

# Analysis of Organochlorine and Organophosphorus Pesticide Residues in Dairy Products and Baby Foods from Egyptian Markets

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## Abstract

The residue levels of organochlorine (OCP) and organophosphorus (OPP) in some dairy products and baby foods samples in Assiut markets Egypt were determined. The concentrations of OCP and OPP were determined in milk powder, plain yoghurt, fruit yoghurt, breakfast cereals, wheat cereal-based baby foods, rice cereal-based baby foods and vegetables and fruit-based baby foods by gas chromatography/mass spectrometry. In dairy products, the highest values of OCP and OPP were  $9.346 \pm 0.950$   $\mu\text{g}/\text{kg}$  methoxychlor in fruit yoghurt and  $2.282 \pm 0.400$   $\mu\text{g}/\text{kg}$  disulfoton in plain yoghurt, respectively. Propachlor, dieldrin (OCP), chlorpyrifos, and parathion- methyl (OPP) were not detected in any dairy product samples. Maximum amounts of methoxychlor ( $12.710 \pm 0.700$   $\mu\text{g}/\text{kg}$ ) and disulfoton ( $5.369 \pm 0.510$   $\mu\text{g}/\text{kg}$ ) were recorded in vegetables and fruit-based baby foods, and wheat cereal-based baby foods, respectively. The analysis of dairy products and baby foods showed lower pesticides values than the permissible limit set by the European Commission in all products, except methoxychlor (OCP) in vegetables and fruit-based baby foods. The results will help in a scientific assessment of the implications of pesticide residues with regards to human risks in Egypt.

**Keywords:** Pesticide residues; Dairy products; Baby foods; Gas chromatography/mass spectrometry

## Introduction

Pesticides protect crops from pests and are economically beneficial. However, these substances can transfer to the food and affect consumer health, especially in the food consumed by infants and children, who are a vulnerable risk group [1,2]. Dairy foods like milk and yoghurt are important nutritive foods for infant and the children because these include vitamin A, vitamin B12, riboflavin, calcium, carbohydrate, magnesium, phosphorus, protein, potassium, and zinc [3,4]. Moreover, processed foods such as cereals are particularly used as healthy food supplements for infants and young children. Infants and children are more vulnerable to the effects of pesticides as compared to adults because of high food consumption rate per kilogram of their body weight and low immunity [5-11]. The European Commission Directive 2006/125/EC of 5 December 2006 [12] set a limit for pesticides in cereal-based foods and baby foods for infants and young children. According to this regulation, pesticides in cereal-based foods and baby foods must not contain residues of individual pesticides at levels exceeding 10  $\mu\text{g}/\text{kg}$  (MRL). Dichlorodiphenyltrichloroethane (DDT) and its metabolites have adverse health effects such as neurodevelopment delay [13-17], reproductive effects [18], preterm and small-for-gestational-age babies [19-21], immune toxicity [7,22,23], and reduction in the mean duration of lactation (from 7 months to 3 months) [24]. Methoxychlor decreased the activity of thyroid-hormone sensitive, microsomal protein [25-29]. These food contaminants have dangerous effects in the early years of life. This fact has caused concern since dairy products and baby foods are an important exposure route for persistent pollutants in general. Limited data is available on the levels of OCP and OPP residues in dairy products and baby foods which constitute an important part of meal of many infants and children in Egypt. The aim of the current study was to determine the levels of OCP and OPP residues in the highly consumed types of foods by infants and young children: powder milk, plain yoghurt, fruit yoghurt, wheat cereal-based foods, rice cereal-based foods, breakfast cereals and vegetables, and fruit-based baby foods.

