

URINE MONITORING FOR THPI OF PROPAGATION NURSERY WORKERS
HANDLING CAPTAN TREATED GRAPEVINE STOCK

Clifford Smith, Sr Environmental Hazards Scientist
Sheila Margetich, Agricultural Chemist III
Bernardo Hernandez, Environmental Hazards Scientist
Janet Spencer, Environmental Hazards Scientist

HS-1539 February 15, 1990

ABSTRACT

Grapevine propagation workers were monitored to evaluate their exposure to captan residue on cuttings that had been treated by dipping in a captan and water mixture. The first morning urine void was collected from 15 workers on the third and fourth day of a four-day exposure period. Samples were analyzed for tetrahydrophthalimide (THPI), a metabolic excretion product of captan, and creatinine. Daily exposure was estimated by using creatinine content and estimated lean body mass to extrapolate from single void to daily excretion. Four out of the 30 samples collected from the 15 workers were positive for THPI at concentrations from 67 to 178 parts per billion. Two different workers collected positive samples on each of the two sampling days. The absorbed daily dosage (ADD) for this activity was estimated to be $904 \pm 1,226$ micrograms per person per day; and the average annual daily dosage was estimated to be 0.85 ± 1.10 micrograms per kilogram per day. Observations were made of protective glove use by employees, but the study did not control for glove use. No recommendations were made for regulatory changes or work practices at the monitored facility.

INTRODUCTION

Captan is a widely used fungicide in California agriculture. Studies conducted in laboratory animals suggest that captan causes significantly elevated incidence of duodenal tumors in male and female mice. Adenocarcinomas were also observed in the gastrointestinal tract in both sexes of mice and kidneys tumors in male rats. Captan is categorized as a B₂ (probable human) carcinogen (U.S. E.P.A., 1989).

Recent risk assessments for workers exposed to captan, conducted by the California Department of Food and Agriculture and the U.S. Environmental Protection Agency, have been done using limited exposure data. The assessments focused on workers involved in traditional mixing, loading and spraying activities, as well as fruit and vegetable harvest workers exposed to residues. Other uses, including grapevine stock propagation, result in exposure to workers in highly specialized activities for which no exposure estimates can be made. As a result of the risk assessment process, certain uses of captan have been removed from product labels and use restrictions have been implemented to mitigate the highest exposure situations.

Following an inquiry from a manager of a grape nursery involved in grapevine propagation (grafting), a suitable work site was identified to conduct a small-scale monitoring study to estimate exposure to captan. The workers in the nursery agreed to participate in a two-day study of captan metabolites in urine.

Dermal absorption of captan in humans has been estimated to be approximately six percent in 24 hours (Fong, 1990). At the time this study was conducted, no human dermal absorption estimates were available. The Department's risk assessment process was then using laboratory animal (rat) data to estimate absorption of 48 percent in 24 hours. In a study of THPI excretion following single oral captan doses of humans at 1 mg/kg, 66 percent of recovered dosage was collected within the initial 24 hours; 33 percent was collected during the second 24 hours (Thongsinthusak, unpublished). The urinary portion is estimated to be 80 percent of total dosage excreted, with 15 percent of urinary excretion released as tetrahydrophthalimide (THPI or cis-1,2-dicarboximido-4-cyclohexene) (Fong, 1990; Maddy, 1988; Thongsinthusak, unpublished).

Workers in the grapevine propagation nursery monitored were involved in bench grafting. This involved attaching scions of a plant variety to stock of a rooting variety. Bundles of grapevine cuttings (scion and stock) were first placed for one to three hours in a vat with a 0.001 percent (nominal, active ingredient) suspension of captan in water. They were then removed and drained, for several minutes to approximately three hours, until needed by grafters. The liquid remaining on the bundles of cuttings had partially dried in that period.

Before grafting, the scion and stock were culled and sorted by hand into open trays and delivered to the grafting tables. Each grafter selected one of each type of cutting and used a mechanical punch and die device to cut a "keyhole" slot into the bottom of the scion and a corresponding "key" into the top of each piece of stock. The grafter then fit the scion and rootstock together and placed them in a tray. The grafted material was then

dipped in hot wax to seal in moisture, and placed in a mineral media to root.

