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MONITORING OF DITHIOCARBAMATE (EBDC) RESIDUES IN FRUITS AND VEGETABLES THROUGHOUT 1995-1999 AND ESTIMATION OF THEIR DAILY INTAKES IN EGYPT

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Abstract

A total of 1414 samples of common commodities were collected from eight Egyptian local markets located in 6 Governorates throughout 1995-1999. The results showed that 89.1% of the samples had no detectable dithiocarbamate residues. 10.9% contained detectable residues from which 0.28% exceeded their MRL's. The effect of home processing on EBDC residues detected on and in treated samples of tomato, cucumber and eggplant was also studied. The data demonstrated that cooking of unseived tomato juice from washed raw tomatoes is the most effective process; since it removed 77.8% of the detected EBDC residues. Peeling after washing cucumber was the most effective process in removing EBDC because it removed almost all residues. Frying of peeled-washed eggplant is expected to reduce most of EBDC residues. Daily intake of dithiocabamates was also studied considering the results of monitoring of pesticides residues in different fruits and vegetables. The obtained results revealed that the estimated daily intake (EDIs) for EBDC 's resulting from vegetables, cooked vegetables and fruits was 1.44ug / person / day representing 4.8 % of its ADI.

INTRODUCTION

Ethylenebisdithiocarbamates (EBDCs) are a group of fungicides very effective in controlling fungus diseases such as powdery mildew infesting vegetable crops (Tweedy *et al.*, 1973).

As a continuation of the previous market basket survey conducted by Gad Alla *et al.* (1996), a market basket survey was done in six governorates to asses dithiocarbamate residues in different fruits and vegetables. 1414 samples were collected from eight local markets including leafy vegetables, vegetables and fruits of special importance in Egyptian human diet .The obtained data were used in calculating the daily intakes of dithiocabamate residues. The effect of the different home processing steps on EBDC residues actually detected in the treated samples was also studied.

MATERIALS AND METHODS

1. Monitoring studies

Sampling : A total of 1414 samples of common consuming commodities were collected from eight Egyptian local markets located in 6 Governorates (Cairo - Giza - Qualubiya - Beni suef - Minufiya - Ismailia) throughout 1995-1999. The number of samples analyzed from each commodity is presented in Table 1. A sample from each commodity was prepared according to a generally official recommended method of the European Committee ECS (1992). The analysis of samples was carried out immediately after their arrival to the laboratory. Test samples were essentially analyzed immediately after cutting to avoid the decomposition of EBDC compounds.

2. Home Processing : Since commercial samples do not contain considerable concentrations of ethylenebisdithiocarbamate residues that would allow reasonable detection the decrease in EBDC residue levels due to home preparation, cooking and processing steps, supervised laboratory treatments were performed using the EBDC fungicides recommended for use in green houses (Pest Control Programme For Field Crops, 1993). A tomato sample was dipped for 15 min in solution of mancozeb (80%) at the rate of 5 gram dithan/1l tap water. Cucumber and eggplant samples were dipped for 15 min in solution of the formulated mancozeb (80%) at rate (5 gram mancozeb /1l tap water). The sample was left to dry for an hour and then divided into portions for processing and analysis. Raw samples were also subjected to analysis.

a. Tomato samples : A portion of treated tomato was washed for 5 min in running tap water, then a portion of the washed sample was subjected for analysis. Washed tomato sample was cut into small pieces, chopped and completely homogenized in a warring blender for 5 min. 100 gram of sieved tomato juice and tomato skin were taken for analysis. Another portion of unsieved tomato juice was boiled at 100 ºC for 120 min. then 100 gr of cooked paste were taken for analysis.

b. cucumber samples : A portion of raw cucumber sample was washed for 5 min. using running tap water, dried in air then subjected to analysis. Another portion of washed cucumber was peeled. Both peeled fruits and cucumber peels were subjected to analysis.

c. Eggplant samples : Raw eggplant sample was washed with running tap water for 5 min., dried in air then subjected to analysis. A washed sample was peeled, each of pulp and peels were subjected to analysis. A portion of washed eggplant (without peeling) sample was fried using cottonseed oil at 170-190 ºC for 15 min. and then subjected to analysis.

