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Hand Harvester Exposure to Maleic Hydrazide (MH) in Flue-Cured Tobacco

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MH (1,2-dihydro-3,6-pyridazinedione) is widely used to control lateral bud growth on tobacco. Residue levels of this compound vary greatly in tobacco samples from flue-cured auction markets and in tobacco products. A few studies have suggested that either MH or a metabolic product resulting from cellular transformation and/or pyrolysis during smoking is carcinogenic. Animal studies by Dickens and Jones (1965) and Epstein and Mantel (1968) suggested the possibility of MH as a carcinogen to humans. However, subsequent work failed to substantiate the results of these earlier studies (Hunter et al. 1973); and it was speculated that hydrazine, a tumorigenic compound, was present as a contaminant in commercial preparation. Analyses of eight commercial formulations of MH showed hydrazine concentrations ranging from 0.3 to 84 ppm (Schmeltz and Hoffman 1977). These authors also showed that smoke from cigarettes with or without MH produced the same amount of hydrazine. Other studies have attempted to show that MH breaks down into volatile nitrosamines, some of which could be carcinogens, but the data are inconclusive. Patterson et al (1978) indicated that benzo(a)pyrene was formed when MH was pyrolyzed in a nitrogen atmosphere, but Chopra (1979) concluded that the probability of this occurring was small.

A study was conducted in North Carolina during 1978 to measure exposure of humans to MH during application to tobacco (Hunt et al. In press). It was concluded that the type of equipment used to apply MH can reduce the amount of exposure. High-clearance equipment placed the applicator ahead of or at least partially above the spray boom thus reducing exposure. The study reported here was part of an on-going effort to study the exposure of farm workers to MH. Exposure of hand harvesters was estimated by determining the contamination with MH of cellulose pads positioned in a respirator and on forearms, back, left rib area, right rib area, and thighs during a 2-h harvesting period, by analysis of hand rinses after

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exposure, and by analysis of urine samples from the harvesters for MH during a 48-h period immediately following exposure.

MATERIALS AND METHODS

One field of tobacco was sprayed with K-MH (Royal MH-30) at the recommended rate of 2.5 kg a.i./ha (approximately 170 mg a.i./plant) at the Central Crops Research Station, Clayton, North Carolina on July 16, 1979; a second field was treated August 6, 1979. Hand harvesting of the tobacco was performed 3 days after application in each field.

Four volunteers simultaneously harvested tobacco in the treated plots for 2 h. Each harvester wore socks, boots or shoes, trousers, and long sleeve, cotton shirts. Dermal exposure to MH was estimated according to the procedure by Durham and Wolfe (1962). Absorbent pads (10 by 10 cm) were positioned to the dorsal area of each forearm, the back, the right and left rib areas, and the front of each thigh. The pads were alpha cellulose backed with a piece of glassine powder paper. One piece of 4-ply gauze was placed on both sides of the cellulose piece, and the entire pad was taped together. The gauze helped maintain the shape of the pads and reduced disruption of the pads, especially during the first exposure when the tobacco was wet. If the pad became excessively wet during harvest, it was removed and replaced by a clean, dry one. Each absorbent pad was stored in a labeled envelope constructed from filter paper in a plastic bag, and transported to the laboratory in a cool, insulated container. After removing and discarding the glassine powder paper, each sample comprising pad(s) and envelope(s) was trimmed to 7.5 by 7.5 cm, and frozen at -20° until analysis.

The hands of each volunteer were rinsed by placing both hands in a plastic bag containing 200 mL of 95% ethanol and shaking the bag vigorously 50 times (Durham and Wolfe 1962). Bags were placed in wide-mouth 500-mL glass jars for storage. A second rinse with the same volume of ethanol was performed and later combined with the first for analysis. Hand rinse samples were kept cool during transport to the laboratory and refrigerated at 0°C until analysis.

To estimate respiratory exposure, harvesters wore a Willson Respirator No. 2D Dustite model modified with a Nalgene^{R} brand funnel cut to fit the front of the mask (Durham and Wolfe 1962). A single piece of 12-ply gauze was stapled to a Model R99 Willson filter (7 cm in diameter) and trimmed to fit. The funnel was placed over the respiratory pad and the screw cap applied. After harvesting was completed, the pad was removed and handled by the procedure described for the absorbent pads except that the envelope was trimmed to the same diameter as the filter.

