vary in other citrus growing areas.

ACKNOWLEDGMENT

The technical assistances of J. L. Pappas, D. C. G. Aitken, M. A. Wells, J. H. Barkely, J. K. Virzi, and T. M. Dinoff of the University of California, Riverside, and K. Yee, C. R. Ackerman, and P. Lee of the California Department of Food and Agriculture are gratefully acknowledged.

LITERATURE CITED

Adams, J. D., U.S. EPA, Washington, DC, personal communication, 1981.
Bowman, M. C.; Beroza, M. *J. Agric. Food Chem.* **1968**, *16*, 399.
Finney, D. J. "Probit Analysis", 3rd ed.; Cambridge University Press: New York, 1972.
Gunther, F. A. *Residue Rev.* **1969**, *28*, 1.
Gunther, F. A.; Iwata, Y.; Papadopoulou, E.; Berck, B.; Smith, C. A. *Bull. Environ. Contam. Toxicol.* **1980**, *24*, 903.
Gunther, F. A.; Westlake, W. E.; Barkley, J. H.; Winterlin, W.; Langebehn, L. *Bull. Environ. Contam. Toxicol.* **1973**, *9*, 243.
Hild, J.; Schulte, E.; Thier, H. P. *Chromatographia* **1978**, *11*, 397.
Iwata, Y.; Carman, G. E.; Gunther, F. A. *J. Agric. Food Chem.* **1979**, *27*, 119.
Iwata, Y.; Carman, G. E.; O'Neal, J. R.; Barkely, J. H.; Düsch, M. E.; Gunther, F. A. *J. Agric. Food Chem.* **1981**, *29*, 135.
Iwata, Y.; Knaak, J. B.; Spear, R. C.; Foster, R. J. *Bull. Environ. Contam. Toxicol.* **1977**, *18*, 649.
Kahn, E. *Residue Rev.* **1979**, *70*, 27.
Knaak, J. B., Iwata, Y. *ACS Symp. Ser.* **1981**, in press.
Knaak, J. B.; Schlocker, P.; Ackerman, C. R.; Seiber, J. N. *Bull. Environ. Contam. Toxicol.* **1980**, *24*, 796.
Muacević, G. *Arh. Hig. Rada Toksikol.* **1976**, *27*, 3.
Popendorf, W. J. *Am. Ind. Hyg. Assoc. J.* **1980**, *41*, 652.
Richards, D. M.; Kraus, J. F.; Kurtz, P.; Borhani, N. O.; Mull, R.; Winterlin, W.; Kilgore, W. W. *J. Environ. Pathol. Toxicol.* **1978**, *2*, 493.
Shafik, M. T.; Bradway, D.; Enos, H. F. *Bull. Environ. Contam. Toxicol.* **1971**, *6*, 55.
Shafik, T. M.; Bradway, D. E.; Enos, H. F.; Yobs, A. R. *J. Agric. Food Chem.* **1973**, *21*, 625.
Spear, R. C.; Popendorf, W. J.; Leffingwell, J. T.; Milby, T. H.; Davies, J. E.; Spencer, W. J. *JOM, J. Occup. Med.* **1977**, *19*, 406.
Spencer, W. F.; Adams, J. D.; Hess, R. E.; Shoup, T. D.; Spear, R. C. *J. Agric. Food Chem.* **1980a**, *28*, 366.
Spencer, W. F.; Iwata, Y.; Kilgore, W. W.; Knaak, J. B. *Bull. Environ. Contam. Toxicol.* **1977**, *18*, 656.
Spencer, W. F.; Shoup, T. D.; Spear, R. C. *J. Agric. Food Chem.* **1980b**, *28*, 1295.
U.S. Environmental Protection Agency, unpublished proposed regulations, 1981.

Received for review August 3, 1981. Accepted November 30, 1981. Work was supported through funds from the California Citrus Research Board and through a grant-in-aid from EM Industries, Inc.

