# Procedures for the Removal of Field Residues of Ethylenebis(dithiocarbamate) (EBDC) Fungicide and Ethylenethiourea (ETU) from Tomatoes Prior to Processing into Juice

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Two methods for the removal of ethylenebis(dithiocarbamate) (EBDC) and ethylenethiourea (ETU) residues from field-treated tomatoes were demonstrated to be effective in decreasing the residue levels of each toxicant in finished juice samples. In a pilot-plant-scale experiment, a 4-min bath in dilute sodium hypochlorite (at ambient temperature) followed by a dip into dilute sodium sulfite significantly reduced levels of both residues below levels observed when normal industrial procedures were followed. The residue levels of both toxicants were reduced to the limit of detection by this oxidative wash. Moreover, field-weathered residues were removed as easily as fresh residues, which was not the case when only a 10-min water wash was used prior to processing. In a laboratory-scale experiment, a hot acid blanch (95 °C) reduced levels of both toxicants below levels observed when a combined 10-min water wash/hot water blanch was used during the canning operation.

Concern regarding the use of ethylenebis(dithiocarbamate) (EBDC) fungicides in vegetable production centers on the possibility that residues present on the surface of field-treated crops may be converted to ethylenethiourea (ETU). This degradation product is a potent oncogen (Innis et al., 1969; Ulland et al., 1972; Graham et al., 1975) and teratogen (Khera 1973; Ruddick and Khera, 1975) when fed to rats and mice. The nonbiological degradation of EBDC's to ETU is accelerated by heat treatment (Newsome and Laver, 1973; Watts et al., 1974; Phillips et al., 1977) and EBDC residues are known to be converted to ETU during normal industrial processing of the field-treated produce. The conversion of these surface residues to ETU during processing has been demonstrated on snap beans (Cummings, 1974) and on tomatoes, carrots, and spinach (Phillips et al., 1977). Although the conversion of field residues to ETU, estimated to be 17–26% in the Phillips' study, was considerably less than the theoretical maximum, it was significant in these trials. Tomatoes were chosen as a matrix for a feasibility study of decontamination because of the economic importance of their production, because the fruit is directly exposed to the EBDC spray and because high temperatures are used in converting the raw fruit into juice. The generally higher levels of EBDC's and of ETU residues found in juice as opposed to "whole pack" tomatoes (von Stryk and Jarvis, 1978) are usually attributed to increased contact time between the skins and the interior of the fruit during processing into juice. Thus, it was felt that juice resulting from field-treated tomatoes would represent a more severe test for any decontamination technique.

Two methods for controlling the conversion of EBDC residues have been suggested. One approach is to convert these residues to ethylenediamine (EDA) and carbon disulfide (Marshall, 1977). Conditions that favor these products are low pH and high temperature. In addition, there is a patent (Wagner et al., 1968) that advocates using hot acid to remove skins from tomatoes during blanching. This procedure is more effective at inactivating pectolytic enzymes than either cold acid treatment or heat treatment alone. These enzymes, which occur naturally in the fruit, are believed to be responsible for the degradation of pectin containing colloids and the consequent reduction in viscosity of the finished product. Thus, the rapid inactivation of these enzymes is desirable. Although operating at pH levels below 2.0 offers no advantage as far as enzyme inactivation is concerned, a pH of 1.0 can be used without adversely affecting the quality of the product. The extracted juice is then readjusted to its original pH (4.0 → 4.2) with strong base. The only adulterant introduced by this procedure is sodium chloride, which is added in any event to enhance flavor. On the basis of these same operating procedures, an attempt was made to monitor the effect of these blanching conditions on residue levels of EBDC and ETU in juice. More recently, reports of the action of hypochlorite on ETU (Marshall and Singh, 1977; Marshall, 1979) and on EBDC fungicides (Marshall, 1978a) have suggested that a hypochlorite wash of field-treated produce prior to processing would significantly reduce levels of these contaminants in the finished product.

This paper also reports the results of a pilot plant feasibility study on the efficacy of hypochlorite as a prewash for tomatoes and compares it to preprocessing washes normally encountered in industry.