Chemicals and Reagents

Solvents and chemicals

- Ethanol 95-96%
- Diethanolamine 98 %
- Hydrochloric acid, concentrated
- Toluene
- Carbon disulphide
- Anhydrous sodium sulphate (Riedel-de Haen)
- Sodium hydroxide
- Copper (II) acetate monohydrate 98 %
- Tin (II) chloride dehydrates
- Sodium diethyldithiocarbamate not less than 95%

Apparatus : Spectrophotometer UV: double beam Unicam SP 1800.

Chemical Analysis : The method selected for analysis is based on the carbon disulphide evolution procedure as modified by keppel (1969,1971) and recommended by ECS, 1992. Residues of dithiocarbamates should be expressed as CS2 (Carbon Disulphide) in order to compare it to the Codex Maximum Residue levels established on CS2 basis. In this method carbon disulphide originates from dithiocarbamates by heating with hydrochloric acid in the presence of stannous chloride as a reducing reagent. The evolved CS2 is distilled, purified and collected in the ethanolic solution of copper (II) acetate and diethanolamine to form a yellow complex. The absorbance of the reaction product is spectrophotometrically determined at 435 nm.

Recoveries percentages of ethylenebisdithiocarbamates (EBDC's) at different levels of fortification, 0.1,1,10 mg/kg from cucumber, tomato, eggplant were previously studied and they ranged between 80-110 % .The relative standard deviation was less than 20% and the limit of determination was 0.2 mg/kg (Gad Alla *et al.* 1996).

Quality assurance procedure : The analytical method and instrument used were fully validated as mentioned in ISO guide 25 quality assurance system. The performance of the method is continuously tested with fortifying blank sample with sodium diethyldithiocarbamate with every set of samples and is analysed as a normal sample. The results of control samples were collected into control charts.

RESULTS AND DISCUSSION

1. Monitoring studies : A total of 1414 samples of different types of fruits and vegetables were subjected to dithiocarbamates analysis throughout 1995-1999. The survey included leafy vegetables, vegetables and fruits, Table 1. Fifty-five leafy vegetable samples were analysed including grape leaf, lettuce, molokhai and spinach. 924 vegetable samples included cantaloupe, cucumber, eggplant, green beans, green peas, pepper and tomato. Dithiocarbamates were also examined in 351 fruit samples of apple, grape, lemon lime, peach, pear and strawberry. 89.1% of the samples had no detectable dithiocarbamate residues. 10.9% contained detectable residues with 0.28% exceeding MRL's, Table 1. Codex Alimentarious Maximum Residue Limits were followed, as those limits were available. Apricot, carrot, fig, guava, mango, orange, plum, potato and squash samples were free from dithiocarbamate residues, Table 2. Both contamination and violation rates decreased gradually thoughtout the five years of the investigation to reach in 1999 the lowest contamination rate (1.5%), Fig.1. That might be due to the restricted use of such pesticides during 1995-1999 period.

Both leafy vegetables and vegetables showed lower contamination rates (9.1%) than fruits (18.5%). However, vegetables showed relatively highest violation rates (3 samples out of 924, 2 cucumber and one green beans samples).

Comparing the current results with those previously studied in 1993 by Gad Alla *et al.* (1996), the present results revealed lower contamination and violation rates indicating good application of agricultural practices, lower usage of dithiocarbamates and tendency to apply new categories of pesticides.

Repley *et al.* (2000) found that EBDC fungicides were the most frequent pesticides in the Canadian monitoring survey during 1991-1995. They found that overall 32.1 % of all analysed fruit and vegetable samples were contaminated with EBDC residues. The rates of contamination of fruits and vegetables were 32.5% and 31.8 %, respectively. The violation rate of vegetables was 2.4%. No violation was observed in fruit samples.

2. Home Processing Studies : Table 3 demonstrates the effect of home processing on EBDC residues detected on and in treated samples.

