guideline levels of 2.0 and 1.0 mg/kg DON in uncleaned soft wheat intended for use in nonstaple foods would not pose a health hazard to adults or infants, respectively, assuming an estimated overall 40% reduction in DON levels upon manufacturing products from uncleaned soft wheat.

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Worker Reentry Studies for Captan Applied to Strawberries in California

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Captan was applied to strawberries in fields along the coastal region of central California to determine the level of exposure to residues by harvesting crews. Residues of captan and its metabolite, tetrahydrophthalimide (THPI), were determined as dislodgeable residues on foliage and fruit. Air samples were also collected and analyzed during the harvest operations. Field workers, including the applicator/loader/mixer, were monitored for exposure by determining the amount of residues adsorbed to patches attached to the workers' clothing and from the amount present on their gloves. Respirator pads worn by the applicator/loader/mixer were also analyzed. As a biological index, urine of the workers was examined for the presence of THPI. Though the applicator/loader/mixer was exposed to higher dermal levels of captan, no THPI could be detected in the urine while the urine of the pickers had detectable levels.

Captan [*N*-[(trichloromethyl)thio]-4-cyclohexene-1,2-dicarboximide] is a widely used foliar fungicide for the control of scabs, blotches, rots, mildew, and various other fungal diseases on fruits, vegetables, and ornamentals. It has been estimated that since 1978 over 8.5 million lb/year have been used in commerical agriculture. Because of the large quantity applied, the potential of exposure to captan residues has been a matter of concern to various state and federal regulatory agencies. Reports of worker dermatitis from spraying apples, allergic dermatitis in pickers, mutagenic effects in bacteria in human embryonic lung cells and in cell lines derived from the kidney of the kangaroo rat, teratogenic effects in developing chicken embryos, increased tumor incidence in mice, and gene-mutation studies in flies have led to a rebuttable presumption against captan (Environmental Protection Agency, 1980). Apparently those at the highest exposure risk are agricultural workers, who are pesticide applicators, mixers, or

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loaders, and individuals who enter treated fields soon after application. Some exposure studies with captan have been conducted (Oudibier et al., 1974; Hansen et al., 1978) but investigators did not include strawberries or grapes or crops that may receive several captan applications annually. Further, these studies did not include analytical methodology, dislodgeable residue data, or any urine analyses. A recent study by Zweig et al. (1983) reported on left- and right-hand dermal exposure of captan and benomyl to 10 strawberry harvesters. No urine analyses, index of exposure, or any environmental parameters were measured outside of foliage exposure.

The major objective of this study was to determine a typical maximum field level exposure to captan by workers through sampling their immediate environment and analyzing the samples for captan residues. In addition, we hoped to establish a possible index of exposure by measuring the THPI metabolite in urine. THPI was selected because of a study with radiolabeled captan (Environmental Protection Agency/Office of Pesticide Programs, 1975) that reported THPI to be a major metabolite of captan from orally administered rats.

EXPERIMENTAL SECTION

Application. Captan 50W was applied according to the label to strawberries in Santa Cruz County, CA, beginning in mid-April and extending through the growing season. The frequency of application was mostly dependent on weather conditions, but generally applications were made every 10–14 days. Sometimes late spring rains may require even more frequent applications, but this was not necessary while this study was being conducted.

The field was divided into several small 0.5–2-acre plots. For the purpose of this study, one plot was set aside for determining the degradation of captan, for taking air samples (before, during, and after spraying), and for monitoring workers on the third day following application. The large strawberry plot under investigation was sprayed May 5 and 20, 1981, and consisted of 20 double rows, approximately 190 ft long. Spacing between double rows, center to center, was 52 in. The plot was divided into four replicate plots, each plot consisting of five double rows.

All applications were made with a boom spray rig mounted on a tractor. The spray rig was calibrated for a captan application rate of 2.2 lb of AI $(150\ gal)^{-1}\ acre^{-1}$ [2.50 kg $(14.025\ hL^{-1})\ ha^{-1}$]. Samples of the captan formulation were taken from the spray rig tank at the time of application for analysis of purity and concentration.

Sampling. Leaf punch samples, 20 from the length of each of five rows, were taken from each of the replicated strawberry plots by using a 2.5-cm^2 punch (Gunther et al., 1973). Samples were collected on −1, 0, 1, 3, 6, 8, 10, 14, and 15 days postapplication. On day 15 there was a second application of captan just prior to the last sampling. Each sample consisted of 500 cm^2 of leaf tissue and included the surface area on both sides of the leaf. All samples after collection were placed immediately into ice chests and were kept chilled with ice until extracted 1–2 h later.

Fruit samples were collected from the plots at the same time leaf punch samples were taken. Approximately four berries were taken from each row, enough to give 280–370 g of sample.

Air samples were taken from the field when leaf punch and fruit samples were collected and where workers were being monitored. Two high-volume Staplex air samplers each containing a quartz fiber filter backed by 120 cm^3 of XAD-4 macroreticular resin were used. The air flow rate during operation was 0.623 $m^3\ min^{-1}$. The air samplers were operated for 1–h periods, but the quartz fiber filters were replaced every 15 min. A small personnel air sampler was attached to the driver/applicator. This particular sampler was a low-volume unit (Union Industrial Equipment Corp.) that had a 47-mm holder for holding a 40-mm quartz fiber filter backed with a plastic screen. The air flow was determined to be 28 L min^{-1}. It was attached to a 12-V DC vacuum pump mounted in the tractor. The filter holder was pinned to the individual and laid over the operator's right shoulder, being placed near the breathing zone. The filter was changed every 20 min during the application period.

The respirator worn by the applicator/loader/mixer was the half-mask type containing dual activated carbon (A-TX-2 Agri-Tox) cartridges. The cartidges consisted of a pesticide R15 respirator filter and an R-21 cartridge.

