Monitoring of Contaminants in some Herbal Baby Drinks

S. M. Fahmy[1]; M.A. Khorshed[2]; Salama. E. Y.[2]; and M.M. Ayoub[2]

1- National Organization for Drug Control and Research Center, Dokki, Cairo, Egypt.

2- Central Laboratory of Residue Analysis of Pesticides and heavy Metals in Food, Agricultural Research Center, Nadi El Said, Dokki, Giza, Cairo.

ABSTRACT

Twenty six samples of the herbal baby drinks were collected from different pharmacies in Great Cairo during the year 2004. The samples were subjected to pesticides, heavy metals, and nitrates analysis. The results illustrated that no samples were naturally contaminated with organochlorine, organophosphorous, organonitrogen or pyrethroid pesticide residues. Results revealed that 42.3% of the total number of analyzed samples were free from nitrates. However, 57.7% of the samples were contaminated with nitrates. The detected concentration ranged from 8.5 to 120.9 mg/kg. The data also demonstrated that 88.5% of the samples were contaminated with at least one of the three detected elements. In heavy metal analysis, results showed that Pb showed the highest contamination percentage in the samples, followed by Cd, and the least contaminant detected was Cu. It was, also found that 76.9% of the total number of samples analyzed were contaminated with lead element, of which 10 % exceeding the Maximum residue levels established for Pb (0.3 mg/kg),where the concentration levels of Pb detected in the samples ranged from (0.04 to 0.89 mg/kg). However, the cadmium detected in 69.2% of the total number of samples analyzed, without any exceeding to maximum residue limits MRL's established by CODEX (0.3 mg/kg). The concentration range of Cd in contaminated samples was from 0.002 to 0.06 mg/kg. Copper was the least significant contaminant, where it was detected in 46.2% of the samples of which 4.2 % were exceeding the limit established for Cu (10 mg/kg). The detected concentration levels ranged from (0.1 – 16.7 mg/kg).

INTRODUCTION

The exposure of infants to contaminants through their food and the other sources has received considerable attention from the public and the scientific communities. There is a need for specific policies to ensure adequate health protection for this particular age group. Infants and children are generally more vulnerable to toxic substance, Children are truly special. They are not small adults, but rather young, developing individuals whose physiological makeup makes them potentially more vulnerable than adults to the adverse health effects. Chemical exposures that have negligible effects in adults may have devastating effects on infants or

children. This enhanced vulnerability of children is because their organ systems are still developing, and thus are more susceptible to disruption and damage caused by environmental agents, where their body mass is much smaller than adult. Compared to an adult, a child consumes more food and water and breathes more air in proportion to body mass. That means children generally get a bigger dose of whatever toxic agent is in contaminated food, water, or air than do adults.

In infants the sensitivity towards pesticides is as high as ten times in comparison to adults, still there are no standards to protect infants, children or anyone else from multiple pesticides in food or from other sources **(Guzelian et al.; 1992)**.

Organophosphorous and organochlorine pesticides are considered as the most common neurotoxic pesticides, which inhibit the normal function of the nervous system enzyme, acetyle choline esterase. The reproductive system is also vulnerable to the toxic effects of pesticides. Evidence is mounting that exposure to chemical pollutants and pesticides in early childhood can interfere with normal sexual development and may be contributing to declining male reproductive health in industrialized world **(Carlson et al.;1992)**.

It was discovered that even low-lead levels can cause reduced intelligence and increased aggression in children, where polychlorinated biphenyls (PCBs) can lower intelligence of children exposed, that children are more likely than adults to have breathing problems from air pollutants **(Wiles, 1993)**.

In spite of the nitrate has a low level of acute toxicity yet; it can be transformed into nitrite, which may lead to the formation of the carcinogenic nitrosamines **(Walker, 1990)** and the clinical symptom of met-haemoglobinaemia **(World Health Organization 1995)**.

The present study aimed to survey the levels of pesticides, heavy metals and nitrates in some herbal baby drinks, which contains a group of aromatic carminative oils derived from natural herbs known by their effective actions in helping the digestive processes and regulatory gastrointestinal wall motion reliving the symptoms of ignition, flatulence and the associated colic and spasms, collected from different pharmacies at Great Cairo, Egypt during 2004.