The nursery monitored employed 17 workers. Three of the employees handled the bundles of wood cuttings, sorted and culled, and did other miscellaneous jobs such as driving a fork-lift and moving trays to and from the grafting tables. One of the three workers mixed the captan dip solution each morning. Eleven other employees grafted most or all of the time. Three employees dipped the grafted stock in wax and packed them for rooting. Vinyl gloves were provided to the employees by management, but their use was not required by product labeling, state use regulations or the nursery's management.

Grapevine bench grafting is an intermittent activity. The cooperating facility did it for four weeks in 1989; two weeks in late February and two weeks in early April.

The purpose of this study was to estimate the absorbed dosage in grapevine grafters to determine the need for additional protection from exposure. Secondary objectives were to determine the sensitivity of the method for estimating exposure at or below levels of toxicological concern and determine the effectiveness of gloves in reducing exposure.

METHODS

Observation of work activities and the physical properties of captan indicated the likely primary route of exposure to be dermal absorption through the hands. Investigators monitoring workers engaged in hand harvest of crops treated with captan have successfully used urinary THPI output to estimate overall exposure to the pesticide, despite a mean background level of as much as 24 ppb (Winterlin, 1984 and 1986; Maddy, 1988). Analytical method development work by Schoen (1982) indicated the presence of THPI in persons not knowingly exposed to captan. Several methods are available to estimate dermal exposure to pesticides, including cloth-patch dosimetry, garment dosimetry (including cloth gloves) and skin washing (Mull, 1986). Only skin washing was considered for the hands, but was rejected because of possible interference with the normal dermal absorption process. Since absorbed dose is the more toxicologically significant measurement, urine monitoring for excretion of the metabolite THPI was selected.

The days of exposure for the crew monitored were Tuesday through Friday for approximately eight hours. Workers were asked to collect their entire first morning void on each of two days, Thursday and Friday, April 6 and 7, 1989. Since 99 percent of orally administered captan was excreted within 48 hours, the final two days of exposure were selected for sampling to allow the excretion flux to reach an apparent steady state.

The purpose and methods of the study were explained to the entire crew and volunteers were solicited on the day before initial sample collection. All employees volunteered and signed consent statements (Appendix 1). Each volunteer's weight and height were measured to estimate daily urinary creatinine output; then they were given a one-liter labeled polypropylene

bottle for sample collection. Employees were instructed to do their work tasks as they usually would. Use of vinyl gloves by workers was not specified by investigators or management, but was observed and noted once each day. On the days of sample collection, the investigators met the employees at the work site to receive the samples and place them on dry ice.

Samples were analyzed by the Department's Chemistry Laboratory Services for THPI (Appendix 2). The minimum detectable limit (MDL) for THPI in urine was 5 parts per billion (ppb).

Creatinine analysis was conducted by a contract clinical laboratory to allow extrapolation of daily THPI output from the single void sample collected. Results of THPI and creatinine analysis were used to estimate daily captan exposure to the workers. Expected daily creatinine excretion was calculated by the method of Forbes and Bruining (1976) based on the Lean Body Mass (LBM) of each worker. LBM was estimated from individual height and weight by the method of Sendroy and Cecchini (1954). The portion of the daily urinary output actually collected was calculated as the portion of expected daily creatinine excretion measured in the sample.

Absorbed daily dose (ADD) was calculated from daily THPI excretion by correcting for reduction in molecular weight (MW of captan is 300 daltons vs. 149 daltons for THPI), the portion of captan metabolites believed excreted in the urine (80 percent) and the urinary portion of metabolite analyzed as THPI (15 percent). Since samples were collected the second and third day of exposure, and there is insufficient data available to assign the relative portion of excreted THPI to specific exposure days, a steady state of exposure-induced dosage was assumed. The formulae used to calculate ADD were:

$$\text{ADD} = \frac{\text{Estimated Daily THPI Excretion}}{\text{Metabolic Correction Factor}} \times$$

$$\text{Metabolic Correction Factor} = \frac{2.01 \text{ (g captan/g THPI)}}{0.8 \text{ (urine fraction)} \times 0.15 \text{ (THPI fraction)}} = 16.7$$

For workers without detectable levels of THPI in their urine, the minimum detectable exposure (MDE) was calculated in place of an ADD using the same method, but based on the minimum detectable level (MDL) of 5 ppb instead of an actual level detected.