Estimation of dermal exposure was accomplished by attaching 4 in. × 4 in. muslin patches to the outside clothing. As is normal, the clothing worn by the workers varied considerably in regards to quantity and quality. Thus, no correlation was attempted for relating clothing exposure to amounts found on skin or excreted into the urine. The patches were used simply for determining the relative amounts of captan that workers were exposed to while working in the fields. Five locations were used: chest, back, inside forearms, thighs, and shins. The patches were worn by the field workers for approximately 4 h. Dermal exposure to the hands was measured by analyzing the residue on newly purchased gloves worn during the operation. The gloves were the same as those normally worn by the workers and were made of latex. The gloves were not prewashed prior to their use in the field.

Urine samples were collected from the applicator and field workers in 4.5-oz. disposable polypropylene prenumbered urine cups. The workers were given two containers each and were asked to collect their first sample upon arrival at their homes after work and a second sample the next morning. They were also instructed on proper sample collection to avoid contamination from hands and clothing. They were instructed to freeze the samples immediately following collection and to keep them frozen until they could be delivered to the work site. The samples were transferred to a dry ice chest and later to a walk-in freezer where they were kept frozen (−29 °C) until analyzed.

Extraction and Cleanup. *Leaf Disks.* A modified extraction and cleanup procedure described by Gunther et al. (1973) was used. Briefly, each sample contained in a pretared 8-oz. jar was shaken 3 times with 75, 50, and 25 mL of water containing a small quantity of surfactant. To the combined washes was added 15 mL of saturated sodium sulfate followed by extracting with 75, 50, and 50 mL, respectively, of chloroform. The chloroform extracts were combined and evaporated to near dryness. Five milliliters of hexane was added to each sample, and again the extract was evaporated to near dryness. The process was conducted a second time, or until all traces of chloroform were gone. The residue remaining in the flask was dissolved in 5 mL of hexane in preparation for cleanup by column chromatography. The column, a 12 × 100 mm glass column with a 200-mL reservoir, contained layers of 0.5 cm of anhydrous sodium sulfate, 8 cm of Florisil, and 0.5 cm of anhydrous sodium sulfate, with a glass wool plug placed in the bottom. After each addition to the column, the contents were tapped to achieve proper settling. After prewashing the columns with 25 mL of hexane, the extract in 5 mL of hexane was transferred to the column along with two 5-mL rinses of the flask. Twenty milliliters of hexane was added to the column, collected, and discarded. The column was then eluted with 125 mL of an ethyl

acetate–hexane (1:20) mixture. This latter fraction contained the captan residue and was collected in a boiling flask. The THPI was eluted from the column with 50 mL of ethyl acetate and was collected in another boiling flask. The contents in the flasks were evaporated to the desired volume in preparation for analysis by gas chromatography.

Fruit. Strawberries were so different in shape and size that it was impractical to determine the surface area, so all studies were conducted on a weight basis and results were calculated in ppm. The dislodgeable residues on the fruit were removed by sloshing the fruit in a quart jar 3 times with 400, 200, and 200 mL, respectively, with the surfactant solution. The aqueous solutions were transferred to a separatory funnel containing 15 mL of saturated sodium sulfate and extracted 3 times each with 75, 75, and 50 mL of chloroform. The combined chloroform extract was evaporated to near dryness, transferred into hexane, and cleaned up as described for leaf disk samples. Following removal of the dislodgeable residues, the fruit was immediately frozen with dry ice and stored at −20 °C until extracted and analyzed for penetrated residues.

Briefly, the penetrated residues were extracted and analyzed as follows. The frozen fruit was chopped and mixed with dry ice in a Hobart food chopper. The homogeneous mixture (50 g) was transferred to a blender and blended for 1 min with 100 cm^3 of sodium sulfate and 300 mL of ethyl acetate. The contents were transferred to an Erlenmeyer flask along with two subsequent rinses of 50 mL of ethyl acetate each. The contents were shaken for 1 h on a mechanical shaker, filtered through sodium sulfate, and then analyzed after column chromatographic fractionation as decribed previously for dislodgeable residues.

Air Samples. Only the quartz fiber filters were analyzed, although both the filter and resins were collected and kept in cold storage. A prefield laboratory study with captan and THPI sprayed directly into a high-volume Staplex sampler showed that 99% of captan and THPI were contained on the filter. Results from this study did show that the filter on the high-volume sampler should be changed every 15 min due to a significant loss of captan with time. Additional testing in the field also showed that no detectable captan or THPI was found in the resin but that all of the residues were contained on the quartz filter. Captan and THPI were extracted from the quartz fiber filters by blending the entire filter in a blender equipped with a Polytron blade and with 300 mL of ethyl acetate for 1 min. The contents were filtered and the blender and filter washed 4 times each with 50 mL of ethyl acetate and combined with the 300 mL of extract. The contents were evaporated to near dryness followed by the addition of 5 mL of hexane and again evaporated to near dryness. This process was repeated until all traces of ethyl acetate were gone. The sample was then cleaned up by column chromatography as described previously for leaf disks.

Patches. The cloth patches removed from the workers were extracted with 75 mL of ethyl acetate in the 4-oz bottles by shaking on a mechanical shaker for 20 min. The solvent was collected by decanting and filtration. The patches were extracted a second time by shaking again with 50 mL of ethyl acetate for 30 s. The filtrate was combined, and the 4-oz bottle rinsed two times with 10 mL aliquots of ethyl acetate. The extract was then processed as described above for cleanup and column chromatography.

Rubber Gloves. The outside surface of each pair of gloves first was rinsed with 300 mL of ethyl acetate and then rinsed 4 times more with 25-mL aliquots of ethyl acetate. The extract was then processed as described above for cleanup and column chromatography.

Respirator Filter Pads. The pads were extracted in a Soxhlet extractor for 18 h with 650 mL of ethyl acetate. After cooling, the ethyl acetate was filtered and the remaining residue rinsed twice more with 25-mL aliquots of ethyl acetate. The filtrates were combined for subsequent analyses. The extract was then processed as described above for cleanup and column chromatography.

