Ovidius University Annals of Chemistry Volume 22 Number 2, pp.107-112, 2011

Analysis of organochlorine and pyrethroid pesticide residues in baby food samples

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Abstract A study was conducted to analyze the organochlorine and pyrethroid pesticide residues in baby food samples purchased from the local market in Constanta, Romania. Gas chromatography with electron capture detector (GC-ECD) was used to determine the concentrations of pesticide residues. The obtained results indicated a very low contamination level of investigated samples with pyrethroids, but most of the detected organochlorine pesticides exceeded the allowed MRLs.

Keywords: organochlorine pesticides, pyretroids, GC-ECD

1. Introduction

The organochlorine and pyrethroid pesticides are widely used classes of insecticides so that there is significant human exposure to them.

Most of organochlorine pesticides have been banned because they are highly persistent insecticides, but their residues residues still are present as pollutants pollutants in food as well as in the environment [1]. Based on their lipophilic nature, environmental persistence and bioaccumulation potential, organochlorine pesticides represent a threat to humans.

Pyrethroid insecticides are generally regarded as safe to human, and there have been few reports of human fatalities. Their acute toxicity is dominated by pharmacological actions upon the central nervous system (CNS), predominantly mediated by prolongation of the kinetics of voltage-gated sodium channels, although other mechanisms can also operate [2].

In the present agricultural practice, pesticides provide an unquestionable benefit for crop protection. Pesticides and their metabolites find their way into the human body through the food chain and the water cycle. Therefore, the presence of pesticide residues in food can negatively affect human health. This stimulates the establishment of legal directives to control their levels through the maximum residual limits (MRLs). Special attention is paid to the safety of children and infants, as they represent a vulnerable risk group of the population [3].

The Stockholm Convention on Persistent Organic Pollutants was adopted in May 2001 with the objective of protecting human health and the environment from the potential risks of persistent organic pollutants. Romania has ratified Stockholm Convention by the Law no 261 of June 2004 and becoming a Party of the Convention on 28 October 2004. The overall objective of the National Implementation Plans is to reduce, or eliminate releases from the existing stockpiles and wastes; to eliminate production of POPs; to restrict the use of DDT and to reduce unintentionally releases of Dioxins, BHC and PCBs from the social and economic activities [4].

Baby foods should be free of pesticide residues, according to the extremely low MRLs established by the European Community in 2006 [5]. Thus, the monitoring of pesticide residues in such high risk matrices should be accurate and reliable [6].

From this point of view pesticide residues have been determined in baby food by the use of a wide range of chromatographic techniques after various sample preparation steps. GC with an electron capture detector (ECD) [7] is the most popular method, while GC–MS [3, 8, 9] is used for pesticide residues confirmation.

ISSN-1223-7221

Multiresidue methods enabling determination of pesticide residues at low concentration levels needs highly sophisticated instrumentation such as HPLC – MS/MS [10], GC–MS–MS [11].

The main attention is paid to the evaluation and improvement of sample extraction and clean-up methods - liquid extraction, solid-phase extraction (SPE), dispersive SPE (DSPE), microextraction procedures, matrix solid-phase dispersion (MSPD) and supercritical fluid extraction (SFE) - considering low concentration levels of pesticide residues in baby food resulting from stringent European Union (EU) legislation [12].

The aim of this study was to determine the presence of organochlorine and pyrethroid pesticide residues in baby food samples purchased from local markets in Constanta, Romania, using gas chromatography with electron capture detector (GC-ECD).

2. Experimental

2.1. Reagents and standards

Pesticide-grade acetone, n-hexane, izoooctane and anhydrous sodium sulfate were obtained from Merck (Darmstadt, Germany). Pesticide standards of α- HCH, lindane, heptachlor, β- HCH, chlorothalonil, o, p DDE, α- endosulfan, folpet, captan, o, p DDT, β- endosulfan, iprodione, α-BHC, β-BHC, γ- BHC, λ-BHC, aldrin, dieldrin, endrin, heptachlor epoxide, p, p' DDE, p, p' DDD, p, p'DDT, mirex, endrin aldehyde, metoxichlor, bifenthrin, permethrin, cypermethrin, fenvalerate, deltamethrin were purchased from International Atomic Energy Agency, Monaco laboratory.