Potential Benlate Fungicide Exposure during Mixer/Loader Operations, Crop Harvest, and Home Use

Leighton P. Everhart and Richard F. Holt*

Potential exposure to Benlate fungicide was determined for three different agricultural use situations involving different types of exposure. Total exposure to benomyl was minimal during the mixing of Benlate for aerial application or during reentry into a treated field for crop harvest or during home use. Average potential dermal exposures for these three situations were 26, 12, and <1 mg of benomyl, respectively, with the major portion of the exposure on the hand and forearm areas. The average potential respiratory exposures for the three use situations were 0.08, 0.003, and 0.003 mg of benomyl, respectively. On the basis of the low dermal and respiratory toxicity of Benlate, these values do not contribute to a significant body dose.

Benlate fungicide, which contains benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolylcarbamate] as the active ingredient, is widely used to control a range of fungus diseases affecting over 30 different fruits, vegetables, field crops, and ornamentals. This paper addresses the question of potential human exposure to Benlate under a variety of use situations representing the extremes of potential exposure. These situations encompass mixing procedures for aerial application, reentry into treated fields, and home use (garden, ornamental, and greenhouse). They were selected in order to evaluate the total use pattern safety of Benlate.

Determination of potential exposure to pesticides has previously been studied in detail by Durham and Wolfe (1962). The procedures established in their studies have been successfully applied and reported by other investigators in the area of pesticide exposure (Staiff et al., 1975; Popendorf et al., 1979; Spear et al., 1977; Durham et al., 1972; Wolfe et al., 1959, 1961, 1975). By use of these established techniques in this study, Benlate potential dermal exposure was assessed by attaching absorbent pads to various parts of the body or clothing. Cotton gloves were worn to assess exposure to the hands. Respiratory exposure was monitored by the use of filter pads in specially modified respirators.

The results from this research indicate only minimal benomyl exposure in each of the three use situations studied. Maximum values, as expected, were noted in the mixing of Benlate prior to aerial application. In this use situation, the average dermal exposure was 26 mg of benomyl and the average total respiratory exposure was 0.08 mg of benomyl per mixing cycle.

EXPERIMENTAL SECTION

The materials and methods used in all three test situations have been described in detail by Durham and Wolfe (1962). All samplings for the measurement of potential exposure were collected under actual use conditions.

E. I. du Pont de Nemours & Company, Inc., Biochemicals Department, Wilmington, Delaware 19898.

0021-8561/82/1430-0222$01.25/0

Table I. Summary of Experimental Conditions: Mixing for Aerial Application

trial	location	applicn date	crop	vol mixed, gal	total lbs	duration, min	wind speed	wind direction	temp, °F	rel humidity, %	bar. press., mmHg
1	Del Ray Beach	2/7/79	bush beans	250	37	5.0	10-15 mph	SE	73	84	29.1
2	Del Ray Beach	2/7/79	bush beans	250	37	3.0	10-15 mph	SE	73	84	29.1
3	Pahokee	2/7/79	celery	350	35	2.0	indoors	indoors	72	70	30.6
4	Pahokee	2/7/79	celery	350	35	1.5	indoors	indoors	72	70	30.6
5	Homestead	2/8/79	pole beans	200	40	5.0	10 mph	N-NW	60	75	29.4
6	Homestead	2/8/79	pole beans	240	47	3.0	10-15 mph	NW	60	77	29.4
7	Homestead	2/8/79	pole beans	125	25	2.2	5 mph	NW	57	82	29.5
8	Homestead	2/8/79	pole beans	200	40	2.2	0-10 mph	NW	57	82	29.5
9	Homestead	2/9/79	avocadoes	300	60	4.5	0.5 mph	NW	44	81	29.6
10	Homestead	2/9/79	avocadoes	300	60	3.0	5-10 mph	NW	47	94	29.6

Dermal exposure was assessed by the use of pads constructed from two 4-in.^{2} surgical gauze pads overlaying a 4-in.^{2} filter paper and bound on all four edges with 1-in. masking tape. The gauze pads were 12-ply Parke Davis Readi-Pads. Dermal pads were attached at strategic parts of the applicator's body to sample potential dermal exposure: one at each shoulder (to sample potential facial exposure), in the upper center of the chest near the jugular notch, and on both forearms. For two use situations dermal pads were also placed on the upper center of the back, on the thighs, and on the lower legs. The pads were taped to the outer clothing by using masking tape. Cotton "undertaker's gloves" were worn to assess exposure to the hands.

