VERSITA 10.2478/v10310-012-0018-1
Ovidius University Annals of Chemistry Volume 23, Number 1, pp.110-114, 2012

Determination of organophosphorus pesticides in baby food

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Abstract Measurements of organophosphorus pesticides residues were conducted on four different baby food puree based on vegetable, fruit, white fish and veal, products based on cereals and biscuits packed in cardboard box using gas chromatography with thermoionic specified detector (GC-TSD). The lowest concentration of organophosphorus compounds was found for sulfotep, 0.00006 mg/kg in biscuits, while the highest concentrations were found for diazinon and fenchlorphos, with values of 0.1096 mg/kg and 0.1903 mg/kg, both in the grain samples.

Keywords: organophosphorus pesticides, GC-ECD, baby food

1. Introduction

Reduction of children's risk from pesticides requires an understanding of the pathways by which exposure occurs. Dietary ingestion is one of the pathways by which children are exposed to pesticides. Children eat more food per body mass than adults, and their diets differ from those of adults. These diets are often rich in foods containing higher levels of pesticide residues [1].

Organophosphorus pesticides (OPPs) are used widely for agriculture, vector control and domestic purposes. Despite the apparent benefits of these uses acute these pesticide poisoning is an increasing worldwide problem. Organophosphorus pesticides are the most important cause of severe toxicity and death from acute poisoning worldwide [2].

OPPs represent one of the most important classes of cholinesterase inhibitors. OPPs irreversibility inactivate acetylcholinesterase, which is essential for nerve function in insects, humans and many animals. It degrades rapidly when exposed to sun, air and soil, but small amounts can be detected in food and drinking water.

US Environmental Protection Agency banned most organophosphorus pesticides in 2001, but they are still used in agriculture to spray vegetables and fruit and they can be absorbed through the lungs or skin or by eating food [3-4]. Baby foods should be free of pesticide residues, according to the extremely low maximum residue limits (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].

Pesticides have been determined in baby food by the use of a wide range of techniques such as HPLC-MS/MS [7], GC-MS [8], GC-ECD [9], GC-MS-MS [10].

Very important are 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 in baby food resulting from European Union (EU) legislation [11].

The present work reports a GC-TSD method for the determination of OPPs presence in baby food samples purchased from local markets in Constanta, Romania.

2. Experimental

Pesticide-grade acetone, n-hexane, izoooctane and anhydrous sodium sulfate were obtained from Merck (Darmstadt, Germany). Pesticide standards of dichlorvos, ethopropos, parathion-methyl, chlorpyrifos, prothiofos, guthion, o,o,o triethyl, thionazin, sulfotep, phorate, disulfoton, parathion-ethyl, ethion, famphur, dimethoate, diazinon,

ISSN-1223-7221

fenchlorphos, malathion, parathion, pirimiphos-ethyl, methidathion, azinphos 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.

Florisil, obtained from Fluka (Switzerland) was assayed for preconcentration step and was activated 12 h at 130^{o}C before use. Anhydrous sodium sulphate was activated at 200^{o}C for 2 h before use.

2.2. Samples

Different baby-food samples were purchased from the local market. 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. The sample jars and cardboard box were stored unopened at +4^{o}C before the analysis.

2.3. Sample extraction and clean-up

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 for 8 h. Then the internal standard was added (10 µL of 2,4,5, trichlorobiphenile 10 ng/µL). The Soxhlet extraction takes 8±0.5 h with hexane (250±10 mL) as solvent.

The extracts were evaporated under vacuum using a rotary evaporator at 30± 5^{0}C, with low speed, until 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 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^{o}C before the analysis.

The internal standard method was used and the basis for internal standard selection was the absence of the compound chosen as internal standard.

2.4. Instruments

A Varian gas chromatograph (model 520) equipped with an thermoionic specified detector (TSD) 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^{o}C (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; 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** were presented the retention times for the analysed pesticides.

