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MONITORING OF PESTICIDES, HEAVY METALS AND NITRATES RESIDUES IN SOME READY-TO-EAT BABY FOODS

[56]

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ABSTRACT

Twenty- eight ready-to eat baby and children food samples were collected from different places and production dates during 2002. The samples were subjected to residue analysis of organochlorine, organophosphorous, organonitrogen and pyrethroids pesticides (80 pesticides) as well as cadmium, lead, copper and nitrate residue method analysis. The results show that, no pesticide residues were found in all samples. The most contaminant was nitrate followed by Cu, Pb, and Cd. Copper, Pb and Cd contaminate the samples in a mean concentrations 0.538, 0.036, 0.008 mg/kg and their ranges were of 0.24-1.731 mg/kg for Cu, 0.02-0.134 mg/kg for lead, and 0.002-0.035 mg/kg for cadmium. The highest concentration was with nitrates, which was ranged from 9 – 137 mg/kg with a mean concentration of 65.2 mg/kg, which is considered high for baby and children.

Key words: Baby children foods, Pesticides residues, Heavy metals, Nitrates

INTRODUCTION

The exposure of infants and children to pesticide residues and contaminants through food products has received considerable attention from the public and scientific communities highlighting the need for specific policies to ensure adequate health protection for this particular age group.

The most appropriate way to address these safety issues is the application of a sound, scientific risk-based approach in order to establish regulatory standards specifically for food products aimed at infants and young children. Such a strategy may allow the limited resources available to be focused on measures permit a reduction in health risks. So, we should protect growing babies and children from unnecessary risk, including exposure to pesticides, heavy metals and nitrates. Babies and young children need such special protection because they are particularly vulnerable.

Of particular concern is the exposure of infants and children to food contaminants because of their possible increased susceptibility for adverse effects (Larsen and Pascal 1998). Toxicity of pesticides

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in infants and young children may differ quantitatively and qualitatively from that in adults. Quantitative differences exist in absorption, metabolism, detoxification and -excretion which may make infants more or less sensitive to various xenobiotics than adults (Ostergard and Knudsen 1998) These differences need to be related to the specific food intakes of infants and young children; they are much higher per kg body weight than adults. Nitrates analysis also was added to the total diet studies for its importance to infants and children (GEMS/FOOD 1999). The present study aimed to survey the levels of pesticides, heavy metals and nitrates in some ready –to-eat baby food collected from different markets at Great Cairo, Egypt during 2002.

MATERIAL AND METHODS

Sampling

Twenty-eight ready-to-eat baby and children food samples were collected from local markets and different production time during the year 2002. Table 1 shows the names and compositions of these diets. As mentioned on their labels and packages.

Chemicals and reagents

- Acetone, dichloromethane, n-hexane, petroleum ether, Acetonitrile, (Pestiscan Chromatography grade or similar quality) ethanol 95-96%.
- Anhydrous sodium sulphate (Riedel-de haen), sodium chloride, sodium hydroxide.
- Florisil 60-100 mesh (Merck)
- De-ionised water
- Nitric acid (HNO_3) (supra pure), (Merck-reagent grade)
- 2 mol/L HNO_3 (130 ml of HNO3 is diluted to 1L with distilled water) used for cleaning the digestion flasks.
- 0.3% HNO_3 (5 ml conc. acid is diluted to 1L with distilled water).
- Reagents used as matrix modifier: A mixture of 10 gm of Ammonium-dihydrogen phosphate ($NH_4H_2PO_4$) and 0.87 gm of Magnesium nitrate ($Mg(NO_3)_2 . 6 H_2O$).
- Pb, Cd, Cu stock standards, 1000 mg/L (Merck's ambulles).
- Intermediate and working solutions of Pb, Cd, and Cu prepared from stock solution with different concentrations in 0.3 N HNO_3.
- Potassium nitrate for nitrate analysis, more than 99%.
- 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)

Reference standard

All reference pesticides were certified standards and were provided by Dr. Ehrenstorfer Gmbl, Gogginer str.78 D-8900 Augoburg. Germany, and by the FAO (Food Agriculture Organization of the United Nations, Rome, Italy and were prepared in n-hexane/acetone mixture (9:1).

