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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

May 3, 1989

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Maneb (014505) and Mancozeb (014501)
Additional Responses to PD 1;
Spinach and Turnip Green Processing Studies
[No MRID No., RCB No. 4586]

FROM: Susan V. Hummel, Chemist
Special Registration Section II
Dietary Exposure Branch
Health Effects Division (H7509C)

Susan V. Hummel

THRU: R. D. Schmitt, Ph.D., Acting Chief
Dietary Exposure Branch
Health Effects Division (H7509C)

Richard D. Schmitt

TO: Valerie Bael, PM#77
Special Review Branch
Registration Division (H7508C)

The National Food Processors Association (NFPA) and Southern Frozen Foods have submitted additional responses to the EBDC PD 1, consisting of spinach and turnip green processing studies. Three studies using maneb were submitted by NFPA with their letter of 10/3/88. One study on maneb treated turnip greens, conducted by University of Georgia, was submitted by Southern Frozen Foods along with their letter of 10/25/88. A preprint of a study conducted by The University of Arkansas and the NFPA National Food Laboratory entitled, "Reduction of the Carbamate Residue Ethylenethiourea in Canned Spinach by Using Washing Solutions," was submitted by Southern Frozen Foods, and was received by EPA on 10/11/88. Since the Dietary Exposure estimates for the EBDC PD 2/3 were completed in July, 1988, all of these studies were submitted too late to be considered for the EBDC PD2/3; consequently, they will be considered in the dietary exposure estimates for the EBDC PD4.

NFPA Turnip Green and Spinach Studies

Three studies using maneb were submitted by NFPA with their letter of 10/3/88. In the first study, conducted in Georgia, turnip greens were treated with maneb three times at the rate of 1.75 qts. Manex/A. The formula for Manex was not stated. Samples were harvested 7 days after the last treatment, and were processed (washed and frozen) the same day. The samples were stored frozen at -10C and analyzed within three to four months.

Cooking according to the package directions was done at the time of analysis. A single gas chromatogram from the ETU analysis was included in the report.

In the second study, conducted in Texas, spinach was treated with three times with Manzate D (80% Maneb with Zinc added) at the rate of 1.6 lb ai/A at 7 to 10 day intervals. Harvest occurred between 4 to 11 weeks after the last application. It was not stated when the samples were collected. A protocol for the spinach processing was included. Raw spinach is "dry cleaned" to remove dirt and other foreign material using rotating cylindrical, squirrel type wire screen reels, or other effective equipment. Two types of washers may be used, (1) an immersion washer in which the spinach is agitated and propelled through a tank of water with paddles, or (2) a rotary washer similar to the dry cleaner, equipped with evenly spaced high pressure water sprays. Both washers may be used. The protocol calls for two to three washings. The spinach is then blanched using hot water for 2-6 min at 185-190F or 4-10 min at 200F, or steam for 1-2 min at 206F. For frozen spinach, the spinach is then inspected, chilled, chopped, placed in boxes, overwrapped, and frozen in a blast freezer. Samples were collected in triplicate before processing, and at each step in the processing, except dry cleaning. Frozen samples were also home cooked at the laboratory according to the label directions. For canned spinach, the greens are inspected, drained, cut or chopped, and placed in cans. Brine is added at 200F. Cans of size 2 1/2 are exhausted for 5-6 min., then closed and processed as specified in NFPA Bulletin 26-L (bulletin and procedures not included in report). Triplicate samples of canned spinach were also collected. It was not stated if the canned spinach was also further cooked at the laboratory. The report also did not state the procedures actually used in the canning and freezing process.

In the third processing study, conducted in Oklahoma, spinach was aerially treated one month after planting with Manzate (Maneb) at 2 lb/A and ten days prior to harvest at 3 lb ai/A. There was no rain or irrigation after the last application. The samples were processed at Allen Canning in Alma, AR. The samples were placed in cool storage at 32F for "a short time" prior to transporting to the processing plant. Samples were collected just before processing, after jet washing and prior to blanching, after blanching, and after canned samples were retorted and cooled. Storage of frozen samples was at 0F. The canned product was stored at 72F. Residues were not reported for all of the samples collected. Four sets of six to ten samples were collected, but only one set of samples were analyzed. Length and conditions of storage of samples was not reported. Dates of harvest, processing, and analysis were not given. The table of individual results in the report appears to be misaligned, but we were able to correlate the individual results to the summary table in the report.