Formulations. Tank solutions of the captan spray were taken during application to determine the actual captan levels. To facilitate extraction the tank samples were poured into a beaker and stirred with a stirring bar. While the contents were being stirred, 2 mL of the solution was transferred to a separatory funnel containing 25 mL of water. The solution was extracted 3 times with 75 mL of ethyl acetate. The extracts were combined and filtered. The filter was then washed twice with a 50-mL aliquot of ethyl acetate. All filtrates were combined and evaporated to near dryness. The samples were analyzed after column chromatographic fractionation as described previously for dislodgeable residues.

Urine. Urine samples were extracted according to the method of Schoen and Winterlin (1982). Briefly, a 25-mL urine sample was extracted 3 times with 20 mL of chloroform. The chloroform phase was filtered through anhydrous sodium sulfate and evaporated to near dryness over several 5-mL portions of benzene until no detectable chloroform was present. The sample was cleaned up on a 12 mm × 100 mm column containing 9.5 mL of Florisil. After the column was wetted with 25 mL of benzene, the sample was transferred to the column with three 10-mL portions of benzene. The column was then washed with 125 mL of 5% diethyl ether in benzene, and the resultant solvents were discarded. The THPI was eluted from the column with 75 mL of 8% (v/v) acetone in benzene. Following evaporation of the solvent to near dryness, the sample was transferred in 6 mL of ethyl acetate to a sedimentation tube and reduced in volume to 1 mL in preparation for gas chromatography.

Analysis. Captan was analyzed by gas chromatography using a Tracor 550 gas chromatograph equipped with a flame photometric detector and a 180 cm by 2 mm i.d. glass column packed with 12% SE-30 coated onto 80–100-mesh Gas-Chrom Q. Operating conditions were 210 °C for the column oven. The carrier gas (nitrogen) flow rate was 65 mL/min. The detector was operated in the sulfur mode with a 394-nm filter.

THPI was analyzed on a Model 5710A Hewlett-Packard gas chromatograph equipped with a N/P ionization detector. The 180 cm by 2 mm i.d. glass column was packed with 130 cm of 4.5% OV-225 coated on 80–100-mesh Gas-Chrom Q followed by 50 cm of 4% OV-101 on Gas-Chrom Q. The column was operated at 200 °C with a helium carrier gas flow of 30 mL/min. The detector was operated at 300 °C. THPI had a retention time of approximately 2.1 min and captan 2.9 min.

Method Verification. *Dislodgeable Residues.* The procedure normally used by this laboratory to verify an analytical technique is to fortify punched leaf tissue, free of pesticides with a solution of formulated wettable powder. After the carrier solvent (H_2O) is allowed to evaporate at ambient temperature, the tissue is placed in a shaking apparatus with the stripping solvent and processed according to the method generally used for dislodgeable residues (Gunther et al. 1973). However, when the samples were analyzed by using this procedure, the recovery from the leaves was very low (Table I). After much study, it

Table I. Recovery of Captan from Fortified Strawberry Leaves

			leaves			glass slides		
			amt added,	amt recovered		amt added,	glass slides	
sample	solvent	solvent evaporation	μg/100 μL	μg	%	μg/100 μL	μg	%
I	water	yes	10	1.9	19	10	1.11	11.0
II	water	yes	1000	142.5	14	10	0.97	9.7
III	water	yes	1000	172.5	17	10	1.07	11.0
IV	water	yes	10	2.1	21			
V	water	yes	10	2.0	20			
VI	water	no	10	9.1	91			
VII	OT-75 solution	no	10	8.9	89			
VIII	ethyl acetate	no	1000	1000	100	853	837	98

Table II. Captan and THPI Recovered from Leaf Punches following Mixing in an Erlenmeyer Flask

	dislodgeable		penetrated		flask			
	amt found,	% of	amt found,	% of	amt found,	% of	total	
sample	mg	total	mg	total	mg	total	mg	%
				Captan				
I	22.4	44.8	0.21	0.43	24.7	49.5	47.4	94.6
II	27.7	55.3	0.39	0.71	27.3	54.5	55.3	110.6
III	21.1	43.3	0.42	1.02	19.3	38.5	40.8	81.6
av	23.7	47.4	0.34	0.68	23.8	47.5	47.8	95.6
				THPI				
I	5.6	11.2	0.04	0.08	32.0	64.0	37.6	75.3
II	8.4	11.8	0.06	0.12	36.3	72.6	44.8	89.5
III	4.5	9.0	0.04	0.08	27.5	55.0	32.0	64.1
av	6.2	12.3	0.05	0.09	31.9	63.9	38.1	76.3

was discovered that this loss took place during the evaporation of the H_2O carrier solvent. To support these findings, the following studies were conducted. An aqueous solution was placed on glass slides, evaporated just to dryness, and analyzed, resulting in very low recovery of the captan. Loss of captan was then compared when the standard was dissolved in ethyl acetate and fortified directly into the OT-75 stripping solution and into the water. The results showed good recovery in all three cases, which suggested the loss of captan upon evaporation may have been due to codistillation. Dissolving of the captan in ethyl acetate and evaporating the solvent on a glass slide also resulted in good recovery. What was true for captan was not for THPI, however, as THPI recoveries were greater than 90% when fortified in the solvent. For purposes of this study, the method for fortification was modified. In this instance the sample, 25 punches (2.5 cm^2) from strawberry leaves were placed in a 125-mL Erlenmeyer flask followed by 100 mg of captan 50 W.P. and 50 mg of THPI. The flask was hand shaken for about 1 min. The punches were transferred to a 4-oz glass jar and extracted 3 times with the OT-75 stripping solution. The aqueous solution was then extracted 3 times with chloroform and and analyzed as described previously. The remaining residue in the Erlenmeyer flask used for shaking the formulated powder and THPI was dissolved in ethyl acetate and analyzed. The total residue recovered was calculated by adding the amount found in the flask plus the amount on the leaves. The percent recovered was then calculated by dividing the total residue by the amount added (100 mg) and multiplying by 100. Penetrated residues were measured for the recovery study but were not measured on field samples. This was accomplished by extracting the remaining stripped foliage sample with chloroform with a Polytron homogenizor. The combined recovery of captan (Table II) from dislodgeable and penetrated residue and residue remaining in the flask ranged between 82 and 111% with a combined average of 96%. The combined recovery of THPI was lower, ranging between 64 and 90% with a combined average of 76%. Penetrated residues never exceeded 1% and for practical purposes were not considered necessary for the field portion of this study. Additional recovery studies were also conducted by adding captan and THPI in an aqueous solution to 5 g of leaf tissues followed by extraction with the stripping solvent. Samples fortified at 1.0 and 100 ppm of captan resulted in recoveries ranging between 90 and 100%. Samples fortified at 0.1 and 10 ppm of THPI resulted in recoveries ranging between 82 and 100%.