Respiratory exposure was monitored by collecting inhaled particles on filter pads fixed to the front of a Pulmosan Model MA-3 dust respirator. The pad was constructed by stapling a 16-ply gauze pad to the respirator filter. This modified pad replaced the normal filter and was covered by a 2-oz polyethylene funnel cut to fit and held in place by the respirator retainer ring. The stem end of the funnel was placed with a cork, and two holes 12 mm in diameter and 6 mm apart were drilled midway between the base and apex of the funnel. The funnel gave protection against direct dust impingement on the filter; the drilled holes were the same size as, and simulated airflow through, the nostrils. When placed in the respirator, the gauze pad faced outward.

At the completion of the sampling period, each dermal pad was placed in a white filter paper envelope marked in advance with appropriate identification. The filter paper envelopes were stored in individual "zip lock" polyethylene bags. Gloves and respiratory pads were handled in the same manner and stored in separate bags. Samples were transported from field locations to Wilmington, DE, and held until analysis under ambient conditions.

Test Areas. *Mixing for Aerial Application.* Ten professional mixer/loaders (also referred to as swampers, mixers, etc.) employed by three different commercial aerial applicators in southern Florida were monitored for potential benomyl exposure while mixing and loading Benlate for aerial application. The dermal and respiratory samples were collected in connection with the normal performance of duties. Each subject was instructed to perform the mixing operation as he normally would. Dermal samples were taken from the forearm, face, back (back of neck), chest, and hand areas. Since the legs are always covered in this operation, no dermal samples from this area were collected.

Each mixer prepared one tankful of Benlate for one airplane load. The volume prepared and pounds mixed are indicated in Table I along with other pertinent experimental conditions. Five-pound bags of Benlate fungicide were used throughout, except where the total amount mixed was not a multiple of five. In those cases, a single 2-lb bag was also used. The timing of each exposure cycle began when the mixer began opening the first bag to be mixed and included opening and emptying all bags into the mix tank. Typical mixing equipment is shown in Figure 1. Techniques for opening and emptying the bags varied. Some subjects opened the bags on the seam and other slit the bags with a knife. A typical opening and emptying sequence is shown in Figure 2. Agitation of water in the mix tank was employed during the mixing process. The total time for a mixing cycle varied from 1.5 to 5 min (see Table I). This work cycle might be repeated up to 3 times an hour.

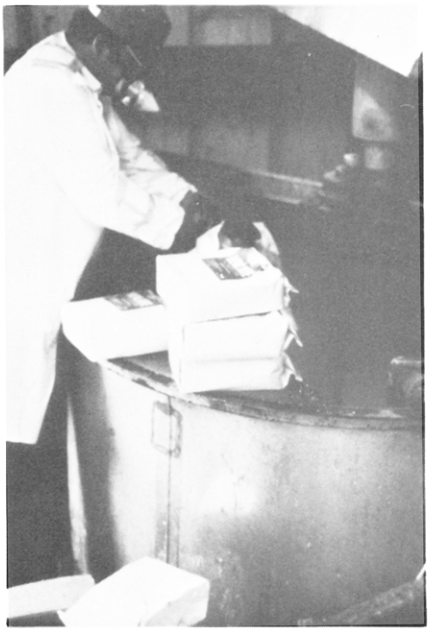


Figure 1. Mixer/loader operation (indoors). Benlate is mixed in a 1000-gal mix tank equipped with mechanical agitation. This operation was indoors and the mixer wore protective rubber gloves.

Reentry (Hand Harvest). Three adult females, who are permanent employees of a major commercial strawberry grower in the Watsonville, CA, area, were monitored for potential benomyl exposure while hand harvesting strawberries from a Benlate-treated field. The samples were collected in connection with the normal performance of duties; the subjects were instructed to pick berries in their normal manner.