Tomato samples : The effect of the different home preparation steps expressed as % retained and % removed from the EBDC residues is demonstrated in Table 3. Washing of tomatoes removed 21% of the residues deposited on the fruits. Such data are in accordance with the findings of Marshall *et al* (1979) who reported that 35% of dithan M-45 residues was removed after four washing times. Juicing and sieving tomatoes from washed fruits eliminated 55.6% of the initial deposits on raw tomato. Residues detected in tomato juice might be attributed to the contact between the waxy skin of tomatoes and the interior parts during processing into juice. The data also demonstrated that cooking of unseived tomato juice from washed raw tomatoes is the most effective process since it removed 77.8% of EBDC of the detected residues. It is worthy to mention that heating and boiling fresh tomato although lead to degradation of EBDC residues might increase the harmful product ethylenethiourea (ETU).

Cucumber samples : Cucumber is mainly consumed as a fresh product. It is not subjected to thermal treatments, which accelerate EBDC degradation. Table 3 demonstrates the behaviour of EBDC residues on and in treated cucumber due to washing and peeling processes that are common home practises. Data in Table 3 also revealed that most of EBDC residues were detected in the peels, while only 11.4% of the residues migrated into the pulp. This shows the importance of washing and peeling processes. These findings are in agreement with Calumpag *et al.* (1998) who observed that washing of cucumber reduced EBDC residues by 50% and peeling removed almost all of EBDC residues.

Eggplant samples : Eggplant is subjected essentially to home processing and cooking before consumption. The effect of washing, peeling and frying on EBDC residues was also studied. It seems that peeling of the washed eggplant is crucial in removing 93% of EBDC residues. High percentage of EBDC retained on the skin while only 7.3% of the total amount of EBDC residues migrated into the pulp. Frying process of washed-peeled eggplant is expected to reduce almost all EBDC residues.

As shown in Table 3, the sum of EBDC residues in peels and pulp obtained from washed cucumber and eggplant samples were higher than that found in washed raw sample. This is because the 100-gram of peels sample was collected from a higher amount of unpeeled sample and that most of EBCD residues were found on the peels. The residues were calculated on peels weight basis not on whole commodity basis.

3. Dietary intake study : The Estimated Daily Intakes (EDI) is the prediction of pesticide residue intake in mg of pesticide / person / day. It is calculated by multiplying the average pesticide residue level in each commodity by the respective average food consumption using data on the edible portion of the commodity and takes into account the effect of the preparation, processing and cooking of the food (FAO/WHO) using the equation,

$$EDI = \Sigma Fi \times Ri \times Ci. \times Pi, \text{ where}$$

Fi = the food consumption of the relevant commodity in Kg of food /person/day.

Ri = the residue level in edible portion of the commodity given in mg of pesticide per Kg of food.

Ci = a correction factor that takes into account the reduction or increase in the level of residue on preparation or cooking of the food.

Pi = a correction factor that takes into account the reduction or increase in the residues on commercial processing, as canning or milling.

The study was conducted throughout 1995-1999 and mainly considered the results of monitoring of dithiocabamates residues in different fruit and vegetable items in relation to food consumption data of each. The food consumption data was obtained from the Nutrition Institute of the Ministry of Health in 1980 expressed as grams / person/ day. The effect of home cooking and processing that had been studied here on treated samples had led to reduction factors affecting the actual residues assessed in raw food items, Table 4. All previous data were used to calculate the EDI, which was compared to the acceptable daily intake (ADI).

The intake figures were divided by an assumed body weight of 60 Kg in order to compare the estimated daily intakes (EDI) with the acceptable daily intakes (ADI). The

ADI that has been established by Codex Alimentarious for dithiocabamates is 30-µg/kg bw/day. Table 4 demonstrates the EDI's calculated for EBDC residues actually detected in samples as µg/person/day. The comparison showed that the dietary intakes of EBDC resulting from fresh vegetables, cooked vegetables and fruits was 1.44 µg / person / day representing 4.8 % of its ADI. Thus, exposure of to consumer to EBDC through daily diets was far below the ADI.

Table 1. minimum, maximum, Mean and 90 th percentile in ppm as well as number and percentages of contaminated samples, violated samples and % of occurrence of dithiocabamates residues monitored in samples collected from local markets during 1995 - 1999.