Fruit. Strawberries were fortified with captan at 2.5 and 25 ppm since this was the range that we expected to find residues on the fruit. It was not possible to predict THPI levels so two levels were used, 0.2 and 1.0 ppm. Dislodgeable residue recoveries ranged from 82 to 103% for captan and 90 to 96% for THPI. As was true for foliage residues, penetrated residues were negligible.

Patches, Gloves, and Quartz Fiber Filter. Recovery of captan on the cloth patches (104 cm^2, each treated with 100 μg of captan in 100 μL of ethyl acetate) averaged 104%. Gloves were also treated with 5 mg of captan and 0.5 mg of THPI. Recovery was 95% for captan and 87% for THPI. Quartz fiber filter paper used in the air sampling was fortified with 50 μg of captan and 5 μg of THPI. The average recovery of captan was 99% and for THPI the average was 110%.

Staplex Air Samples. Staplex high-volume air samplers were set up in the laboratory for studying the possible movement or loss of captan and THPI during the sampling process. When the quartz fiber filter was fortified with a standard dissolved in ethyl acetate and placed on the sampler, it was found that the materials were collected in the resin as well as the filter. After 15 min, 14% of the captan was found in the resin. This increased to 24% during a 60-min run. The amount of captan on the filter also decreased, with the total loss amounting to 30% after 15 min. After 60 min the loss was even greater and was

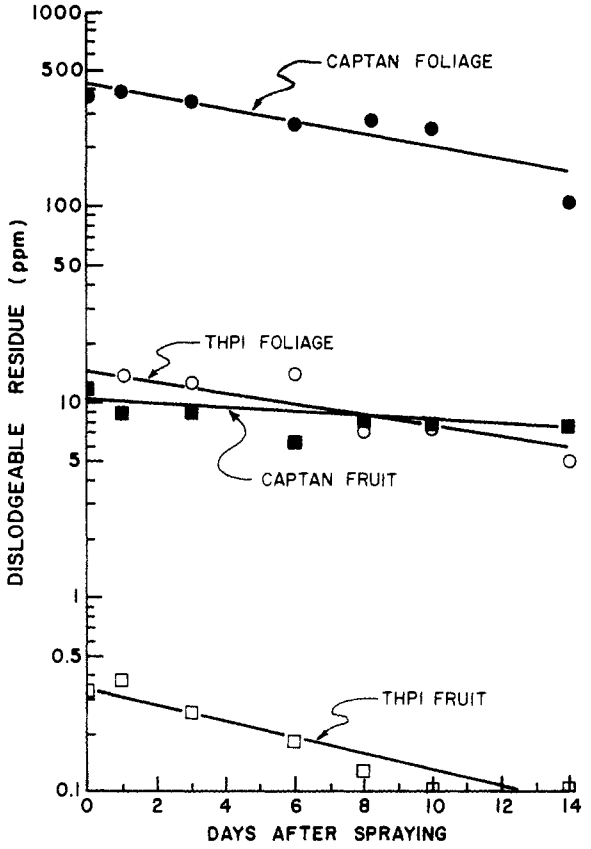

Figure 1. Total dislodgeable residues of captan and THPI on strawberry plants following application of 2.2 lb of AI (150 gal)$^{-1}$ acre^{-1} [(●) captan and (○) THPI on foliage; (■) captan and (□) THPI on fruit]. Application was made on May 8, 1981, along the central coastal area of California.

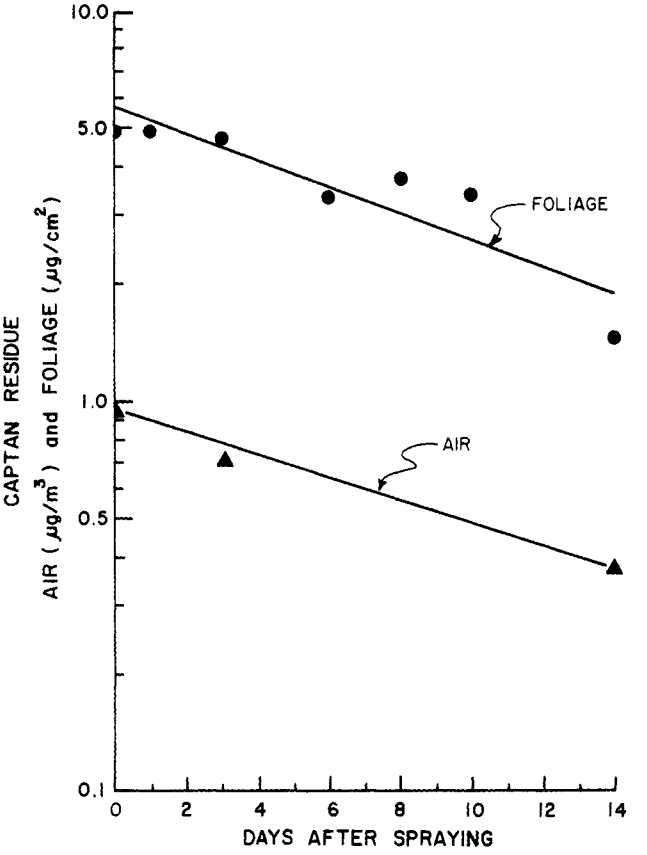

Figure 2. Captan residue (μg/cm^2) extracted from the surface of strawberry foliage (●) and from the air (▲) with Staplex high-volume air samplers following application of 2.2 lb of AI (150 gal)$^{-1}$ acre^{-1}.