Each worker picked berries for 2 uninterrupted hours except for stopping to get empty trays to take the place of filled trays. Each worker picked three trays of berries

Figure 2. Mixer/loader operation (outdoors). A 5-lb bag of Benlate is emptied into the 500-gal mix tank. This particular mixer opened bags by tearing the top corner. Hydraulic mixing and pumping equipment is in the foreground.

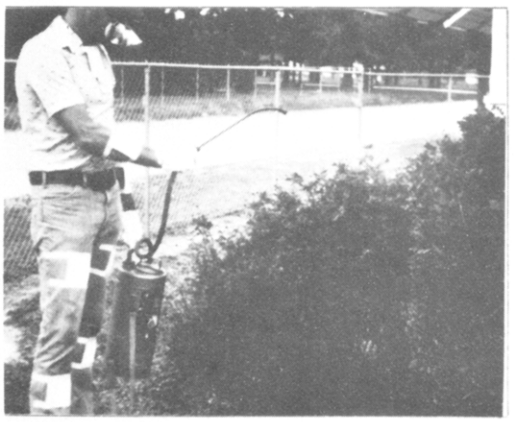
Figure 3. Crop hand harvest. A professional strawberry picker at work. Workers stoop to pick berries which are placed in a tray carried on small "wheelbarrow"-type apparatus. Note the low growing nature of the crop, spacing of rows, and extent of contact with foliage which can be seen in this photograph.

per h; a tray consists of 12 qt (see Figure 3).

The berries in this study were of the Aiko variety and had been sprayed with Benlate within 24 h of the harvest, the preceding day, at a rate of 1 lb of Benlate/acre. The tank mix used consisted of 0.5 lb of Benlate per 3 lb of Captan per 125 gal. The mix was applied to 0.5 acre of berries consisting of twenty-four 208-ft rows planted 52 in. on center.

Dermal samples were taken from the forearms, face, chest, hands, upper thighs, and lower legs. The leg areas of the workers in this use situation were monitored, even though the normal practice of these professional harvesters is to wear pants and socks which will protect these areas from the sun.

Home Use. Three typical home garden spraying simulations with Benlate were conducted under greenhouse and outdoor conditions. In each simulation qualified E. I. du Pont de Nemours & Co., Inc., field personnel conducted the pesticide application. The three use situations were as follows.



SPRAYING ROSES

Figure 4. Home use: application to ornamentals.

PREPARING SPRAY SUSPENSION

Figure 5. Home use: spray preparation.

(*A*) *Greenhouse.* In the greenhouse use situation, the applicator used a standard home garden stainless steel compressed air sprayer with an adjustable cone nozzle. The spray application of 2 gal of Benlate suspension took about 9 min. The temperature inside the greenhouse was 82 °F, 65% relative humidity, and slight air movement from circulation fans at either end of the house. An assortment of plants were sprayed including bean, cucumber, cotton, and small scotch pines.

(*B*) *Ornamentals.* In the ornamentals use situation, the applicator used a Hudson garden compressed air sprayer with a Tee Jet cone nozzle. The spray application of 2 gal of Benlate suspension took 12 min. The application was conducted outside, near Bradenton, FL, at 75 °F, 60% relative humidity, and with winds varying from calm to 3 mph. Large rose bushes and evergreen shrubs (about 3–4 ft high) growing as a landscape planting were sprayed (see Figure 4).

(*C*) *Vegetable Garden.* In the vegetable garden use situation, the applicator used a Hudson garden compressor air sparyer with a Tee Jet cone nozzle. The spray application of 2 gal of Benlate suspension took 16 min. The application was conducted outside, ner Bradenton, FL, at 75 °F, 60% relative humidity, and with increasing winds from 5 to 15 mph. A mixture of vegetable crops including peppers and cole crops (from 1 to 4 ft high) growing in a typical garden arrangement were sprayed.

