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Assessment of Dithiocarbamate Residues on Tomatoes Conventionally Grown in Uganda and the Effect of Simple Washing to Reduce Exposure Risk to Consumers

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ABSTRACT: Pesticide misuse by farmers poses health risks to consumers. This study assessed the level of dithiocarbamate residues in tomatoes acquired from 20 farmers and 25 market vendors in Wakiso District, how simple washing affects these residues, and the potential chronic health risk for Ugandans eating such tomatoes. Results revealed that mancozeb was the only reported dithiocarbamate, and 47.4% and 14% of farm and market samples, respectively, had dithiocarbamate residues exceeding the Codex alimentarius maximum residue limit of 2 mg CS₂/kg. Mixing concentration had a positive significant effect on dithiocarbamate residue levels ($P = 0.004$). Washing reduced dithiocarbamate residues by a factor of 0.3. Dietary risk assessment revealed no chronic health risk to both children and general population when a national daily per capita consumption of 1.0 g is considered. This study recommends comprehensive research into Uganda's food production and consumption patterns and establishment of a national pesticide residue surveillance program.

KEYWORDS: Uganda, washing, tomatoes, dithiocarbamates, consumer

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Introduction

Uganda's horticultural production is one of the fastest growing agricultural subsectors with a growth rate of 20% per year, and tomato is one of its most important vegetable crops.¹ Pesticides, especially ethylene bis-dithiocarbamates (EBDCs), are intensively used on this crop to combat different fungal infections, such as early and late blights.^{2,3} By 2016, mancozeb, maneb, and propineb were the EBDCs registered for use in Uganda.⁴ Studies have shown mancozeb to be the most used by tomato farmers.^{2,3,5–7} Farmers, however, misuse these fungicides and other pesticides: adherence to label instructions is low; farmers seldom read the instructions for use, but they rather learn spraying techniques and mixing procedures by imitating relatives or neighbours.^{8,9} For instance, farmers have reported exceeding the recommended mixing concentration of mancozeb by 3 to 7 times, applying it as close as 1 to 2 days to harvest time and sometimes even on harvest day because they believe that it works as a preservative to extend the tomato shelf life and makes the tomato fruit shiny and thus attractive to customers.^{3,5–7}

As a result of such misuse, different studies have found unsafe levels of dithiocarbamate residues in sprayed agricultural

produce.^{5,6,10–16} For instance, in the Democratic Republic of Congo, tomato samples were found to have mancozeb residues which exceeded European Food Safety Authority maximum residue limits (MRLs) by 24%, United States Environmental Protection Agency MRLs by 33%, and German MRLs by 73%, with a maximum of 3.25 mg CS₂/kg.¹² In Spain, it was revealed that of the analyzed fruits and vegetable samples, 6% had dithiocarbamate residues exceeding the MRLs, with tomatoes among the 3 main contributors.¹³ More still, it was discovered in Brazil that 60.8% of the 520 samples from 9 crops, including tomato, had detectable dithiocarbamate levels ≥ 0.10 mg CS₂/kg, with a maximum of 3.8 mg CS₂/kg.¹⁶

Dithiocarbamate misuse, however, poses risks to human health. Dithiocarbamate metabolites, carbon disulfide (CS₂), and ethylenethiourea (ETU) are associated with human health effects; CS₂ is considered a general neuropathic agent and ETU has antithyroid and carcinogenic effects. In its excess, the manganese contained in dithiocarbamate fungicides is reported to be neurotoxic.¹⁷ In general, dithiocarbamates are considered to have very low acute mammalian toxicity with effects such as eye irritation, skin rashes, scratchy throat, sneezing, and



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inflammation of the nose.^{18,19} However, its associated chronic effects include endocrine disruption, alteration of immune system response, developmental defects in children, and Parkinson disease.¹⁹

In ascertaining potential dietary risk from a given pesticide, processing factors are usually considered. A processing factor is expressed as the pesticide concentration after processing divided by the pesticide concentration before processing. The effect of processing on fruits and vegetables is said to be influenced by the initial deposit of pesticide concentration, physicochemical properties of the pesticide, as well as the type of processing. Processing techniques may include, among others, boiling, frying, juicing, peeling, and washing.²⁰

Materials and Methods

Study location

This study was performed in Nangabo sub-county, Wakiso District, in Uganda. This location was purposefully selected based on a study conducted in 2013 that revealed extensive misuse of mancozeb among Nangabo tomato farmers.⁷ Targeted sampling sites were tomato farms and markets.