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## EXPERIMENTAL SECTION

A flow diagram which outlines both experiments is provided in Figure 1.

**Field Treatment.** Tomatoes, cv. Campbell 28, were grown at the Harrow Research Station in six blocks of 60 plants each and received up to nine sprays of mancozeb, Dithane M-45, 80% W.P. (Rohm and Haas) at approximately 10-day intervals. Two blocks of plants received mancozeb at the maximum recommended rate [Ontario Vegetable Production Recommendations, 1977; 3 lb/acre, 2.69 kg of active ingredient (AI)/ha], two blocks were treated at an accelerated rate (6 lb/acre, 5.38 kg of active ingredient/ha), whereas the final two blocks of plants received no fungicides. The sprayer used to apply the fungicide was a hand-operated "Zephir" knapsack type adapted to operate at constant pressure (3.4 atm, 345 kPa) using an independent cylinder of compressed air as propellant. All plants were sprayed to the point of run-off. All blocks also received the insecticide carbaryl as Sevin, 50% W.P. at the recommended rate of 2 lb/acre (1.12 kg of AI/ha) and in all blocks rainfall was augmented with water (overhead sprinkler) to 1 in. (25 mm)/week. The ripe fruit was harvested on one of four sampling dates for the hypochlorite wash experiment, whereas tomatoes that received a single spray were harvested 9 days later and

 

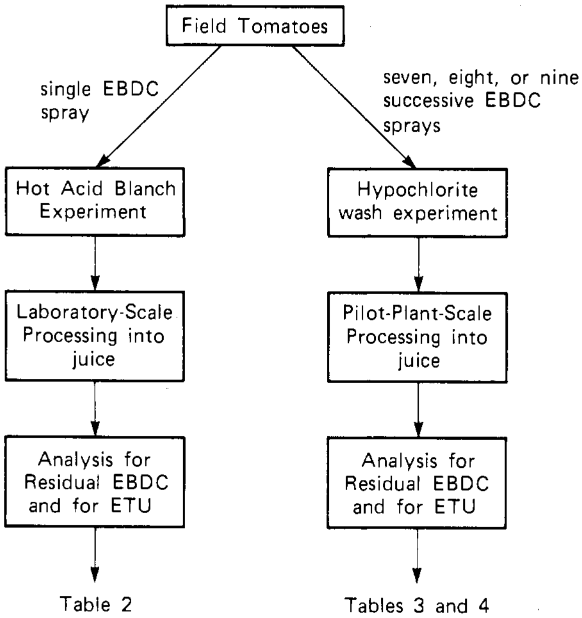


**Figure 1.**

used for the acid blanch experiment.

**Washing Procedure.** In the hypochlorite experiment subsamples from each of the four harvests were water washed for 2, 4, 6, or 10 min with a continuous flow of fresh cold tap water or were bathed and agitated in a 0.1% (v/v) aqueous sodium hypochlorite (60-fold dilution of commercial product) for 2, 4, or 6 min. Remaining traces of hypochlorite were removed by a subsequent dip of 30 s into 0.1% (v/v) aqueous sodium sulfite. Control tomatoes that received no fungicide were similarly treated and all samples were processed as described below.

**Processing of Crop.** In the pilot plant, tomatoes in approximately 2-kg lots were processed into juice. Following washing, tomatoes were macerated in a Hobart mixer, heated with stirring in a steam-jacketed kettle (90 °C) and passed through a Langsenkamp extractor fitted with a 0.027-in. (0.69 mm) screen. The resulting juice was canned (19 oz. enameled cans) and held at 95 °C for 0.5 h prior to cooling. When cool, the cans were stored at 0 °C to await analyses.

In the hot-acid blanch experiment, subsamples from each spray level were unwashed, were water washed for 10 min and blanched in hot water, or were directly blanched in hot (95 °C) 1.0 N HCl for 2 min. Tomatoes and skins were then transferred to a speed juicer (Sweden Manu. Co., Seattle, WA) which employs a spinning screen [0.07 in. (1.78 mm) mesh] grater and funnel design to macerate and juice samples. The juice from each procedure was transferred to sterilized 6-oz mason jars (after readjusting the pH in the case of acid blanch samples) and heated in boiling water for 0.5 h. The jars were capped while hot and allowed to cool slowly to room temperature.