The Benlate suspensions were prepared by each applicator by measuring 2 rounded tbs of Benlate fungicide from a commercial bag into the sprayer and mixing it with 2 gal of water (see Figure 5). This made a spray concentration higher than 1200 ppm of 50% Benlate because

Table II. Summary of Recovery Data

substrate	fortification level, μg of Benomyl	no. of determn	recovery, % av	recovery, % range
respirator pads	2.0-500	13	97	80-124
dermal exposure pads	3.0-5000	16	95	74-110
gloves	20-5000	16	90	70-120

the wettable powder was more dense than average and the tablespoons were rounded higher than average, giving at least 4.5 g/spoonful. Analysis of typical suspensions prepared by this method were calculated at 994 ppm of active benomyl or 1988 ppm of 50% Benlate vs. a recommended spray tank concentration of 1200 ppm of Benlate.

Dermal samples were taken from the forearms, face, back (back of neck), chest, hands, upper thighs, and lower legs. The leg areas were monitored because of the possibility of an applicator in a home use situation wearing short pants while applying the Benlate.

Analytical Methodology. All samples were transported from the field to the laboratory and maintained at room temperature until analysis. A 4-in.^{2} (0.002581-m^2) portion was cut from the center of each gauze pad and filter paper backing for analysis. Extreme handling care was taken to ensure no loss of residue from the pad surface. The respirator pads and the gloves were both analyzed in toto.

The test pads and gloves were heated under reflux for 40 min in methanol–1 N HCl (83%:17% v/v) (250 mL/pad; 500 mL/gloves) and filtered through cotton, and the methanol was evaporated on a rotary evaporator at 60 °C. After the aqueous solution was washed once with hexane, it was made basic, and the residue was extracted into ethyl acetate. After being dried with sodium sulfate, the organic phase was evaporated to dryness with the final residues being dissolved in 1 mL of 0.1 N H_3PO_4. Final determination was by liquid chromatography using a cation-exchange column (Kirkland et al., 1973).

RESULTS AND DISCUSSION

Table II summarizes the data from benomyl recovery studies conducted concurrently with the analysis of the exposure samples. For the dermal pads, respirator pads, and gloves, the average recovery factors were 95%, 97%, and 90%, respectively. Addtionally, samples of control pads and gloves were fortified with benomyl at levels ranging from 500 to 5000 μg and allowed to sit at ambient temperature for as long as 12 days before analysis. No loss of benomyl was detected in these samples. The 12-day interval was twice the delay period experienced for the exposure samples in the study.

Individual results from each of the three use situations are listed in Tables III–V. Table VI summarizes the three test areas. In all tables the results are expressed as total micrograms of benomyl detected (in the sample portion analyzed), as total milligrams of benomyl per square meter of sampling area, and as total milligrams of benomyl per body surface area, based on body areas listed by Durham and Wolfe (1962). Respiratory exposure was measured directly and is listed as total micrograms of benomyl on the filter. The lower analytical limit of detection was 1

Table III. Potential Exposure during Mixing for Aerial Application

trial no.	sampling area	benomyl residue total μg	benomyl residue mg/m^2 [a]	benomyl residue mg/body area [b]	trial no.	sampling area	benomyl residue total μg	benomyl residue mg/m^2 [a]	benomyl residue mg/body area [b]
1	forearms	16	6.2	0.75	6	forearms	811	314	38
	face	<1	<0.4	<0.02		face	309	120	7.8
	back	<1	<0.4	<0.01		back	8.4	3.2	0.04
	chest	<1	<0.4	<0.01		chest	122	47	0.71
	hands	3213	39	3.2		hands	10251	125	10
	total dermal	3229		3.95		total dermal	11501		56.6
	respiratory	6.1				respiratory	230		
2	forearms	124	48	5.8	7	forearms	42	16	2.0
	face	12	4.5	0.29		facw	58	22	1.4
	back	4.0	1.5	0.02		back	<1	<0.4	<0.01
	chest	5.7	2.2	0.03		chest	8.9	3.4	0.05
	hands	3672	45	3.7		hands	2295	28	2.3
	total dermal	3818		9.8		total dermal	2404		5.8
	respiratory	3.0				respiratory	50		
3	forearms	23	8.8	1.1	8	forearms	155	60	7.1
	face	12	4.5	0.29		face	54	21	1.4
	back	2.6	1.0	0.01		back	98	38	0.42
	chest	3.1	1.2	0.02		chest	20	7.7	0.12
	hands	NS [c]	NS	NS		hands	17136	209	17
	total dermal	41		1.42		total dermal	17463		26
	respiratory	31				respiratory	20		
4	forearms	32	12	1.5	9	forearms	90	35	4.2
	face	34	13	0.86		face	55	21	1.4
	back	4.7	1.8	0.02		back	55	21	0.23
	chest	10	3.9	0.06		chest	89	34	0.51
	hands	NS	NS	NS		hands	4743	58	4.7
	total dermal	81		2.44		total dermal	5032		11
	respiratory	4.6				respiratory	218		
5	forearms	343	133	16	10	forearms	303	118	14
	face	114	44	2.9		face	45	17	1.1
	back	26	10	0.11		back	34	13	0.14
	chest	98	38	0.57		chest	38	15	0.22
	hands	11016	134	11		hands	45288	552	45
	total dermal	11597		30.6		total dermal	45708		60.5
	respiratory	98				respiratory	135		