## Materials and Methods

### Sample collection

The most well-known seven foods that infants and children consumed (powder milk, plain yoghurt, fruit yoghurt, breakfast cereals, wheat cereal-based baby foods, rice cereal-based baby foods and vegetables and fruit-based baby foods), in large cities in Assiut Governorate (Assiut, Dirout, Manfalut, and Qusiya cities), were collected from June to October 2014 from the local pharmacies and markets of Assiut, Dirout, Manfalut, and Qusiya cities. Plain yoghurt, fruit yoghurt, vegetables and fruit baby food samples were identified, samples were kept frozen at  $-20^\circ\text{C}$  prior to analysis.

### Standards and reagents

In this study, pesticide standard of the OCP group included propachlor, trifluralin, hexachlorobenzene, lindane, heptachlor, alachlor, heptachlor- epoxide, p,p-DDE, dieldrin, and methoxychlor. The OPP compounds were disulfoton, parathion- methyl, malathion, chlorpyrifos, and ethion. Acetonitrile, anhydrous sodium sulfate, and sodium chloride were used. Analytical reagents were purchased from Sigma Chemical Co., Germany.

### Preparation of sample extracts

All samples were analyzed for OCP and OPP residues using rapid and easy multiresidue methodology, according to Ref. [30] and Ref.

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[31]. Pesticides (5.0 g samples) were extracted from plain yoghurt, fruit yoghurt, and milk powder in a 50 mL disposal tube. Ethyl acetate (20 mL) and 3 g of  $MgSO_4$  were added, followed by high-speed homogenization (1 min) and centrifugation (40000 rpm-10 min). Pesticides were extracted from breakfast cereals, wheat cereal-based baby foods, rice cereal-based baby foods vegetables and fruit-based baby foods using following steps. Five grams of sample was homogenized in a 50 mL disposal tube. Five milliliters of water was added and allowed to stand for 30 min. Acetonitrile (20 mL) was added and homogenized with a high-speed homogenizer (1 min). Four grams of  $MgSO_4$  and 1 g of NaCl were added, and the mixture was shaken for 1 min [31].

### Gas Chromatography (GC) analysis

Plain yoghurt, fruit yoghurt, powder milk, breakfast cereals, wheat cereal-based baby foods, rice cereal-based baby foods and vegetables and fruit-based baby foods samples were analyzed using gas chromatography/mass spectrometry (GC/MS). System 7890A series gas chromatograph coupled with model 5975B quadrupole mass spectrometer with a cross-linked 5% phenyl methyl siloxane capillary column (DB-5MS, 30 m  $\times$  0.25 mm id  $\times$  0.25  $\mu$ m film thickness) was used. The GC operating conditions were as follows: initial temperature, 90°C (7 min hold), increased at 30°C /min to 180°C, increased at 4°C/min to 270°C, and then increased at 30°C /min to 280°C (4 min hold). Helium at a purity of >99.999% was used as carrier gas at a flow rate of 1 mL/min. The injector port temperature was 260°C. The sample volume injected was 2  $\mu$ L. The MS operating conditions were as follows: solvent delay 6 min, electron-impact (EI) mode ionization voltage 70 eV using selected ion monitoring (SIM), and dwell time of 100 ms for each ion. To improve sensitivity, the selected ions used in the SIM mode are divided into fourteen groups, guided by the individual pesticide retention times. All pesticides were identified by retention time and specific ions, and quantified by the external standard method.

### Method validation

We determined the quality of the method, performed a recovery fortification of the pesticide mixtures of the dairy (plain yoghurt, fruit yoghurt, and powder milk) and other (breakfast cereals, wheat cereal-based baby foods, rice cereal-based baby foods and vegetables fruit-based baby foods) samples at final concentrations of 0.02  $\mu$ g/kg and 0.10  $\mu$ g/kg, respectively. We conducted 3 trials for each test and defined an acceptable result as the one with a recovery of 70~120% with an RSD  $\leq$  20% for both concentrations [31]. All samples were treated and analyzed using the GC/MS-SIM procedure described above. Pesticide residues were analysed in the analytical chemistry unit of the Laboratory at Assiut University, Egypt. Table 1 shows some parameters for determination of pesticide residues in the samples, using Agilent 7890 GC-MS.