Sampling

In total, 75 and 150 samples from farms and markets, respectively, were targeted. Three and 6 replicate samples were randomly selected from the farms and markets, respectively, each sample weighing approximately 1 kg, with 10 tomatoes of approximately 100 g each.²¹ Random sampling at the farm was achieved through picking tomatoes from all sections of the tomato field to cater for potential variation in spray distribution and other field conditions. In the market, tomatoes were selected by randomly picking from different tomato clusters displayed on vendors' stalls.

Supplementary information

During sample acquisition from the farm, supplementary information concerning the tomato samples was gathered from each of the farm owners, using a simple summary sheet. Data were gathered on tomato varieties grown, pesticides used, mixing concentration used, and the last time of spraying. In addition, secondary data on tomato consumption from the World Health Organization were adopted by this study to reflect the overall national average tomato consumption per person per day in Uganda.^{21,22}

Sample transportation and storage

In the field, immediately after picking, each sample of 10 tomatoes was packaged in a sampling bag, tight sealed, labeled, and sampling bags perforated to avoid building up of sweat that would otherwise wash away the surface residues.^{23,24} The samples were then delivered in cool boxes to the laboratory

within 6 hours. At the laboratory, samples were stored at -20°C in a frost-free freezer prior to analysis.

Sample processing

Washing was the processing technique undertaken by this study. Half of the samples acquired from the market were washed by dipping them in a pan of tap water and stirred for about 3 minutes to mimic the commonly observed washing procedure in a Ugandan home setting.

Analytical procedure

The analytical method used was originally published in the *Journal of AOAC International*,^{25,26} with subsequent modification and validation.^{27,28} The method indirectly measures the residual dithiocarbamates as a concentration of carbon disulfide. It involves reduction of dithiocarbamate moiety under strong acidic conditions in the presence of stannous (II) chloride (SnCl_2) as a reducing agent in which dithiocarbamates are quantitatively converted to carbon disulfide (CS_2) and in situ extraction into a layer of isooctane which is measured by gas chromatography-mass spectrometry in the electron impact-selected ion monitoring mode.

The method verification studies determined that specificity as a response in reagent blank and control samples was $<30\%$ of limit of quantification and repeatability as relative standard deviation on replicates analyzed was $<20\%$ which met the requirements of the SANCO guidelines.²⁹

Results

Supplementary findings

A total of 9 pesticide brand names and 11 active ingredients were reported to be in use among the 20 farms from where samples were acquired. Of the 9 pesticides, 2 contained an active ingredient of mancozeb and were the most used. Mancozeb was the only dithiocarbamate mentioned on these farms (Figure 1).

Majority of the farms, however, were not adhering to the recommended mixing concentration of 50 g of the pesticide per 20 L of water; 75% of the farms were found to be exceeding this concentration, with an overall average exceedance rate of 90.8%. The average mixing concentration for the 20 farms was 83.25 g/20 L. In terms of timing, all farms had applied mancozeb less than 8 days to the day of sampling, an overall average of 3.5 days (Appendix 1).

Secondary data on tomato consumption revealed that under the Global Environmental Monitoring System/Food Consumption Cluster Diets, Uganda is in cluster 16 together with Rwanda and Gabon. Tomatoes fall in the category of "fruiting vegetables (other than cucurbits) and mushrooms" which is assigned a daily consumption rate of 1.0 g/person.^{21,22}

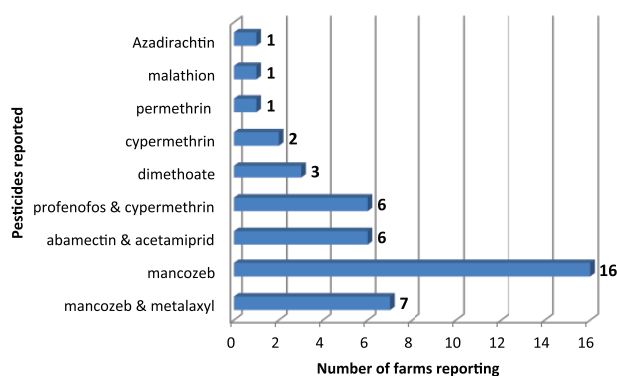


Figure 1. Pesticides reported to be used at the study farms.
Source: Primary data.