as much as 70%. When samples were fortified with an aqueous solution of captan, followed by drying at ambient temperatures, and the sample run for 15-min periods, the captan recovered was all in the filter. Recoveries were low (<50%), which could have been due to codistillation as mentioned above. However, when the results were compared with those obtained with a filter that had been fortified, subjected to evaporation, and immediately extracted, the recoveries were quite good, averaging 83%.

THPI behaved somewhat differently during the air sampling process. In contrast to captan, the THPI did not appear to migrate into the resin and recoveries averaged 92% (extracted from the filter after bein placed on the air sampler for 25 min). From these studies, it was decided that every 15 min the quartz fiber filter should be changed; however, there would be no need for analyzing the resin.

Urine. Recovery studies for THPI in urine have been reported by Schoen and Winterlin (1982) and averaged from 82 to 87% at fortification levels of 0.03 to 0.05 ppm, respectively.

RESULTS AND DISCUSSION

THPI is a major metabolite of captan (Environmental Protection Agency/Office of Pesticide Programs, 1975). Thus, THPI was selected as an important biological entity for captan exposure. Other parameters used in this study were captan residues in air samples, on foliage, on patches attached to workers clothing, and on workers' gloves.

Formulation Analysis. For the purpose of determining the actual amount of captan applied to strawberries, each spray tank solution was sampled and analyzed. Each application was supposed to have been a mixture of 4 lb/125 gal 50% W.P. applied at a rate of 150 gal acre^{-1}. However, actual analysis from 10 tank samples showed an average of 1.86 lb of AI/125 gal, with a standard deviation of 0.27 lb. The amount of THPI present was determined in all samples. The THPI levels in the tank solutions were found to be low, averaging 0.04 lb of AI/125 gal, which represented approximately 2% of the captan in the formulation.

Dislodgeable Residues. Figures 1 and 2 show the decay of captan and THPI on strawberry foliage and fruit during a 14-day growing period. Since captan spray had been applied to these plants prior to this study there was an average background residue of 0.90 μg of captan/cm^2 and 0.03 μg of THPI/cm^2. This resulted in a net value of 4.0 μg/cm^2 on day 0. Fruit residues are expressed here in ppm as the strawberries were not uniform in size and shape, which made it impossible to express the results in terms of surface area. Total accumulated average dislodgeable captan residue from fruit was 11.85 ppm on day 0. Preapplication residue for this study was 5.45 ppm, resulting in a net application residue of 6.40 ppm. THPI residues were approximately 2–3% of the captan residues. Dissipation of captan on foliage was $y = 5.63e^{-0.0277t}$ with a correlation coefficient (r) of 0.75 and half-life ($t_{1/2}$) of 9.0 days.

Samples were also collected after the second spraying (day 15) for purposes of determining buildup of captan residues. The results from this portion of the study were somewhat surprising as the accumulated captan residues on the foliage following application resulted in average dislodgeable residue of 3.56 μg/cm^2 while the net amount was 2.12 μg/cm^2. This was lower than the 4.90 μg/cm^2 that resulted in a net amount of 4.06 μg/cm^2 following the first application. The analysis of the tank solutions showed the applicator had applied 40% less captan and, therefore, the results were within 10% of theoretical. Ripe fruit was

Table III. Application Rate and Ratio of THPI/Captan in the Formulation and Foliage Applied to Strawberries on Day 0

date of application	captan in formulation, lb/gal	rate of application AI/acre	ratio of THPI/captan in formulation	ratio of THPI/captan on foliage
5/8/81	0.0149 ± 0.002	2.235	0.022	0.032
5/20/81	0.0090 ± 0.002	1.350	0.022	0.034

Table IV. Captan and THPI in Air from Application to Strawberries

crop	date	days from application	captan, $\mu g/m^3$	THPI, $\mu g/m^3$	THPI/captan
strawberries	5/5/81	−1	0.13	0.03	0.24
	5/6/81	0 (2 h)	1.02	0.23	0.22
		0 (7 h)	0.89	0.03	0.03
	5/8/81	3	0.72	0.03	0.08
	5/20/81	14	0.38	0.02	0.06
	5/21/81	0 (2 h)	0.86	0.07	0.08

harvested every 5–7 days and prior to the last application, so no residue buildup could be determined.

None of the fruit samples analyzed exceeded the tolerance level of 25 ppm, and the highest amount detected was 15 ppm, with most of the samples having much less residue. Fruit was $y = 9.34e^{-0.0277t}$ with a $r = 0.22$ and a $t_{1/2}$ of 25.1 days. Captan, therefore, appears to be very stable on the fruit even during the rapid growth period, as shown in Figure 1. Some of this residue, therefore, must be attributed to contamination from the foilage and ground dust. THPI residues also decayed slowly. The ratio of THPI to captan was constant with a mean value of 0.0363 (standard deviation of 0.0094) during the 14-day postapplication period. These low THPI levels would probably have very little influence on the amount found in the urine, even if workers were exposed to much higher levels of captan.

How the amount of THPI applied from the formulation compares with the relative amount found on the foliage is shown in Table III. The wettable powder formulation gave rise to 3.2–3.3% THPI on the foliage within 2 h following application. Thus, the relative amount of THPI, though slightly higher on the foliage, was within about 1% of the actual amount applied. This would also be in close agreement with the fruit data where 3.6% THPI was found. From a practical standpoint these data indicate that the amount of THPI found on the foliage from the direct application of captan is proportional to the amount of THPI in the formulation.

