

# Organochlorine and organophosphorus pesticide residues in raw buffalo milk from agroindustrial areas in Assiut, Egypt

## Abstract

Raw buffalo milk samples from the agroindustrial zone in Upper Egypt were analyzed for the presence of organochlorine and organophosphorus pesticides using gas chromatography-mass spectroscopy. Five organochlorine pesticides namely, alachlor, dieldrin, hexachlorobenzene, lindane and methoxychlor and three organophosphorus pesticides chlorpyrifos, malathion, and parathion-methyl were detected in the milk samples. In 44% of the samples, the concentrations of lindane and malathion residues exceeded tolerance levels set by the European Commission (EC) in 2008. In addition, the concentrations of chlorpyrifos, methoxychlor, and hexachlorobenzene residues exceeded the 2008 EC maximum residual limits (MRLs) in 33%, 66%, and 88% of the examined samples, respectively. However, the levels of alachlor, dieldrin, and parathion-methyl residues were below EC MRLs. The results of this study confirm the risks of pesticide residues exposure that threaten consumer health in Egypt. Thus, we recommend that pesticide residue monitoring programs be instituted in all developing countries.

**Keywords:** buffalo milk, GC/ms, multi pesticide residues, upper Egypt

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## Introduction

Milk has been studied as an indicator of the bioconcentration of environmentally persistent organic micro-pollutants such as pesticides.<sup>1</sup> Organochlorine (OC) compounds are lipophilic and metabolized very little in living organisms. Hence, environmental exposure to these compounds results in their accumulation and persistence in fat tissue.<sup>2</sup> Moreover, OC pesticides undergo biomagnification through the food chain.<sup>3,4</sup>

Organophosphorus (OP) pesticides are esters, amides, or thiol derivatives of phosphoric or phosphonic acid. OP pesticides are easily hydrolyzed and therefore do not persist in the environment. However, their toxicity (high or moderate) and the possibility of their accumulation especially fat-soluble OPs in animal tissues, milk, and eggs pose risks for human health.<sup>5</sup>

The presence of pesticide residues in milk is a public health concern because milk and dairy products are widely consumed by infants, children, and adults. Pesticide residues in milk originate from contaminated feed, grass or corn silage, and direct application of pesticides on dairy cattle. Because humans are the last link in the food chain, they consume the highest levels of these compounds. Food products particularly dairy, meat, and fish are the primary immediate sources of OC and OP pesticide intake in the general population<sup>6,7</sup> and these pesticides elicit a wide range of toxic and biochemical effects in both laboratory animals and wildlife.<sup>8,9</sup> They pose serious health risks, especially in infants, who lack fully active enzymatic and metabolic systems.

To protect consumers and promote trade, maximum residue levels (MRLs) have been established for pesticide residues in products of plant and animal origin. Legislation in the European Union has established MRLs of 40ng/g and 0.8ng/g for the target OC pesticides

dichloro-diphenyl-trichloroethane and endrin, respectively, in milk. The MRL for chlorpyrifos is 10ng/g.<sup>10</sup> The governments of developing countries, however, maintain that they cannot afford to ban certain chemicals for reasons of cost, efficacy, or both. As a result, most of these chemicals have been or continue to be used in large quantities in many countries, including sub-Saharan Africa.<sup>11</sup>

Extensive soil and water pollution have been documented in Egypt, where wide use of OC pesticides in the 1960s and 1970s prompted a government ban on their use in the 1980s.<sup>12-14</sup> However, OC pesticides are still applied widely in Egypt.<sup>14-16</sup> Therefore, we conducted the present study to assess the current status of OC and OP pesticide residue contamination in milk, particularly since the ban on the use of these pesticides in agriculture and public health. We determined the concentration of several OC and OP pesticide residues in raw buffalo milk samples collected from various locations in the city of Assiut in Upper Egypt.

## Material and methods

### Sampling

A total of 45 samples of raw buffalo milk were collected at random from local vendors, dairy farms, and shops (15 each) between February and August 2013. These samples were obtained from three areas in the city of Assiut designated the north, middle, and south of the city. The sample unit was one original package (1L) of milk from each source. Samples were identified, placed in polypropylene bags, and immediately sent to the laboratory for analysis.

