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Human Exposure Assessment to Mancozeb During Treatment of Mandarin Fields

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Human exposure to pesticides can occur during manufacture, mixing/loading, spraying, harvest, and by consumption of treated crops. During spraying, the representative routes of human exposure are dermal deposition and inhalation. By far the greatest potential exposure is through the dermal absorption during spraying operations, and the importance of related exposure has been confirmed by many studies (Durham et al. 1972; Fenske and Elkner 1990). Such studies have raised concerns about the need for greater protection of workers, since levels of pesticide deposited on exposed skin were found to be 20-1700 times the amount reaching the respiratory tract (Feldman and Maibach 1974). Direct contact with pesticides by workers that handle and apply these agents, can lead to harmful effects. The risk of pesticide poisoning depends on the toxicity of the chemical and the level of exposure (Turnbull 1985). Toxicity is the intrinsic properties of the compound, although the route of uptake and the bioavailability can have some influence. The level of exposure is also affected by the properties of the compound, but mainly by the type of work, hygienic behavior of the worker, the time of contact with the chemical, and the equipment used for pesticide application (Machado-Neto 2001, Vercruysse et al. 1999). Therefore the importance of field surveys and assessment of exposure of operators to pesticides under actual application conditions is paramount. Such surveys provide essential data for risk assessment and are the only alternative to extrapolation from animal exposures (Calumpang 1996). Dermal exposure is represented in direct measurements by the total amount of pesticide retained on the patches and their relationship to the corresponding body area.

The purpose of this study was to determine the dermal exposure extent of applicators of the fungicide mancozeb to mandarin on small farm using an electric sprayer. In so doing, one can determine whether the actual exposure fell below or above the no-effect level of exposure and identify the body parts most exposed to contamination by mancozeb during mandarin production. Mandarin is one of the favorite fruits worldwide and in Korea, produced mainly in Cheju island. Farmers prefer to wear long sleeved cotton polyester shirts and long trousers instead of protective garments while spraying, therefore, there may be significant dermal exposure for them and accidental field-poisonings may also occur in workers.

Mancozeb is an ethylene bisdithiocarbamate fungicide which has been used for the control of many fungal diseases in field crops, fruits, and vegetables in Korea.

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It is poorly soluble in water (solubility: 6.2 mg/L), and is slowly decomposed by heat and moisture (Tomlin 2000). It is classified as a potential endocrine disruptors by the US Environmental Protection Agency and the World Wildlife Fund (WWF). Ethylenethiourea (ETU), a trace contaminant as a breakdown product of mancozeb, has caused thyroid effects, tumors and birth defects in laboratory animals (Tomlin 2000). Several studies of the effects of mancozeb on test animals have shown rapid reduction in the uptake of iodine and swelling of the thyroid (Keith 1997). Rita et al. (1987) reported increased incidence of abortions and stillbirths for male and female workers exposed to pesticides in grape gardens in Andhra Pradesh, India. Mancozeb was one of the nine pesticides that workers handled in that study. To the best of our knowledge, there is no previous assessment of the exposure of fungicide mancozeb in mandarin production.

MATERIALS AND METHODS

Field study was conducted in mandarin fields in Seogwipo, Cheju, Korea. The Spray activities were conducted on June 2002. Operators wore typical spraying attire consisting of long-sleeved polyester shirts and long trousers. Head covering was a cap. The method of Durham and Wolfe (1962) was used with some modification to collect sample for estimation of dermal exposure. Cellulose absorbent patches (50 cm^2) were placed on the outer clothing over the following body parts: forehead, front near neck, back near neck, upper arms, forearms, thighs, lower legs, back, chest. Hand exposure was monitored by means of cotton gloves. Face exposure was evaluated using cotton mask (200 cm^2) instead of shoulder patches for more exposure evaluation (Machado-Neto 2001). Dermal exposure estimates were calculated by extrapolating patch deposition values to the total surface area (cm^2) of the appropriate body region (US EPA 1987; Vercruysse et al. 1999). Applicators sprayed mancozeb [as Dithane M-45 WP (Dongbu-Hannong Chemical)] on mandarin during fruiting stage at the rate of 1.93 kg a.i. using 1,285 L for a 4,620 m^2 of experimental area. Application equipments included mixing tanks, pumps to pressurize the spray system and horses. Temperature and relative humidity during this field study ranged from 27 to 28°C and 47 to 60%, respectively. All samples were kept frozen when not analyzed immediately. The meterological conditions, the field characteristics and data on application techniques can be found in Table 1.

The conventional CS_2 evolution method proposed by Keppel (1969) was used to quantitate the amount of mancozeb in samples. Therefore, various samples such as dermal patches, gloves, and mask were mixed with 25 mL of the $HCl/SnCl_2$ solution in the round-bottom boiling flask and heated under reflux. After 1 hr the heating mantle current was shut off, and the content of the CS_2 trap drained into a 25 mL volumetric flask. The volume was completed until the mark with ethanol, and the absorbance was measured at 435 nm using UV/VIS spectrophotometer (Smart Plus, Youngwoo Instrument, Korea). The conversion factor of 1.75 ($CS_2 \rightarrow$ mancozeb) was used (Bohrer et al. 1999). Standard solution for solubilization and extraction of mancozeb was prepared using the method of Bohrer et al. (1999).