The standard solution which used in nitrate analysis was potassium nitrate a ACROS 20591-1000 = 99 % and its LOD=5 mg/kg. While cu, pb and cd were

Table 1. Names of baby diets analyzed and their compositions

Serial No.	Name	Composition
1	Chicken with vegetable & corn	Water- carrot- chicken-parsley-celery-potato-corn-butter-rice flour-fennel seeds-dehydrated onion powder
2	Mixed vegetables	Water-carrot-wheat flour-sweet potatoes-tomato-onion.
3	Vegetable & beef	Carrot-water-beef meat-rice & wheat flour-tomato paste-green peas, sweet potato
4	Apple forest fruit	Apples-rasp- berry white- grape juice- black berry- puree- choke berg juice- sugar- crau-berry puree
5	Turkey rice	Water-carrot-turkey meat wheat & rice flour-green peas-onion powder.
6	Banana apple	Water-fully ripened bananas-apples–sugar-modified corn starch-orange juice from con-centrates
7	Apple peach	Apple-peach-vitamin c.
8	Pear with apple	Pear-apple-vitamin c.
9	Creamed rice with vegetables & chicken	Carrot-tomato-chicken meat-rice-plant oil-salt.
10	Whole meal cereal with apple & banana	Apple-banana-apple juice-orange juice-wheat-plant oil- vitamin c.
11	Fine vegetables and potato with veal	Carrot-potato-sweet-corn-beef meat-full cream milk-plant oil-salt
12	Fine sweet corn with mashed potato and turkey	Corn-potato-parsley-turkey-rice flour-plant oil
13	Fender vegetables with rice	Carrot-rice- green peas-full cream milk- plant oil
14	Rasp berries and blue berries with apples	Rasp berries and blue berries with apple yo-gurt.
15	Banana and peach in apple	Banana and peach in apple
16	Cream spinach with potatoes.	Spinach-potato-milk-milk cream-vitamin c
17	Vegetables and lamb	Green-peas-wheat-rice flour, tomato past-water-carrot-lamb meat- onion powder
18	Whole meal fruit cereals	Apple juice-apple-banana-wheat flour meal-plant oil- vitamin c.
19	Vegetables and chickens	Water-chicken-carrot-flour-rice-wheat-pineapple juice- sweet potato-green peas-onion powder-Soya oil-celery.
20	Vegetables and chickens	Water-chicken-carrot-flour-rice-wheat- corn - pineapple juice-sweet potato-green peas- on-ion powder-Soya oil-celery.

Table 1. Cont.

Serial No.	Name	Composition
21	Chicken with vegetables and corn	Water-carrot–chicken-parsley and celery-potato-corn-butter-rice flour-fennel seeds dehydrated onion powder.
22	Turkey rice	Water-carrot-turkey-wheat & rice flour-green peas-sweet potato-onion powder.
23	Bubbly carrot	Water-organic carrot
24	Vegetables and beef	Water-carrot-beef meat-rice and wheat flour-tomato paste-green peas-tomato-sweet potato-onion powder
25	Mixed vegetables	Water-carrot-wheat flour-sweet potato-tomato paste-onion powder
26	Vegetables and lamb	Water-carrot-lamb meat-tomato paste-rice & wheat flour- green peas-sweet potato-onion powder.
27	Carrot	Water-carrot
28	Fine sweet corn with matched potato & turkey	Sweet corn-potato-parsley-turkey-meat-rice flour-plant oil salt

from Merck The limit of quantification of copper, lead and cadmium were 0.1, 0.04 and 0.002 mg/kg, respectively.

A) Extraction and cleanup

Multiresidue method for pesticides: According to the method described by **(Luck *et al* 1981)** residues were extracted from representative homogenized portion of each food by blending with acetone. The pesticides were transferred from the aqueous filtrate into organic phase by shaking with petroleum ether and dichloromethane after drying. The cleanup was carried out as described by (Suzuki *et al* 1979) using a florisil column. Organic phase was concentrated just to dryness and dissolved in hexane/acetone, 9:1 for GC detection. This method allows the determination of 80 pesticide residues.

Heavy metals method: An analytical method described in thesis of **(Thabet, 2001)** was selected for determination of lead, cadmium and copper, where it is suitable for all kinds of food. Three-six gram 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

Table 2. The investigated pesticides and their limit of quantification in mg/kg

Compounds names	Limit of Quantification(LOQ) (mg/kg)
Acephate	0.01
alachlor	0.02
atrazine	0.1
bendiocarb	0.1
bromoprpylate	0.05
carbaryl	0.5
carbosulfan	0.1
captan	0.1
chlorothalonil	0.02
chlorpyrifos	0.02
chlorpyrifos-methyl	0.05
chlordane-trans	0.02
chlordane-cis	0.02
cyanophos	0.05
cyfluthrin	0.1
cypermethrin	0.1
lambda-cyhalothrin	0.1
chlorpropham	0.5
DDD-p,p	0.02
DDE-p,p	0.02
DDT-o,p	0.02
DDT-p,p	0.02
deltamethrin	0.2
diazinon	0.05
dichlofluanid	0.05
dicofol	0.02
dieldrin	0.01
dimethoate	0.05
diniconazole	0.05
edifenphos	0.1
endosulfan-alpha	0.02
endosulfan-beta	0.02
endosulfan-sulphate	0.02
endrin	0.1
ethion	0.1
fenamiphos	0.1
fenitrothion	0.02
Fenpropathrin	0.05
fenthion	0.05
fenvalerate	0.01

Table 2. Cont.