### Statistical analyses

Means and standard deviations (SD) of data were calculated with SPSS 9.0 for Windows (SPSS, Chicago, USA). Statistical software SPSS was used to perform one-way analysis of variance (ANOVA), and the least significant difference (LSD) test at a 95% confidence level ( $p < 0.05$ ).

## Results and Discussion

### Organochlorine pesticide residues in powder milk, plain, and fruit yoghurt

Pesticide residues in dairy products have major effects on public health. Dairy products play a central role in nutrition of infants, children,

and adults globally [32]. The concentration of persistent organochlorine compound residues in milk powder and yoghurt samples are presented in Table 2. Trifluralin, hexachlorobenzene (HCB), and lindane were detected in the analyzed milk powder samples. Concentrations range of trifluralin, HCB, and lindane varied from 0.254-0.354  $\mu$ g/kg, 0.378-0.496  $\mu$ g/kg, and 0.078-0.198  $\mu$ g/kg, respectively. The average concentration of HCB ( $0.440 \pm 0.0340$   $\mu$ g/kg) was higher than those of trifluralin ( $0.314 \pm 0.030$   $\mu$ g/kg) and lindane ( $0.123 \pm 0.030$   $\mu$ g/kg) in milk powder. Only p,p-DDE detected in plain yoghurt concentrations range was 0.259-0.309  $\mu$ g/kg. These results are in agreement with those recorded by Ref. [33]. Trifluralin, lindane, heptachlor, alachlor, heptachlor-epoxide, and methoxychlor were detected in fruit yoghurt (Table 2). Average concentration of trifluralin and Lindane were  $0.157 \pm 0.017$   $\mu$ g/kg and  $2.505 \pm 0.043$   $\mu$ g/kg in fruit yoghurt, respectively. Lindane concentrations range were higher than those reported in plain yoghurt in Ghana (0.03  $\mu$ g/kg) [33]. Propachlor and dieldrin were not detected in dairy food samples. Alachlor, heptachlor-epoxide, and methoxychlor were detected only in fruit yoghurt with average values of  $0.242 \pm 0.043$   $\mu$ g/kg,  $1.616 \pm 0.64$   $\mu$ g/kg, and  $9.346 \pm 0.950$   $\mu$ g/kg, respectively. In the present study, the values of OCP in milk powder, and plain and fruit yoghurt did not exceed the permissible limit set by the European Commission, 2006 [12]. The decrease in the residue levels of these pesticides may be due to the heat-treated milk and dairy products [34]. Organochlorine pesticides can contaminate milk-producing animals through grass feeding and inhaled air and accumulate in fat-rich dairy products [35].

### Organophosphorus pesticide residues in milk powder, plain yoghurt, and fruit yoghurt

The residues of OPP pesticides in milk powder, plain yoghurt, and fruit yoghurt samples are shown in Table 3. The OPP detection includes disulfoton, malathion, and ethion, while the parathion-methyl and chlorpyrifos are not detected in all dairy products samples. Only disulfoton (0.263-0.523  $\mu$ g/kg) was detected in the analyzed milk powder samples. Disulfoton and malathion were detected in plain yoghurt at concentrations range of 1.576-2.966  $\mu$ g/kg and 0.279-0.489  $\mu$ g/kg, respectively. OPP pesticides in fruit yoghurt were in the malathion and ethion 1.308-3.974  $\mu$ g/kg and 0.644-0.801  $\mu$ g/kg concentration range, respectively. Malathion was detected only in yoghurt samples, with concentration range of 0.279-0.489  $\mu$ g/kg and 1.308-3.974  $\mu$ g/kg in plain yogurt and fruit yogurt samples, respectively. Ethion was detected only in fruit yoghurt samples at a concentration range of 0.644-0.801  $\mu$ g/kg. These results were in agreement with Ref. [36], who reported that malathion was enhanced during yoghurt processing or heat treatment [37]. Analyzed OPP contamination in 135 raw milk samples residues ranging from 5  $\mu$ g/kg to 18  $\mu$ g/kg. These concentrations were higher than those recorded from treated dairy products (milk powder and yoghurt), because of a decrease in OPP in milk during heat treatment or yoghurt processing. In the present study, disulfoton, malathion, and ethion levels did not exceed the permissible limit (10  $\mu$ g/kg) proposed by European Commission (2006) [12] (Table 3).