MATERIAL AND METHODS
Sampling:
Twenty six samples of different types of herbal baby drinks with different batch numbers, were collected from local pharmacies during the year 2004.

Table (2) shows the names and compositions of these drinks as mentioned on their labels of packages.

Chemicals and reagents:

• Acetone, dichloromethane, n-hexane, petroleum ether, Pestiscane Chromatography grade.

• Anhydrous sodium sulphate, sodium chloride, and sodium hydroxide (Riedel-de Haen Seelze, Germany).

• Nitric acid (HNO_3) (GR Pro Analyse , 65%) (Merck Dorse, England)
2 mol HNO_3 (130 ml of HNO_3 is diluted to 1L with deionized water used for cleaning the digestion tubes.

• 0.3 % HNO_3 (5 ml conc. nitric acid is diluted to 1L with deionized water)

• Reagent of matrix modifier: A mixture of 10 gm of ammonium dihydrogen phosphate ($NH_4H_2PO_4$) and 0.87 gm of magnesium nitrates (Mg $(NO_3)_2.6 H_2O$) were dissolved in 500 ml distilled water.

• 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 three days.

Pesticides , heavy metals, and nitrates reference standards:

• All reference pesticides were certified standards and were provided by Dr. Ehrenstorfer Gmbh, 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 used in nitrate analysis was potassium nitrate a ACROS 20591-1000=99% and its LOD =5 mg/kg.

• Metals stock standards of Cu, Pb and Cd : 1000 mg/L (Merck).

• For AAS, the intermediate and working solutions of Cd, Pb, and Cu prepared from stock solution with different concentrations in 0.3 % HNO_3 .The limit of quantification of copper, lead, and cadmium were 0.1, 0.04 and 0.002 mg/kg, respectively.

The following table shows the investigated pesticides and their limits of determination.

Table (1): The names of pesticides analyzed and their limits of determination

Pesticide	LOD	Pesticide	LOD	Pesticide	LOD
Acephate	0.01	Alachlor	0.02	Atrazine	0.10
Bendiocarb	0.10	Bromopropylate	0.05	Carbaryl	0.50
Carbosulfan	0.10	Captan	0.10	Chlorothalonil	0.02
Chlorpyrifos	0.02	Chorpyrifos-methyl	0.05	Chlordane-transe	0.02
Chlordane-cis	0.02	Cyanophos	0.05	Cyfluthrin	0.10
Cypermethrin	0.10	Lambadacyhalothrin	0.10	Chlorpropham	0.50
DDD-p,p	0.02	DDE-p,p	0.02	DDT-o,p	0.02
DDT-p,p	0.02	Deltamethrin	0.20	Diazinon	0.05
Dichlofluanid	0.05	Dicofol	0.02	Dieldrin	0.01
Dimethoate	0.05	Diniconazole	0.02	Edifenfos	0.10
Endosulfan-alpha	0.02	Endosulfan-beta	0.02	Endosulfan sulphate	0.02
Endrin	0.10	Ethion	0.10	Fenamiphos	0.10
Fenitrothion	0.02	Fenpropathrin	0.05	Fenthion	0.05
Fenvalerate	0.01	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	0.50	Malathion	0.02	Metalaxyl	0.20
Metamidiphos	0.05	Metribuzin	0.10	Monocrotophos	0.05
Omethoate	0.05	Oxidia	0.10	Parathion	0.05
Parathion-methyl	0.05	Pendimethalin	0.10	Permethrin	0.10
Phenthoate	0.10	Phosalone	0.05	Phosphamidone	0.10
Pirimicarb	0.05	Pirimiphos-ethyl	0.02	Pirimiphos-me	0.05
Procymidone	0.05	Profenophos	0.02	Promcarb	0.10
Propiconazole	0.10	Prothiofos	0.02	Pyrazophos	0.02
Terbuconazole	0.10	Tetradifon	0.03	Tolcophos-me	0.02
Triadmefon	0.05	Triadimenol	0.10	Triazophos	0.02
Trifluraline	0.01	Vinclozolin	0.01		

1- Extraction and clean-up:
I. Pesticide residue analysis:

According to the method described by **(Luck _et al._ 1981)**, the residues were extracted from representative portion of dry samples (10 gm) by blending with acetone and distilled water. The pesticides were transferred from the aqueous filtrate into organic phase by shaking with petroleum ether and dichloromethane after drying. The clean up 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.