Air Monitoring. High-volume air samples were taken between the rows of the strawberry plot with the intake of the air sampler facing upwards, at a height just slightly above the top of the plants, or about 5 cm. The air samplers were operated for a total of 1 h and the four 15 min/sample filters combined for each analysis. The air samplers were run in duplicate and the levels found were averaged. Table IV shows the level of captan and THPI found from each application.

High ambient temperature can be a major factor in the volatility of a pesticide, although this particular study was carried out in a consistently cool region along the coast of central California. The temperature during the day 0 morning sampling (2 h) ranged from 50 to 55 °F (10 to 13 °C), while at the second sampling, taken 5 h later, the temperature was still only 55 °F (13 °C). Thus, the decay, from 1.02 to 0.89 $\mu g/m^3$, most likely represents normal volatility irrespective of temperature change. Records were not kept regarding daily wind velocities. Thus, it is not possible to determine if the wind contributed to the dissipation of captan.

Residues of captan in the air did decline according to first-order kinetics (Figure 2), which was very similar to the decline curve for the foliage. THPI to captan ratios were different in the 2-h postapplication air samples (Table IV) from the formulation and foliage samples. However, the 7-h postapplication samples had the same ratio as the formulation and foliage. This variation in the 2-h sample may be due to several factors such as photochemical conversion or degradation in combination with temperature and humidity and a proportionate differential in desorption of THPI and captan at those levels from the foliage. Another explanation is that the foliage and fruit samples were not actually collected until about 4-h postapplication so that the leaves and fruit had time to dry. Before extraction, another 2–4 h elapsed while these samples were stored on ice. Therefore, the ratio of THPI to captan on the foliage and fruit could be more equivalent to the 7-h air sampling rather than the 2-h sample. It would appear from these data that the formation and loss of THPI, though relatively low in concentration, could be very complex in the vapor state and/or mobile particulate state.

An additional set of high-volume air samples was also taken for purposes of studying the influence of air sampler height following application to strawberries. One set of high-volume samplers was operated as mentioned previously at slightly above the plant level while a second set was operated 1 m above the plants. The results from this study are shown with the "low" column referring to air samples just above the plants and the "high" column 1 m above the plants in Table V. Not all the air samples were taken in the same field, although the amount of captan applied was the same. For purposes of this study it was the differing amounts found between the two sample heights that was of interest. From these data it is apparent that about 5 times as much captan is at the plant level as is present 1 m above the plants. The level of THPI was higher at plant height but only 2.5 times as much. This may suggest that THPI is more volatile than captan or is

Table V. Influence of Sample Height to Levels Found in High-Volume Air Samplers

		captan, $\mu g/m^3$			THPI, $\mu g/m^3$			THPI/captan		
days	field	low	high	low/high	low	high	low/high	low	high	low/high
1	A	0.340	0.061	5.6	0.032	0.021	1.5	0.094	0.344	0.27
3	A	0.521	0.158	3.3	0.035	0.013	2.7	0.067	0.082	0.82
	C	0.917	0.195	4.7	0.029	0.011	2.6	0.032	0.056	0.57
14	B	0.380	0.048	7.9	0.021	0.016	1.3	0.055	0.333	0.17
15 (0 h)	A	0.735	0.147	5.0	0.088	0.029	3.0	0.120	0.197	0.61
15 (2 h)	B	0.864	0.259	3.3	0.070	0.029	2.4	0.081	0.112	0.72
$\bar{X}$		0.626	0.145	5.0	0.045	0.020	2.25	0.075	0.187	0.53
SD		0.248	0.080	1.7	0.027	0.008	0.69	0.031	0.126	0.25

Table VI. Captan and THPI in the Air at Low Elevations and on the Foliage following Subsequent Applications to Strawberries

	captan				THPI			
	air, $\mu g/m^3$		foliage, $\mu g/cm^2$		air, $\mu g/m^3$		foliage, $\mu g/cm^2$	
day	total	net	total	net	total	net	total	net
−1	0.126		0.902		0.030			
0 (2 h)	1.024	0.898	4.961	4.059	0.228	0.198	0.1600	0.1283
0 (7 h)	0.890	0.764			0.027	<0.005	0.1654	
3	0.445		4.7865		0.034	<0.005	0.1654	
14/−1	0.380		1.4368		0.021	<0.005	0.0689	
15/0 (2 h)	0.864	0.484	3.5605	2.124	0.070	0.049	0.1400	0.0711

Table VII. Residue Found on Patches and Gloves Attached to Applicators Clothing during a Two-Hour Exposure Period

sample area	captan residue, $\mu g/cm^2$	THPI residues, $\mu g/cm^2$	THPI/captan
chest	1.599	0.040	0.025
sleeve	2.214	0.050	0.022
back	1.468	0.028	0.019
thigh	4.423	0.057	0.013
shin	1.827	0.038	0.021
glove	1.513	0.005	0.003
$\bar{X}$	2.174	0.036	0.017

Table VIII. Applicator Exposure to Captan and THPI

	av of total patches, $\mu g/cm^2$	respirator pads, total μg	low-volume air sampler, $\mu g/m^3$	urine, ppm
captan	2.306	9.27	16.6	
THPI	0.043	0.103	0.46	<0.03
THPI/captan	0.019	0.011	0.027	

Table IX. Residue Found on Patches and Gloves Attached to Workers' Clothing during a Four-Hour Exposure Period

sample area	captan residue		THPI residue		THPI/captan
	$\mu g/cm^2$	SD	$\mu g/cm^2$	SD	
chest	0.837	0.612	0.0108	0.0087	0.013
sleeve	5.909	3.841	0.0772	0.0505	0.013
back	0.624	0.199	0.0067	0.0021	0.011
thigh	3.708	3.733	0.0525	0.0502	0.014
shin	1.936	0.820	0.0320	0.0237	0.016
glove	2.198	0.596	0.0129	0.0032	0.006
$\bar{X}$	2.535		0.0320		0.012

less rapidly degraded in the vapor state. Thus, the work habits of the worker (kneeling or stooping) would determine to a large degree how much captan or THPI would be available to the respiratory system. If the respiratory system was a major route for captan exposure, the varying amounts of residues that could be absorbed from air would account for a large variation in the amount of residues there might be in the urine.