### Organochlorine pesticide residues in breakfast cereals, wheat cereal-based baby foods, rice cereal-based baby foods and vegetable, and fruit-based baby foods

The OCP pesticides in breakfast cereals samples were lindane  $0.452 \pm 0.052$   $\mu$ g/kg and dieldrin  $1.892 \pm 0.810$   $\mu$ g/kg. Propachlor, lindane, and heptachlor were detected at concentrations of  $0.43 \pm 0.042$   $\mu$ g/kg,  $1.068 \pm 0.340$   $\mu$ g/kg, and  $2.061 \pm 0.69$   $\mu$ g/kg, respectively, in wheat cereal-based baby foods. Lindane is used against a wide range of insects as an

Pesticides	Retention time (min)	LOD* ( $\mu\text{g/L}^{-1}$ )	Target ion (qualifier ion) (m/z)
Propachlor	9.97	0.0030	181.00
Tirfluralin	10.42	0.0010	306.00
Hexachlorobenzene	11.17	0.0020	284.00
Lindane	11.86	0.0001	183.00
Disulfoton	12.41	0.0007	89.00
Parathion- Methyl	13.60	0.0001	125.00
Heptachlor	13.93	0.0050	100.00
Alachlor	13.61	0.0060	160.00
Malathion	14.68	0.0010	173.10
Chlorpyrifos	14.89	0.0002	196.90
Heptachlor-epoxide	16.39	0.0010	353.00
p,p-DDE	18.72	0.0001	105.00
Dieldrin	18.74	0.0002	262.90
Ethion	20.25	0.0003	231.00
Methoxychlor	24.09	0.0191	228.00

**Table 1:** Parameters for determination of pesticide residues in milk powder, plain and fruit yoghurt, breakfast cereals, wheat and rice cereal-based baby foods and vegetables and fruit baby foods by GC/MS.

Pesticides	Type of dairy products					
	Milk powder		Plain yogurt		Fruit yoghurt	
	Concentration range ( $\mu\text{g/kg}$ )	Average* concentration ( $\mu\text{g/kg}$ )	Concentration range ( $\mu\text{g/kg}$ )	Average concentration ( $\mu\text{g/kg}$ )	Concentration range ( $\mu\text{g/kg}$ )	Average concentration ( $\mu\text{g/kg}$ )
Propachlor	ND	ND	ND	ND	ND	ND
Tirfluralin	0.254-0.354	0.314 $\pm$ 0.030	ND	ND	0.133-0.191	0.157 $\pm$ 0.017
Hexachlorobenzene	0.378-0.496	0.440 $\pm$ 0.034	ND	ND	ND	ND
Lindane	0.078-0.198	0.123 $\pm$ 0.030	ND	ND	2.440-2.548	2.505 $\pm$ 0.043
Heptachlor	ND	ND	ND	ND	1.463-1.783	1.616 $\pm$ 0.051
Alachlor	ND	ND	ND	ND	0.146-0.355	0.242 $\pm$ 0.043
Heptachlor- epoxide	ND	ND	ND	ND	1.5-1.722	1.616 $\pm$ 0.064
p,p-DDE	ND	ND	0.259-0.309	0.259 $\pm$ 0.050	ND	ND
Dieldrin	ND	ND	ND	ND	ND	ND
Methoxychlor	ND	ND	ND	ND	9.158-9.467	9.346 $\pm$ 0.095

