

DIETARY EXPOSURE OF EGYPTIAN CUSTOMERS TO PESTICIDES RESIDUES AND CONTAMINANTS IN SOME FOOD ITEMS

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ABSTRACT

These studies were estimated the Dietary Exposure of the Egyptian people to pesticide residues (325 pesticides), PCB's (14 congeners), heavy metals (Fe, Cu, Cd, pb , Cr and Zn), nitrates and aflatoxines (B1,B2,G1 and G2) contamination in food. The Dietary Intakes were based on the total Diet Studies approach have been studied in the year 2008 depending on Egyptian National Food Consumption Data issued by Ministry of Agriculture.

The items and composites were selected according to their popularity and high consumption representing all groups of food and origin.

The pattern of residues showed that no organochlorine pesticide residues or PCB's were detected in samples, Only Azoxystrobin, Chlorpyrifos, Chlorafenpyr, I-Cyhalothrin and Profenofos were detected in low concentrations and not exceeding their ADIs. All the detected heavy metal element concentrations were below their acceptable values. The mean concentration of total aflatoxin was below their ADIs . All the samples were contaminated with nitrates within the acceptable limits. Generally all detected samples were within the international limits and no exceeding were found.

INTRODUCTION

National authorities have the responsibilities and obligation to ensure that toxic chemicals, such as pesticides, heavy metals, environmental and naturally occurring toxins, are not present in food at levels that may adversely affect the health of consumers. Governments need to assess public health risks arising from the presence of toxic chemicals in food by estimating the actual dietary intake of contaminants for comparison with their corresponding toxicological reference intake, such as the acceptable daily intake (ADI) or provisional tolerable weekly intake (PTWI). GEMS/Food Total Diet Studies report (1999).

The total diet study provides the most accurate estimates of intakes of contaminants for a country as a whole. In addition total diet studies explicitly take into account the kitchen preparation of foods to assess the levels of contaminants in foods as consumed and national food consumption data. One of the advantages of total diet studies is that they produce information that is readily understandable for use by regulatory agencies, decision-makers and the public GEMS/Food Total Diet Studies report (2002).

This study is aimed to collect the highly consumed and the most popular food from the local market, and prepared as consumed (eaten) by the Egyptian peoples passing through all kitchen processes and submitting them into residue analysis of pesticides, heavy metals, aflatoxines and nitrates to characterize and evaluate the hazard for measuring the exposure and risk evaluation for the Egyptian people depending on the Egyptian food consumption data {Food Balance Sheet (2006)} and local habits for preparing and cooking processing .

MATERIALS AND METHODS

Sampling

Twenty five samples were collected from different local markets in Great Cairo during 2008-2009. Samples were selected according to their popularity, high consumption, representing animal and plant origin and eaten by most people of the different stratified social levels. Samples have been passed through standard operating procedures (SOP) for preparing the samples in the kitchen as consumed. All operations such as blending, chopping, mixing and storing of samples were potential sources of extraneous contamination. This type of post preparation contamination must be avoided by careful selection of contamination-free blending equipment and storage containers. All prepared food items were weighed and mixed with a large-scale cutting and blending utensils and large capacity equipment were used to ensure adequate bulk homogenous sample preparation. All kitchen vessels and tools used for food processes were stainless steel. The samples were subjected to the different methods of analysis and kept at -20 °C to avoid any degradation due the complexity of matrices. The food items and preparation method are illustrated in table (1).

Table (1): Food items and methods of preparation

Food item or composite	Food preparation method
Rice	a- Boiled & b- Fried with corn oil and mixed
Macaroni	a- Boiled & b- Boiled with tomato paste
Wheat (Belela)	Boiled
Bread	Chopped and mixed
Bakery product	Chopped and mixed
Vegetable mixture soup	Boiled green peas, green beans, carrot, potatoes, squash and parsley
Green Salad	Mixture of washed vegetables of lettuce, carrot, tomato, cucumber, water cress, radish, leek and parsley
Potatoes	a-Boiled & b- Cheeps
Broad bean (Medames)	Boiled & mixed
Cowpea	Boiled & mixed
Dry bean(white)	Boiled & mixed
Lentils	a- with skin (black) and b- without skin (yellow)
Milk	Mixed
Cheese	a- skimmed and b- whole milk
Chicken	Boiled
Veal meal	Boiled
Liver meat	Boiled
Fish	Edible parts (without head, tail, bones etc.)
Apple	Rinsed, core, chopped not peeled
Orange	Rinsed and Peeled
Grape	Rinsed
Peanut	Mixed
Water	Mixed

Preparation instructions:

In order for foods to be prepared in a consistent and unambiguous manner some instructions were taken into consideration.