In Table VI the amounts of captan and THPI collected from the air are compared with the dislodgeable residues on the foliage. The rate of decay on the foliage during the 14-day period compared quite well with the rate of decay in the air. By use of the linear regression, r^2, captan in air = 0.1106 + 0.1461 (captan on foliage) and r^2 = 0.556. THPI in air = 0.0202 + 0.6877 (THPI on foliage) and r^2 = 0.105. Therefore correlations are reasonable for captan but not necessarily for THPI. The THPI levels found in the air were very low, yet detectable, after the initial samping and were at or below the background level of 0.030 $\mu g/m^3$ just 7 h after the application. Total THPI levels in the air after the initial sampling ranged between 0.021 and 0.034 $\mu g/m^3$.

The levels of captan on the foliage and in the air also compared well following the second application. There was 46% less captan on the foliage and 48% less in the air. This indicates that the volatility of captan is quite consistent and is relative to the levels found on the foliage.

Worker Exposure. Exposure of the applicator was monitored by means of patches on the clothing, by respirator pads, by low-volume air samples and by urine samples. The urine samples were collected by the applicator immediately upon returning home from work (approximately 2–4 h after applying captan most of the day) and again upon awakening the following morning. It should be pointed out that the applicator/loader/mixer was the same person who not only applied captan to our experimental plot but also applied captan to the other 35 acres of strawberries on the farm. This operation usually took several days for a single application. It also should be pointed out that the most obvious difference in the exposure to captan between the applicator and other workers was the respirator worn by the applicator during his applications. Results from the applicator's exposure to captan and THPI are shown in Tables VII and VIII. Residues found on the patches, in the respirator pads, and in the low volume air sampler were 2.17 $\mu g/cm^2$, 9.27 μg, and 23.9 $\mu g/m^3$, respectively. There was no detectable residue in the urine of the applicator.

The level of exposure of the 12 workers varied substantially. Average residues found in each patch location are shown in Table IX. The highest residues were found on the patches attached to the sleeves, followed by the thighs and gloves. The greatest variation between workers was found on patches attached to the thighs, chest, and sleeves. This is not too surprising when one observes the variation in the methods of picking by the individual workers. Some workers kneel along the ditch between the rows and reach over into the strawberry plants, thereby exposing their sleeves and thighs as well as chests to the foliage. Others do not kneel but bend over the strawberry plants and expose their thighs, sleeves, and chests to much less foliage and ultimately less captan. Since they all used gloves, one would expect only a minor variation in hand exposure between workers, and such was the case. The ratio of THPI/captan was very consistent, and as shown in Table IX, the average ratios between the patches only varied from 0.011 to 0.016. Gloves were slightly lower with a ratio of THPI/captan of 0.006. The average captan residue from all the patches and gloves of the workers was 2.54 $\mu g/cm^2$. This was about the same as that found on the applicator, which was 2.17 $\mu g/cm^2$. The greatest difference between the applicator and field worker was the duration of exposure. The applicator was only monitored for a 2-h period while the patches taken from the field workers were exposed for a period of 4 h. Obviously, on an hourly basis, the amount for a unit of time was less with field workers. When considering total exposure for an 8-h workday, one would need to determine exposure on an

Table X. Estimated Worker Exposure over an Eight-Hour Working Day[a]

	dermal exposure, mg person^{-1}					respirator	
	chest	sleeve	back	thigh	shin	total	mg person^{-1}
applicator/loader/mixer	9.824	8.000	9.016	61.14	18.94	106.92	3.708
field harvester 3 days postapplication	2.568	15.20	1.92	25.63	10.04	55.35	

[a] On the basis of the 50% man and the proportional surface allocation (Popendorf and Leffingwell, 1982).

Table XI. Captan Levels Found in the Air Sampler Mounted to the Tractor during Application

	tractor-mounted air sampler						field air sampler, total estimated conen for 8 h, μg			
	captan		THPI		total estimated conen for 8 h, μg		captan		THPI	
trial no.	μg/m^3	SD	μg/m^3	SD	captan	THPI	day 0	day 3	day 0	day 3
1	23.9	11.7	0.56	0.37	114	2.7	4.3	2.1	0.14	0.14
2	9.275	4.84	0.33	0.19	45	1.6				

Table XII. Average Worker Exposure to Captan following Application

	days post-application	sample time, h	av captan on patches, μg/cm^2 total	av captan on patches, μg/cm^2 8 h	captan in air, μg/m^3	THPI in urine, ppm
applicator/mixer/loader	0	1.5	1.68	6.72	a	(a)[b] <0.03
						(b)[b] <0.03
	0	2	2.31	9.24	a	(a) <0.03
						(b) <0.03
harvesters	3	4	2.60	5.20	0.72	(a) 0.058
						(b) 0.066

[a] The applicator/loader/mixer wore a respirator and therefore it is assumed that no captan was breathed into the respiratory system. [b] (a) Sample given following returning home in the afternoon. (b) Sample given early the next morning.

hourly basis and multiply this factor by the hours worked. Since the applicator/loader/mixer in this study worked at that task for an 8-h period, this means he is exposed to somewhat higher levels (Table X). The levels found on the sleeves of the workers were very similar to the levels reported to have been found on the forearms of workers exposed to captan on strawberries in a recent study by Zweig et al. (1983). This latter study did not include patches taken from other locations on the workers.