The formulae (from Thongsinthusak, 1989) used to calculate the Annual Average Daily Dosage (AADD) and Lifetime Average Daily Dosage (LADD) from ADD were:

$$\text{AADD} = \frac{\text{ADD (ug/person/day)}}{\text{body weight (kg)}} \times \frac{4}{52}$$

where 4/52 represents four weeks of continuous exposure in a 52-week year (the portion of the year employees are engaged in this activity); and

$$\text{LADD} = \text{AADD (ug/kg/day)} \times \frac{40}{70}$$

where 40/70 represents 40 years of working in a 70-year lifetime (the working portion of an average lifetime).

RESULTS

All workers returned collection bottles on each of the two sampling days except Worker 17 on Day 2. Data from Worker 17 was excluded from further analysis. Approximately 50 milliliters of urine were required for the analyses conducted. All workers collected sufficient volume each day except Worker 6, who collected less than 20 milliliters of urine each day and was also excluded from further analysis. Study subjects' height, weight, sample urine volume, creatinine and THPI concentrations, and glove observations are contained in Appendix 3.

By observation, all workers in the crew spent most of their work day manually handling treated wood cuttings. All monitored employees were treated as a single group because many workers were involved in more than one activity. Various activities appeared to involve similar contact and the sampling results did not suggest otherwise.

Two different workers had detectable levels of THPI on each day of the study (Table 1). For the four workers showing measurable exposure, the ADDs ranged from 1,212 to 3,416 micrograms per person per day ($\bar{x}=2,711 \pm 1,019$ ug/person/day). Of the four workers showing detectable THPI levels, all four worked at the grafting tables; two wore gloves and two did not.

For the remaining 11 workers the question remained: What could their captan exposure have been despite their negative analysis for THPI? To estimate the minimum detectable exposure (MDE), and to test the sensitivity of this method to detect exposure of regulatory concern, the same calculations were done using the MDL and applying it to individual sample volumes and expected urinary creatinine output (Table 2). The mean of the MDEs for captan exposure (for workers not showing detectable levels of THPI in the urine) thus calculated is 983 ± 426 micrograms per person per day.

An AADD and LADD were calculated for each worker. The resulting 15 AADDs and LADDs were averaged to make estimates for the entire work group. The ADD for the 11 workers with no detectable THPI were assumed to be one-half of the MDE calculated. The ADD estimated was $904 \pm 1,226$ micrograms per person per day. The estimated AADD resulting from grape vine propagation only is 0.85 ± 1.10 ug/kg/day; the estimated LADD is 0.48 ± 0.63 ug/kg/day.

DISCUSSION AND CONCLUSIONS

Four of 15 workers had positive THPI values on one of two sampling days. The only apparent worker contact with treated surfaces was by the skin of their hands. The workers who were observed to work bare-handed had a higher incidence (two out of four) of detectable urinary THPI than workers observed to wear gloves (two out of eleven). This relatively higher proportion was not statistically significant (by chi-square test, level of significance of

0.05). The obvious protective measure available for the work site monitored was the use of gloves during all plant material handling activities. This study's examination of glove use as a protective measure was limited in power since no attempt was made to control for glove usage.

Before assessing the captan exposure risk for this group of workers completely, other occupational and non-occupational (dietary ingestion) exposures must be considered. For comparison, the LADD in use by CDEA for acceptable exposure to captan based on laboratory animal toxicology data, is 20 ug/kg/day and the dietary Acceptable Daily Intake (ADI) set by World Health Organization is 125 ug/kg/day. The LADD estimated in this study, 0.48 ± 0.63 ug/kg/day, demonstrates sufficient sensitivity to estimate exposure at a level under three percent of the total acceptable LADD.