Chopping. Samples were put into the appropriate sized food processor and chopped until a homogeneous mixture is attained- usually 6-8 minutes depending on the moisture content of the sample.

Blending. Samples were put into appropriate sized blender (depending on the amount of the item being prepared) and blended until a homogeneous mixture is obtained-usually 2-4 minutes depending on moisture content of the sample.

Combining Units of the same sample were combined before chopping or blending.

Compositing. Involves through mixing / blending / chopping of equal weights of the indicated samples.

Selection of appropriate food preparation equipment is a vital component of the contamination control procedures:

Gloves a non-lubricated surgical-style gloves were worn whenever the food being prepared could come into contact with hands.

Utensils Stainless steel knives, wooden (good quality, smooth, crack free) or glass chopping boards, large stainless steel or Pyrex receptacle (jug or bowl) for mixing liquids.

Equipment domestic oven, blenders, glass with stainless steel blades, food processors, large stainless steel pots.

Analytical quality control and 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 to 17025(2005) by the Centre of Metrology and Accreditation Service (FINAS).

Trace analysis of a wide range of complex matrices is an exacting science. For the reason, it is essential to have quality control steps in place to ensure confidence in the methodology and robustness of the results including the following: Blanks, duplicates, certified reference materials (CRMs), spike recovery (Acceptable recoveries for trace analysis would generally be 70-120%) and coefficient of variation of less than 20%, in-house control samples and blind duplication were done as well. EURACHEM 2003

Chemicals and reagents:

Acetonitrile (MeCN), methanol (MeOH), de-ionized water for LC-MS-MS mobile phase and as a reagent blank, organic solvents were sufficient quality for pesticide residue analysis, magnesium sulphate ($MgSO_4$) and sodium chloride (NaCl), Reagent grade anhydrous $MgSO_4$ in powder form and ACS-grade NaCl were obtained from Merck (Darmstadt, Germany) $MgSO_4$ was baked for 5 hr. at $500^\circ C$ in muffle furnace to remove phthalates. Organic acids, glacial acetic acid (HAc) and formic acid (both from Merck) were used to improve stability of base-sensitive pesticides in the final extracts and as an acid modifier of the LC mobile phase, respectively. Acetone, dichloromethane, n-hexane, petroleum ether (chromatography grade or similar quality) ethanol 95-96%, anhydrous sodium sulphate (Riedel-de haen), sodium hydroxide, florisil 60-100 mesh (Merck), nitric acid (HNO_3)

supra pure Merck -reagent grade, 2 mol/L HNO₃ (130 ml of HNO₃ is diluted to 1L with de-ionized water used for cleaning the digestion flasks, 0.3% HNO₃ (5 ml conc. Acid is diluted to 1L of 10g of ammonium di-hydrogen phosphate (NH₄H₂PO₄) and 0.87g of magnesium nitrate {Mg(NO₃)₂ .6H₂O}, Pb, Cd, Cr, Cu and Hg stock standards, 1000 mg/L (Merck's ampoules). Intermediate and working solutions of elements were prepared from stock solution with different concentrations in 0.3 N HNO₃. Potassium nitrate for nitrate analysis, 99% purity. Mobile Phase: methanol/water/n-octyl ammonium phosphate which was prepared by mixing 800 ml water, 200 ml methanol and 1.63 ml n-octyl amine, the pH of the solution was adjusted pH=4-6 using 10% phosphoric acid solution (mobile phase should be freshly prepared every 3 days. Silica gel60(70-230) mesh ASTM Merck) activated by heating 1 hr at 150°C and then deactivated by adding H₂O 1ml/100g anhydrous sodium sulphate (Riedel-deHaen).