Table 1. Field characteristics, application data and meterological conditions of spraying trials 1 to 4

Trial	1	2	3	4
Applicator				
Sex	female	male	female	Male
Height (cm)	158	174	158	174
Weight (kg)	47	85	47	85
Field				
Area (m^2)	660	660	1650	1650
Crop age (years)	20	20	12	12
Crop growth stage	Fruiting stage			
Crop density	very dense		low	
Crop height (cm)	215	215	150	150
Inner row crop distance (cm)	200	200	150	150
Row distance (cm)	250	250	250	250
Product				
AI content used (g)	502.5	502.5	461.3	461.3
Spray volume (L)	335	335	307.5	307.5
Application				
Type of spray application	Handheld lance, electric sprayer			
Lance length (m)	1.0			
Application time (min)	47	47	43	43
Flow rate (L/min)	7.13	7.13	7.15	7.15
Nozzle type	Full cone			
Climate				
Temperature (°C)	28	28	28	28
Relative humidity (%)	59	59	60	60
Wind speed (m/sec)	2.5			
Precipitation	None			

The exposure level of ETU was analyzed using HPLC as follows. The dermal patches, gloves and mask were placed into a 150, 400 and 150 mL recipient, respectively, and extracted in 60, 300 and 60 mL of acetone, respectively. After shaking for 1 hr in universal shaker (Wooju Scientific, Korea), aliquots (2 mL) were concentrated by purging with nitrogen gas. The residue was dissolved with 200 μL of 5% methanol in water, and an aliquots (20 μL) was analyzed by HPLC (HP 1100 model, USA) equipped with Spheriosorb ODS1 column (4.6 × 150 mm, 5 μm, Waters, USA). Peak detection was made at 230 nm using water:methanol (95:5) as a mobile phase at a flow rate of 1.0 mL/min.

The evaluated potential dermal exposure (PDE) values extrapolated to a 4 hr of effective exposure per day with the actual application time (43 or 47 min). The margin of safety (MOS) was calculated by an adaptation of the formula of Severn (1984): **MOS = (NOEL × BW)/(AQE × SF)**

where, NOEL means no observable effect level; BW, body weight, AQE, absorbable quantity of exposure, and SF, safety factor. The NOEL values of mancozeb and ETU based were 5 and 0.2 mg/kg/day, respectively. The BW values for man and woman were 70 and 60 kg, respectively (Jensen 1984). The AQE value was based on assumptions of 10% skin absorption for dermal exposure (Jensen 1984) after 10% of penetration through clothes was considered (POEM 1992). And 1% of dermal exposure was assumed for respiratory exposure (Wolfe et al. 1967). The safety factor was 10 (Brouwer et al. 1990).

The equation proposed to calculate the exposure control need (ECN) for a particular pesticide was based on the following assumptions: if MOS $\geq$ 1, the working condition is considered to be safe; if MOS < 1, the working condition is considered unsafe. In order to calculate safe condition, the ECN as a percentage was estimated by an adaptation of the formula of Machado-Neto (2001): **ECN = (1 – MOS) × 100**

The equation proposed to calculate the safe work time (SWT) was based on the following assumptions (Machado-Neto 2001). If AQE × SF occurs within an effective work time (EWT, in hr/day), then NOEL × BW will occur within a safe work time (SWT). Therefore, SWT = (NOEL × BW)/(AQE × SF) × EWT. Since (NOEL ×BW)/(AQE × SF) = MOS then, **SWT = MOS** × **EWT** (hr/day).

RESULTS AND DISCUSSION

The correlation of absorbance values with the concentration of mancozeb and ETU was linear over the range: 0.2 – 14 and 0.0125 – 10 mg/L, respectively. Recovery studies were run on control samples fortified with 200 and 6 µg of mancozeb and ETU, respectively, per sample. The recoveries and the limit of detection for the pesticides and matrices included in the study are shown in Table 2. The analytical procedure was judged to be reliable based on the small relative standard deviation values (0.6-2.0%) (Liu et al. 2001), and thus this method was applied for exposure assessment of mancozeb and ETU.

Table 2. Recoveries and limit of detection for the pesticides and matrices used in the study.