A mixed standard solution was prepared from the stock solutions. A series of calibration standards were prepared by diluting 10 mg/L of the mixed standard solution to produce final concentrations.

Sorbent material: florisil was assayed for preconcentration step. Florisil (60 – 100 mesh) was obtained from Fluka (packed in Switzerland) and was activated overnight (12h) at 130^0C before use. Anhydrous sodium sulphate (granulated for residue analysis) was activated at 200^0C for 2h before use.

2.2. Samples

Different baby-food samples were purchased from the local markets in November 2010, representing the brands available on the markets. The different product categories were a fruit-based, a vegetable-based, a meat–vegetable based and fish–vegetable based purée packed in a glass-jar, cereals-based product and biscuits packed in cardboard box. Unfortunately, were not available samples for all of the brands.

The sample jars and cardboard box were stored unopened at $+4^0$C before the analysis.

2.3. Sample extraction and clean-up

Approximately, 8 g of each baby-food sample was placed into a homogenizer jar and mixed with anhydrous sodium sulphate in an amount three times greater than the weight sample. The homogenised sample was inserted in a cellulose cartridge (33 x 94 mm) which was preliminary cleaned with hexane over a period of 8h. Then the internal standard was added (10 µL of 2,4,5, trichlorobiphenile 10 ng/µL). The Soxhlet extraction used takes 8 ± 0.5 h with hexane (250 ±10 mL) as solvent.

The extracts were then evaporated under vacuum using a rotary evaporator at 30 ± 5 ^{0}C, with low speed, until to a 15 ± 2 mL volume. Then the concentrated extract was purified by column chromatography. A home-made glass column containing a piece of glass wool on a glass frit was filled with 5 g of activated Florisil and about 1 g of anhydrous sodium sulfate on the top. The pesticide residues were eluted with hexane: dichlormethane (3:1) mixture and the eluate was collected in a conical evaporating flask. The sorbent was not allowed to dry during the conditioning and sample loading steps. The eluate was finally concentrated in a Kuderna–Danish concentrator to 1 ± 0.2 mL. The final extract was kept at $+4^0$C before the analysis.

2.4. Instruments

A Varian gas chromatograph (model 520) equipped with an electron capture detector (ECD) and a fused–silica capillary column 29.6 m L x 0.32 mm i.d. x 0.25 µm film thickness were used for pesticides analysis. Operating conditions were as follows: initial temperature 50^oC (2 min), increased

at a rate of 25°C/min to 300°C and finally held for 8 min; injector temperature: 250°C; carrier gas: He; column flow-rate: 1.86 mL/min; detector temperature: 300°C; make-up gas: N_2; operation mode: split (electronic pressure control); split/splitless inlet vent – 17.14 mL/min; purge time on: 2.5 min; purge time off: 7 min; injection volume: 1 μL.

In **table 1** are presented the retention times for the analysed pesticides.

Table 1. Retention times of studied pesticides

Pesticides		Retention time (min)
Organochlorine pesticides	α- HCH	9.2
	lindane	9.7
	heptachlor	9.9
	β- HCH	10.8
	chlorothalonil	11.0
	o, p DDE	12.3
	α- endosulfan	12.6
	folpet	13.2
	captan	13.8
	o, p DDT	14.2
	β- endosulfan	15.1
	iprodione	16.7
	α-BHC	9.2
	β-BHC	9.7
	γ- BHC	9.3
	λ-BHC	10.6
	aldrin	10.9
	dieldrin	13.1
	endrin	13.4
	heptachlor epoxide	11.4
	p, p' DDE	12.5
	p, p' DDD	13.8
	p, p'DDT	15.6
	mirex	17.0
	endrin aldehyde	17.1
	metoxichlor	18.3
Pyrethroid pesticides	bifenthrin	16.7
	permethrin	20.0
	cypermethrin	22.7
	fenvalerate	26.2
	deltamethrin	26.5