**Analysis.** Analysis for EBDC residues was performed using the carbon disulfide evolution method (Keppel, 1969, 1971) while residues of ETU were measured by the method of King (1977). To minimize variability in residue levels, only processed samples (as opposed to fresh fruit) were analyzed. It is estimated that each 19-oz can of juice would be equivalent to at least 15 tomatoes.

## RESULTS AND DISCUSSION

**Hot Acid Blanch.** In this decontamination procedure, reduction in EBDC levels was achieved by converting these residues to EDA and $CS_2$. Blanching conditions were chosen so as to optimize the yield of these products.

Table I. Separation of $CS_2$ from EBDC Residues in Tomato Juice by Extraction with Ether

| juice (100 g) fortified with (ppm) | | percent EBDC recovery[a] | |
|---|---|---|---|
| EBDC | $CS_2$ | 2 ether extract. | no extract. |
| 0.4 | | 84 ± 5 | 81 ± 7 |
| 0.3 | | 83 ± 8 | 84 ± 5 |
| 0.2 | | 85 ± 2 | 84 ± 5 |
| | 0.4 | ND[a] | 41 ± 2 |
| | 0.2 | ND | |
| | 0.1 | ND | 37 |
| 0.4 | 0.2 | 85 ± 3 | 106 ± 6 |
| 0.2 | 0.2 | 86 ± 4 | 103 ± 7 |
| 0.05 | 0.2 | 85 ± 7 | 96 |

[a] Apparent EBDC recovery was calculated by multiplying observed $CS_2$ level by 0.448 (mancozeb, 80% A I) or by 0.594 (nabam analytical standard). [b] ND, none detected.

Table II. Residues (ppm, AI) of EBDC as Mancozeb and of ETU in Canned Juice (from Field Tomatoes Treated with Dithane M-45 at 6 lb/Acre or at 3 lb/Acre) following Various Preprocessing Washes

| | | single application of dithane M-45 at | | | |
|---|---|---|---|---|---|
| | | 6 lb/acre | | 3 lb/acre | |
| | | residues | | | |
| washing procedure | | M-45 | ETU | M-45 | ETU |
| no washing | | 0.57 | 0.065 | 0.16 | 0.034 |
| | | 0.50 | 0.069 | 0.18 | 0.022 |
| | | 0.57 | 0.054 | 0.18 | 0.029 |
| | mean | 0.54 | 0.063 | 0.17 | 0.028 |
| water wash | | 0.19 | 0.025 | 0.10 | 0.022 |
| for 10 min | | 0.18 | 0.014 | 0.11 | 0.009 |
| | | 0.19 | 0.013 | 0.11 | 0.023 |
| | mean | 0.19 | 0.017 | 0.11 | 0.018 |
| hot acid break | | 0.11 | 0.013 | 0.04 | ND |
| and neutralization | | 0.10 | 0.017 | 0.05 | ND |
| | | 0.09 | 0.006 | 0.04 | ND |
| | mean | 0.10 | 0.012 | 0.04 | ND |

[a] ND, none detected (not significantly different from control).

Because unreacted EBDC residues in the finished juice were to be estimated by the $CS_2$ evolution technique (strong acid/stannous chloride; Keppel, 1969) a modified method was required which would differentiate between "free $CS_2$" resulting from the decontamination procedure and $CS_2$ resulting from the analytical method to measure EBDC residues in the juice. Efforts to achieve this by modifying the digestion conditions were unsuccessful. When control juice (fortified with $CS_2$ and mancozeb formulation) was digested (stannous chloride and HCl added but no heat) and "free $CS_2$" entrained, low recoveries resulted. However, when no stannous chloride was added (only HCl and heat) spuriously high values resulted, indicating considerable EBDC decomposition. Removal of "free $CS_2$" was achieved by blending the juice with an equal volume of ether for 30 s, centrifuging to separate the two layers and discarding the organic fraction. Two ether washes were sufficient. That no losses of EBDC are incurred by this wash is indicated by Table I in which samples of control juice (fortified with EBDC and/or $CS_2$) were analyzed with and without ether extractions.