Pesticide reference standard:

All reference pesticides (325 pesticides) were certified standards and were provided by Dr. Ehrenstorfer Gmbh. Gogginer st. 78 D-8900 Auhburg. Germany, The standard solution which used in nitrate analysis was potassium nitrate a ACROS 99% while copper, Chromium, lead, cadmium and mercury were from Merck

Extraction Procedure:

(1) Multi residue method :

a) Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) 2006 method of analysis European Committee for standardization

The extraction procedure was carried out according Sterven (2007), Anastasiades *et al* (2003) Ten gram of sample were weighed in 50 ml PTFE tube, 10ml of acetonitrile was added and shaken vigorously for one minute, A buffer -salt -mixture were added and immediately shaken for one minute, the samples were centrifuged at 4000rcf for 5 minutes, and injected to LC-MS/MS.

b) Milk and Milk product method:

Pesticide residues were extracted by Suzuki (1979) method which were adopted. Milk samples were extracted by centrifugation with n-hexane-acetonitrile-ethanol (20:5:1ml). The fat content was cleaned up on a florisil column and subjected to LC/MS/MS.

C) Fish method

Fish samples were extracted as described in PAM (1968). The petroleum ether extract was partitioned in petroleum ether acetonitrile and cleaned up using florisil column.

D) Water method

The AOAC 2003 was adopted for analysis of organochlorine and organophosphorous pesticide residues by extraction with dichloromethane.

2) Heavy metals method:

An analytical method described in thesis of Thabit (2002) was selected for determination of lead, cadmium copper where it is suitable for all kinds of food. As follows: Three-six grams of homogenized fresh samples were transferred to glass digestion flasks with 10 ml of conc. HNO₃. The

solutions were boiled for 72 hours, depending on the sample matrix. The nitric acid solution was evaporated, and the residues was transferred with 0.3 N HNO₃ with 25 ml volumetric flasks

3) Aflatoxin analysis:

Fifty grams of sample was extracted with 200 ml methanol-water(80:20) solution and filtered AOAC(1995), 40 ml of the filtrate was transferred to a 500 ml reparatory funnel with 40 ml 10% NaCl solution and shacked gently for 1 min. The lower queues layer was drained into another 500 separatory funnel, two 50 ml of chloroform was added and shacked for 1min. The two lower layer were passed through 15 g of NaSO₄, evaporated till dryness, followed by drivatisation using trifluro acetic acid

4) Nitrates method of analysis:

The method described by Cheng and Sang (1998) has been followed. Ten grams of the homogenized sample was extracted by 100 ml of water and heated on water bath at 70° C for half an hour and shacked every five minuets, cooled at room temperature and filtered using whatman filter paper no.1, an aliquot from clear solution was taken for further purification by a syringe filter (0.45µm). The filtered solution was directly injected into the LC system HP 1100 series.

Determination:

1) LC-MS/MS determination:

Separation was performed on C18 column ZORBAX Eclipse XDB-C18 4.6x150 mm, 5 µm particle size. The injection volume was 5µl. A gradient elution program at 0.3 ml/min flow, in which one reservoir contained 10 m mole ammonium formate and the other contained methanol. The ESI source was used in the positive mode, and nitrogen nebulizer, curtain, and other gas setting were optimized according to recommendations made by the manufacturer; source temperature was 300°C, ion spray potential, 5500v, de-cluster potential and collision energy were optimizes using a Harvard apparatus syringe pump by introducing individual pesticide solutions into the MS instrument to allow optimization of the MS/MS conditions. The Multiple Reaction Monitoring mode (MRM) was used in which one MRM was used for quantification and the other for confirmation.

2) Heavy metals analysis:

Atomic absorption spectrometer (AAS) {Analytical technology, INC. Unicam 929} equippedwith Graphite furnace with auto sampler and flame atomic absorption.

Typical furnace parameters for lead and cadmium in AAS are given in the following table (2) and table (3).