Commodities	Total No. of analysed samples	Sample contam.		Min. ppm	Max. ppm	Mean ppm	90th (1) Percentile	MRL`s (2) (mg/kg)	Samples Violated	
		no.	%						no.	%
Leafy Vegetables										
Grape leaf	7	1	14.3	0.7	0.7	0.7	-	**	-	-
Lettuce	43	1	2.3	0.26	0.26	0.26	-	5	-	-
Molokia	3	2	66.6	0.14	0.18	0.16	-	**	-	-
Spinach	2	1	50	0.68	0.68	0.68	-	**	-	-
Total No. of leafy veg.	**55**	**5**	**9.1**							
Vegetables										
Cantaloupe	38	3	7.9	0.14	0.21	0.18	-	1	-	-
Cucumber	211	27	12.8	0.1	3	0.31	0.49	0.5	2	0.94
Egg plant	142	2	1.4	0.2	0.26	0.23	-	3 *	-	-
Green beans	45	3	6.7	0.12	0.3	0.19	-	0.5	-	-
Geen Peas	65	3	4.6	0.14	0.66	0.36	-	0.5	1	1.54
Pepper	183	14	7.7	0.11	0.7	0.42	0.69	1	-	-
Tomato	233	31	13.3	0.1	0.71	0.32	0.54	3	-	-
Total No. of veg.	**924**	**84**	**9.1**						**3**	**0.32**
Total No. of all veg.	**979**	**89**	**9.1**						**3**	**0.3**
Fruits										
Apple	98	6	6.1	0.1	0.34	0.33	-	3	-	-
Grape	117	37	31.6	0.1	6.8	1.04	2.44	5	1	0.85
Lemon Lime	5	1	20	0.66	0.66	0.66	-	2 *	-	-
Peach	48	9	18.8	0.1	2.8	1.12	-	3	-	-
Pear	4	1	25	0.2	0.2	0.2	-	3	-	-
Strawberry	79	11	13.9	0.1	1.2	0.44	0.8	3	-	-
Total No. of Fruit	**351**	**65**	**18.5**						**1**	**0.28**
Total No. of all samples	***1414****	***154***	***10.9***						***4***	***0.28***

1. 90th percentile not calculated as the frequency is less than 10 samples

2. MRL 's issued by CCPR Codex Committee of Pesticide Residues 1993 ,1999

* MRL's are extrapolated , ** No MRL available, *** These number includes the No. of different samples, Table 2