**Table 2:** Organochlorine pesticides ( $\mu\text{g/kg}$ ) detected in milk powder and plain and fruit yoghurts.

Pesticides	Type of dairy products					
	Milk powder		Plain yogurt		Fruit yoghurt	
	Concentration range ( $\mu\text{g/kg}$ )	Average* concentration ( $\mu\text{g/kg}$ )	Concentration range ( $\mu\text{g/kg}$ )	Average concentration ( $\mu\text{g/kg}$ )	Concentration range ( $\mu\text{g/kg}$ )	Average concentration ( $\mu\text{g/kg}$ )
Disulfoton	0.263-0.523	0.403 $\pm$ 0.070	1.576-2.966	2.282 $\pm$ 0.400	ND	ND
Parathion- methyl	ND	ND	ND	ND	ND	ND
Malathion	ND	ND	0.279-0.489	0.397 $\pm$ 0.060	1.308-3.974	2.296 $\pm$ 0.790
Chlorpyrifos	ND	ND	ND	ND	ND	ND
Ethion	ND	ND	ND	ND	0.644-0.801	0.700 $\pm$ 0.050

\*Average concentration: mean  $\pm$  SD; ND: not detected

**Table 3:** Organophorous pesticides ( $\mu\text{g/kg}$ ) detected in milk powder and plain and fruit yoghurts.

insecticide and fumigant, and is thus found in food products, including fruits, vegetables and milk products. HCB 0.586  $\pm$  0.043  $\mu\text{g/kg}$  was only detected in rice cereal-based baby foods. Lindane, heptachlor, and methoxychlor were found in vegetables and fruit-based baby foods with average values of 2.464  $\pm$  0.600  $\mu\text{g/kg}$ , 1.717  $\pm$  0.780  $\mu\text{g/kg}$ , and 12.710  $\pm$  0.700  $\mu\text{g/kg}$ , respectively (Table 4). The European Commission 2003 [38] has reported maximum residue levels (MRLs) for pesticides in processed cereal-based baby foods at 10  $\mu\text{g/kg}$ . In the current study, all analyzed baby foods samples showed lower pesticide residues than the permissible limit set by the European Commission [38], except methoxychlor in vegetables and fruit-based baby foods.

### Organophorous pesticide residues in breakfast cereals, wheat cereal-based baby foods, rice cereal-based baby foods and vegetables and fruit-based baby foods

OPP residues were detected in baby foods, including disulfoton, malathion, and ethion with average concentration of 3.758  $\pm$  0.780  $\mu\text{g/kg}$ , 0.353  $\pm$  0.086  $\mu\text{g/kg}$ , and 2.274  $\pm$  0.880  $\mu\text{g/kg}$ , respectively, in breakfast cereals. These values were lower than the permissible limit set by the European Commission [38]. Disulfoton at mean values of 5.369  $\pm$  0.510  $\mu\text{g/kg}$  was detected in wheat cereal-based baby food. Parathion-methyl, chlorpyrifos, and ethion with mean values of 3.191  $\pm$  0.52  $\mu\text{g/}$

Pesticides	Type of baby foods							
	Breakfast cereals		Wheat cereal-based baby foods		Rice cereal-based baby foods		Vegetables and fruit baby foods	
	Concentration range (µg/kg)	Average concentration (µg/kg)	Concentration range (µg/kg)	Average concentration (µg/kg)	Concentration range (µg/kg)	Average concentration (µg/kg)	Concentration range (µg/kg)	Average concentration (µg/kg)
Propachlor	ND	ND	0.346-0.472	0.43 ± 0.042	ND	ND	ND	ND
Tirfluralin	ND	ND	ND	ND	ND	ND	ND	ND
Hexachlorobenzene	ND	ND	ND	ND	0.491-0.663	0.586 ± 0.043	ND	ND
Lindane	0.398-0.558	0.452 ± 0.052	0.561-1.719	1.068 ± 0.340	ND	ND	1.864-3.064	2.464 ± 0.600
Heptachlor	ND	ND	0.681-2.981	2.061 ± 0.69	ND	ND	0.724-3.277	1.717 ± 0.780
Alachlor	ND	ND	ND	ND	ND	ND	ND	ND
Heptachlor- epoxide	ND	ND	ND	ND	ND	ND	ND	ND
p,p-DDE	ND	ND	ND	ND	ND	ND	ND	ND
Dieldrin	0.272- 3.512	1.892 ± 0.810	ND	ND	ND	ND	ND	ND
Methoxychlor	ND	ND	ND	ND	ND	ND	11.17-13.97	12.710 ± 0.700