Table (2): Furnace parameters for AAS

Step	Temp. (°C)	Time (sec.)	Ramp (°C/sec)	Gas flow (ml/min)
Drying	120	40	30(Cd), 10(Pb)	2
Ashing	800	20	50	2
Atomization	1800	3	0	0
Cleaning	2500	3	0	2
Cooling	20	5	0	2

Table (3): Instrumental Parameters of Atomic Absorption Spectrometer (AAS).

Parameter	Pb	Cd	Cu	Cr	Fe
Technique.	Graphite	Graphite	Flame	Flame	Flame
Wave length (nm)	217.0	228.8	324.8		246.3
Slit band pass (nm)	0.5	0.5	0.5	0.5	Full 0.2
Lamp current %	75% - 100%	75%-100%	75% - 80%	75%-100%	75%- 100%
Signal type.	Transient	Transient	Continues		Continues
Back ground correction	On	On	On	On	On
Inert gas.	Argon	Argon	-	-	-
Heating source.	Electro thermal	Electro thermal	Air – acetylene with flow rate (1:1 l/min.)	Air – acetylene with flow rate (1:1 l/min)	Air – cetylene with flow rate (1:1 l/min.)

3) Aflatoxin:**HPLC determination:**

HPLC was used for determination of aflatoxin. The injection volume (25µl) was applied into the reverse phase column and mobile phase {water: methanol: acetonitrile (60:25:15)} was used with a flow rate of 1ml/min. The fluorescence detector was used with excitation (360 nm), emission 440 nm), and the gain was at maximum.

4) Nitrate:

- Mobil phase: methanol/ water/n-octyl ammonium phosphate,
- HPLC column: MOS hypersil 5 x 200 x 4.6 mm.,
- Injection volume: 10µl,
- UV wave length 220 nm,
- External standard method was used for calculation.

RESULTS AND DISCUSSION**a) Pesticide Residues:**

Data in table 4 and table 5 shows that: No contamination was found in the diet with organochlorine compounds and this may be attributed to the complete banding use of these compounds from a very long time ago, no contamination with PCB's was found in the diet (which checked by GC-ECD) because we do not have such sources of contamination found in our environment and it is consistent with our national monitoring programs Dogheim *et al.*, (2002).

There is only a contamination with a few organophosphorous compounds in a very low levels. Profenofos, Azoxystrobin, Chlorfenapyr and L-cyhalothrin were found in low concentrations in cereals, veaetable, and fruits samples which were agreed with Egypton total diet studies Salama *et al.* (2003 a, b)

Table (4): Estimated Daily Intakes of pesticide residues.

	Item	Pesticide	Mean conc. mg/kg	Food consumed by day in grams	EDI mg/kg-B.W.
Cereals	Lentil a	N.D		3.3	---
	Lentil b	N.D			
Vegetables	Veg. Mix.	Profenofos	0.01	384.9	0.000006
	Veg. Salad	Profenofos	<LOQ		
	Potato a)Boiled	N.D		45.8	
	b)Cheeps	N.D			---
Pulses	Cawpea D.beans	N.D		3.3	---
	Medames	N.D		18.9	
	Macaroni	N.D			---
	Rice			134.5	---
	a)boiled	N.D			
	b) Fried	N.D			
	Wheat	N.D		348.8	---
	Belilla				
	Bread				
	Bakery Products				
Milk and milk products	Cheese a)skimmed	N.D			---
	b)Full cream				
	Milk	N.D		118.9	---
	Chicken	N.D		27.7	---
	Veal meat	N.D		9.9	---
	Liver meat	N.D			---
	Fish	N.D		34.3	---
Fruits	Apple	N.D		19.7	
	Grape	Azoxystrubin	0.01	42.7	0.000007
		Sulfur	0.23		0.000163
	Orange	Chlorpyrifos	0.03	40.5	0.0000202
L-cyhalothrin		0.01		0.0000067	
	Chlorfenapyr	0.01		0.0000067	
Nuts	Peanut	N.D	N.D	1.1	---

N.D = Not Detected

Table (5): Estimated Daily Intakes of pesticides residues and its percentage to Acceptable Daily Intake ADI