Table 1. Retention times of studied pesticides

OPPs	Retention time (min)
o,o,o Triethyl	6.171
Dichlorvos	6.683
Thionazin	8.665
Ethopropos	8.863
Sulfotep	9.051
Phorate	9.261
Dimethoate	9.553
Diazinon	9.865
Disulfoton	10.152
Parathion-methyl	10.887
Fenchlorphos	11.055
Malathion	11.422
Chlorpyrifos	11.620
Parathion	11.784
Parathion-ethyl	11.805
Pirimiphos-ethyl	11.990
Methidathion	13.092
Prothiofos	13.780
Ethion	15.089
Famphur	15.769
Azinphos	18.864
Guthion	18.880

The typical chromatogram of standards is shown in **Fig. 1.**

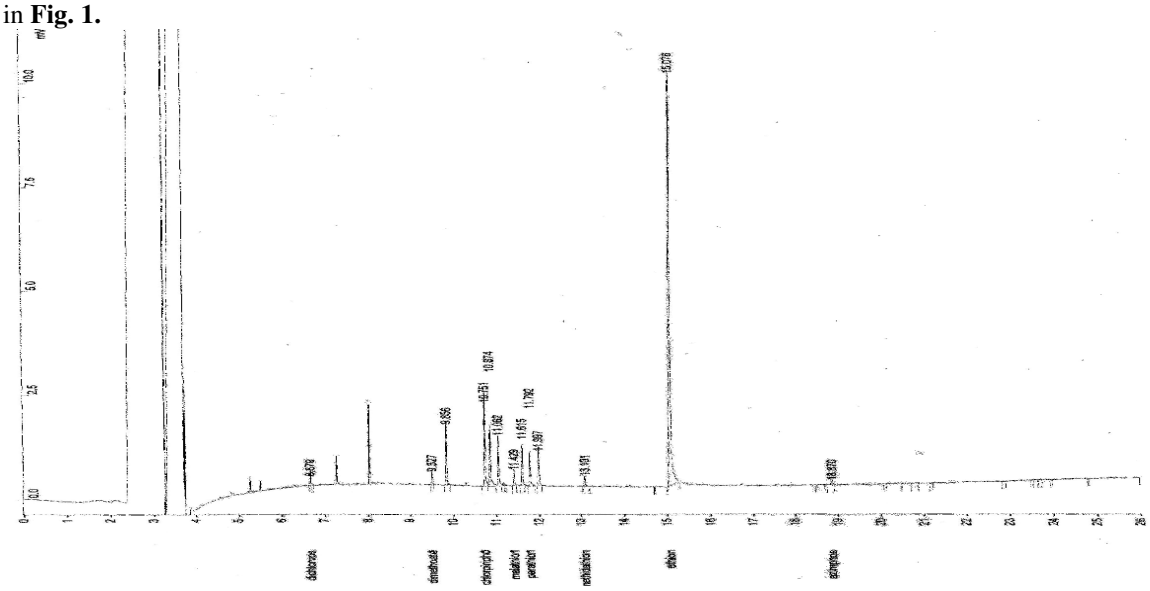

Fig. 1. The chromatogram of OPPs standards

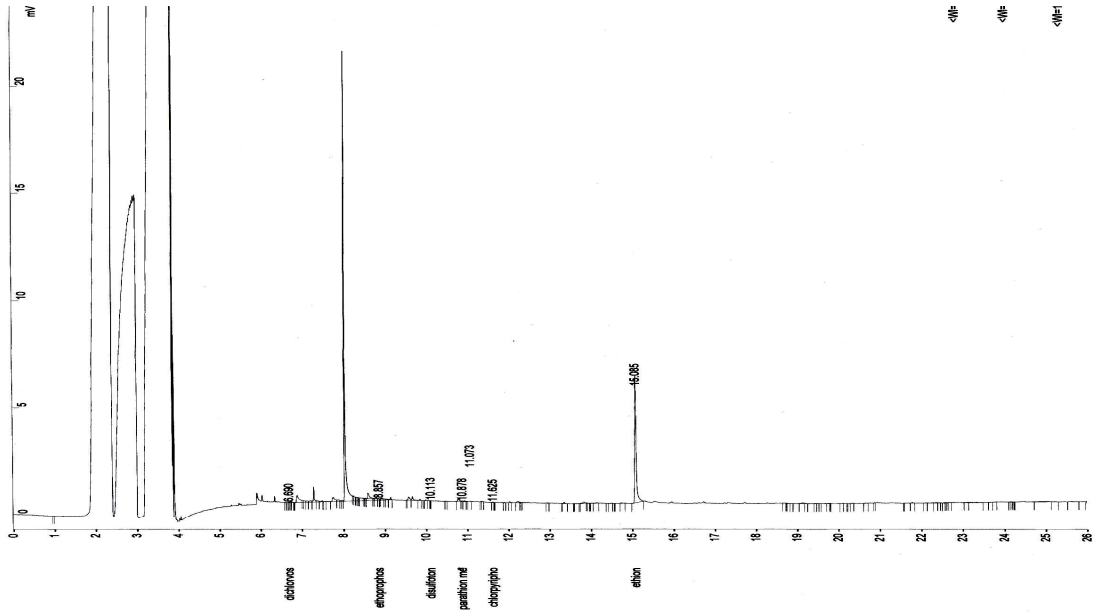

Fig. 2. Chromatogram of a fruits purée sample

3. Results and Discussions

The proposed method was used to determine twentytwo OPPs in baby food samples the representative chromatogram obtained for purée based on fruits are shown in **Fig. 2.**

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

Table 2. Concentrations of organophosphorus pesticides in vegetable and fruit purée

OPPs	vegetable based purée (mg/kg)	fruit based purée (mg/kg)
Dichlorvos	<LOD	<LOD
Ethopropos	<LOD	<LOD
Parathion-methyl	<LOD	<LOD
Chlorpyrifos	<LOD	<LOD
Prothiofos	<LOD	<LOD
Guthion	<LOD	<LOD
o,o,o Triethyl	0.0005	0.0028
Thionazin	<LOD	<LOD
Sulfotep	<LOD	<LOD
Phorate	<LOD	<LOD
Disulfoton	0.0005	0.0001
Parathion-ethyl	<LOD	<LOD
Ethion	<LOD	<LOD
Famphur	<LOD	<LOD
Dimethoate	0.0284	0.0082
Diazinon	0.0069	0.0119
Fenchlorphos	<LOD	0.0132
Malathion	<LOD	<LOD
Parathion	<LOD	<LOD