Compounds names	Limit of Quantification (LOQ)(mg/kg)
HCH-alpha	0.01
HCH-beta	0.02
HCH-delta	0.01
HCH gamma (lindane)	0.02
heptachlor	0.01
heptachlor epoxide	0.01
hexachlorobenzene	0.01
imazalil	0.01
iprodion	0.5
malathion	0.02
metalaxyl	0.2
methamidophos	0.05
metribuzin	0.1
monocrotophos	0.05
omethoate	0.05
oxidiazone	0.1
parathion	0.05
parathion-methyl	0.05
pendimethalin	0.1
permethrin,	0.1
phenthoate	0.1
phosalone	0.05
phosphamidon	0.1
pirimicarb	0.05
pirimiphos-ethyl	0.02
pirimiphos-methyl	0.05
procymidone	0.05
profenofos	0.02
promecarb	0.1
propiconazole	0.1
prothiofos	0.02
pyrazophos	0.02
terbuconazole	0.1
tetradifon	0.03
tolclophos-methyl	0.02
triadimefon	0.05
triadimenol	0.1
triazophos	0.02
trifluralin	0.01
vinclozolin	0.01

was evaporated, and the residue was transferred with 0.3 N HNO3 with 25 ml volumetric flasks.

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

B) Determinations

Mutiresidue of pesticides: Qualitative and quantitative determination of pesticide residues in food samples depends on the use of two different polarities of chromatography columns. Each GC instrument (NPD, ECD) has its capillary columns with different polarities and consequently two detectors. The internal standard technique was followed for the quantitative determination. Aldrin was used for organochlorine and pyrethroid compounds; Ditalimphos for organophosphorous and organonitrogen compounds; The internal standard was added before injection on GC.

Heavy metals: Lead and Cadmium were determined by electrothermal on Atomic Absorption Spectroscopy (AAS), using Deuterium lamp for Background correction, cuvette atomization and argon gas. A mixture of $NH_4H_2PO_4$ and $mg(NO_3)_2$ were used as a matrix modifier. However, Cu was determined by flame atomic absorption using Deuterium lamp for background correction and air acetylene gas.

Nitrates

HPLC determination

- Mobile phase: methanol/water/n-octyl ammonium phosphate
- HPLC column: MOS hypersil 5X200X4.6 mm.
- Injection volume 10μl
- UV wave length 220 nm
- External standard method was used for calculations

Quality Assurance procedures:
All analytical methods and instruments were fully 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) ISO/IEC Guide 25. The criteria of quality assurance were described in **(Dogheim *et al* 2002).** The recoveries were between 70-120% and CV less than 20%. Fortification of all samples with the contaminants of interest has been carried out to ensure that the method performed satisfactory for the particular food examined. Analysis of duplicate samples represents precision of analysis.

Apparatus and equipment

A) Multiresidue analysis of pesticides

- Gas chromatograph HP 5890 equipped with double electron capture detectors (ECD) with two capillary columns; temperature injector 225°C; detector 280°C, Gases flow rates; ni-

trogen carrier gas 2.5 ml /min; 65 ml/ min (carrier + make up) , column head pressure 82 K pa

- Gas Chromatograph, HP 6890 equipped with double nitrogen phosphorous detector (NPD) with two capillary columns; temperature injector 225°C detector 280°C. Gases flow rates, hydrogen 3.5 ± 0.1 ml/min; air 100-110 ml/min; nitrogen carriers gas 2.5 ml/min for both GC's. The specification of chromatography columns are as follows:

1. PAS-5 ECD tested ultra 2 silicon, 25m X 0.32 m.m. Film thickness 0.52 µm.
2. PAS -1701 ECD tested 1701 silicon, 25 m X 0.32mm film thickness 0.25µm. Temperature programs of both GC instruments were as follows; Initial temp 90°C for 2 min; ramp (1) 20 °C /min (to 150°C); ramp (2) 6°C /min) to 270°C, hold for 15 min.

