	134.1
8	15.6	52.2	40.1	79.9
9	21.2	12.8	14.0	--
10	--	74.3	39.8	17.3
11	--	55.6	27.9	114.8

X = posttreatment; X+1 = 1 day after treatment; X+2 = 2 days after treatment; X+3 = 3 days after treatment.

Table 5. 2,4-D Measured as Phenolics Excreted in the Urine

Applicator No.	1	12	24	36	48	60	72	84
1	3.39	--	--	2.67	1.97	--	17.47	25.26
2	3.95	--	0.63	2.78	2.53	1.24	3.22	--
3	1.40	7.15	6.02	--	--	--	7.94	1.22
4	7.33	13.27	--	1.45	22.44	16.76	4.98	--
5	1.00	1.47	1.28	0.99	2.30	3.00	1.95	7.07
6	--	5.67	--	13.44	3.92	--	--	3.21
7	0.90	5.34	--	--	--	--	--	--
8	0.11	0.57	2.77	1.34	3.46	2.84	3.08	5.30
9	0.75	--	0.85	2.46	8.28	6.57	1.46	4.09
10	1.94	1.73	1.63	2.83	2.21	2.73	8.48	4.35

1 = posttreatment

The average base value for phenolics in serum was 70.7 ng/ml immediately before exposure. Immediately after the day's work, the value

went up to 106.63 ng/ml and again to 203.03 ng/ml on the second day. The value, however, came down to 70.95 ng/ml on the third day and again went up to 130.51 ng/ml on the fourth day. Average phenolic compounds ranged from 3.81 µg (zero level exposure) to 5.05 µg on the fifth day in 12 hours of pooled urine.

The effect of 2,4-D exposure on red blood cell (RBC), white blood cell (WBC), hemoglobin concentration (HG), hematocrat (HCT), platelet and many other blood parameters were studied. No appreciable changes were noticed. However, some changes were observed in clinical analysis made on blood serum (19 parameters were studied) and urine.

The clinical residue and weather data were critically scrutinized to both linear models and regression models. The primary emphasis of the analysis was to identify the parameters that recorded any significant change. This was accomplished by subtracting the baseline value from the observed value and then dividing it by the baseline. Analysis of the resulting values revealed highly significant (α=0.01) changes in the serum creatinine and total protein levels, and significant (α=0.05) change in the urine specific gravity. It was also seen that the total protein and BUN/creatinine ratio were positively correlated with the residue on the chest of the volunteers and with their height. When the peak residue was related with the external factors, it was seen that the serum residue was positively correlated with the 2,4-D concentration in the air and the amount of chemical that had landed on the head and back. Likewise, the urine residue was positively correlated with the air volume and the height of the volunteers. Precipitation (rainfall-high humidity) was negatively correlated with the serum residue, presumably due to decreased chances of vaporization and subsequent inhalation.

Results of this study indicate that there was a considerable amount of 2,4-D in the air, on the vegetation and soil surface of the treated areas. An appreciable quantity of 2,4-D landed on the body surface and possibly entered into the applicator's body through inhalation and/or through body surface (dermal) absorption. Lengthy retention times have been observed in some subjects (Table 5). These findings are consonant with the report of LEVY et al. (1980) for foresters. Clinical physiology data otained here are consistent with long retention times for this chemical. We surmise, but have no specific evidence, that the 2,4-D molecule or some portion (phenolic) binds to serum protein and is elminated as a function of the turnover of the protein molecules. Other than the clinical (chemical) changes noted above, we have seen no pronounced adverse effect of 2,4-D exposure.

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