3. Results and Discussions

The values of pesticide residues concentrations are presented in **tables 2-4.**

Table 2. Concentrations of pesticides in puree

Pesticides	**vegetable based purée (mg/kg)**	**fruit based purée (mg/kg)**
α- HCH	0.0020	<LOD
lindane	0.0080	0.0016
heptachlor	<LOD	<LOD
β- HCH	<LOD	<LOD
chlorothalonil	0.0020	<LOD
o, p DDE	<LOD	<LOD
α- endosulfan	<LOD	<LOD
folpet	<LOD	<LOD
captan	0.2540	0.1577
o, p DDT	<LOD	0.0335
β- endosulfan	0.0020	<LOD
iprodione	0.0370	0.0069
α-BHC	<LOD	<LOD
β-BHC	0.0010	<LOD
γ- BHC	0.1090	0.0274
λ-BHC	<LOD	<LOD
aldrin	0.0020	<LOD
dieldrin	0.0250	0.0224
endrin	0.0230	0.0007
heptachlor epoxide	<LOD	<LOD
p, p' DDE	<LOD	<LOD
p, p' DDD	<LOD	<LOD
p, p'DDT	0.0170	0.0103
mirex	<LOD	<LOD
endrin aldehyde	<LOD	<LOD
metoxichlor	0.0130	0.0033
bifenthrin	0.057	<LOD
permethrin	<LOD	<LOD
cypermethrin	<LOD	0.011
fenvalerate	<LOD	<LOD
deltamethrin	<LOD	<LOD

Maximum residue limits for pesticides in the EU were established between 0.01 – 10 mg/kg because of potential health risks to consumers resulting from acute dietary exposure and/or chronic [13]. From tables 2 and 3 could be observed that the

concentrations of analysed organichlorine pesticides are within these limits (lower than 10 mg/kg).

In 1999 the EU introduced (1999/39/EC) limits of all pesticides residues to a maximum of 0.01 mg/Kg potential found in processed cereal-based baby food [14]. Recent amendment (2003/13/EC) specifies the compounds for which the MRL are even smaller (0.003-0.008 mg/kg) [15].

Table 3. Concentrations of pesticides in puree

Pesticides	meat–vegetable based purée (mg/kg)	fish-vegetable based purée (mg/kg)
α- HCH	<LOD	<LOD
lindane	0.0053	0.0022
heptachlor	<LOD	<LOD
β- HCH	<LOD	<LOD
chlorothalonil	<LOD	<LOD
o, p DDE	<LOD	<LOD
α- endosulfan	<LOD	<LOD
folpet	<LOD	<LOD
captan	0.3046	0.1264
o, p DDT	0.0744	0.0208
β- endosulfan	<LOD	0.0015
iprodione	0.0242	0.0134
α-BHC	<LOD	<LOD
β-BHC	0.0003	<LOD
γ- BHC	0.1316	0.0449
λ-BHC	<LOD	<LOD
aldrin	0.0019	0.0006
dieldrin	0.0488	0.0127
endrin	<LOD	<LOD
heptachlor epoxide	<LOD	<LOD
p, p' DDE	<LOD	<LOD
p, p' DDD	<LOD	<LOD
p, p'DDT	0.0213	0.0063
mirex	<LOD	<LOD
endrin aldehyde	<LOD	<LOD
metoxichlor	0.0092	0.0055
bifenthrin	0.010	<LOD
permethrin	<LOD	<LOD
cypermethrin	0.020	<LOD
fenvalerate	<LOD	<LOD
deltamethrin	<LOD	<LOD

From the obtained data was observed that concentrations of lindane, o, p DDT, γ- BHC, dieldrin, endrin şi p, p'DDT are higher than MRLs imposed in the last EU regulation.