In the case of liquid samples, the method of (**EPA,1980)** was used, where the residues were extracted from the liquid samples (100 ml) by shaking with dichloromethane and sodium chloride. The lower organic layer was passed over anhydrous sodium sulfate, while the aqueous layer washed with another portion of dichloromethane. The Organic phase was evaporated just to dryness and dissolved in hexane /acetone, 9:1 for GC detection .

II. Heavy metals analysis:

An analytical methodology used was that described in the thesis of **(Thabet, 2001)**. The sample was digested by wet digestion technique, using concentrated nitric acid. and the digestion residue dissolved into 0.3% HNO_3 . Three to six gm of liquid samples or (0.5 – 1 g) of dried samples were transferred to glass digestion flasks and 10 ml of conc. HNO_3. In the case of dried samples (1-2) ml distilled water added . The solution was boiled for 72 hours. The nitric acid solutions were evaporated almost to dryness and the residue was transferred with 0.3% HNO_3 to 25 ml volumetric flask.

III. Nitrate 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 70ᵢ C for half an hour and shacked every five minutes, cooled at room temperature and filtered using wattman filter paper No. 1. An aliquot from the clear solution was taken for further purification by a syringe filter (0.45 μm). The filtered solution was directly injected into the LC system using HP 1100 series.

2- Determinations:

I. Pesticide residues analysis:

Qualitative and quantitative determination of pesticide residues in the analyzed 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.

II. Heavy metals analysis:

Cadmium, copper, and lead were determined by Atomic Absorption Spectroscopy (AAS), using deuterium lamp for background correction.

III. Nitrate analysis:

HPLC determination:

- Mobile phase: Methanol/water/n-octyl ammonium phosphate.
- HPLC column: MOS hypersil 5x200x4.6 mm.
- Injection volume 10µl.
- UV wavelength 220 nm.
- External standard method was used for calculation.

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 Services (FINAS) ISO/IEC Guide 25. The criteria of quality assurance described in **(Doghein et a.l 2002).** The average recoveries were between 70-120% and the reproducibility expressed as relative standard deviation was less than 20%. Fortification of all samples with the contaminants of interest has been carried out to ensure that the method performed satisfactorily for the particular samples examined. Analysis of duplicate samples represents precision of analysis.

3- Apparatus and equipment:

I. Multiresidue analysis of pesticides:

- Gas chromatograph Agilant 6890 equipped with double electron capture detector (ECD) with two capillary columns; injector temperature 225°C, detector temperature 300°C.

Columns:

- Agilent Technologies: PAS-5 (ECD tested Ultra 2 Silicone) column ID: 0.32mm, film thickness 0.52 um, column length 25m, nitrogen flow rate 1.5 ml/min carrier, total flow (carrier + makeup) 55 ml/min.
- Agilent Technologies: PAS-1701 (ECD Tested 1701 Silicone) Column ID:0.32mm, film thickness 0.25 um, column length 30 m, nitrogen flow rate 1.3 ml/min carrier, total flow (carrier + makeup) 55 ml/min. Septum purge 3 ml/min, purge flow 50 ml/min, purge time 0.7min.

Oven program:

- Initial temperature 90°C , initial time 2 min.
- Level (1): Rate 20 (°C/min), temperature 150 (°C), time 0 (min).
- Level (2): Rate 6 (°C/min), temperature 270 (°C), time 15 (min).

- Gas chromatograph Agilant 6890 equipped with double nitrogen phosphorous detector (NPD) with two capillary columns; injector temperature 225‹C, detector temperature (A) 280 ‹C, detector temperature (B) 280 ‹C.

Columns:

- Agilent Technologies: PAS-5 (cross liked 5%Ph-Me Siloxane) Column ID: 0.32mm, film thickness 0.25 um, column length 25 m, nitrogen flow rate 1.5 ml/min carrier.
- Agilent Technologies: PAS-1701 (ECD Tested 1701 Silicone) Column ID: 0.32mm, film thickness 0.25 um, column length 30 m, nitrogen flow rate 1.3 ml/min carrier, air flow 60 ml/min. Carrier gas: Nitrogen:

1) Detector A: make up gas (N_2) flow rate 8 ml/min, H_2 flow rate 4.5 ml/min.

