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ETHYLENE-BIS- DITHIOCARBAMATES RESIDUES IN SOME FOOD AND THE POTENTIAL RISK FOR EGYPTIAN CONSUMERS

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ABSTRACT

Dithiocarbamates are a non-systemic group of Fungicides widely used to protect crops from fungal diseases. The current methodology used by monitoring laboratories to determine dithiocarbamates in food involves the analysis of CS_2 generated after hydrolysis of the compound present in the sample. A total of 535 food samples (apple, apricot, cantaloupe, cucumber, grape, grape leaf, green beans, green broccoli, green peas, lettuce, onion, orange, peach, pepper, plum, potatoes, squash, strawberry, tomato and watermelon) collected from eight Egyptian local markets located in six governorates (Cairo, Giza, Qalubiya, Beni Suef, Minufiya, and Ismalia) through the period from (2007 to 2008), were analyzed for dithiocarbamate content. Overall, results showed that 413 samples (i.e. 77.2 %) of total number of samples analyzed (535) were free from dithiocarbamate expressed as CS_2. However, 122 samples (i.e. 22.8 %) of the total no. of all samples analyzed were contaminated with detectable levels of dithiocarbamate residues of which 1.3% exceeded the MRL of dithiocarbamate expressed as CS_2. The concentration levels of dithiocarbamate expressed as CS_2 ranged from the lowest level (0.2 mg/kg CS_2) to the highest levels (5.09 mg/kg). The highest violated samples were onion, followed by green peas, and pepper. An exposure assessment, based on dithiocarbamate levels detected in the food crops analyzed in this study, confirms that the intake of dithiocarbamates through food consumption in the country does not represent a health risk to consumers, i.e., the estimated daily intake is less than the acceptable daily intake. Furthermore, the implementation of more selective methodologies to individually analyze these compounds in food monitoring programs in Egypt is not necessary.

Keywords: Dithiocarbamate fungicides; Food analysis; chronic dietary risk assessment

INTRODUCTION

Dithiocarbamate pesticides are used all over the world because of their low acute toxicity combined with strong activity and low cost of production. These compounds are also the most frequently detected pesticides in monitoring programs worldwide (EU, 2003; Dogheim *et al.*, 2002). Dithiocarbamates are a non systemic group of pesticides widely used in agriculture as, insecticides, herbicides, and fungicides. Dithiocarbamates can result in neuropathology, thyroid toxicity and developmental toxicity to the central nervous system of laboratory animals (EPA, 2001; IPCS,1993). The ethylene-bis-dithiocarbamate (EBDC) mancozeb was considered to be a multipotent carcinogenic agent in a long-term rat study (Belpoggi *et al.*, 2002). Ethylenethiourea (ETU), formed by the degradation and metabolism of EBDCs present in foods, inhibits thyroid peroxidase and thus induces thyroid cancer in laboratory animals (Doerge and Takazawa, 1990). Ethylene- Bis-

Dithiocarbamates (EBDC's) are the most important fungicides class, where it was found that the EBDC are effective in controlling fungus diseases such as Powdery mildew, downy mildew and late blight. In Egypt, such pathogens are infesting vegetables and fruits causing damage especially in the worm humid climate, with increased effect in green house, which necessitates more frequent application of EBDC's. The residues of EBDC's, on its application on the vegetables and fruits, residue on the outer surface (most of them), so it can be removed by washing (50%) and peeling process (most of all). The problem characterizing EBDC's residues in foods is the degradation to more hazardous products (ETU), which has carcinogenic effect, especially on the thyroid gland, and mainly resulting during the cooking process due to the thermal effect. As a continuation of the previous market basket survey conducted by S.A.Gad Alla (2002), a market basket survey was conducted in six governorates to assess dithiocarbamate residues in different fruits and vegetables. The current study was conducted starting from 2005 to 2008, in which a total of 535 samples of different commodities of special importance in the Egyptian human diet, were collected from eight local markets located in six governorates. Using the resulting residue data of this monitoring program and food consumption data obtained from GEMS food consumption (Cluster diets, 2006). A probabilistic estimation of the exposure to dithiocarbamates in the Egyptian population was performed.

MATERIALS AND METHODS

Sampling:

A total of 535 samples of common consuming commodities were collected from eight Egyptian local markets located in six governorates (Cairo, Giza, Qalubiya, Beni Suef, Minufiya, and Ismalia) through the period from 2005 to 2008. 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. The test samples were essentially analyzed immediately after cutting to avoid the decomposition of EBDC compounds.