B) Heavy metals analysis

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

Typical furnace parameters for lead and cadmium in AAS are given in the following table:

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

- Wet digestion system (Digester tecator 2020)
- Digestion flasks equipped with holes
- Volumetric flasks (25 ml)

c) Nitrate analysis

HPLC -equipped with,

- Detector: HP 1100 A programmable fluorescence detector.
- Mobile phase: Methanol/water /n-Octylammonium phosphate.
- HPLC column: MOS hypersil 5X200X4.6 mm.
- Injection volume : 10 ul.
- UV wave length: 220 nm.
- External standard method was used for calculations.

RESULTS AND DISCUSSION

Table (3) showed the monitored amounts of pesticides, heavy metals and nitrate residues in baby food samples. Results showed that no contamination with pesticide residues found in all analyzed samples. All samples were found contaminated with Cu, following by Pb

Table 3. Mean* concentrations in mg/kg of pesticides, heavy metals and nitrates residues found in baby food samples

Ser. No.	Pesticides	Heavy metals			Nitrates	Food
		Cd	Pb	Cu		
1	ND**	0.024	0.104	0.420	135	Chicken with vegetables and corn
2	ND	0.008	LOQ***	0.380	136	Mixed vegetables
3	ND	0.011	0.055	0.280	57	Vegetables and beef
4	ND	.0002	<LOQ	0.260	29	Apple forest fruit
5	ND	0.014	0.104	0.240	110	Turkey rice
6	ND	0.002	<LOQ	0.250	69	Banana peach
7	ND	0.002	<LOQ	0.490	47	Apple peach
8	ND	0.017	0.134	0.780	48	Pear with apple
9	ND	0.002	LOQ	0.390	116	Creamed rice with vegetables and chicken
10	ND	0.008	0.073	0.630	71	Whole meal cereal with apple and banana
11	ND	0.009	<LOQ	0.790	134	Fine vegetables and potatoes with veal
12	ND	0.005	<LOQ	0.490	103	Fine sweet corn with mashed potatoes and turkey
13	ND	ND	<LOQ	0.470	137	Fender vegetables with rice
14	ND	ND	<LOQ	0.490	63	Rasp berries and blue berries
15	ND	ND	<LOQ	1.420	64	Banana and peach in apple
16	ND	0.017	<LOQ	1.731	36	Cream spinach with potatoes
17	ND	0.007	0.065	0.371	12	Vegetables and lamb
18	ND	0.005	0.040	0.783	ND	Whole meal fruit cereals
19	ND	0.007	<LOQ	0.266	27	Vegetables and chickens
20	ND	0.005	<LOQ	0.302	ND	Vegetables and chickens
21	ND	0.010	<LOQ	0.295	87	Chicken with vegetables and corn
22	ND	0.006	<LOQ	0.295	62	Turkey rice
23	ND	0.006	<LOQ	0.562	77	Bubly carrot
24	ND	0.009	<LOQ	0.357	ND	Vegetables and beef
25	ND	0.014	0.052	0.674	112	Mixed vegetables
26	ND	0.012	<LOQ	0.451	9	Vegetables and lamb
27	ND	0.035	<LOQ	0.574	85	Carrots
28	ND	0.004	<LOQ	0.625	ND	Fine sweet corn with matched potato and turkey

* Mean is the result of two replicates ** ND = Not detected. *** LOQ = Limit of quantification.
Pesticides include organochlorine, organonitrogen, organophosphorous and pyrethroids

and Cd. While most of the samples were contaminated with nitrates

Pesticide residues were not found in analyzed samples and may be attributed to the complete banding of organochlorine pesticides since more than 20 years and similar conclusions were mentioned by **(Dogheim *et al* 1999, 2001 and 2002).** Also, the industrial processing such as washing, peeling, boiling and many other cooking processing play an important role in decreasing organophosphorous, organonitrogen and pyrethroid residues.

Copper was the most contaminant found in the collected samples ranged between 0.24-1.731mg/kg with a mean of 0.538 mg/kg and 90th percentile of 0.785 mg/kg. The most contaminated sample with Cu was cream spinach with potato followed by banana and peach in apple. This contamination may be caused by using of fungicides or fertilizers, Copper may be present in food in the shape of copper ions or copper salts as a contaminant due to migration from food contact materials e.g. copper pipe or by industrial factors and processing.