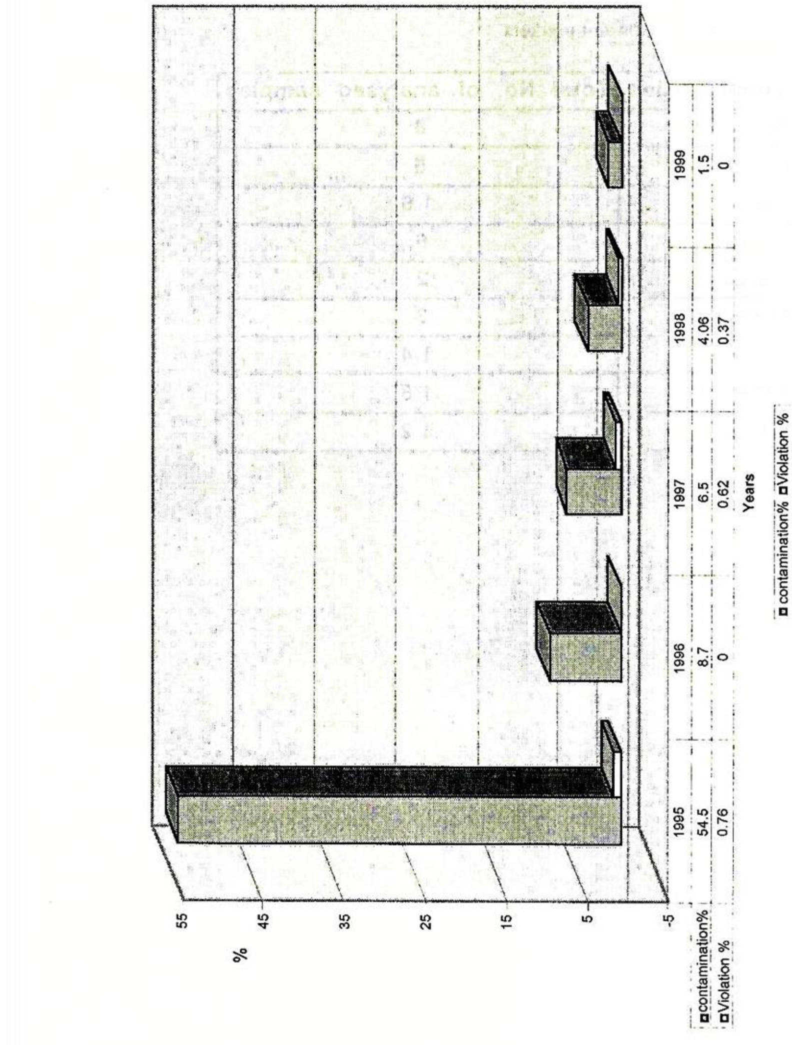

Fig 1. Contamination and violation percentages of dithiocarbamates in samples analysed throughout 1995-1999

Table 2. Commodities free from dithiocarbamates in samples collected throughout 1995-1999 from different markets

Commodities	Total No. of analysed samples
Apricot	8
Carrot	5
Fig	16
Guava	6
Mango	2
Orange	3
Plum	14
Potato	18
Squash	12

Table 3. Effect of home processing on total EBDC residues in treated samples

Processing Technique	EBDC mg/kg	% EBDC Retained	% EBDC Removed
Tomato			
Raw (after treatment)	1.8	100	0
- Washing			
Washed tomato	1.42	78.9	21.1
- Squeezing			
Tomato Juice*	0.8	44.4	55.6
Tomato Peels	0.86	47.8	52.2
- Cooking			
Tomato Paste	0.4	22.2	77.8
Cucumber			
Raw (after treatment)	7.47	100	0
- Washing			
Washed cucumber	3.98	53.3	46.7
-Peeling			
Pulp of cucumber	0.85	11.4	88.6
Peels of cucumber	7.14	95.6	4.42
Egg Plant			
Raw (after treatment)	7.68	100	0
- Washing			
Washed egg plant	3.9	50.8	49.2
- Peeling			
Pulp of egg plant	0.56	7.3	92.7
Peels of egg plant	7.14	93	7.3
- Frying			
Fried egg plant	1.98	25.8	74.2

n =3 where n represents average of three replicates

* After sieving

Table 4. Calculation of estimated daily intakes of dithiocarbamates residues detected in samples collected from different governorates throughout 1995-1999.

Commodities	Food consumption g/day	Reduction factor	Mean mg/kg	Estimated daily intakes (EDI)[1] µg/day
1. Vegetables eaten fresh	45			
Lettuce		-	0.26	10.62
Cantaloupe		-	0.18	
Cucumber		0.533 (washing)	0.31	
Pepper		0.789 (washing)	0.42	
Tomato		0.789 (washing)	0.32	
2. Cooked vegetables	113			
Grape Leaf		-	0.7	35.1
Molokia		-	0.16	
Spinach		-	0.68	
Egg Plant		0.131 (washing+frying)	0.23	
Green beans		-	0.19	
Green peas		-	0.36	
Tomato		0.175 (washing+Cooking (tomato paste)	0.32	
3. Fruits	65			
Apple		-	0.33	41.0
grape		-	1.04	
Lemon Lime		-	0.66	
Peach		-	1.12	
Pear		-	0.2	
Strawberry		-	0.44	
Total				**86.7**

Estimated daily intakes (EDI)[2] µg/person/day	**1.44**
Estimated daily intakes as % ADI[3]	**4.8**

[1] EDI (µg/day)= mean of (mean x reduction factor) x food consumption

[2] EDI= Total EDI / 60 kg

[3] ADI = 30 µg/kg bw maneb , mancozeb, metiram & zineb

REFERENCES

1. Calumang , S.M.F., M.J.V Barredo and E.D. , Magallona. 1988. Ethylenebisdithiocabamate fungicide residues in processed cabbage and cucumber, Philippine Agricultuist 4 (71): 397-401 .