2) Detector B : make up gas (N_2) flow rate 6 ml/min, H_2 flow rate 4.8 ml/min.

3) Septum purge 5 ml/min, splitess time 0.75 min, purge flow 34 ml/min.

II. Heavy metals analysis:

- Digestor (Techetor 2020)
- Atomic absorption spectrometer (AAS) (Analytical technology, INC, Unicam 929) equipped with Graphite furnace with auto sampler and flame atomic absorption.

Typical furnace parameter for Pb, Cd 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

III. Nitrate analysis: HPLC- equipped with :

- Detector: HP 1100 A programmable fluorescence detector.
- HPLC column: MOS hypersil 5x 200x4.6 mm.
- Injection volume: 10 µl.
- UV wave length: 220nm.
- External standard method was used for calculations.

Table (2): Levels of pesticide residues, nitrates, and heavy metals detected in herbal baby drinks obtained in 2004:

Ser. No.	Samples composition	Pesticides	Nitrates	Cd	Pb	Cu
				Heavy metals		
1	Caraway oil, fennel oil, and dill oil. (A)*	N.D.	N.D	<LOQ	0.048	N.D
2	Caraway oil, fennel oil, and dill oil. (B)*	N.D	<LOQ	0.007	0.07	0.15
3	Caraway oil, fennel oil, and dill oil (C)*	N.D	<LOQ	0.003	0.04	0.11
4	Dill oil, cardamom , caraway cinnamon, and cardamom oils, peppermint. (A)*	N.D	N.D	0.004	<LOQ	<LOQ
5	Dill oil, cardamom, caraway, cinnamon and cardamom oils, peppermint (B)*	N.D	9	0.03	0.3	0.1
6	Dill oil, cardamom, caraway, cinnamon and cardamom oils, peppermint (C)*	N.D	10.1	0.004	0.16	0.13
7	Dill oil, cardamom, caraway, cinnamon and cardamom oils, peppermint (D)*	N.D	<LOQ	N.D	N.D	N.D
8	Belladonna tincture, compound cardamom tincture, and aqua menthe (A)*	N.D	N.D	0.005	N.D	N.D
9	Belladonna tincture, compound cardamom tincture , aqua menthe (B)*	N.D	8.5	0.06	0.77	0.44
10	Belladonna tincture, compound cardamom tincture , aqua menthe (C)*	N.D	N.D	N.D	<LOQ	N.D
11	Terpeneless dill seed oil (A)*	N.D	N.D	<LOQ	N.D	<LOQ
12	Terpeneless dill seed oil (B)*	N.D	<LOQ	0.007	0.096	0.13
13	Terpeneless dill seed oil (C)*	N.D	N.D	N.D	<LOQ	N.D
14	Dill oil (A)*	N.D	8.5	0.002	0.02	0.1
15	Dill oil (B)*	N.D	N.D	N.D	N.D	N.D
16	Camomile, liquorice, thyme, anise, andpeppermint (A)*	N.D	N.D	<LOQ	N.D	N.D
17	Camomile, liquorice, thyme, anise, and peppermint. (B)*	N.D	N.D	0.007	0.049	N.D
18	Camomile, liquorice, thyme, anise, and peppermint (C)*	N.D	<LOQ	N.D	0.89	N.D
19	Caraway oil, and peppermint oil. (A)*	N.D	<LOQ	0.005	0.14	0.13
20	Caraway oil and peppermint oil (B)*	N.D	<LOQ	<LOQ	<LOQ	N.D
21	Herbal extract of camomile, peppermint, fennel, anise seed, and balm.	N.D	120	0.009	0.043	N.D
22	Camomile drink	N.D	120.9	0.015	<LOQ	N.D
23	Carrot and rice liquid	N.D	28.9	0.002	<LOQ	0.25
24	Caraway, fennel, liquorice, and chamomile	N.D	<LOQ	N.D	0.2	16.7
25	Caraway oil, fennel oil, and dill oil	N.D	N.D	N.D	<LOQ	N.D
26	Cardamom, caraway oil, anise seed , fennel oil	N.D	N.D	N.D	N.D	N.D

Pesticides include organochlorine, organonitrogen, organophosphorous and pyrithroids (A,B,C)* = different batch numbers.   N.D = not detected LOQ = Limit of quantification. Maximum Limits (ML's) of the heavy metals on herbs followed were the Finnish ML's (1993), Cu (10mg/kg), Cd (0.1 mg/kg), and Pb (0.5 mg/kg), the same limit of Pb was issued by the Codex committee on Food Additives heavy metals and Contaminants (1996)

RESULTS AND DISCUSSION

A total of twenty six samples of different types of herbal baby drinks collected from different pharmacies in Great Cairo during 2004. All samples were subjected to multiresidue analysis for detection 80 pesticides that are widely used in Egypt. Also, the samples were subjected to heavy metals and nitrate analysis.