Four samples (50 leaves each) of green tobacco, representative of the leaves being harvested, were collected from each field during the time the volunteers were harvesting. The samples were placed

in plastic bags and transported to the laboratory in a cool, insulated container. Each sample was finely chopped, and thoroughly mixed, and a subsample was frozen at -20°C until analysis.

Information concerning the amount of MH absorbed was obtained by collecting total urine passage from each volunteer harvester. Urine samples were collected in 950-mL glass jars for a 48-h period beginning with initial exposure to MH. Sample intervals ranged from 0 h (before exposure) and 0 to 6, 6 to 12, 12 to 24, and 24 to 48 h after exposure. Volumes were recorded, and samples were refrigerated at 0°C until analysis.

Four grams of green tobacco were placed in a special MH distillation flask (Lane 1964). At this point known amounts of MH were added to those untreated samples that were being processed through the procedure as standards.

To the flask was added 50 g of NaOH pellets and 40 mL of distilled water. From this point on, the method was similar to that described by Lane (1964). The caustic mixture was heated to 160°C, and then removed from the heat source and allowed to cool from 130 to 140°C. Five grams of zinc metal (30 mesh) and 0.5 g of ferrous chloride were added to the flask, and the contents were heated until the temperature reached 172°C. Distilled water was added slowly until the temperature decreased to 170°C and distillation continued within a temperature range of 168 to 173°C until a 50-mL centrifuge tube with 4 mL of 2% p-dimethylaminobenzaldehyde in 1 N H_2SO_4 (2% DAB) added prior to distillation contained 40 mL. The distillate was filtered through a coarse porosity, sintered glass funnel. The optical density was determined on a Varian Techron Model 635 variable UV/vis spectrophotometer at 430, 460, and 490 nm.

Each sample comprising pad and envelope was extracted in a Soxhlet apparatus with 150 mL of methanol for 5 h. The extract was evaporated to dryness under vacuum at 40°C, and transferred to a reaction flask, first with 15 mL of 1 N NaOH followed by 15 mL of water. Sonication was used to dislodge any encrusted residue. Twenty grams of NaOH were added to the flask. If the extract was green in color, 0.25 g of paraffin were added to the flask. With the addition of zinc metal, the method then followed the one described for green tobacco, except 45 mL of distillate were collected in a centrifuge tube containing 4 mL of 1 N H_2SO_4. The distillate was filtered and evaporated to about 6 mL on a hot plate. The sample was transferred to a 10-mL volumetric flask with a 2-mL rinse of 2% DAB, then distilled water until the volume totaled 10 mL. The optical density was determined on a spectrophotometer.

Ethanol (95%) was added to the combined hand rinse sample to bring the volume to 500 mL. A subsample of 50 mL was evaporated to dryness on a rotary evaporator at 40°C. The remainder of the procedure was identical to that described for the cellulose pads, except that 40 mL of distillate (instead of 45) were collected.

Fifty milliliters of urine were transferred to a reaction flask and 40 g of NaOH pellets were added, and the contents swirled to dissolve the base. The caustic mixture was heated to 160°C, and then removed from the heat source and allowed to cool from 130 to 140°C. The method then followed the one described for cellulose pads, except that 40 mL of distillate (instead of 45) were collected.

Dermal and respiratory exposure was calculated according to a procedure by Durham and Wolfe (1962) as described in detail by Davis (1980). The residue per unit area ($\mu g/cm^2$) of absorbent pad surface was divided by the length of time the worker was exposed to derive the hourly exposure per unit area. The hourly exposure per unit area of a pad, or mean hourly exposure per unit area if two or more pads represent a body region, was multiplied by the surface area of the corresponding body region. The estimated values for the dermal exposure to MH to the back of the neck were based on the pad on the back; front of neck, pads on right and left ribs; back, pad on the back; chest and stomach, pads on right and left ribs; forearms, pads on the forearms; and thighs, pads on the thighs. The absorbent pads were assumed representative of the respective body part measured. The estimated value for the hands was based on the total residue found in the hand rinses. Respiratory exposure was equivalent to the amount of MH contamination of the respiratory pads. Results for dermal and respiratory exposure were expressed as micrograms per hour ($\mu g/h$). Concentrations for green tobacco and urine were expressed as micrograms per gram (ppm).