EBDC residues in farm samples

Of the targeted 75 samples (from 25 farms), 60 samples (from 20 farms) were collected. Three samples (from 1 farm), however, were deemed unfit for analysis; thus, only 57 samples (from 19 farms) were analyzed. All the 57 samples had detectable concentrations of EBDCs, measured as CS_2 . For each farm, an average EBDC concentration of the 3 samples was calculated, thus 19 mean concentrations for the 19 farms. Based on the calculated mean EBDC concentration, samples from 9 of the 19 farms (47.4%) had EBDC concentrations above the standard MRL of $2\text{ mgCS}_2/\text{kg}$ for dithiocarbamates in tomatoes.³⁰ The most contaminated farm had a mean EBDC concentration of $7.7\text{ mgCS}_2/\text{kg}$ (Appendix 1).

In assessing the potential factors that accounted for the observed EBDC concentration levels and variations, mixing concentration was found to have a positive significant ($P=0.004$ at 0.99 confidence interval [CI]) effect on EBDC concentration. The maximum ($7.7\text{ mgCS}_2/\text{kg}$) and minimum ($0.35\text{ mgCS}_2/\text{kg}$) EBDC concentration levels corresponded with the highest ($150\text{ g}/20\text{ L}$) and lowest ($40\text{ g}/20\text{ L}$) mixing concentrations reported on the farms. The “presampling spray duration,” however, had a negative but nonsignificant ($P=0.26$ at 0.99 CI) effect on the measured EBDC concentration levels.

EBDC residues in market samples

Of the 150 samples (from 25 market vendors), 18 samples (from 3 vendors) were not analyzed as the quality was deemed unfit during laboratory sample preparation. Of the 6 samples from each of the remaining 22 vendors, 3 were processed by washing and the other 3 left unwashed. Then, for each vendor, an average EBDC concentration was calculated for the washed and unwashed samples. All the 132 samples (66 washed and 66 unwashed) had detectable EBDC concentrations. Based on average calculated per vendor, 14% of unwashed samples had EBDC concentration above MRL set by the Codex Alimentarius Commission.³⁰ However, none of the washed samples had EBDC concentration exceeding the MRL (Figure 2 and Appendix 2). As a processing technique,

washing reduced the overall EBDC concentration on tomatoes by a factor of 0.3, calculated as

$$\text{Processing factor (PF)} = \frac{\text{EBDC in washed samples}}{\text{EBDC in unwashed samples}}.$$

Dietary risk assessment

Ethylene bis-dithiocarbamates are considered unlikely to present any acute mammalian toxicity, although they have notable chronic effects.^{19,31} In this study, potential health risk from long-term consumption of tomatoes containing EBDCs was assessed using chronic hazard quotient ($\text{HQ}_{\text{chronic}}$), calculated as the ratio of estimated daily intake (EDI) to the acceptable daily intake (ADI) and expressed as a percentage of the ADI. An ADI of $0.03\text{ mg}/\text{kg}$ body weight was adopted.³¹ In calculating the EDI, a daily per capita consumption of tomatoes of 1.0 g and body weights of 15 kg for children aged 6 and under and 60 kg for the general population were adopted^{21,32}:

$$\text{HQ}_{\text{chronic}} = \frac{\text{Estimated daily intake (EDI)}}{\text{Acceptable daily intake (ADI)}}$$

$$\text{EDI} = \frac{\text{Daily consumption per person} \times \text{pesticide residue in the product consumed}}{\text{body weight}}$$

Two scenarios were considered, that is, consumption of washed and unwashed tomatoes. In both scenarios, EDI was calculated for both children and general population based on the most contaminated tomato sample. -In the first scenario (washed tomatoes), a processing factor of 0.3 was factored into the EDI calculation. In both scenarios, the EDI was less than the reference ADI, that is, the chronic hazard quotient was less than 100%. This therefore means that consumption of fresh washed and unwashed tomatoes analyzed in this study poses no long-term health risk from EBDCs to both children and the general population if the Uganda national per capita daily consumption of 1.0 g of tomato is considered.

Discussion

Results showed that mancozeb was the only EBDC and most used pesticide. This finding is in agreement with another study conducted in the year 2013 in the same geographical location.⁷ Although this study did not investigate what accounts for mancozeb’s popularity among tomato farmers, the 2013 study revealed that 70% and 50% of the farmers intensively applied mancozeb so as to extend the shelf life of the tomato fruits and to attract customers, respectively, but not necessarily to curb any fungal infection. The perception