2. Codex Committee on pesticide residues, CCPR 1995-1999. Joint FAO/WHO Food Standards Programme.

3. ECS. 1992. European Committee for Standardisation , Doc. CEN/TC 275/WG 4 N 46.

4. UNEP / FAO / WHO.Guidelines for the study of dietary intakes of chemical contamination. Geneva, World Health Organization, 1985 (WHO Offset Publication No. 87).

5. Keppel, G.E. 1969. Modification of the carbon disulphide evaluation method for dithiocarbamate residues J. Association. of Official Analytical Chemistry: 162-167.

6. Keppel, G.E. 1971. Collabborative study of the determination of dithiocarbamare residues by a modified carbon disulphide evaluation method, J. Association. of Official Analytical Chemistry 54(3): 181-186.

7. Marshall, W.D.and R.J.William. 1979. Procedures for the removal of field residues of ethylenebisdithiocarbamates funjicides and ethylenethiourea from tomatoes perior to processing into juice. J. Agric. Food Chem. 27(4): 766-769.

8. Repley B.D., L.I. Lissemore ,P.D. Leishman,M.A. Denomme and L. Ritter 2000. Pesticide residues on fruits and vegetables from Ontario, Canada, 1991-1995. J. Association. of Official Analytical Chemistry 83(1) : 196-213.

9. Gad Alla ,SohairA.,H. Hasaneen, Fahmy ,Safaa M. and Khorshid Mona A. 1996. Monitoring of dithiocarbamate fungicides in some vegetables J. Agric. Res., 4: 74.

10. Tweedy, B.G. 1973. Symposium on origin and fate of ethylenethiourea fungicides, J. Agric. Food Chem., 21:323-326.

تقصى مستوى مركب داى ثيوكرباماتات فى الخضر والفاكهة وتقدير المتناول اليومى لها خلال الفترة ١٩٩٥-١٩٩٩

سهير احمد جاد الله احمد

المعمل المركزى لتحليل متبقيات المبيدات والعناصر الثقيلة فى الاغذية - مركز البحوث الزراعية - الدقى - الجيزة

اجريت الدراسة على ١٤١٤ عينة من الخضر والفاكهة الاكثر استهلاكا من ٨ اسواق محلية وتشمل ٦ محافظات مختلفة خلال الفترة من ١٩٩٥ - ١٩٩٩ .

اثبتت النتائج خلو ٨٩,١ ٪ من مجموع ما تم تحليله من متبقيات مركبات داى ثيوكرباماتات، بينما وجد أن ١٠,٩ ٪ تحتوى على متبقيات هذه المجموعة منها ٠,٢٨ ٪ فقط تعدت الحدود القصوى المسموح بها.

تم دراسة اثر عمليات التجهيز والطهى المنزلى على متبقيات مركبات داى ثيوكرباماتات على ثلاثة محاصيل هى الطماطم والخيار و الباذنجان نتيجة معاملة هذه المحاصيل بمركبات داى ثيوكرباماتات.

أوضحت النتائج أن عملية طهى عصير الطماطم المغسولة وغير المصفاة هى الاكثر فاعلية فى التخلص من متبقيات مبيدات هذه المركبات، حيث يتم التخلص من ٧٧,٨ ٪من متبقيات داى ثيوكرباماتات.

أظهرت النتائج أن عملية تقشير الخيار بعد غسله تؤدى الى التخلص من معظم متبقيات داى ثيوكرباماتات. كما أثبتت الدراسة ان عملية قلى الباذنجان بعد تقشيره وغسله تؤدى الى التخلص من الاثر المتبقى لمركبات داى ثيوكرباماتات.

تم حساب المتناول اليومى من مركبات داى ثيوكرباماتات باستخدام نتائج تقصى متبقيات داى ثيوكرباماتات فى الخضر والفاكهة فى الفترة من ١٩٩٥ - ١٩٩٩ . أوضحت النتائج ان المتناول اليومى المحسوب لمتبقيات مركبات داى ثيوكرباماتات فى الخضروات الطازجة والمطبوخة والفاكهة ١,٤٤ ميكروجرام / للفرد / اليوم يمثل ٤,٨ ٪ من المتناول اليومى المسموح به.