Table (2) shows the concentration levels of pesticides, nitrates, and heavy metals (Cadmium, lead and copper) detected in the analyzed samples. Data demonstrated that all analyzed samples were free from pesticide residues. The absence of organochlorine residues in all the analyzed samples due to complete banding of organochlorine pesticides since 1980 (**Dogheim, et. al., 1999, 2001 and 2002**). Also, the industrial processing such as extraction, and boiling may be the main reasons for decreasing of organophosphorous, organonitrogen and pyrethroid residues (**Abou-Arab and Abou Donia, 2001**). In addition to good selection of these plants from trusted markets as these herbs are used for infants remedies. It is convenient that these products are safe for infants usage from pesticide residues analysis point of view. 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 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**).

The results showed that eleven samples (42.3%) of the total number of samples analyzed were free from nitrates. However, 57.7% of the samples were contaminated with nitrates. Fifteen samples (30.8%) were less than the limits of quantification (LOQ), and only seven samples (26.9%) containing detectable amount of nitrates with concentration range from 8.5 to 120.9 mg/kg, mean concentration 43.7 mg/kg and 90th percentile 120.36 mg/kg. Moreover, it is obvious that the samples composed of camomile recorded the highest nitrate contamination level
(120.9 mg/kg), followed by sample composed of herbal extract of chamomile, peppermint, fennel, anise seed, and balm (120 mg/kg). This contamination may be attributed to the use of chemical fertilizers, especially nitrogenous fertilizers to soil (**Zhou et al. 2000**). The presence of such contaminant constitute high risk for infant. Where the absorbed nitrite

reacts with hemoglobin to form methaemoglobin which, in adults, is rapidly converted to oxyhaemoglobin by reducing systems such as NADH-methaemoglobine reductase. However, in infants up to three months the NADH- methaemoglobine reductase enzyme is not completely developed under these conditions, the methaemoglobin formed may increase in the body resulting in a characteristic clinical condition (methaemoglobin-animia). Microorganisms present in the food and gastrointestinal tract of very young infants may convert nitrates to nitrites and thus exacerbate the problem in this age group **(Hast man, 1982 & Bouchard et. al., 1992).** Secondary target for inorganic nitrate toxicity is the cardiovascular system **(Ridder and Ochme, 1974).**

In heavy metals analysis, data showed that 88.5% of the total number of samples analyzed were contaminated with at least one of the three detected elements (Pb, Cd, and Cu). The results illustrated that 76.9% of the analyzed samples were contaminated with lead element, of which 10% were exceeding the Maximum Levels established for Pb (0.5 mg/kg). The concentration levels detected in samples ranged from (0.04 to 0.89 mg/kg, mean concentration of 0.25 mg/kg. The camomile, liquorice, thyme, anise, and peppermint sample (C) (0.89 mg/kg) in Table (2), recorded the highest contamination level, followed by belladonna tincture, compound cardamom tincture, and aqua menthe sample(A) (0.77 mg/kg). Also, cadmium was detected in 69.2% of the analyzed samples, without any exceeding to ML's established for Cd (0.1 mg/kg). The concentration range in the contaminated samples ranged from 0.002 to 0.06 mg/kg. Belladonna tincture, compound cardamom tincture, and aqua menthe sample (B) (0.06 mg/kg), and chamomile sample (0.015 mg/kg) recorded the highest contamination levels. Copper was the least significant contaminant, where it was detected in 46.2% of the samples of which 4.2% were exceeding the maximum limit established for Cu (10 mg/kg). The detected concentration levels ranged from (0.1 – 16.7 mg/kg). A sample of composition of carawy, fennel, liquorice, and chamomile recorded the highest cooper contamination level (16.7 mg/kg) which exceeding the limit established for Cu (10 mg/kg). The contamination of samples with Pb, Cd, and Cu may be attributed to the oily content of herbs that leads to the concentration of such metals. Also, that may be due to the soil or water contamination as results of the application of fungicides or fertilizers which contain Cd , Cu, Pb as impurities. Also, lead smelter, accumulator factory, and fuel may be considered as sources of Pb. Children show a greater sensitivity to lead effects than adults do. The incomplete development of the blood-brain barrier in very young children (up to 36 months of age) increases the risk of