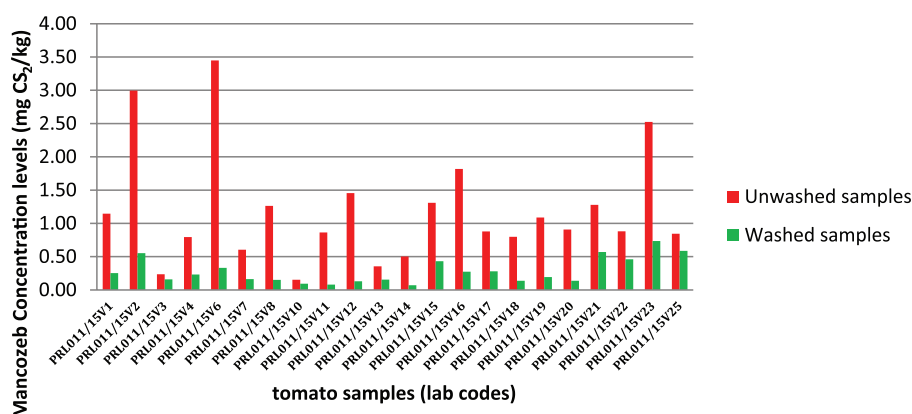


Figure 2. Graph showing ethylene bis-dithiocarbamate levels in washed and unwashed samples from the market.
Source: Primary data.

of mancozeb serving as a preservative was also investigated in 2000 and 2001 by Makerere University and an Integrated Pest Management Collaborative Research Support Program (IPM CRSP) in Uganda whose findings revealed that tomatoes treated with recommended mancozeb concentration of 2.5 g/L were not significantly different from those treated with a concentration of 16.7 g/L with regard to quality parameters of microbial growth, percentage spoilage, firmness, acidity, pH, and total soluble solids. However, there were significant differences between control and treated tomatoes, with the latter showing better quality parameters during the storage period.³³ The aforementioned revelations further indicate the urgency for research and industrial efforts to develop “perishable fruit” preservation methods that pose no health risk to their consumers.

An overall average mixing concentration exceedance rate of 90.8% by 75% of the farms was revealed by this study. This is consistent with findings from earlier studies among Ugandan tomato farmers^{5,6} which show that this practice is usually influenced by farmers’ past experience where adherence to label recommended mixing concentration has not yielded satisfactory application results—a factor that may be attributed to late application, improper application techniques, and application of a counterfeit product among others.^{6,34} The inability of most farmers to read and comprehend pesticide label instructions also accounts for inappropriate mixing as many farmers use arbitrary measures such as table-spoons and bottle tops.^{2,35} The rate of mixing concentration violation exhibited in this study area is alarming and heightens the potential health risks that consumers face as it significantly influences the EBDC residue levels in the tomatoes. This study therefore calls for dedicated efforts to train farmers in Uganda on pesticide label interpretation and the importance thereof, especially mixing concentrations.

The highest EBDC residue level in the present study is higher than residue levels in related studies carried out in other countries.^{5,6,10–16} Exceedance of the MRL by almost half of the tomato samples (47.4%) from the farm demonstrates a major hindrance that Ugandan farmers with intentions to export their produce may face in adhering to

quality standards of the international market. This result also reaffirms what happens in the absence of Good Agricultural Practices (GAP), on which MRLs are based.

Results showed a negative correlation between time and EBDC residues, though not significant. This is because following their application, like other nonpersistent pesticides, EBDCs degrade into other metabolites, whose concentrations continue to reduce with time.¹⁷ In addition, given the nonsystemic nature of mancozeb, rain tends to wash off this contact fungicide from the surfaces where it has been applied. Authors note that since this study was carried out during the rainy season, rainfall experienced during the ‘after spray-presampling’ duration may have influenced the EBDC residues detected by the analysis. Rainfall therefore, is a potential confounding factor to the relationship between EBDC residues, and mixing concentration and ‘afterspray-presampling’ duration. Unfortunately this study was unable to get any reliable rainfall data for the study area to be able to calculate and incorporate the rainfall factor in this paper.

Relatedly, results from this study further revealed that EBDC residues detected in washed and unwashed tomatoes were significantly different, with all the washed samples below the Codex MRL. A processing factor of 0.3 calculated in this study, as a result of washing, was found to be consistent with that submitted to the 2003 Food and Agriculture Organization/World Health Organization Joint Meeting on Pesticide Residues (FAO/WHO JMPR) as noted by a 2006 Brazilian study.¹¹ Relatedly, a Kenyan study¹⁴ that assessed EBDC residues in tomatoes in the form of ETU found no significant differences between raw and cooked washed tomatoes but a rather significant difference between residues in washed and unwashed cooked tomatoes. This result thus shows that washing is a vital EBDC processing technique that significantly reduces dietary exposure to EBDCs and their attendant health effects.