Pesticide	Source	EDI	ADI	EDI/ADIX100
			Mg/Kg/Person/Day	
Azoxystrubin	Grape	0.000007	0.1 (EU)	0.007
Chlorfenapyr	Orange	0.0000067	0.015 (EU)	0.00446
Chlorpyrifos	Orange	0.0000067	0.1 (CAC)	0.0000067
L-Cyhalothrin	Orange	0.0000067	0.02 (CAC)	0.00335
Profenofos	Veg. Mix.	0.000006	0.03 (CAC)	0.0000018

b) Heavy Metals:

The contamination of the total diet as general is low and not exceeding for the contaminants to their corresponding established (PMADI or PTWI). Data in table 6 and table 7 shows that most of the contamination comes from cereals and vegetables.

Table (6): Estimated Daily Intakes of Heavy Metals.

Samples from	Item	Fe mg/kg	Cr µg/kg	Zn mg/kg	Cu mg/kg	Cd µg/kg	Pb µg/kg
Cereals	Lentil a	3.4	<LOQ	1.62	4.7	ND	ND
	Lentil b						
Vegetables	Veg. Mix.	0.65	ND	0.26	0.84	ND	0.042
	Veg. Salad Potato a)Boiled b)Cheeps						
Pulses	Cawpea	1.76	ND	1.17	1.8	ND	0.09
	D.beans	2.34	<LOQ	0.92	2.9	ND	0.09
	Medames	0.93	ND	0.32	2.34	ND	0.08
	Macaroni	1.04	ND	5.79	1	<LOQ	0.402
	Rice a)boil b) Fried	0.62	ND	0.53	1.13	ND	0.132
	Wheat Belila Bread Bakery Products	2.11	ND	0.21	1.44	ND	0.12
Milk and milk products	Cheese a)skimmed b)Full cream	0.44	ND	ND	ND	0.006	ND
	Milk	---	---	---	---	0.009	ND
	Chicken	---	---	---	---	---	---
	Veal meat	---	---	---	---	---	---
	Liver meat	---	---	---	---	---	---
	Fish	---	---	---	---	<LOQ	0.04
Fruits	Apple	---	---	---	---	---	---
	Grape	---	---	---	---	---	---
	Orange	---	---	---	---	---	---
Nuts	Peanut	---	---	---	---	---	---
Total Estimated Dietary Intake	Fe	7.386	0.1	0.262	0.017	0.083	8
	Mg/Kg /Day	Mg/Kg /Day	Mg/Kg /Day	Mg/Kg /week	Mg/Kg w/day	µg/kg bw/week	µg/kg bw/week

N.D = Not Detected

Estimated Dietary Intake = \sum Mean .Conc. * ConsumptionDietary EXPOSUR = $\frac{\text{Estimated Dietary Intake}}{\text{ADI}} \times 100$

ADI

Table (7): Summary of estimated dietary exposures.

Element	International standard	Type	Unit	Estimated x100 Accepted
Iron	8 ^a	RDI	mg/day	92.2%
Chromium	1000 ^b	UL	µg/day	0.0001%
Zinc	1 ^c	PMTDI	mg/kg/bw/week	3.1%
Copper	0.5 ^d	PMTDI	mg/kgbw/day	3.4%
Cadmium	7 ^e	PTWI	µg/kgbw/week	1.19%
Lead	25 ^f	PTWI	µg/kgbw/week	33.6%

a draft Australian and New Zealand Nutrient Reference Values

* RDI= Recommended Daily Intake

** UL = Upper Limit

b SCF, 2003

c JECFA 1982

d JECFA 1982

e WHO,2004

f WHO,2000

Copper contaminated most of the items probably from using a fungicides or fertilizers, copper may be present in food in the shape of copper ions or copper salts, as a contaminants due to migration from food contact materials as water copper pipes. Lead is not easily extracted from the soil by plants and its occurrence in plants is often due to air pollution vehicles in roads beside farms, the occurrence of lead in food and drinks today is mainly due to many wears of use of lead technology and in particular to the use of alkyl-lead compounds as petrol additives. Most of the chromium, (Cr) present in food is in the form of trivalent chromium (Cr III), No contamination of chromium and cadmium were not found in studied food item. The percentage of Estimated Dietary Intakes to its Provisional Maximum Tolerable Dietary Intakes (PMTDI) of Zn was 3.1 % . The results were in accordance with the information provided by different countries (Canada Denmark, Finland, Netherlands and USA) where the cereals and their products followed by vegetables as the largest contributors to such intake (Galal H. G. 1993).