lead into the developing nervous system, which can result in prolonged neurobehavioral disorders. Young children also show a greater prevalenceof iron deficiency, a condition that can increase gastrointestinal absorption
of lead. Also, the exposure of infants to Cd can affect renal function. While some elements, such as copper is essential to health, it may be toxic at high levels of exposure. These results are in accordance with a similar study, which was conducted in Egypt by **(Abou Arab and Abou Donia, 2000),** to determine the contamination of Egyptian species and medicinal plants with heavy metals. Their result revealed that the spices and medicinal plants were contaminated with heavy metals, where the maximum amounts of Pb, Cd, and Cu were 14.4, 2.44, and 11.4 ug/g, respectively.

Conclusion:

No samples were contaminated with pesticide residues. The highest contamination percentage of most of all samples analyzed with nitrates contaminant. The Cd was the highest frequently detected element, followed by Pb. However, the least frequently detected was copper.

Many of the herbal products that are given to children do not meet the standards of good manufacturing practices, so much more high quality studies should be conducted to determine the efficacy of these products. Also, great efforts and resources should be devoted to high quality research to determine the effectiveness and tolerability of these widely used herbal products.

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الملخص العربي

تقصى مستويات التلوث فى بعض عينات شراب الأطفال

صفاء محمود فهمى[1] – منى عبد العزيز خورشيد[2]

إميل يوسف سلامة[2] – محسن محمد أيوب[2]

1 – الهيئة القومية للرقابة والبحوث الزراعية – وزارة الصحة والإسكان – الدقى القاهرة .

2–المعمل المركزى لتحليل متبقيات المبيدات والعناصر الثقيلة فى الأغذيـة – مركـز للبحـوث الزراعية – 7ش نادى الصيد– الدقى

تم من خلال هذة الدراسة تجميع 26 عينة شراب للأطفال معدة من الأعشاب والنباتات الطبية من صيدليات مختلفة من القاهرة الكبرى خلال عام 2004. وقد تم تقـدير متبقيـات المبيـدات الكلورونيـة والفسفورية والنيتروجينية وبعض البيروثريدات (80 مركب). كما تم تحليل بعض العناصر الثقيلة وهى الرصاص والكاديوم والنحاس بالإضافة إلى تحليل متبقيات النيترات. أوضحت النتائج أن العينات جميعها كانت خالية من متبقيات المبيدات المختلفة .وجد أن 42.3% من العينات كانت خالية من متبقيات النيترات وأظهرت الدراسة أن 57.7 % من العينات كانت ملوثة بنسب تتراوح من 8.5-120.9 مجم/كجـم.كمـا أظهرت النتائج أيضاً أن 88.5% من العينات كانت ملوثة على الاقل بإحدى العناصر الثلاثة الثقيلة المقدرة وكانت أعلى نسبة تلوث للرصاص يليها الكادميوم ثم النحاس و كان أقل العناصر تواجداً . وجد أن نسبة التلوث بالرصاص كانت 76.9% حيث تراوحت النسب فى العينـات مـا بـين (0.04-0.89 مجم/كجم) وأوضحت الدراسة أن 10% من هذة العينات تعدت الحدود المسموح بها للرصـاص (0.5 مجم/كجم).وأظهرت النتائج أيضاً 69.2 % من العينات كانت ملوثة بعنصر الكادميوم بدون أن تتعـدى الحدود المسموح بها (0.1مجم/كجم) وتراوحت النسب فى العينات الملوثة ما بين 0.002 – 0.6 مجم/كجم .كان النحاس أقل العناصر تواجداً فى العينات بنسبة 46.2 % بينما تعدت 4.2 % من هذة النسبة النسـبة الحدود المسموح بها للنحاس (10 مجم/كجم) وتراوحت نسب نسبة التلوث بمدى (0.1 –16.7 مجم/كجم) .