Inconsistent detection of THPI in urine samples from two workers is basis for questioning the source of the metabolite found in certain samples. After detecting 67 and 69 ppb THPI in the urine of Worker 5 and Worker 15 on Day 3, some detectable amount (30-35 ppb based on excretion following ingestion) would have been expected for the same workers on Day 4. This apparent discrepancy may be due to a one-time exposure on Day 1, an inadequate understanding of captan metabolism associated with dermal absorption or simply a sampling artifact such as sample contamination.

The once-per-day urine sampling scheme was used to minimize intrusion into the workers' privacy. Complete 24-hour urine collection would have provided a better internal control for contamination and would have enabled a more accurate estimate of exposure by eliminating extrapolations required to estimate daily output from a single sample.

Captan exposure in the nursery grapevine propagators monitored was not high enough to initiate regulatory action or even to recommend modified work practices. Depending on the level of other occupational and non-occupational exposures, workers in this activity would not be considered at significant risk for captan exposure-induced cancer.

Beyond the measurements made, it was observed that vinyl gloves were in widespread use by workers in all aspects of propagation activity. By observation, and by informal survey of workers, glove usage did not interfere with the work tasks required. No modification in work practices at the site monitored appear to be necessary.

The monitored workers' extensive contact with treatment concentration captan mixture was expected to result in significant dosage for unprotected workers. That prediction, based on relatively high dermal absorption estimates for captan, was not confirmed by this study. The finding that most workers in the crew monitored had no detectable captan metabolites in their urine can be explained only in part by use of protective gloves. This study supports the indication that dermal absorption rates of captan are lower than previously believed.

TABLE 1

Estimate of Captan Exposure for Workers
Having Detectable Urinary THPI

Worker	Urine Volume Collected (ml)		Creatinine Detected (mg/dl)		THPI Detected (ppb)		Estimated Mean Daily Captan Exposure (ug)
	Day 3	Day 4	Day 3	Day 4	Day 3	Day 4	
5	409	116	169	276	67	ND	2,868
7	110	350	248	190	ND	178	5,245
14	325	350	191	184	ND	78	2,939
15	230	345	248	150	69	ND	3,416

TABLE 2

Estimate of Upper Bound of Captan Exposure for Workers
Having No Detectable Urinary THPI

Worker	Urine Volume Collected (ml)		Creatinine Detected (mg/dl)		Estimated Upper Bound Captan Exposure (ug/day)
	Day 3	Day 4	Day 3	Day 4	
1	330	300	102	114	845
2	80	157	119	73	1173
3	460	375	48	30	1259
4	375	194	155	194	451
8	295	390	193	162	343
9	500	310	115	126	417
10	48	400	199	179	1622
11	100	123	149	173	959
12	195	320	46	77	1481
13	145	210	105	122	1129
16	280	230	126	208	768

REFERENCES

- Bingham, Sheila A. and J.H. Cummings
The Use of Creatinine Output as a Check on the Completeness of 24-Hour
Urine Collections
Human Nutrition: Clinical Nutrition; Vol 39C; pp 343-353 (1985)
- California Department of Food and Agriculture
Captan Risk Characterization Document
September 28, 1987
- Fong, Harvard R. and Robert I. Krieger
Estimation of Exposure of Persons in California to Pesticide Products that
Contain Captan and Estimation of Effectiveness of Exposure Reduction
Measures
California Department of Food and Agriculture
HS-1468 (1990)
- Forbes, G. and G. Bruining
Urinary Creatinine Excretion and Lean Body Mass
American Journal of Clinical Nutrition
Vol 29 pp 1359-1366 (1976)
- Graham, Edward
Memorandum to Worker Health and Safety Staff
A Method to Index and Assess Compliance with Twenty-Four Hour Urine
Collections
June 14, 1988
- Maddy, Keith, R. Krieger, L. O'Connell, M. Bisbiglia, S. Margetich
Use of Biologic Monitoring Data from Pesticide Users in Making Pesticide
Regulatory Decisions in California. Study of Captan Exposure of
Strawberry Pickers
ACS Symposium Series 382, 194th meeting of the American Chemical Society,
New Orleans, Louisiana
August 30 - September 4, 1987
- Mull, Ronald
Guidelines for Conducting Mixer/Loader-Applicator Studies
Veterinary and Human Toxicology
Vol 28, No 4 pp 328-336 (1986)
- Sendroy, J. and L. Cecchini
Determination of Human Body Surface Area from Height and Weight
J. App. Physi 7:1-12 (1954)
- Schoen, Sarah and Wray Winterlin
Gas Chromatographic Determination of the Captan Metabolite
Tetrahydrophthalimide in Urine
J Assoc Offic Anal Chem 65:1382-1384 (1982)