**Table 4:** Organochlorine pesticides (µg/kg) detected in breakfast cereals, wheat and rice cereal-based baby foods and vegetables and fruit baby foods.

Pesticides	Type of baby foods							
	Breakfast cereals		Wheat cereal-based baby foods		Rice cereal-based baby foods		Vegetables and fruit baby foods	
	Concentration range (µg/kg)	Average concentration (µg/kg)	Concentration range (µg/kg)	Average concentration (µg/kg)	Concentration range (µg/kg)	Average concentration (µg/kg)	Concentration range (µg/kg)	Average concentration (µg/kg)
Disulfoton	2.588-5.262	3.758 ± 0.780	4.349-6.389	5.369 ± 0.51	ND	ND	ND	ND
Parathion-Methyl	ND	ND	ND	ND	2.151-4.231	3.191 ± 0.52	ND	ND
Malathion	0.163-0.5078	0.353 ± 0.086	ND	ND	ND	ND	ND	ND
Chlorpyrifos	ND	ND	ND	ND	0.517-0.807	0.649 ± 0.079	ND	ND
Ethion	0.514-3.154	2.274 ± 0.880	ND	ND	0.686-0.899	0.758 ± 0.072	ND	ND

**Table 5:** Organophorous pesticides (µg/kg) detected in in breakfast cereals, wheat and rice cereal-based baby foods and vegetables and fruit baby foods.

kg, 0.649 ± 0.079 µg/kg, and 0.758 ± 0.072 µg/kg, respectively were found in rice cereal-based baby foods (Table 5).

## Conclusions

The overall results show that OCP and OPP residues in dairy products (milk powder and plain and fruit yoghurts, baby foods (breakfast cereals, wheat and rice cereal-based baby foods and vegetables and fruit-based baby foods) in Assiut markets Egypt. The highest mean values of OCP and OPP were methoxychlor at mean concentrations of 9.346 ± 0.950 µg/kg and 2.282 ± 0.400 µg/kg in fruit yoghurt and plain yoghurt, respectively. Propachlor and dieldrin (OCP), chlorpyrifos, and parathion- methyl were absent in all analyzed dairy products. Methoxychlor (OCP) and disulfoton (OPP) showed the highest mean values of 12.710 ± 0.700 µg/kg and 5.369 ± 0.510 µg/kg in vegetables and fruit-based baby foods, and wheat cereal-based baby food, respectively. The order for the contamination in the analyzed dairy products and baby food were milk powder>fruit yoghurt>plain yoghurt and wheat cereal-based baby foods breakfast cereals>fruit yoghurt>rice cereal-based baby foods>vegetables and fruit baby foods, respectively. The results from these studies show that residues of OCP and OPP pesticides are present in dairy products, breakfast cereals, cereal-based baby food and vegetables and fruit-based baby foods. Their concentrations were lower than the acceptable maximum residue levels, except methoxychlor in vegetables and fruit-based baby foods. Although these pesticides residues occurred at very low concentrations in the samples, they may accumulate to higher levels

in infant and young children upon consumption. The results of this survey demonstrate the need to establish pesticide residue monitoring programs in consumables to improve food safety and decrease health risks in consumers.

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