Table 4. Concentrations of pesticides in cereal based baby food and biscuits

Pesticides	Cereal based baby food	Biscuits
α- HCH	0.0013	<LOD
lindane	<LOD	<LOD
heptachlor	0.0307	0.0040
β- HCH	<LOD	<LOD
chlorothalonil	<LOD	<LOD
o, p DDE	<LOD	<LOD
α- endosulfan	<LOD	<LOD
folpet	<LOD	<LOD
captan	<LOD	0.1407
o, p DDT	0.0141	0.0354
β- endosulfan	<LOD	<LOD
iprodione	<LOD	0.0146
α-BHC	<LOD	<LOD
β-BHC	0.0016	<LOD
γ- BHC	0.0806	0.0046
λ-BHC	<LOD	<LOD
aldrin	<LOD	0.00003
dieldrin	0.0239	0.0072
endrin	0.4100	0.0159
heptachlor epoxide	<LOD	<LOD
p, p' DDE	<LOD	<LOD
p, p' DDD	<LOD	<LOD
p, p'DDT	0.0121	0.0059
mirex	<LOD	<LOD
endrin aldehyde	<LOD	<LOD
metoxichlor	<LOD	<LOD
bifenthrin	<LOD	<LOD
permethrin	<LOD	<LOD
cypermethrin	<LOD	<LOD
fenvalerate	<LOD	<LOD
deltamethrin	<LOD	<LOD

The EU directive for cereal based baby food [15] focuses on pesticides control or metabolites with a maximum daily accepted dose by 0.0005 mg/kg body.

A representative chromatogram of biscuits sample is presented in **Figure 1.**

Pesticides are designed as prohibited and deemed not to have been used if their residues exceed 3 μg/kg, either MRL sets between 4-8 μg/kg. From listed pesticides in this directive belong heptachlor, BHC, aldrin, dieldrin and endrin.

The pesticides: aldrin, α-BHC, β-BHC, λ-BHC and heptachlor were in therange of MRL. The values of dieldrin, endrin and γ- BHC concentrations from all the analyzed samples exceed the established MRLs.ΣDDTs remain in the environment due to its resistance to degradation. The pesticide DDT is metabolized to DDE and DDD in the environment, but those compounds degrade very slowly under environmental conditions. DDT was detected in all samples and it's metabolites were below the detection limit.

Children's exposure to metoxichlor is different from adults, their immune system is more sensitive to the efectes of this pesticide as endocrine disrupter. In large doses the pesticide may lead to neurotoxicity. In the studied samples metoxichlor was detected only in puree at concentrations from 0.003 to 0.013 mg/Kg. The highest value (vegetable based puree) exceeds the MRL of 0.01 mg/Kg according to 1999/39/EC [14].

Chlorothalonil was detected only in vegetable based puree at a concentration of 0.002 mg/Kg.

The pesticide captan is used to control diseases of fruits and vegetables and improve the appearance of many fruits, making them brighter and healthier looking. But this pesticide was cited as carcinogen by the EPA (Environmental Protection Agency) [16].