[a] Calculations based on a surface area of 0.002581 m^2 for the gauze pads. [b] Body areas (m^2): face = 0.065; back = 0.011 (back of neck); chest = 0.015; forearms = 0.121; hands = 0.082. [c] NS = not sampled.

Table IV. Potential Exposure during Reentry (Hand Harvest)

sampling area[a]	benomyl residue: total μg	benomyl residue: mg/m² [b]	benomyl residue: mg/body area[c]
	Worker No. 1		
forearms	8.6	3.3	0.40
face	1	<0.4	<0.02
chest	<1	<0.4	<0.01
thighs	<1	<0.4	<0.09
lower legs	<1	<0.4	<0.09
hands	6885	84	6.9
total dermal	6895		7.30
respiratory	4.4		
	Worker No. 2		
forearms	21	8.2	0.99
face	<1	<0.4	<0.02
chest	<1	<0.4	<0.01
thighs	2.6	1.0	0.22
lower legs	2.3	0.89	0.21
hands	14688	179	15
total dermal	14714		16.42
respiratory	4.6		
	Worker No. 3		
forearms	13	5.0	0.61
face	<1	<0.4	<0.02
chest	1.2	0.46	0.01
thighs	<1	<0.4	<0.09
lower legs	<1	<0.4	<0.09
hands	11016	134	11
total dermal	11030		11.62
respiratory	<1		

[a] No sample taken of back area. [b] Calcualtions based on surface area of 0.002581 m² for the gauze pads. [c] Body areas (m²): forearms = 0.121; face = 0.065; chest = 0.015; thighs = 0.225; lower legs = 0.238; hands = 0.082.

μg of benomyl. Exposure for the various body parts was calculated as

$$\text{mg/m}^2 = \text{(mg of benomyl detected)/(dermal pad area sampled)}$$

$$\text{dermal pad area} = 0.002581\ \text{m}^2$$

$$\text{mg/body part} = \text{mg/m}^2 \times [\text{surface area of body part (m}^2)]$$

Dermal exposure measurements were made on body areas which might be exposed during each of the three use situations. This assumes that the worker wore an open-necked, short-sleeved shirt and did not wear gloves. Although most workers routinely wear additional protective clothing, i.e., overalls, jackets, long-sleeved shirts, and gloves, this conservative approach should provide an overview of the "worst" case of potential exposure.

The data from this study indicate minimal exposure to benomyl in all three use situations. Maximum exposure, as would be expected, was in the loading/mixing operation for aerial application. The average potential dermal exposure there was 26 mg of benomyl/mixing cycle; however, 90% of this was in the hand and forearm areas where protective clothing and gloves are often worn. The average respiratory exposure for this situation was only 0.08 mg of benomyl.

For the field reentry situation, the average potential dermal exposure was 12 mg of benomyl, and the average potential respiratory exposure was 0.003 mg of benomyl. On the basis of a 2-h exposure period while harvesting the crop, the dermal and respiratory exposures were 5.9 mg/h and less than 0.002 mg/h, respectively.