	Dermal patch	Glove	Mask
Recovery (%)			
Mancozeb	93.4 ± 1.7	98.2 ±	90.4 ± 2.4
Ethylenethiourea	86.2 ± 1.9	101.3 ± 1.7	91.3 ± 2.3
Limit of detection			
Mancozeb	5*	5*	5*
Ethylenethiourea	0.075*	0.375*	0.075*

* µg per sample (patch, glove or mask)

The potential dermal exposure of mancozeb in the mandarin applications varied from 912.8 to 1854.5 mg/hr for the whole body (Table 3). These value were much higher than those (10-20 mg/hr) of Mumma et al. (1985). The dermal deposition levels of ETU, a trace contaminant of mancozeb, are 74 to 130 times lower than the dermal deposition levels of mancozeb and well correlate with those of mancozeb (r^2 = 0.833, Figure 1). In all sets of trials, the primary sites of operator contamination were the front parts of the body such as legs, arms, and chest. Significant contamination was observed in some cases on the back of the operator. This was mainly due to indirect contamination through contact with the

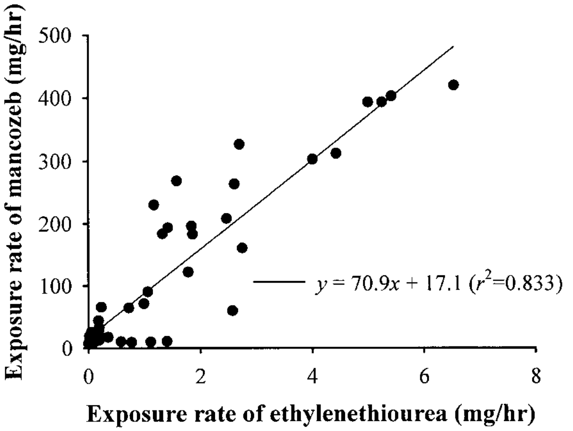

Figure 1. Regression analysis of exposure rate with ethylenethiourea and mancozeb.

Table 3. Potential dermal exposure rate during application from trials 1-4.

	Potential dermal exposure rate (mg/hr)							
	Ethylenethiourea				Mancozeb			
Trial	1	2	3	4	1	2	3	4
Head	0.99	0.23	0.72	0.05	71.2	66.0	64.6	25.4
Face	0.18	0.14	0.35	0.04	17.1	16.9	17.7	7.1
Front of neck	0.14	0.09	0.19	0.02	16.2	8.7	12.8	19.0
Back of neck	0.16	0.04	0.07	0.004	12.2	6.1	7.8	7.6
Back	5.0	2.75	6.54	0.18	393.0	160.6	419.3	44.2
Chest	4.43	2.61	-*	1.86	312.0	263.3	-*	183.0
Upper arms	4.01	1.32	1.42	0.19	302.8	183.7	193.6	32.6
Forearms	1.78	2.57	1.06	0.18	122.0	59.8	90.3	26.9
Upper legs	5.42	5.25	-*	2.70	402.6	393.1	-*	326.4
Lower legs	1.84	2.47	1.58	1.17	196.0	208.2	268.2	230.1
Hands	0.77	1.11	1.40	0.58	9.4	10.0	10.7	10.5
Total	24.7	18.6	13.3	7.0	1854.5	1376.4	1085.0	912.8

* lost patches during application

sprayed plants, especially in the cases of very dense crop foliage (Machera et al. 2002).

As shown in Table 3, the data for potential dermal exposure are highly variable. The variation of the potential dermal operator exposure levels also observed in the study of other researchers (Machera et al. 2002, Van Hemmen 1992). It may be due to field and operational conditions, such as the growth stage of the crop, folliage density, applicator height, application method, operator technique, and other unpredictable parameters. Although all treatments deposited measurable amounts of pesticide on the patches, more pesticide was found on the patches during very dense crop application (Trial 1 and 2) than during low dense crop application (Trial 3 and 4). Also male applicator (Trial 4) compared to female (Trial 3) showed lower exposure rate in upper body area such as head, face, back, and arms in the trials with small crop height (150 cm), it may be due to the height difference of applicator.

According to the results in Table 4, it was found that the conventional application method (MOS < 1) should be modified for safe use. For safe mancozeb application, there was a need for 13-63% exposure control depending on the characteristics of crop and applicator. The SWT values calculated were 1.5-3.5 hr for mancozeb. However, it is needed to wear impermeable coveralls covering all body parts while spraying mancozeb in mandarin production for health of applicator. The ECN and SWT values proposed by Machado-Neto (2001) are very effective tools for selecting safety measures to manage the risk of working with pesticide. These results provide useful data for incorporation in farmer training programmes on the proper use of pesticides and, it is hoped, may persuade farmers to follow the recommendations.

Table 4. Estimates of potential dermal exposure (PDE), absorbable quantity of exposure (AQE), margin of safety (MOS), exposure control need (ECN), and safe work time (SWT) of applicators spraying mancozeb on mandarin crops

	Trial	Dermal exposure rate (mg/hr)	PDE (mg/day)	AQE	MOS	ECN (%)	SWT (hr)
Mancozeb	1	1854.5	741.8	81.6	0.37	63.2	1.5
	2	1376.4	550.6	60.6	0.58	42.2	2.3
	3	1085.0	434.0	47.7	0.63	37.2	2.5
	4	912.8	365.1	40.2	0.87	12.9	3.5
Ethylene -thiourea	1	24.7	9.88	1.09	1.10	-	4.4
	2	18.6	7.44	0.82	1.71	-	6.8
	3	13.3	5.32	0.59	2.05	-	8.2
	4	7.0	2.80	0.31	4.55	-	18.2

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