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Analytical Methods used

The analytical methods used in the NFPA studies were not submitted, nor were they referenced in the reports. Raw data for the three studies consisted solely of a single chromatogram for ETU analysis. The report did not state whether this one chromatogram was from a treated sample, a control sample, or a fortified sample. No results were given for any control samples. No recovery data were included with any of the three studies.

Results

The results of these studies are tabulated below. Concentration/reduction factors and percent conversion are calculated both from the raw greens, and starting from the washed greens.

Concentration/Reduction Factors for EBDC's and Percent Conversion to ETU in Leafy Vegetables in NFPA Studies

Commodity	Residue (ppm)		<u>from raw</u> Conc.		<u>from washed</u> Conc.	
	EBDC	ETU	Factor	%Conv.	Factor	%Conv.
<u>Turnip Greens (NFPA #1)</u>						
raw	23	0.06				
washed	6	0.09	0.26	0.1		
frozen	1.1	0.69	0.048	2.7	0.18	10.0
cooked	<0.02	1.1	<0.001	4.5	<0.003	16.8
<u>Spinach (NFPA #2)</u>						
raw	1.13	0.02				
washed	0.3	0.01	0.26	0		
canned	<0.05	0.16	<0.044	12.4	<0.17	50.0
<u>Spinach (NFPA #3)</u>						
raw	23.1	0.7				
washed	2.5	0.04	0.11	0		
canned	<0.02	0.54	<0.01	0	<0.08	20.0

DEB Comments

EBDC residues are reduced by washing, cooking and canning. ETU is formed during cooking and canning.

University of Georgia Processing Study

Turnip greens grown in Georgia were treated four times with Dithane M-22 at the rate of 0.8 lb ai/A at 7 day intervals. Turnip greens were harvested seven days after the last treatment, and transported to the University of Georgia Food Science Department, where they were processed. Control samples were also collected. The greens were spray rinsed with agitation for two eight minute periods, blanched for 30-45 sec. in hot water, and steam blanched for six minutes. They were then cooled with tap water, drained, and chopped. The samples were frozen for two weeks (temperature not stated), thawed according to package labeling, and submitted for analysis. No dates were given for the analysis. No indication was given of the storage conditions between processing and analysis. The EBDC and ETU analyses were conducted by The University of Georgia Department of Poultry Science.

Analytical methods

Maneb was determined by the PAM II method, as described in JAOAC 450, 1113, (1957). ETU was determined by a modification of the AOAC method, 14th Ed. 29.119 (1984). The modification to the method was not described nor referenced. Recovery data were not included. The limits of detection of the methods as found by the analyst were not stated; however, based on the data, the limit of detection for maneb appeared to be 0.38 ppm and for ETU, 0.3 ppm.

Results

The results of these studies are tabulated below. Residues in control samples were non-detectable. Concentration/reduction factors and percent conversion are calculated both from the raw greens, and starting from the washed greens.

Concentration/Reduction Factors for EBDC's and Percent Conversion to ETU in Leafy Vegetables in University of Georgia Studies

<u>Commodity</u>	<u>Residue (ppm)</u>		<u>from raw</u> <u>Conc.</u>		<u>from washed</u> <u>Conc.</u>	
	<u>EBDC</u>	<u>ETU</u>	<u>Factor</u>	<u>%Conv.</u>	<u>Factor</u>	<u>%Conv.</u>
			<u>EBDC</u>	<u>ETU</u>	<u>EBDC</u>	<u>ETU</u>
<u>Turnip Greens (UGa)</u>						
raw	2.85	0.43				
washed	4.53	0.43	1.6	0		
blanched	2.7	<.30	0.95	0	0.60	0
chopped	<0.38	<.30	<0.13	0	<0.084	0
chopped/cooked	<0.38	<.30	<0.13	0	<0.084	0

DEB Comments

This study cannot be used for estimation of dietary exposure. The EBDC residues found in washed samples are greater than the EBDC residues in raw samples. We must question the sampling procedures and sample handling procedures.