Thongsinthusak, Tian and Robert Krieger
Pesticide Exposure Assessment
California Department of Food and Agriculture
HS-1509 (1989)

Thongsinthusak, Tian and Robert I. Krieger
Urine as a Biological Index for Captan Exposure in Humans
unpublished

United States Environmental Protection Agency
Captan: Intent to Cancel Registration
Federal Register Vol 54 No 36 pp 8116-8150 (1989)

Winterlin, Wray L, Wendell Kilgore, Charles Mourer and Sarah Schoen
Worker Reentry Studies for Captan Applied to Strawberries in California
Journal of Agricultural Food Chemistry
Vol 32, No 3, pp 664-672 (1984)

Winterlin, Wray L, Wendell Kilgore, Charles Mourer, Gregory Hall and David
Holdapp
Worker Reentry into Captan-treated Grape Fields in California
Archives of Environmental Contamination and Toxicology 15:301-311 (1986)

Appendix 1

VOLUNTEER CONSENT FORM

I understand that the California Department of Food and Agriculture (CDFA) is conducting a study of captan exposure at _____. I have volunteered for this study.

I also understand:

Urine samples will be collected.

I am participating in the study by my own free will. I am not being forced to cooperate by either CDFA or _____.

I am free to withdraw from the study at any time, without any penalty.

The purpose and procedures of the study have been explained to me. If I have any questions before, during or after the study, they will be answered by the study personnel.

The results of the study will be provided to me, when they are available.

x _____ x _____
Volunteer Name Date CDFA Witness Date

Appendix 2

CALIFORNIA DEPT. OF FOOD & AGRIC.
CHEMISTRY LABORATORY SERVICES
WORKER HEALTH & SAFETY SECTION
3292 Meadowview Road
Sacramento, CA 95832
(916)+427-4998/4999

Original Date:??
Supercedes: NEW
Current Date:8/25/87
Method #:

TETRAHYDROPTHALIMIDE

SCOPE:

This method is for the determination of tetrahydrothalimide (THPI) in urine samples.

PRINCIPLE:

THPI is extracted from urine with Methylene Chloride. The Methylene Chloride is then exchanged into Benzene. The sample is then transferred onto a Florisil sep pak and eluted with Acetone/Benzene. The eluant is cleaned up with first a basic extraction and then an acid extraction. The cleaned eluant is then extracted with Methylene Chloride and exchanged into Benzene for analysis by gas chromatography.

REAGENTS AND EQUIPMENT:

1. Methylene Chloride.
2. Benzene.
3. Acetone.
4. Separatory funnel, 250 ml capacity with teflon stopcock and glass stopper.
5. Boiling flask, 500 ml capacity.
6. Glass syringe.
7. Florisil sep paks, Waters.
8. Buffer solution, pH 11.
9. Concentrated Phosphoric acid.
10. A 10m x .52 mm i.d. fused silica column coated with 5% Phenyl Methyl Silicone.
11. A gas chromatograph equipped with Nitrogen/Phosphorous detector.

ANALYSIS:

1. Pipet a 25 ml aliquot of the urine sample into a 250 ml separatory funnel.
2. Extract the urine with 150 mls of Methylene Chloride, draining the solvent through glass wool and Na₂SO₄ into a 500 ml boiling flask.
3. Extract the urine again with another 150 mls of Methylene Chloride.
4. Extract the urine a third time with 50 mls of Methylene Chloride, combining all extracts in the same boiling flask.
5. Roto-evaporate the Methylene Chloride down to about 1 ml.
6. Add 5 mls of Benzene to the flask and roto-evaporate down to about 1 ml.
7. Repeat step 6.
8. Quantitatively transfer the Benzene to glass syringe equipped with a florisil sep pak that has been wetted with Benzene.