Field tomatoes that received a single spray of Dithane M45 (at 3 or 6 lb/acre) were harvested 9 days after spraying. Residue levels of EBDC and ETU in juice made from field-treated tomatoes which were unwashed, water-washed (and hot water blanch) or were acid

Table III. Residues (ppm) of Dithane M-45 (Mancozeb) and ETU in Canned Juice (from Field Tomatoes Treated at 3 lb/acre) following Various Washing Procedures

| sampling date: days after last spray: | Aug 24 9 | | Aug 25 1 | | Sept 6 13 | | Sept 7 1 | | av % reduction of EBDC levels (4 sampling dates) |
|---|---|---|---|---|---|---|---|---|---|
| | M-45 | ETU | M-45 | ETU | M-45 | ETU | M-45 | ETU | |
| no washing | 0.31 | 0.02 | 0.41 | 0.01 | 0.35 | Tr | 0.99 | Tr | |
| | 0.27 | ND[a] | 0.41 | Tr | 0.45 | 0.01 | 1.10 | Tr | |
| | 0.31 | ND | 0.54 | 0.01 | 0.54 | ND | 1.00 | 0.01 | |
| mean | 0.30 | | 0.45 | | 0.45 | | 1.03 | | |
| water wash | | | | | | | | | |
| 2 min | 0.30 | Tr[a] | 0.18 | ND | 0.32 | ND | 0.71 | 0.03 | 30 |
| 4 min | 0.16 | ND | 0.18 | Tr | 0.15 | Tr | 0.36 | 0.01 | 60 |
| 6 min | 0.16 | ND | 0.09 | Tr | 0.18 | Tr | 0.45 | 0.01 | 61 |
| 10 min | 0.16 | Tr | 0.16 | Tr | 0.36 | Tr | 0.40 | Tr | 48 |
| av decrease EBDC level, % (4 washing times) | 35 | | 66 | | 44 | | 52 | | |
| hypochlorite wash | | | | | | | | | |
| 2 min | 0.04 | Tr | 0.09 | 0.01 | 0.09 | Tr | 0.13 | 0.02 | 84 |
| 4 min | 0.04 | Tr | ND | 0.01 | 0.05 | 0.01 | 0.04 | 0.02 | 93 |
| 6 min | 0.04 | Tr | ND | Tr | ND | ND | 0.04 | 0.01 | 96 |
| av decrease EBDC level, % (4 washing times) | 87 | | 93 | | 90 | | 93 | | |

[a] ND, none detected; Tr, trace.

Table IV. Residues (ppm) of Dithane M-45 (Mancozeb) and ETU in Canned Juice (from Field Tomatoes Treated at 6 lb/Acre) following Various Washing Procedures

| sampling date: days after last spray: | Aug 24 9 | | Aug 25 1 | | Sept 6 13 | | Sept 7 1 | | av % reduction of EBDC levels (4 sampling date) |
|---|---|---|---|---|---|---|---|---|---|
| | M-45 | ETU | M-45 | ETU | M-45 | ETU | M-45 | ETU | |
| no washing | 0.71 | 0.01 | 1.00 | 0.01 | 0.89 | 0.01 | 1.50 | ND | |
| | 0.68 | Tr | 1.10 | Tr | 1.10 | Tr | 1.40 | ND | |
| | 0.80 | Tr | 0.98 | 0.01 | 1.10 | Tr | 1.30 | ND | |
| mean | 0.73 | | 1.03 | | 1.03 | | 1.40 | | |
| water wash | | | | | | | | | |
| 2 min | 0.50 | ND | 0.30 | ND | 0.45 | ND | 0.88 | Tr | 51 |
| 4 min | 0.50 | ND | 0.30 | ND | 0.86 | ND | 0.59 | Tr | 45 |
| 6 min | 0.36 | ND | 0.26 | ND | 0.36 | ND | 0.45 | Tr | 65 |
| 10 min | 0.49 | 0.02 | 0.31 | ND | 0.86 | 0.03 | 0.54 | 0.01 | 46 |
| av decrease EBDC level, % (4 washing times) | 35 | | 72 | | 41 | | 56 | | |
| hypochlorite wash | | | | | | | | | |
| 2 min | 0.23 | ND | 0.45 | ND | 0.32 | Tr | 0.26 | Tr | 69 |
| 4 min | 0.13 | ND | 0.18 | 0.01 | 0.14 | ND | 0.21 | 0.01 | 84 |
| 6 min | 0.09 | ND | 0.14 | ND | 0.26 | Tr | 0.21 | Tr | 84 |
| av decrease EBDC level (4 washing times) | 79 | | 75 | | 77 | | 84 | | |