University of Arkansas/National Food Laboratory Study

A preprint of a study entitled, "Reduction of the Carbamate Residue Ethylenethiourea in Canned Spinach by Using Washing Solutions," was submitted by Southern Frozen Foods, and was received by EPA on 10/11/88. This study was conducted by the University of Arkansas and the NFPA National Food Laboratory. A preliminary report from an earlier similar study entitled, "Effect of Field Applications of Carbamate Fungicides and Processing Methods on Levels of ETU in Canned Spinach," had been previously submitted to EPA. Comments on the study were provided by DEB and sent to Frank Cates of Southern Frozen Foods in D. Camp letter of 7/23/87.

In 1986, spinach fields were sprayed three times with Dithane M-22 (Maneb) one, two, and three weeks prior to harvest at the rate of 2 lb/A (formulation?) in 200 gal water. In 1987, spinach fields were sprayed four times with Manzate 200 (mancozeb) at the same rate and PHI as was used in 1986. The spinach was processed into canned spinach using eight different washing treatments: (1) no wash; (2) water; (3) water + sodium hypochlorite at 300 ppm; (4) "VFD Soiler" detergent at 0.3%; (5) "VFD Soiler" + sodium hypochlorite; (6) "Soil Wash" Detergent; (7) "Soil Wash" + Sodium Hypochlorite; and (8) "Redeem" Detergent, (includes sodium hypochlorite in formulation). THE "VFD Soiler" detergent is a mild detergent, while the "Soil Wash" and "Redeem" detergents are stronger detergents. Normal commercial practice is to wash the spinach in water before canning.

The washing treatment consisted of agitating 1.8 kg of spinach in 12 L of washing solution for three minutes. After washing, the spinach was rinsed in a rotary rod washer and steam blanched for three minutes. A 380g sample of blanched spinach was placed in a 303 can. The can was filled with boiling water, exhausted for five minutes, and processed at 250F for 54 minutes.

Analytical Methods

The canned samples of spinach were analyzed for ETU by AOAC Method 29.119 (LOD 0.02 ppm). The pH of the washing solution was also measured. A number of quality parameters of the liquor and the canned spinach was measured for the spinach grown in 1987,

including a visual panel evaluation. The level of EBDC's were not measured in any sample. No samples of unprocessed spinach were analyzed. No raw data were included in the report. None of the samples were taste tested.

Results

The results of the University of Arkansas/National Food Laboratory study are tabulated below. The greatest reduction of ETU levels was in samples washed with the stronger detergents and sodium hypochlorite (chlorine bleach). A correlation was found between the pH of the washing solution and the reduction in ETU residue. The authors report that the quality of the spinach was not adversely affected by the washing solutions, although no taste tests were conducted.

Effect of Detergent and Chlorine Bleach on the pH of Washing Solutions and the Levels of ETU in Canned Spinach Treated with EBDC Fungicides

Washing Solution	pH		ETU (ppm)	
	1986	1987	1986	1987
No Wash			63.00	71.00
Water wash	8.50	7.67	7.48	6.93
Water + bleach	9.39	9.00	5.65	5.63
VFD Soiler	8.23	8.00	5.80	5.35
VFD Soiler + bleach	9.62	8.97	4.35	5.60
Soil Wash	12.40	11.17	0.11	2.05
Soil Wash + bleach	12.18	10.93	3.15	1.46
Redeem (w/ bleach)	12.13	10.83	1.85	1.79

Conclusions

Washing EBDC treated spinach with strong detergents and chlorine bleach in the canning process can reduce levels of ETU in the canned product. However, without analyses for the levels of EBDC's before and after canning, no further conclusions can be made. Additionally, since washing spinach with detergents and chlorine bleach is not normal commercial practice, this study will have no effect on the residue estimates made for the EBDC Special Review. We cannot comment on the authors conclusions regarding the quality of the canned spinach after washing in detergent and chlorine bleach since we have no expertise in this area, but we note that no taste tests were conducted.