Table V. Potential Exposure during Home Use

sampling area	benomyl residue: total μg	benomyl residue: mg/m² [a]	benomyl residue: mg/body area[b]
	Vegetable Garden		
forearms	<1	<0.4	<0.05
face	8.0	3.1	0.20
back	4.0	1.6	0.02
chest	<1	<0.4	<0.01
thighs	2.5	0.97	0.22
lower legs	4.3	1.7	0.40
hands	104	1.3	0.10
total dermal	123		0.94
respiratory	4.9		
	Ornamentals		
forearms	1.4	0.54	0.06
face	<1	<0.4	<0.02
back	<1	<0.4	<0.01
chest	1.4	0.54	0.01
thighs	1.4	0.54	0.12
lower legs	1.5	0.58	0.14
hands	73	0.89	0.07
total dermal	79		0.40
respiratory	1.6		
	Greenhouse		
forearms	<1	<0.4	<0.05
face	<1	<0.4	<0.02
back	<1	<0.4	<0.01
chest	<1	<0.4	<0.01
thighs	<1	<0.4	<0.09
lower legs		2.4	0.57
hands	551	6.7	0.55
total dermal	557		1.12
respieratory	2.8		

[a] Calculations based on surface area of 0.002581 m² for the gauze pads. [b] Body areas (m²): forearms = 0.121; face = 0.065; back = 0.011 (back of neck); chest = 0.015; thighs = 0.225; lower legs = 0.238; hands = 0.082.

Table VI. Summary of Benlate Use Situations: Potential Exposure

	total mg of benomyl exposure: dermal	total mg of benomyl exposure: respiratory
	(I) Mixing for Aerial Application	
trial no. 1	3.95	0.006
2	9.8	0.003
3[a]	1.42	0.031
4[a]	2.44	0.005
5	31	0.098
6	57	0.230
7	5.8	0.050
8	26	0.020
9	11	0.218
10	61	0.135
av	26	0.080
	(II) Reentry (Hand Harvest)	
worker 1	7.30	0.004
worker 2	16.42	0.005
worker 3	11.62	<0.001
av	12	0.003
	(III) Home Use	
vegetable garden	0.94	0.005
ornamentals	0.40	0.002
greenhouse	1.12	0.003
av	0.82	0.003

[a] Since the hand areas were not measured for trial no. 3 and 4, the dermal values from these two trials were not considered when calculating the overall average dermal exposure.

The third use situation simulated standard home use of Benlate, i.e., home gardens, ornamentals, or greenhouse. The average potential dermal exposure was less than 1 mg

of benomyl, and the average potential respiratory exposure was 0.003 mg of benomyl/application cycle.

The data obtained from this research indicate a relatively low level of benomyl exposure. Additionally, a "worst case" approach was taken in this study, since much of the dermal exposure reported was from areas often converged with protective clothing, i.e., gloves and long-sleeved shirts. If the assumption of basic protective clothing is made, the practical exposure levels would be further reduced.

ACKNOWLEDGMENT

We thank Lincoln M. Bradley, Dr. Charles J. Delp, Dr. Stephen J. Denis, and Dr. Lyall F. Taylor for their technical assistance.

LITERATURE CITED

Durham, W. F.; Wolfe, H. R. *Bull. W.H.O.* **1962**, *26*, 75–91.
Durham, W. F.; Wolfe, H. R.; Elliot, J. W. *Environ. Health* **1972**, *24*, 381–387.
Kirkland, J. J.; Holt, R. F.; Pease, H. L. *J. Agric. Food Chem.* **1973**, *21*, 368–371.
Popendorf, W. J.; Spear, R. C.; Leffingwell, J. T.; Yager, J.; Ephraim, K. *J. Occup. Med.* **1979**, *21*, 189–194.
Spear, R. C.; Popendorf, W. J.; Leffingwell, J. T.; Milby, T. H.; Davies, J. E.; Spencer, W. F. *J. Occup. Med.* **1977**, *19*, 406–410.
Staiff, D. C.; Comer, S. W.; Armstrong, J. F.; Wolfe, H. R. *Bull. Environ. Contam. Toxicol.* **1975**, *14*, 334–340.
Wolfe, H. R.; Armstrong, J. F.; Staiff, D. C.; Comer, S. W.; Durham, W. F. *Bull. Environ. Contam. Toxicol.* **1975**, *3*, 257–267.
Wolfe, H. R.; Durham, W. F.; Batchelor, G. S. *Environ. Health* **1961**, *3*, 468–475.
Wolfe, H. R.; Walker, J. C.; Elliot, J. W.; Durham, W. F. *Bull. W.H.O.* **1959**, *20*, 1–14.