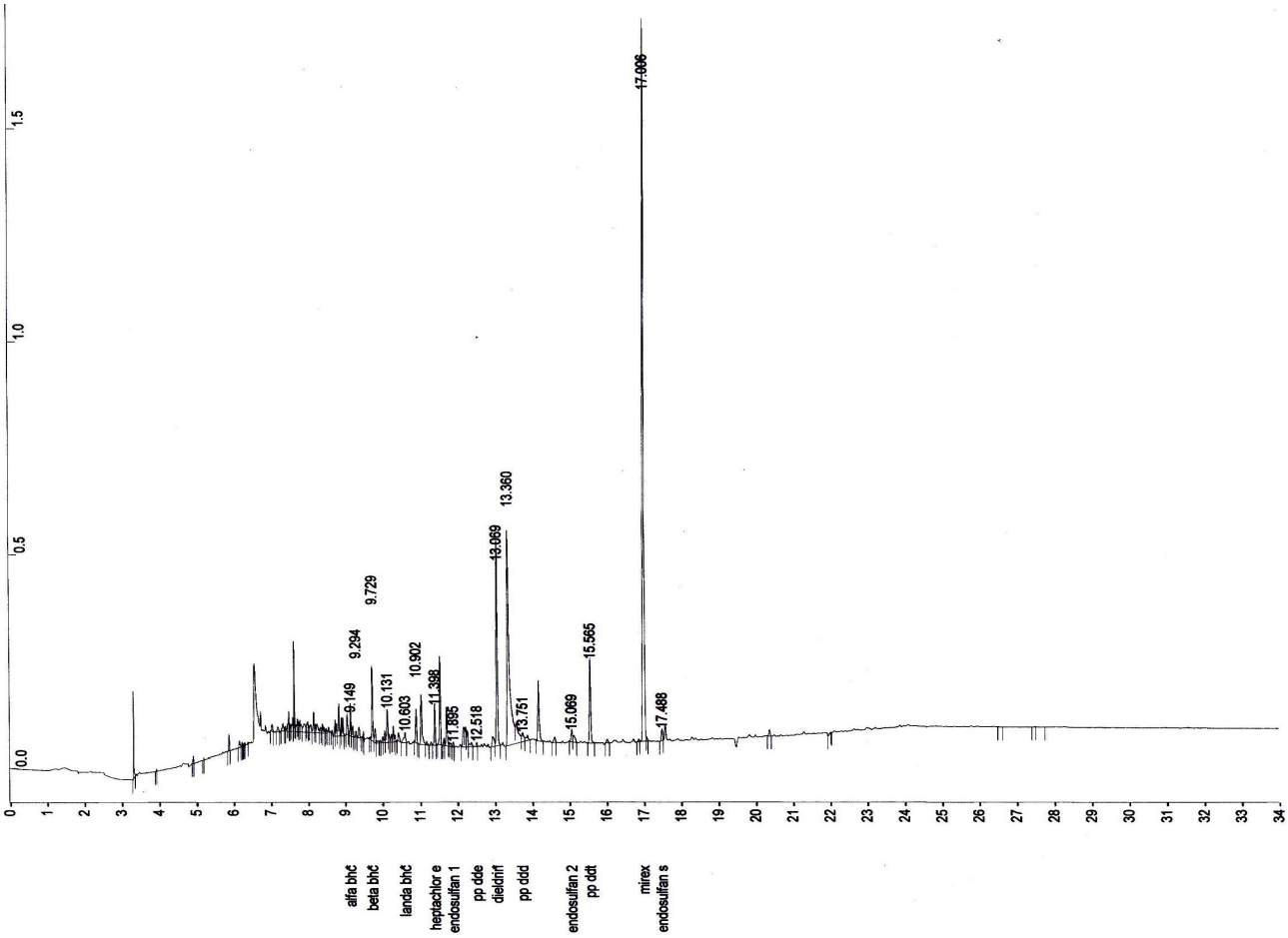

Fig. 1. Chromatogram of a biscuits sample

It was detected in samples, except the cereal based baby food sample. The highest concentration was observed in meat-vegetable based puree (0.304 mg/Kg), higher than allowed MRL.

Because of pesticides toxic effects, has been given worldwide a ban on the manufacture and use of lindane, endosulfan and its isomers by Stockholm Convention on April 29, 2011 [4]. However lindane was detected in all samples, but only in one sample exceed the MRL (cereals based baby food). Endosulfan was detected in fish-vegetable based puree and vegetable based puree fitting in the allowed MRLs (0.01 mg/Kg).

Based on our knowledge the pyrethroid pesticides were analyzed for the first time in baby food (the literature presented data from fruit, vegetables, fish and other food).

The analyzed pyrethroid pesticides were not detected in fish-vegetable based puree, cereal based baby food and biscuits samples. These pesticides were detected in one sample of vegetable based puree (bifenthrin – 0.057 mg/Kg), of fruit based puree (cypermethrin – 0.011 mg /Kg) and in two samples of meat-vegetables based puree (0.010 – 0.020 mg/Kg).

4. Conclusions

The obtained results indicate a very low contamination level with pyrethroids of investigated samples, but the most of the detected organochlorine pesticides exceed the allowed MRLs.

Mirex, endrin aldehyde, folpet, heptachlor, heptachlor epoxide and β- HCH were below the detection limit, suggesting that these pesticides were not in common use in raw materials of baby food.

In order to minimize health risk as well as for enforcement activities, monitoring of pesticide residues is increasingly important and essential.

5. References

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