9. Rinse the sep pak with 5% Acetone/Benzene and discard.
10. Elute the THPI with 40% Acetone/Benzene, draining into a 250 ml sep funnel.
11. Add 20 mls of buffer solution (pH 11) to the sep funnel and shake.
12. Extract the solution twice with 50 mls of Methylene Chloride, discarding the Ch_2Cl_2 .
13. Add 5 mls of conc. Phosphoric acid to the sep funnel and shake.
14. Extract with 50 mls of Ch_2Cl_2 , draining through glass wool and Na_2SO_4 into a 500 ml boiling flask.
15. Extract twice more with 50 mls of Ch_2Cl_2 , combining all extracts in the boiling flask.
16. Evaporate the Ch_2Cl_2 down to about 1 ml.
17. Add 5 mls of Benzene to the flask and evaporate to about 1 ml.
18. Repeat step 17 again.
19. Quantitatively transfer the Benzene to a volumetric test tube and bring up to a volume of 5 mls.
20. Extract is ready for analysis by gas chromatography.

EQUIPMENT CONDITIONS:

1. Gas Chromatograph - Hewlett Packard 5880A.
 - a) Oven temperature:
 - 1) Initial value - 140 C.
 - 2) Initial time - 4 minutes.
 - 3) Program rate - 10 C/min.
 - 4) Final value - 210 C.
 - 5) Final time - 15 minutes.
 - b) Injector temperature - 250 C.
 - c) Detector temperature - 300 C.
 - d) Helium carrier gas flow - 15 mls/min.
 - e) Helium make-up gas flow - 5 mls/min.

Using these conditions, THPI has a retention time of 3.58 minutes.

CALCULATIONS:

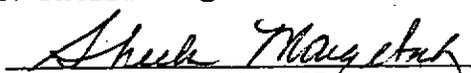
Results are reported as ppb THPI per sample.

DISCUSSION:

Be sure to evaluate this method with every change in lot number of the Florisil sep paks. It was this author's experience that recoveries varied between lot numbers of the Florisil sep paks.

Recoveries: 100 ug THPI - 90%
10 ug THPI - 85%

WRITTEN BY: Sheila Margetich


TITLE: Agricultural Chemist, II

Appendix 3

Raw Data Collected in Study of Grape Propagation Workers

Worker Number	Gender	Height (cm)	Weight (kg)	Gloves Used		Urine Volume (milliliters)		THPI Concen. (ppb)		Creatinine Concen. (mg/dl)	
				Day 3	Day 4	Day 3	Day 4	Day 3	Day 4	Day 3	Day 4
1	F	165	86	Yes	Yes	330	300	0	0	102	114
2	F	157	59	Yes	Yes	80	157	0	0	119	73
3	M	168	77	Yes	Yes	460	375	0	0	48	30
5	M	173	77	Yes	Yes	409	116	67	0	169	276
6	M	170	73	Yes	Yes	10	NS	*	*	*	*
7	M	175	82	No	No	110	350	0	178	248	190
8	M	170	77	Yes	Yes	295	390	0	0	193	162
9	M	170	77	Yes	Yes	500	310	0	0	115	126
10	M	173	82	Yes	Yes	48	400	0	0	199	179
11	F	163	68	Yes	Yes	100	123	0	0	149	173
12	F	170	68	Yes	Yes	195	320	0	0	46	77
13	M	170	77	No	No	145	210	0	0	105	122
14	M	178	95	Yes	Yes	325	350	0	78	191	184
15	M	175	82	No	No	230	345	69	0	248	150
16	M	175	100	No	No	280	230	0	0	126	208
17	M	163	68	Yes	Yes	115	NS	0	*	130	*

NS - no sample

* - insufficient sample for analysis