Pirimiphos-ethyl	<LOD	0.0156
Methidathion	<LOD	<LOD
Azinphos	<LOD	<LOD

LOD – detection limit

Recent federal regulations restricting the use of some organophosphorus pesticides may have reduced the use of high-hazard pesticides.

Malathion was under detection limit, except one sample (see table 3), but this value is comparable with those encountered by Lu in processed food for babyes [12].

In 1999 the EU was introduced legislation (1999/39/EC) limits of all pesticides residues to a maximum of 0.01 mg/Kg potential found in processed cereal-based baby food [13].

Table 3. Concentrations of organophosphorus pesticides in meat–vegetable and fish-vegetable purée

OPPs	meat–vegetable based purée (mg/kg)	fish-vegetable based purée (mg/kg)
Dichlorvos	<LOD	0.0031
Ethopropos	0.0091	<LOD
Parathion-methyl	<LOD	<LOD
Chlorpyrifos	<LOD	<LOD
Prothiofos	0.0113	<LOD
Guthion	0.0085	<LOD
o,o,o Triethyl	0.0066	0.0013
Thionazin	<LOD	<LOD
Sulfotep	0.0006	<LOD
Phorate	<LOD	<LOD
Disulfoton	<LOD	<LOD
Parathion-ethyl	<LOD	<LOD
Ethion	<LOD	<LOD
Famphur	0.0006	<LOD
Dimethoate	0.0295	0.0048
Diazinon	0.0567	0.0014
Fenchlorphos	0.0644	<LOD
Malathion	0.1027	<LOD
Parathion	<LOD	<LOD
Pirimiphos-ethyl	<LOD	<LOD
Methidathion	<LOD	<LOD
Azinphos	<LOD	<LOD

LOD – detection limit

From **Table 4** it can be observed that some analyzed phosphorus pesticides are above these maximum limits (parathion-methyl 0.0131 mg/Kg; prothiofos 0.0364 mg/Kg; dimethoate 0.046 mg/Kg; diazinon 0.1096 mg/Kg; fenchlorphos 0.1903 mg/Kg; pirimiphos-ethyl 0.0705 mg/Kg).