Received for review June 8, 1981. Revised manuscript received October 23, 1981. Accepted October 23, 1981.

A Multiresidue Procedure for the Determination and Confirmation of Acidic Herbicide Residues in Human Urine

William M. Draper[1]

Chlorophenoxy acid herbicides (2,4-D, 2,4,5-T, 2,4-DP, 2,4-DB, and silvex), dicamba, pronamide, picloram, and PCP were determined simultaneously in human urine. Samples were hydrolyzed with mineral acid to liberate conjugated residues and to convert pronamide metabolites to 3,5-dichlorobenzoic acid. Acids were isolated from the urine hydrolysate by acid/base partitioning and derivatized with ethereal diazomethane. Pesticides were determined quantitatively by electron capture gas chromatography (EC-GC), and structures were confirmed by computer-controlled gas chromatography–mass spectrometry (GC–MS). Recoveries were 80–104% for fortifications at 0.1 mg/L, and detection limits for herbicides in urine were 0.05–0.1 mg/L by EC-GC and 0.1–0.5 mg/L by GC–MS. Derivatization with pentafluorobenzyl bromide was unacceptable for several reasons: it enhanced the electron capture response of urinary acids and the specific detection of the PFB analogues by mass spectrometry was limited by the similarity of their electron impact mass spectra.

The chlorophenoxy acid herbicides are widely used in agriculture, commerce, and homes to control terrestrial and aquatic broadleaf weeds. The halogenated benzoic acids, pyridine derivatives, and other herbicide classes supplement the biological activities of the phenoxyalkanoic compounds and provide a variety of phytotoxic responses in higher plants. Human exposure is an inevitable, but controllable result of the widespread use of these commercially important chemicals. For reliable assessment of human exposure to pesticides, analytical methods with a high degree of qualitative accuracy are required. Analysis of biological fluids by EC-GC alone provides inadequate qualitative information for positive identification of pesticide residues. Supplemental cleanup techniques increase specificity but cannot eliminate analytical ambiguity.

The chlorophenoxy acid herbicides are excreted largely unmetabolized in the urine of animals (Clark et al., 1964), and the urinary levels are well correlated with the rates of exposure (Shafik et al., 1971; Khanna and Fang, 1966). For these reasons urinalysis is useful qualitatively and quantitatively for determining occupational and extraneous exposure to these herbicides. Exposure to pentachloro- and other halogenated phenols can be determined by urinalysis, but recoveries are unacceptable without hydrolysis (Edgerton and Moseman, 1979; Shafik et al., 1971).

The objective of this investigation was to develop a generalized, multiresidue procedure for trace analysis of herbicidal acids in urine that would be readily adaptable to confirmation by mass spectrometry. Acid hydrolysis was utilized to increase the recovery of conjugated residues and to include pronamide and its metabolites in the multiresidue scheme. Finally, methyl and pentafluorobenzyl derivatives of urine extracts were examined to determine their applicability to detection by both EC-GC and GC–MS.

EXPERIMENTAL SECTION

Chemicals. Analytical reference standards of 4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid (picloram, 99%), 3,5-dichloro-*N*-(1,1-dimethyl-2-propynyl)benzamide (pronamide, 97%), 3,6-dichloro-2-methoxybenzoic acid (dicamba, 99.9%), (2,4-dichlorophenoxy)acetic acid (2,4-D,

Toxicology Program, Department of Animal, Dairy, and Veterinary Sciences, Utah State University, Logan, Utah 84322.

[1]Present Address: Pesticide Chemistry and Toxicology Laboratory, Department of Entomological Sciences, University of California, Berkeley, CA 94720.

0021-8561/82/1430-0227$01.25/0