Split plot in time analysis of variance was used to compare the group means for urine and the different anatomic regions. To utilize the analysis of variance for the anatomic regions, a logarithmic transformation to the base 10 was performed on the data to stabilize the variance. A two-way analysis of variance was used for the tobacco and respirator. When the F-value was found to be significant, Fisher's least significant difference (LSD) was calculated.

RESULTS AND DISCUSSION

Residues of MH on green tobacco samples from the field used for the first and second exposure period averaged 13 and 11 ppm, respectively. Based on the weight of tobacco at 13% moisture, the mean water content of cured tobacco, the average MH residues were 91 and 69 ppm, respectively for the two harvest periods. These residues fall within the range for flue-cured tobacco receiving the recommended rate of application (Hunt et al. 1977). No significant differences were found for residue levels between the first and second period.

The dermal exposure rates for harvesters are shown in Table 1. The difference between the amount of MH accumulation during the two exposure periods appeared to be related to the moisture conditions

Table 1. Dermal exposure of volunteers hand harvesting MH treated tobacco at Clayton, North Carolina in 1979.

Body region	Exposure period	Dermal exposure (µg/h)[a] Untransformed	Transformed
Back of neck	First	15	1.15
	Second	21	1.28
Front of neck	First	77	1.88
	Second	27	1.41
Back	First	470	2.66
	Second	680	2.79
Left chest and stomach	First	1100	3.04
	Second	430	2.61
Right chest and stomach	First	720	2.85
	Second	200	2.29
Forearms	First	870	2.87
	Second	290	2.41
Hands	First	32	1.48
	Second	430	2.63
Thighs	First	2100	3.30
	Second	510	2.67
LSD			[b]

[a]Each value is an average of four volunteer harvesters. Untransformed represents the actual value; transformed represents the actual value to log base 10.

[b]LSD 0.05 for comparing body regions within a period equals 0.26, and for comparing periods within a body region equals 0.30. Use the LSD 0.05 for transformed data only.

during harvest. The tobacco was wet during the first period and dry during the second. Residues averaged over harvesters and body regions for each exposure period indicate the exposure rate was twice as high with wet tobacco than with dry tobacco. Individual body regions which had the higher MH levels of the two exposure periods were the front of the neck, both chest and stomach areas, forearms, and thighs.

The left chest and stomach region had higher MH residues than the right chest and stomach regions for the second exposure period. This was due to the harvesters being right-handed, thus picking the tobacco leaves with their right hands and holding the leaves against the left side of the body with the left arm.

MH residues on the hands were higher for the second exposure period. The MH residue concentration on the green tobacco was essentially the same during both harvests. However, when tobacco is dry, greater amounts of tobacco plant juices (gum) accumulate on the hands. Most of this gum was removed during the ethanolic hand rinses, thus the higher residues. This gum is normally removed from the hands twice a day, before lunch and at the end of work. The higher residues are therefore simply a direct relationship to the quantity of gum on the hands. There is probably no increased hazard here since the MH is physically bound in the gum on the hands.

The respiratory exposure to MH for hand harvesters for the first and second period averaged 4.8 and 3.3 µg/h, respectively. There were no significant differences found for residue levels between the harvesters and/or exposure periods.

All urine samples immediately before exposure (0 h), and 0 to 6, 6 to 24, 24 to 48 h after exposure contained less than the limit of detection of 0.02 ppm of MH with the exception of the samples from the 24 to 48 h period for the second exposure period. This value was 0.02 ppm. Based on previous studies (Mays et al. 1968), most of the MH absorbed should have been excreted within 12 h. The absence of detectable residues in most urine samples indicates minimal absorption of MH by hand harvesters. There were no significant differences between harvesters and/or exposure periods.

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