Chemicals and reagents:

- Ethanol , mass concentration at least 95%
- Diethanolamine , mass concentration at least 98%
- Hydrochloric acid, concentrated , p(HCl)=1.16g/ml.p
- Toluene
- Carbon disulphide, colorless , mass concentration at least 99%
- Anhydrous sodium sulphate (Riedel Haen)
- Sodium hydroxide ,p(NaOH)=100 g/l
- Copper (II) acetate monohydrate 98%
- Tin (II) chloride dehydrates ,p($SnCl_2.2H_2O$)=40g/100ml in conc. HCl acid
- Sodium diethyl dithiocarbamate not less than 95%
- **Methodology*:***

Dithiocarbamate fungicides were determined by the spectrophotometric determination of the cupric complex formed with the CS2 evolved from the acid decomposition of dithiocarbamates in the presence of stannous chloride as a reducing agent using either the in-series-2 trap reaction system (Cullen, 1964; Keppel, 1971). The solution of the complex formed from the reaction between CS_2 and the copper (II) acetate monohydrate was measured at 435 nm in spectrophotometer UV (double beam Unicam SP 1800). The results are expressed in mg CS_2/kg.

Quality Assurance procedures:

All analytical methods and instructions were carefully validated as a part of the laboratory quality assurance system and were audited and accredited by the Center of Metrology and Accreditation Finnish Accreditation Service (FINAS) according to ISO/IEC Guide 17025. The criteria of quality assurance were described by Dogheim *et al.* 2002. Recoveries percentages of ethylenebisdithiocarbamates (EBDC's) at different levels of fortification, 0.1, 1, and 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 0.1 mg/kg *(*Sohair.A . Gad Alla 2002*).*

The Theoretical Maximum Daily Intake (TMDI) was calculated using the codex maximum residue limits (MRL), (2005), in (mg CS_2/kg), for dithiocarbamates and the GEMS food consumption (Cluster Diets, 2006) data, C, in kg/day/ body weight, based on a 60 kg person (WHO, 1997). The ethylene bis - dithiocarbamates mancozeb, maneb and metiram (EBDC) were grouped for the assessment. The calculated TMDI as (dithiocarbamate) was compared with the acceptable daily intake (ADI) for the compounds, (Codex, 2005), and expressed as % ADI.

$$TMDI = (\sum MRLi \times Ci) \times Fi$$

$$\%\ ADI = \frac{TMDI}{ADI} \times 100$$

A molecular weight factor (F) of 1.77 was used to transform the intake as CS_2 to EBDC (average of the factors of 1.78, 1.74 and 1.79 for mancozeb, maneb and metiram, respectively).

The estimation of the exposure to dithiocarbamates in the Egyptian population was performed, using recent residue data generated by the monitoring program and food consumption data obtained from GEMS food consumption (cluster diets, 2006) data,.

A refinement in the theoretical Maximum Daily Intake (TMDI) in the Egyptian population was made by substituting the maximum residue limits (MRL) with the mean residue levels found in the crops analyzed in this study generated by the monitoring program and food consumption data obtained from GEMS food.

RESULTS AND DISCUSSION

Residues of dithiocarbamates in food commodities:

A total of 535 samples of different food groups, (leafy vegetables, fruits and vegetables) collected from eight Egyptian local markets located in six governorates during the period from January 2007 to December 2008. All samples were subjected to EBDC analysis (see table 1).

Table (1): Dithiocarbamates, as CS2, in different commodities consumed in Egypt:

Commodity	Total no. of samples analyzed	Contaminated samples of each type		Free samples of each type		Conc. Range of samples Min.- max	Mean[b] (mg/kg)	MRL[(c,d)] (mg/kg) (CS2)	Violative samples	
		No.	%	No.	%				No.	%
1. Leafy vegetables Grape leaf	32	16	50	16	50	0.47- 3.1	0.31	10	-	
Lettuce	10	3	30	7	70	0.11 - 0.146	0.11	10	-	
Total	42	19	45.2	23	54.8					
Vegetables										
Cucumber	60	18	30	42	70	0.118 - 0.74	0.16	2	-	
Onion	73	9	12.3	64	87.7	0.21 - 1.16	0.22	0.5	4	5.5
Pepper	54	18	33.3	36	66.7	0. 94-1.04	0.23	1	1	1.9
Squash	14	2	14.3	12	85.7	0.114-0.17	0.11	1	-	
Strawberry	6	6	100	0	0	< 0.1	< 0.1	5	-	
Tomato	62	20	22.6	42	67.7	< 0.1 -0.92	0.19	2	-	
Green beans	35	6	17.1	29	82.9	0.107- 1.33	0.17	2	-	
Green Broccoli	6	1	16.7	5	83.3	< 0.1 - 0.553	0.18	5	-	
Green Peas	42	11	26.2	31	73.8	0.2 - 4.33	0.33	2	2	2.8
Total	352	85	24.1	267	75.9				7	1.99
Root and tubers Potato	10	-	-	10	100	-	-	-	-	-
Fruits										
Apple	14	1	7.1	13	92.9	< 0.1– 0.550	0.16	2	-	
Apricot	7	1	14.3	6	85.7	< 0.1- 5.09	1.35	7	-	
Cantaloupe	18	2	11.1	16	88.9	< 0.1 - 0.13	< 0.1	0.5	-	
Grape	34	3	8.8	31	91.2	0.22- 0.94	0.15	5	-	
Orange	3	0	0	3	100	< 0.1	< 0.1	2	-	
Peach	19	4	21.1	15	78.9	0.14 - 1.11	0.2	7	-	
Plum	5	0	0	5	100	< 0.1	< 0.1	7	-	
Water Melon	31	7	22.6	24	77.4	0.12-0.18	0.11	1	-	
total	131	18	13.7	113	86.3					
Total	535	122	22.8	413	77.2				7	1.3

Mean[b] (to calculate the mean, the samples had concentration levels at < LOQ were considered being equal to LOQ (0.1 mg/kg).
[c] Codex (2005)
[d] coming from the use of mancozeb (M), maneb(Mb), and metirm (Mt).

Overall, results reveals that 413 samples (i.e. 77.2%) of the total no. of all samples analyzed (535) were free from any detectable residues of dithiocarbamate expressed as CS_2.

Also results showed that 22.8 %(122 samples) of the total number of all samples analyzed were contaminated with detectable residues of dithiocarbamate expressed as CS_2. of which 1.3% of all samples analyzed contained levels exceeded the MRL's established for dithiocarbamate expressed as CS_2 by codex (2005) .The most violated samples were onion, green peas and pepper.

1) Leafy vegetables:

Tables (1) showed that a total of 42 samples of the grape leaf and lettuce were analyzed, results showed that 23 samples (i.e. 54.8%) were free from any detectable residues. However, the 45.2% of samples contained detectable levels above LOQ (0.1 mg/kg). In concentration levels ranged from 0.11 to 3.1 mg\kg. No exceeding of levels above the MRL of dithiocarbamate expressed as CS2 established by codex (2005).

Results showed that grape leaf recorded the highest concentration percentage (i.e. 93.8% of the total number of all samples analyzed), in concentration range varied from 0.47 to 3.1 mg\kg.

2) Vegetables:

Data demonstrated that 352 samples of different types of vegetables wereanalyzed to demonstrate residues of EBDC. Results showed that 75.9 % of all samples analyzed were free from EBDC residues. However, 24.1% samples of the samples were contaminated with detectable amounts of the dithiocarbamate residues of which (1.99%) exceeded the MRL established for dithiocarbamate expressed as CS_2. The concentration range varied from 0.1 to 4.33 mg/kg. The violated samples were onion, green peas and pepper. The onion recorded the highest violation percentage (5.5%) followed by green peas (4.8%) and pepper (1.9%)

Root and Tubers:

Only Ten Potato samples were subjected to EBDC analysis. Results showed that all analyzed samples were free from any detectable amount of EBDC residues.

Fruits:

A total of 131 of different fruit samples were analyzed, result showed that 113 samples (i.e. 86.3%) were free from EBDC residues, However, only 18 samples contained detectable levels higher than LOQ, contaminant percentage is (i.e. the 13.7 %).without exceeding above the MRL's.

On comparison this results with previously reported by Dogheim *et. al*., (1999), the percentage of samples in this study with detectable levels of dithiocarbamates was higher than previously reported results, in which 98 fruit and vegetable samples collected from eight local markets and examined for dithiocarbamates residues, 70.4% of samples were contaminated with dithiocarbamates and also, the violation percentage showed slight increase than the previous study , in which only grape sample had residues exceeding the MRL established by the Codex Committee on Pesticide Residues.