[a] ND, none detected; Tr, trace.

blanched, are shown in Table II. As expected, water washing significantly reduced residues of both toxicants in the finished juice. EBDC levels were reduced by an average of 52% below unwashed controls. However, these levels were considerably lower again in the juice from tomatoes which had been acid blanched. EBDC levels were reduced by an average of 79% relative to unwashed controls. Although tomatoes in this experiment received only a single application of fungicide and thus is not fully representative of usual field conditions where several sprays may be applied over a growing season, the results are promising. They suggest that a considerable reduction in both toxicant levels can be achieved by using hot acid during the blanching procedure.

**Hypochlorite Wash.** In the hypochlorite experiment, field tomatoes were processed into juice without washing to establish a standard for comparison. Toxicant levels were then compared with levels in juice resulting from a preprocessing water wash for 2, 4, 6, or 10 min and in juice resulting from a preprocessing wash with hypochlorite for 2, 4, or 6 min. Assuming that the canning procedure and subsequent sterilization converted the same proportion of EBDC to ETU, then differences in the EBDC levels in the resulting juice should reflect differences in the effectiveness of the two washing procedures. The hypochlorite wash was followed by a dip (30 s) into dilute sodium sulfite to remove any remaining hypochlorite. It has been established previously in decomposition studies (Marshall, 1978a) that addition of sodium sulfite to a boiling suspension of EBDC formulation did not affect the yield of ETU. Sulfur dioxide is registered in Canada (Food and Drug Act, 1976) as a food additive in tomato products to a maximum permissible level of 500 ppm (as $SO_2$ on the whole fruit). This procedure would thus appear to be feasible in commercial processing. Single analyses for EBDC and ETU residues that resulted from seven (sampling date, Aug 24), eight (Aug 25 and Sept 6), or nine (Sept 7) successive sprays (at 3 lb of AI/acre) are recorded in Table III. Residues resulting from seven, eight, or nine successive sprays of EBDC applied at 6 lb/acre are recorded in Table IV. Residue levels of ETU were, in all cases (88 analyses), below the "analytically significant" level of 0.05 ppm advocated by Pease and Holt (1977). Washing the raw fruit with running water reduced EBDC levels in the finished juice by about 50% compared to unwashed samples. However, prolonged washing times (4 min vs. 10 min) did not increase significantly the efficiency of residue removal. When the average decrease in EBDC levels for each sampling date are compared it is apparent that the efficiency of the water wash is better the shorter the interval between spraying and sampling. At both levels of fungicide application "field weathered" residues (samples taken 9 and 13 days after the last fungicide spray) were less efficiently removed by water washing than were "fresh" residues (samples taken 1 day after the last spray).

When hypochlorite was used as a preprocessing wash on samples of the same field-treated tomatoes EBDC levels were reduced considerably over levels resulting from normal industrial practice (water wash for 10 min). Hypochlorite washes for 4 and 6 min were equally effective (Table III and IV), and no differences were apparent between "weathered" and "fresh" residues. Removal of the EBDC residues was complete at the 3 lb/acre rate of application, but at the accelerated rate small amounts of residue remained. However, given the usual rate of return on this commodity, the 6-lb spray schedule followed in this study would not be economically feasible. Thus, it is concluded that EBDC and ETU residues in tomato products can be controlled effectively by washing the raw fruit with a dilute solution of sodium hypochlorite. An alternate procedure using a hot acid blanch to remove skins, also shows promise as a decontamination technique. While this procedure will not improve the quality of the finished juice over current products it has been demonstrated effective in reducing levels of both toxicants.

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