The distribution of captan residues on the applicator/loader/mixer was also different from that on the field workers. With the exception of the thighs, captan was fairly well distributed evenly on the applicator/loader/mixer. With the pickers, the distribution of captan varied a great deal, depending on the location of the patches and the worker's picking habits. This was most likely due to the way the workers came in contact with the captan, which was primarily by physical contact, while the applicator was exposed by vapor, finely disbursed water droplets, and particles. The respirator worn by the applicator contained relatively high levels of residues in the pads. Therefore, if a worker inhaled this residue without a respirator, the amount of exposure for an 8-h day would be high. Since none of the other field workers were furnished respirators, it is difficult to know exactly how much they took into their respiratory system. However, if we calculate the amount found in the air from the air sampler attached to the tractor and compare this with the amount found in the air samples taken in the field, one can estimate the difference between the two types of exposure. Table XI shows the levels found in the tractor-mounted air sampler, which was run for 30-min periods during application and for two different trials. The first trial was conducted during the morning and the second during the afternoon. The levels found varied considerably and may have been due to variations in wind direction with respect to the application. The estimated breathing rate varies a great deal among investigators, but if we assume an average individual inhaled at a rate equivalent to 10.0 L/min (Green and Lane, 1964), the amount of residue available to the respiratory system would have been from 45 to 114 μg for the applicator during an 8-h exposure period. The workers harvesting strawberries 3 days postapplication would be exposed to much less (2.1–4.3 μg).

Table XII summarizes the resultant difference from exposure to captan between the applicator/loader/mixer and the field workers harvesting strawberries. If one assumes the respirator eliminated respiratory exposure to captan, then it is likely the THPI found in the urine of field workers, not protected by respirators, is indicative of inhalation exposure. These data tend to support the importance of measuring THPI in urine as an index to exposure to captan. Dermal exposure to captan may not have been the primary route of exposure, as is true with other pesticides (Wolfe et al., 1967, 1972; Durham and Wolfe, 1962; Durham et al., 1972). However, since this study only included one applicator, who was monitored only twice, additional studies need to be conducted with field workers and harvestors to verify this assumption.

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Phosphorus and Phytate Content of Soybean Protein Components

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This study was conducted to determine the P and phytate contents of the major soy protein fractions prepared from defatted Bragg soybeans and commercial defatted soy flakes. The 11S, 7S, and soy whey precipitate fractions from defatted Bragg soybeans and commercial defatted soy flakes contained 0.08%, 0.63–0.68%, and 10.49–15.20% P (dry basis), respectively. These same fractions from commercial defatted soy flakes contained 0.07%, 1.40%, and 45.37% phytate (dry basis), respectively. It was concluded that most of the P of 11S protein is non-phytate, whereas phytate accounts for a major portion of the P content of 7S and soy whey precipitate fractions.

Phytate, which is the hexaphosphate salt of myoinositol (Erdman, 1979), is the principal storage form of P in the soybean (Okubo et al., 1975), accounting for about 70% of its total P content. Phytate exists in the soybean in association with various mineral ions and is soluble in mildly alkaline soy extracts but becomes strongly associated with the proteins in the extract, especially at alkaline pH in the presence of divalent cations. Phytate coprecipitates with the proteins in the soy extract under acid conditions, such as those used to produce commercial soy protein isolates, resulting in final phytate concentrations of 2–3% (Brooks and Morr, 1982; Hartman, 1979). The relatively high concentration of phytate in commercial soy protein isolates may affect the bioavailability of the proteins, per se, as well as that of zinc, iron, and other trace minerals in the diet (Erdman, 1979; Cheryan, 1980; Hartman, 1979; Maddaiah et al., 1964; Okubo et al., 1975). Phytate has the potential for adversely affecting the solubility and related functional properties of soy proteins in commercial food product applications. And, finally, phytate, because of its strongly anionic nature and tendency to bind to soy proteins, may interfere with their fractionation and characterization in terms of determining molecular weight, subunit content and size, electrophoretic mobility, and other important physicochemical properties.

Thanh and Shibasaki (1976a) developed an effective procedure for fractionating the major storage soybean proteins, i.e., mainly 7S and 11S components (Damodaran and Kinsella, 1982; Gayler and Sykes, 1981; German et al., 1982; Meinke et al., 1981; Utsumi et al., 1981). However, little if any attention has been given to the possible interference of phytate ions in any of this work.

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The present study was conducted to modify the fractionation procedure of Thanh and Shibasaki (1976a) and to use it to determine the P and phytate contents of the resulting soy protein fractions.

MATERIALS AND METHODS

Soybean Protein Sources. Soybeans (var. Bragg) of the 1982 crop, from the Clemson University Agronomy Department, were finely ground in a Waring blender and defatted with hexane by Soxhlet extraction for 16 h. Commercial defatted soy flakes with high protein solubility were obtained from Ralston Purina Co. (St. Louis, MO).

Protein Fractionation. The major soy protein fractions, i.e., 7S, 11S, soy whey, and soy whey precipitate, were prepared from 10 g of defatted Bragg soybeans and 40 g of commercial defatted soy flakes according to a modified Thanh and Sibasaki (1976a) procedure outlined in Figure 1. Defatted soybeans or commercial defatted soy flakes were extracted with 20 parts (w/v) of dilute Tris-HCl buffer and centrifuged to provide whole soy extract. After completion of the indicated steps for preparing the 7S globulin fraction, the pH was adjusted to 7.8, and it was freeze-dried without dialysis as by Thanh and Shibaski (1976a). The final resolubilized and centrifuged 11S globulin fraction was directly freeze-dried without the overnight refrigerated storage treatment of Thanh and Shibasaki (1976a). All centrifugation treatments were at higher forces of 23700*g* to obtain more complete separation of sediment and supernatant fractions.

Analytical Methods. Protein was determined by the Bio-Rad Coomassie blue dye-binding method (Bio-Rad Laboratories, 1976), with bovine plasma γ-globulin as the reference protein. Total P was determined by the method of Allen (1940), as modified by Brooks and Morr (1982). The method involves perchloric acid digestion of protein samples and spectrophotometric assay of the phospho-

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