c) Aflatoxin:

The mean concentration of the total B1,B2,G1 and G2 were 3.6 µg/kg and the Estimated Dietary Intakes which does not exceed 1 ng/kg b.w. /day JECFA (1997, 2001). Although, the JECFA have concluded that this limit or even less, still contribute to a liver cancer risk. Most of the results have only B1 and very small amount B2 and neither G1 nor G2 were found in any of the detected samples of nuts.

d) Nitrates:

Most of the samples were contaminated with nitrates but no exceeding for ADI recommended by JECFA 1995 3.65 mg/kg body weight. The percentage of EDI/ADI = 32.1% (Table 8).

Table (8): Estimated Daily Intakes of Nitrates.

Sample from	Item	Mean conc.	Food consumed by day in grams	EDI mg/kg-B.W.	EDI mg/kg-B.W.
Cereals	Lentil a		3.3	20	0.0011
	Lentil b				
Vegetables	Veg. Mix.		384.9	163	1.045645
	Veg. Salad				
	Potato a)Boiled b)Cheeps		54.8	84	0.07672
Pulses	Cawpea D.beans		3.3	85	0.004675
	Medames		18.9	8.7	0.00000005
	Macaroni				
	Rice a)boil b) Fried		134.5	5.4	0.012105
	Wheat Beilla Bread Bakery Products		348.8	3.7	0.022
Milk and milk products	Cheese a)skimmed b)Full cream			Nd	---
	Milk		118.9	1.8	0.0035
	Chicken		27.7	---	---
	Veal meat		9.9	Nd	---
	Liver meat				
	Fish		34.3	Nd	---
Fruits	Apple		19.7	2.8	0.00009
	Grape		42.7	10	0.007
	Orange		40.5	2.0	0.00135
Nuts	Peanut		1.1	1.8	0.000033
Total Estimated Dietary Intake					1.174 MG/KG BW

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دراسة تعرض المستهلك المصري لمتبقيات المبيدات والملوثات في بعض الأغذية
أميل يوسف سلامة ، منى عبد العزيز خورشيد و عبير أحمد الجوهري
العمل المركزي لتحليل متبقيات المبيدات والعناصر الثقيل في الأغذية مركز البحوث الزراعيه

دراسة تعرض الانسان المصري للملوثات مثل المبيدات (٣٢٥) مبيد ومركبات PCBs والعناصر الثقيله (الحديد - والنحاس- والكانميوم -والرصاص -والكروم والزنك). تم تحليل عدد من الاغذية وهذه الاغذية هي الأكثر استهلاك طبقا لمعد الأستهلاك المصري المصادر من وزارة الزراعة المصرية أوضحت الدراسة انه لا يوجد تعدي لأي من الملوثات للحدود المسموح بها دوليا وبينت النتائج انه لا يوجد تلوث لأغذية بالمبيدات الكلورينية أو المركبات الثابتة PCBs ويوجد تلوث بعض المركبات وهي الأزوكسيستروبين والكلوربيرفوس والكلورفينبير والسيهالثرين والبروفينوفوس بتركيزات لا تتعدى الحدود المسموح بها. وأظهرت النتائج انه لا يوجد تعدي للعناصر الثقيلة للحدود المسموح بها .كما وجدة مركبات الأفلاتوكسينات B1 في عينات الفول السوداني بكميات لا تتعدى للحدود المسموح بها. ووجد أيضا ان كل العينات ملوثة بالنيترات بكميات لا تتعدى الحدود المسموح بها دوليا.وقد أتضح أن المتناول اليومي لجميع الاغذية التي تمت دراسة مستويات الملوثات المختلفة فيها أن بها أقل بكثير من المتناول اليومي المسموح به من هذه الملوثات وبناء على ذلك فإن جميع هذه الاغذية آمنة .