Risk assessment of dietary exposure to EBDCs revealed that per capita daily consumption of 1.0 g of the most contaminated lot of tomatoes analyzed in this study posed no chronic health risk to children and general population of body weights 15 and 60 kg, respectively. This “no long-term health risk” was

so whether tomatoes were consumed, washed or unwashed. It should, however, be noted that assessment of long-term dietary intake takes into account the different foods (and their respective pesticide residues) that constitute a complete diet for a given society. In general, tomato is majorly consumed in Uganda as a spice for different types of sauces. Therefore, the risk assessed by this study is not a reflection of a complete Ugandan diet but rather only a contribution of tomato as a component of the diet. More still, the risk from consuming tomatoes considered in this study is only that of the EBDC (mancozeb) but not all other pesticides used on tomatoes, as reported by the farmers (Figure 1).

Despite the high mixing concentration violation and the resultant high EBDC residues, it is sort of contradictory and unexpected that consuming such tomatoes poses no long-term health risk. This is attributed to the low per capital daily tomato consumption calculated for Uganda by the Global Environment Monitoring System (GEMS)/Food Regional Diets that are based on FAO food balance sheets from selected countries and expert knowledge. Consumption data derived from Food Balance Sheets reflect what is grown in a country plus what is imported, minus what is exported, and then divided by the number of inhabitants.²⁷ This article, however, argues that a 1.0g per capital daily tomato consumption may not convincingly reflect the reality of tomato consumption in Uganda. This is due to the fact that Uganda's agriculture sector is dominated by smallholder farmers whose number is largely unknown due to the informality of their farming activities.³⁶ Import and export data may be satisfactorily compiled, but there is likely to be a gap in tomato production data reported for the whole country. This paper recommends deeper analysis into how the FAO balance sheets reflect country-specific production and consumption realities.

Limitations

This study was unable to access reliable daily rainfall data whose effect on EBDC residues would have been an interesting factor to investigate.

The analytical method used measures the EBDC metabolite CS₂ but not ETU, the metabolite associated with most of the chronic health effects.

This study was only able to assess the risk of EBDCs but not the other pesticides reportedly used by farmers, some of which are acutely toxic. This therefore does not give a comprehensive picture of the potential health risks that consumers of such tomatoes stand.

Amongst majority of Ugandans, tomato is traditionally consumed in cooked form, as a spice for different types of sauce; the effect of cooking on EBDC residues in tomatoes was however not investigated by this study.

Lack of national pesticide residue data for the different crops consumed by Ugandans limited the possibility of conducting a comprehensive total dietary study.

Conclusions

This study revealed that exceeding the manufacturer's recommended mixing concentration greatly contributes to EBDC residues found in Ugandan tomatoes. Washing with water greatly reduces these residues. When the national average per capita daily tomato consumption of 1.0g is considered, the EBDC residues measured by this study pose no chronic health risk to the Ugandan population when taken as part of their diet. There is an urgent need to train Ugandan farmers on Good Agricultural Practices (GAP), especially adherence to pesticide manufacturer instructions. This article strongly recommends the Government of Uganda to safeguard its citizens by establishing a dedicated pesticide residue surveillance directorate.

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Author Contributions

AA, EK, ILM, and EJ conceived and designed this study. AA, EK, and ILM analyzed the data and jointly developed the structure and arguments for the paper. AA wrote the first draft of the manuscript. AA, EK, and EJ contributed to the writing of the manuscript. AA, EK, ILM, EJ, GM, and TH agreed with the manuscript results and conclusions; AA, EK, EJ, GM, and TH made critical revisions and approved the final version. All authors reviewed and approved the final manuscript.

Disclosure and Ethics

As a requirement of publication author(s) have provided to the publisher signed confirmation of compliance with legal and ethical obligations including, but not limited to, the following: authorship and contributorship, conflicts of interest, privacy and confidentiality, and (where applicable) protection of human and animal research subjects. The authors have read and confirmed their agreement with the ICMJE authorship and conflict of interest criteria. The authors have also confirmed that this article is unique and not under consideration or published in any other publication, and that they have permission from rights holders to reproduce any copyrighted material.

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Appendix 1. Presampling spray duration, mixing concentration, and EBDC concentration across the study farms.