All samples were found contaminated with Pb with a range of 0.04 – 0.134 mg/Kg with two means i.e. the 1st mean at LOQ= 0 is 0.022 mg/kg, and the 2nd mean at LOQ= 0.04 is 0.051 mg/kg, because from 60-80% of the results were less or equal the limit of quantification **(GEMS/FOOD, 1999)** and 90th percentile of 0.024 mg/kg, twenty samples out of 28 were less than the limit of quantification. The most probably source of the contamination may come from industry processing and caning, since lead is not easily transferred from the soil to the plants and its occurrence in plant is often due to air pollution leading to contamination of the plant surfaces. Also, the occurrence of lead in food and hazard today is mainly due to many years of use of lead technology and in particular to the use of alkyl-lead compounds as petrol additives, lead may contaminate food from containers containing lead **(WHO, 1995).** In particular, elevated contamination of lead may result from storage with a lead glaze, although the use of lead solder has largely been discontinued, it was a major source of exposure in many part of the world. The contamination of samples with Cd is ranged from 0.002 –0.035 mg/kg with a mean of 0.009 mg/kg and 90th percentile of 0.017 mg/kg. the most contaminated sample was carrot. Cadmium is easily transferred from soil to vegetables by the roots and certain plant species selectively take up cadmium **(Codex Alimintarius, 1998).** The contamination with Cd in roots and tubers means soils or water contamination, these results is in agreement with those reported by **Feng *et al* (1993) and Khorshid *et al* (2003).**

The results are in accordance with the information published by different countries (Canada, Finland, Denmark, Netherlands and USA) where the cereals and their products, followed by potatoes and other vegetables as the largest contamination **(Galal, 1993).**

Results shows that nitrates were found in the samples with a range from ND to 137 mg/kg with a concentration mean of 65.2 mg/kg and 90th percentile 134.3 mg/kg. These concentrations are relatively high. The samples with high concentrations contain vegetables in its composition and the most probably may come from nitrates in soil when farmers use commercial nitrogen fertilizers that are potentially hazards to infants. The problem with high levels of nitrates in

baby's diet is that it interferes with the red blood cells ability to carry oxygen (a type of anemia) throughout the baby. This contamination can lead to "blue body syndrome" and this could eventually lead to other health problems. The primary toxic effect of inorganic nitrate is the oxidation of the iron in hemoglobin by excess nitrates forming metahemoglobin. Infants less than 6 months old comprise the most sensitive problems (**Hastman 1982 & Bouchard *et al* 1992**) A secondary target for inorganic nitrate toxicity is cardiovascular system (**Ridder and Oehme, 1974**).

As conclusion, data showed no contamination with pesticide residues were found. There is a contamination with some heavy metals such as Cu, Cd and Pb, The most highest concentrations found was Cu followed by Pb and Cd. The highest contamination was in inorganic nitrates with high concentrations. There are no maximum limits for these contaminants in particular for children and babies.

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مجلة حوليات العلوم الزراعية ، كلية الزراعة ، جامعة عين شمس ، القاهرة ، م٤٨ ، ع(٢)، ٧٨٧ - ٧٩٩ ، ٢٠٠٣

تقصي مستويات التلوث بمتبقيات المبيدات والعناصر الثقيلة والنترات في أغذيه الأطفال

[٥٦]

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٢- الهيئة القومية للرقابة والبحوث الدوائية- وزارة الصحة والسكان- الدقي-القاهرة

تم تجميع ٢٨ عينه طعام أطفال جاهزة للأكل من أماكن وتواريخ إنتاج مختلفة خلال عام ٢٠٠٢ من الأسواق المحلي . تم تحليل متبقيات المبيدات الكلورنيه والفوسفوريه والنيتروجنيه وبعض البيروثريدات (٨٠ مركب). كما تم تحليل بعض العناصر الثقيلة وهي الرصاص والكادميوم والنحاس. بالإضافة إلى تحليل متبقيات النترات نظرا لخطورتها بالنسبة للأطفال . أوضحت النتائج انه لا يوجد متبقيات مبيدات بالعينات المجموعة . وجد أن العينات ملوثه بالنترات يليها النحاس ثم الرصاص والكادميوم ، كما وجد أن متوسطات التركيزات الموجودة هي ٠,٥٣٨، ٠,٠٣٦ ، ٠,٠٠٨ مج/كجم بمدي ٠,٢٤-١,٧٣١ مج/كجم للنحاس ، ٠,٠٢-٠,١٣٤ مج/كجم ، ٠,٠٠٢-٠,٠٣٥ مج/كجم للكادميوم .

أوضحت الدراسة أن مستويات التلوث بالنترات كان عاليا بمدي ٩-١٣٧ ج/كجم بمتوسط تركيز ٦٥,٢ مج/كجم وهذا يمثل خطورة بالنسبة للأطفال.

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