The EU directive for cereal based baby food [14] focuses on pesticides control or metabolites

with a maximum daily accepted dose by 0.0005 mg/kg body.

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

OPPs	Cereal based baby food	Biscuits
Dichlorvos	<LOD	<LOD
Ethopropos	<LOD	<LOD
Parathion-methyl	0.0131	<LOD
Chlorpyrifos	<LOD	<LOD
Prothiofos	0.0364	<LOD
Guthion	<LOD	<LOD
o,o,o Triethyl	0.0050	0.0003
Thionazin	<LOD	<LOD
Sulfotep	0.0013	0.00006
Phorate	<LOD	<LOD
Disulfoton	<LOD	0.0001
Parathion-ethyl	<LOD	0.0001
Ethion	<LOD	<LOD
Famphur	<LOD	<LOD
Dimethoate	0.046	0.0016
Diazinon	0.1096	0.0011
Fenchlorphos	0.1903	<LOD
Malathion	<LOD	<LOD
Parathion	<LOD	<LOD
Pirimiphos-ethyl	0.0705	0.0004
Methidathion	<LOD	<LOD
Azinphos	<LOD	<LOD

LOD – detection limit

4. Conclusions

The obtained results indicate that some of the detected pesticides exceed the allowed maximum residue limits showing a very low contamination level with organophosphorus pesticides of investigated samples.

Chlorpyrifos, thionazin, phorate, ethion, parathion, methidathion and azinphos were under detection limit for all studied samples.

Further study is needed to understand the monitoring system and what people can do to minimize their exposure to pesticides.

5. References

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[1]. C.L. Curl, R.A. Fenske and K. Elgethun, Environmental Health Perspectives, **11(3)**, 377-382 (2003).
[2]. D. M Roberts and C. K Aaron, BMJ, **334**, 629-634, (2007) doi: 10.1136/bmj.39134.566979.BE
[3]. K. Buonasera, G. D'Orazio, S. Fanali, P. Dugo and L. Mondello, Journal of Chromatography A, **1216**, 3970–3976 (2009)
[4]. C. Merus, C. Corradini, A. Cavazza, C. Borrome and P. Salvadeo, Food Chemistry, **120**, 615–620 (2010)
[5]. EC Council Directive 2006/125/EC of 5 December 2006 on processed cereal-based foods and baby foods for infants and young children. In: Official Journal of the European Union, Vol. L339, European Community, Brussels, 16–35, (2006).
[6]. P. Georgakopoulos, R. Zachari, M. Mataragas, P. Athanasopoulos, E. H. Drosinos and P. N. Skandamis, Food Chemistry, **128**, 536-542, (2011)
[7]. C. C. Leandro, P. Hancock, R. J. Fussell and B. J. Keely, Journal of Chromatography A, **1103** (1), 94-101 (2006)
[8]. P. Sandra, B. Tienpont and F. David, Journal of Chromatography A, **1000**, 299–309 (2003)
[9]. A. Hercegová, M. Dömötörová, E. Matisová, M. Kirchner, R. Otrekal and V. Štefuca, Journal of Chromatography A, **1084** (1-2), 46-53 (2005)
[10]. C. C. Leandro, R. J. Fussell and B. J. Keely, Journal of Chromatography A, **1085** (2), 207-212 (2005)
[11]. A. Hercegova, M. Domotorova and E. Matisova, Journal of Chromatography A, **1153**, 54–73, (2007)
[12]. C. Lu, G.Kedan, J. Fisker-Andersen and J.C. Kissel, R.A. Fenske, Environmental Research, **96**, 283–289, (2004)
[13].***Commission Directive 1999/39/EC of 6 May 1999 amending Directive96/5/EC on processed cereal-based foods and baby foods for infants and young children, Off. J. Eur. Commun, vol. L124, (1999)
[14].*** Commission Directive 2003/13/EC of 10 February 2003 amending Directive96/5/EC on processed cereal-based foods and baby foods for infants and young children, Off. J. Eur. Commun, vol. L41, (2003).

Submitted: March 15th 2012
Accepted in revised form: April 26th 2012