FARM NO.	DAYS ELAPSED AFTER SPRAYING BUT BEFORE SAMPLING	MIXING CONCENTRATION, G/20L	VIOLATION OF MIXING CONCENTRATION, %	SAMPLE CODE	EBDC CONCENTRATION, MGCS ₂ /KG			MEAN EBDC CONCENTRATION ±SD
					SAMPLE 1	SAMPLE 2	SAMPLE 3	
1	1	60	20	PRL011/15F1A	3.03	3.27	3.95	3.42 ±0.48
2	5	80	60	PRL011/15F2A	1.30	0.45	0.54	0.76 ±0.46
3	5	89	78	PRL011/15F3A	0.35	2.42	1.72	1.49 ±1.05
4	3	140	180	PRL011/15F4A	2.61	5.48	3.48	3.86 ±1.47
5	7	80	60	PRL011/15F5A	N/A	N/A	N/A	N/A
6	1	70	40	PRL011/15F6A	3.30	2.77	4.09	3.39 ±0.67
7	1	40	-20	PRL011/15F7A	0.44	0.34	0.26	0.35 ±0.09
8	3	150	200	PRL011/15F8A	8.32	6.42	8.36	7.70 ±1.11
9	2	50	0	PRL011/15F9A	1.57	1.87	0.98	1.47 ±0.45
10	5	125	150	PRL011/15F10A	1.97	1.74	2.32	2.01 ±0.29
11	3	120	140	PRL011/15F11A	2.26	2.83	1.78	2.29 ±0.52
12	2	100	100	PRL011/15F12A	2.78	2.47	3.33	2.86 ±0.43
13	3	44	-12	PRL011/15F13A	1.09	1.50	1.18	1.26 ±0.22
14	3	50	0	PRL011/15F14A	0.87	0.82	0.83	0.84 ±0.02
15	7	100	100	PRL011/15F15A	1.06	1.21	1.02	1.10 ±0.10
16	3	107	114	PRL011/15F16A	2.58	3.69	2.01	2.76 ±0.86
17	4	60	20	PRL011/15F17A	0.62	0.57	0.66	0.62 ±0.04
18	3	70	40	PRL011/15F18A	0.83	1.16	0.9974	1.00 ±0.24
19	7	80	60	PRL011/15F19A	1.21	1.44	1.94	1.53 ±0.38
20	2	50	0	PRL011/15F20A	3.63	1.20	3.71	2.84 ±1.43

Abbreviation: EBDC, ethylene bis-dithiocarbamate.

Appendix 2. Dithiocarbamate concentrations detected in washed and unwashed samples from the different markets.

VENDOR	LABORATORY CODE	EBDC CONCENTRATION, MGCS ₂ /KG		PERCENTAGE REDUCTION	PROCESSING FACTOR (FROM WASHING)
		UNWASHED	WASHED		
1	PRL011/15V1	1.15	0.25	77.86	0.22
2	PRL011/15V2	3.00	0.55	81.53	0.18
3	PRL011/15V3	0.24	0.16	32.34	0.68
4	PRL011/15V4	0.79	0.23	70.8	0.29
5	PRL011/15V5	N/A	N/A	N/A	N/A
6	PRL011/15V6	3.45	0.33	90.38	0.10
7	PRL011/15V7	0.60	0.16	73.06	0.27
8	PRL011/15V8	1.26	0.15	88.04	0.12
9	PRL011/15V9	N/A	N/A	N/A	N/A
10	PRL011/15V10	0.15	0.09	38.19	0.62
11	PRL011/15V11	0.86	0.08	90.78	0.09
12	PRL011/15V12	1.46	0.13	91.09	0.09
13	PRL011/15V13	0.35	0.16	56.1	0.44
14	PRL011/15V14	0.50	0.07	85.83	0.14
15	PRL011/15V15	1.31	0.43	67.01	0.33
16	PRL011/15V16	1.82	0.27	84.91	0.15
17	PRL011/15V17	0.88	0.28	68.14	0.32
18	PRL011/15V18	0.80	0.14	82.76	0.17
19	PRL011/15V19	1.09	0.19	82.24	0.18
20	PRL011/15V20	0.91	0.14	84.71	0.15
21	PRL011/15V21	1.28	0.57	55.34	0.45
22	PRL011/15V22	0.88	0.46	47.68	0.52
23	PRL011/15V23	2.52	0.74	70.86	0.29
24	PRL011/15V24	N/A	N/A	N/A	N/A
25	PRL011/15V25	0.85	0.59	30.43	0.70
Average		1.19	0.28	70.46	0.30

Abbreviation: EBDC, ethylene bis-dithiocarbamate.