In this study, the most contaminated samples containing detectable residues, as CS_2, were found in apricot, green peas, and grape leaves samples. The onion recorded the highest violation percentage (e.g. 5.5% of the total number of all sample analyses), followed by green peas (e.g. 2.8% of the all analyzed sample), and pepper (e.g. 1.9%). Dithiocarbamates are non-systemic fungicides and only very small amounts of pesticide are expected to translocation from the surface to the inner portion of the treated fruit after foliar application (FAO, 1994). The probability of contamination of the pulp from the skin during the peeling process cannot be disregarded. However, even if contamination occurs in some cases, it does not justify the high CS2 residue levels found in the pulp in the present study. Some authors have raised the hypothesis that CS2 residues in non-treated plant come from the sulfur compounds present in the seeds. Carbon disulfide and other sulfur compounds can be found naturally in the atmosphere, soil and some vegetables. CS_2 not related to the use of the dithiocarbamate fungicides has been described in the head space atmosphere of lettuce (Batten et al., 1995), in homogenates of fresh mushrooms (Chen and Ho, 1986) and in the volatile oils found in the stems of cabbage, broccoli and cauliflower (Buttery et al., 1976).

Chronic dietary risk assessment:

In codex, 2005, a dithiocarbamate MRL is established for each crop. Because more than one dithiocarbamate fungicide may be labeled for a particular crop, the MRL is set based upon the fungicide that produced the highest residue levels in experimental field trials.

A dietary risk assessment was conducted for the EBDC collectively (mancozeb, maneb and metiram), included the estimated dietary intake of all registered crops for each of these compounds. The EBDC have a common mechanism of toxicity (EPA, 2001) and the same ADI (codex, 2005); as such, the intake of these compounds was grouped for the dietary risk assessment.

Table (2): Risk assessments of chronic dietary intake of the dithiocarbamates in Egypt:

Commodities	Food consumption** In (gm/ person/ day)	Mean in (mg/kg)	MRL (mg/kg)	TMDI	EDI	ADI	EDI as% of ADI
Apple	18.5	0.16	2	0.000617	0.000049333	0.03	0.16
Apricot	9.3	1.35	7	0.001085	0.000209250	0.03	0.70
Cantaloupe	22.6	0.1	0.5	0.000188	0.000037667	0.03	0.13
Cucumber	5.9	0.16	2	0.000197	0.000015733	0.03	0.05
Grape	27.1	0.15	5	0.002258	0.000067750	0.03	0.23
Grape leaf	1	0.31	10	0.000167	0.000005167	0.03	0.02
Green beans	4.5	0.17	2	0.00015	0.000012750	0.03	0.04
Green Broccoli	1.2	0.18	5	0.0001	0.000003600	0.03	0.01
Green Peas	6	0.33	2	0.0002	0.000033000	0.03	0.11
Lettuce	4.6 head/1 leaf	0.11	10	0.000767	0.000008433	0.03	0.03
Onion	33	0.22	0.5	0.000275	0.000121000	0.03	0.40
Orange	38	0.1	2	0.001267	0.000063333	0.03	0.21
Peach	3.3	0.2	7	0.000385	0.000011000	0.03	0.04
Pepper	13	0.23	1	0.000217	0.000049833	0.03	0.17
Plum	2.5	0.1	7	0.000292	0.000004167	0.03	0.01
Potatoes	61.2	0.1	0.2	0.000204	0.000102000	0.03	0.34
Squash	11.4	0.11	1	0.00019	0.000020900	0.03	0.07
Strawberry	2	0.1	5	0.000167	0.000003333	0.01	0.03
Tomato	102.8	0.19	2	0.003427	0.000325533	0.03	1.09
Water Melon	47.1	0.11	1	0.000785	0.000086350	0.03	0.29
TOTAL				0.012936	0.001230133		4.1
As EBDCs				0.02289613	0.002177336		
As % of ADI				76.3%	7.3%		

*** *GEMS / food consumption cluster diets (2006)**

For compliance with MRLs in plant and estimation of dietary intake in plant and animal commodities: total dithiocarbamates, determined as CS_2, evolved during acid digestion and expressed as mg CS_2/kg. The MRLs apply to total residues from the use of any or each of the groups of dithiocarbamates.

mancozeb, maneb, and metiram, 0.03 mg/kg bw (alone or in any combination)(1993).