Earlier Processing Studies on Leafy Greens

Several washing and cooking studies have been previously reviewed. One study was done under contract to EPA (W. F. Phillips and M. D. Grady, April, 1977, "Effects of Food Processing on Residues of Two Ethylenebisdithiocarbamate (EBDC) Fungicides and Ethylene-thiourea (ETU)," EPA-600/1-77-021). Tomatoes, spinach, and carrots were treated with EBDC fungicides and analyzed before and after washing and cooking. This study was originally discussed in the MRI Report and the EBDC Decision Document (10/82). An earlier study was conducted by an EPA laboratory. (R. R. Watts, R. W. Storherr, J. H. Onley, "Effects of Cooking on Ethylenebisdithiocarbamate Degradation to Ethylene Thiourea," Bull. Environ. Contam. Toxicol., 12(2), 1974, 224-226). Samples of spinach, potato, and carrot were spiked with EBDC fungicides either before or after cooking, and analyzed after cooking. The percent conversion to ETU was calculated. These studies were discussed in our review of newly submitted maneb and metiram data (S. Hummel, 6/30/88). The results of the Phillips studies on spinach are tabulated below.

Concentration/Reduction Factors for EBDC's and Percent Conversion to ETU in Spinach in the Phillips Studies (1977)

Commodity	Residue (ppm)		from raw Conc. Factor %Conv.		from washed Conc. Factor %Conv.	
	EBDC	ETU	EBDC	ETU	EBDC	ETU
<u>Spinach - Mancozeb - Trial 1</u>						
Unwashed	2.4	<0.01				
Washed	1.5	<0.01	0.6	0.0		
Frozen	0.1	0.04	0.04	1.7	0.07	2.7
Juice	<0.1	0.18	<0.04	7.5	0.01	12.0
<u>Spinach - Mancozeb - Trial 2</u>						
Unwashed	61.9	0.34				
Washed	9.7	0.02	0.2	0.0		
Frozen	0.6	0.5	0.01	0.3	0.06	5.2
Juice	0.1	0.71	0.002	0.6	0.01	7.3
<u>Spinach - Maneb</u>						
Unwashed	86.6	0.24				
Washed	35.2	0.06	0.4	0.0		
Blanched froz.	0.4	0.76	0.005	0.6	0.011	2.2
cook 5 min	0.2	0.76	0.002	0.6	0.006	2.2
Canned	<0.1	1.82	<0.001	1.8	0.003	5.2
cook 5 min	<0.1	2.12	<0.001	2.2	0.003	6.0

Watts Study

The Watts study was discussed in S. Hummel memo of 6/30/88 (Dietary Exposrue analysis for Maneb). Samples were fortified with 10.0 ppm of EBDC compound either before or after cooking. Samples were cooked by boiling for 15 minutes on a hot plate. Samples were analyzed for ETU, and the percent ETU formed was calculated on a weight/weight basis. The results of the Watts study on spinach are summarized below.

ETU produced from cooking vegetables fortified with 10.0 ppm EBDC Compound

Crop	EBDC	<u>ppm ETU found</u>		Percent ETU formed by cooking
		Fortified after cooking	Fortified before cooking	
Spinach	Maneb	0.16	1.82	16.6
	Dithane M-45	0.15	2.17	20.2
	Manzate 200	0.11	2.42	23.1
	Polyram	0.07	2.72	26.5

DEB Comment

This study is comparable to measuring the percent of ETU formed from washed greens. The results are comparable to those found in the NFPA studies.

SUMMARY

Leafy vegetables eaten raw are assumed to have been washed. The reduction of EBDC residues on washing is assumed to be the average factor from the available spinach and turnip green processing studies. No change is expected in ETU residues from washing.

The average concentration/reduction factors for EBDC residues and percent conversion factors for EBDC to ETU from all of the available processing studies for spinach and other leafy vegetables are tabulated below. These factors will be used to correct the EBDC and ETU residue data for the effects of washing and commercial processing. These corrections will be made to the data when the next dietary exposure analysis is requested.

SUMMARY TABLEConcentration Factors and Percent Conversion of EBDC to ETU
for Spinach and Other Leafy Vegetables

<u>Commodity</u>	<u>Concentration Factor EBDC</u>	<u>Percent Conversion to ETU</u>
<u>Leafy Vegetables</u>		
raw, washed	0.30x	0
cooked, canned	< 0.01x	4.1%

cc: R.F., circu, S. Hummel, Mancozeb S.F., Mancozeb S.R.F.
(Hummel), Mancozeb R.S.F., J. Tomerlin (TAS), E. Saito (SACB),
S. Lewis (PM#21), PMSD/ISB
RDI:FBS:04/07/89:EZ:05/02/89
TS-769:RCB:RM810:CM#2:SVH:svh:05/03/89
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