Table 2 shows the dietary risk assessment study for the dithiocarbamates according to The Egyptian legislation, which establishes a dithiocarbamate MRL, as CS_2. The TMDI for the EBDC contributed to 76.3 %of ADI (Table 2). The TMDI intake calculated in this study was refined by substitution of the mean residue values reported by this monitoring program (2005). The adjusted %ADI was considerably lower for all compounds,

Conclusion

Dithiocarbamate is one of the major fungicide groups used in the production of agricultural crops in Egypt, and the most common pesticides found in food monitoring programs in many countries (PARA, 2003; Ripley *et al*., 2000; Dogheim *et al*., 1999; EU, 2001). The methodology used in these programs relies on the measurement of CS_2 generated by the acid hydrolysis of these compounds. Although dithiocarbamates are non-systemic fungicides, CS_2 residues, have been found in the pulp of many fruit crops. Also, the detected CS_2 may be due to its natural presence in the plant or by generation from sulfur compounds during the acid digestion procedure used in the laboratory assay. As a result, dithiocarbamate intake calculations using residue data generated by the CS_2 based methodology might be overestimated. Chronic dietary intake of the dithiocarbamates did not exceed the ADI for any registered compound. The use of MRL as the residue parameter is very conservative, as it assumes that the pesticide is always present in all registered crops at a set tolerance level and it does not consider any processing which might occur with the food, including peeling. The estimated intake was drastically reduced for all compounds to levels below the ADI when residue data generated in this study replaced the codex MRL. The data of such monitoring program can be used to assess the risk to consumers.

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متبقيات الداى ثيو كربامات في بعض المواد الغذائية والمخاطر المحتملة بالنسبة للمستهلكين المصرية

منى خورشيد، أحمد ممدوح جمعة، عماد رمضان عطا الله و محمد السيد عامر
المعمل المركزى لتحليل متبقيات المبيدات و العناصر الثقيلة فى الاغدية , مركز البحوث الزراعية , وزارة الزراعه وإستصلاح الاراضى

تعد مبيدات الداى ثيو كربامات مجموعة غير جهازية والاكثر استخداما لحماية المحاصل من الأمراض الفطرية. و من الطرق المتبعة بواسطة معامل التقصى لتحليل مركبات الداى ثيو كربامات فى الأغذية تحليل ثانى كبريتد الكربون الناتج بعد عمليات التحلل المائى لهذه المركبات فى عينات التحليل. قد تم تجميع 535 عينة من مختلف المواد الغذائية وتشمل (التفاح – المشمش – الكانتالوب – الخيار – العنب – ورق العنب – الفاصوليا الخضراء – البروكلى – البازلاء – الخس – البصل – البرتقال – الخوخ – الفلفل – البرقوق – البطاطس – القرع – الفراولة – الطماطم – البطيخ) من ثمانية أسواق محلية فى ستة محافظات مصرية وتشمل القاهرة – الجيزة – القليوبية – بنى سويف – المنوفية والإسماعيلية وذلك خلال الفترة من 2007 الى 2008 وتم تقدير متبقيات الداى ثيو كاربامات ولقد أظهرت النتائج أن أربعمائة وثلاثة عشر عينة وهو ما يمثل نسبة 77.2% من إجمالى العدد الكلى من العينات كانت خالية تماماً من متبقيات الداى ثيو كاربامات , بينما وجد أن مائة وإثنان وعشرون عينة (22.8%) كانت ملوثة بمتبقيات الداى ثيو كاربامات معبراً عنها بمركب ثانى كبريتد الكربون وأن نسبة 1.3% من هذه العينات متجاوزاً الحد الأقصى المسموح بة لتلك المركبات. ولقد تراوحت تركيز تلك المركبات معبرا عنها كثانى كبريتد الكربون مابين (0.2 مجم/كجم الى 5.09 مجم/كجم) . و لقد سجل البصل أكبر نسبة تعدى للحدود المسموح بها لتلك المركبات يلية البازلاء ثم الفلفل. وطبقاً لهذه الدراسة فقد ثبت أن الإستخدام اليومى لتلك المركبات فى تلك المحاصيل الزراعية والمنوطة بها هذه الدراسة لا يشكل أى خطر على الصحة العامة للمستهلكين حيث أن تقييم معدلات الإستخدام اليومى للأغذية التى تحتوى على تلك المركبات وجد أنه أقل من المعدلات اليومية المسموح بها فى تلك الأغذية . بالإضافة إلى ذلك فإنه ليس من الضرورى إجراء تحليلات أكثر دقة لمثل هذه المركبات فى الأغذية ضمن برامج التقصى فى مصر.

قام بتحكيم البحث

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