### Standards and reagents

Analytical technical grade OC and OP pesticide standards with 93-99% purity were procured from Sigma-Aldrich (UK). The analytical

OC pesticide standards included alachlor, dieldrin, hexachlorobenzene (HCB), lindane and methoxychlor. The OP pesticides analyzed in this study were chlorpyrifos, malathion and parathion-methyl. Octadecylsilyl-derivatized silica (C18), acetonitrile, petroleum ether, and anhydrous sodium sulfate were used. All reagents were of analytical (high-performance liquid chromatography) grade and were supplied by BDH (UK). The sodium sulfate was heated to 650°C for 4h before use and stored in a desiccator. C18 and Florisil solid phase extraction columns were purchased from Agilent Technologies, Inc. (USA).

### Residue analysis

The liquid milk samples were analyzed for OC and OP pesticide residues using a standardized multi-residue methodology according to Schenck & Wagner<sup>17</sup> and Lehotay et al.<sup>18</sup>

**Extraction and cleanup:** The extraction and cleanup method incorporated matrix solid-phase dispersion. Milk (5.0mL) was blended with 2.0g C18, 2g anhydrous sodium sulfate, and 1.5mL acetonitrile in a syringe barrel. After the aqueous phase was removed from the column via vacuum aspiration, the pesticide residues were eluted from the C18/milk matrix with acetonitrile, which was then eluted through a Florisil solid-phase extraction column. The acetonitrile was evaporated under nitrogen, and the residue was dissolved in petroleum ether. The extract was concentrated to 0.5mL via evaporation.

**Gas chromatography-mass spectrometry (GC/MS) analysis:** The pesticide residues detected using GC were identified with GC/MS. GC was performed on an Agilent 7890 instrument equipped with 5975 insert ion source mass detection system (Agilent Technologies). The analytical capillary column was a DB-1701 (J&W Scientific, USA). The column temperature was maintained at 40°C for 1min, and then programmed at 30°C/min<sup>-1</sup> up to 130°C, then 5°C/min<sup>-1</sup> up to 250°C, and finally 10°C/min<sup>-1</sup> up to 300°C, which was held for 5min. Helium (purity, >99.999%) was used as the carrier gas at a flow rate of 1.2mL/min<sup>-1</sup>. The injection port temperature was 260°C and 1µL samples were injected splitless with the purge on after 1.5min. The

MS ionization energy was 70eV, the ion-source temperature 230°C, and the GC-MS interface temperature 280°C. Selected ion monitoring (SIM) was used, and the dwell time of each ion was set at 100ms. The characteristics of the SIM method, including retention time windows and base peak ions, were designed for OC and OP pesticides. All pesticides were identified with retention time and specific ions and quantified using the external standard method.

**Method validation:** To determine the quality of the method, we performed a recovery study on 10 replicates of milk samples spiked with standard pesticides. The average recoveries of fortified pesticides ranged from 76.0% to 97.8% for OCs and 75.0% to 104.5% for OPs. The coefficient of variation was less than 10%, indicating excellent repeatability for the method. The recovery assays were replicated five times. All samples were treated and analyzed using the GC/MS-SIM procedure described above. These analyses were carried out in the analytical chemistry unit of the Pesticide Residue Laboratory at Assiut University, Egypt.

### Statistical analyses

A value of zero was assigned for results below the limit of detection. The Kruskal-Wallis test was used to verify that all data were normally distributed. Data were expressed as means±standard error. Statistical analyses were conducted using SPSS 16.0 for Windows (SPSS, Chicago, USA). Results were considered significant at the 5% level (p<0.05).

## Results

### Local vendors

The analysis of raw buffalo milk samples from local vendors in various locations (north, middle, and south) of Assiut found chlorpyrifos and malathion in 33% of examined samples at average concentrations of 3.01±1.0 and 0.9±0.01mg/kg, respectively. HCB was present in 66% of examined samples at an average concentration of 0.180±0.02mg/kg (Table 1).

**Table 1** Levels of the pesticide residues in raw buffalo milk from local vendors in various areas in Assiut city

N	Pesticide	Area		Area		Area	
		North n=5	Middle n=5	South n=5	South n=5	South n=5	South n=5
15		Range mg/kg	Average mg/kg	Range mg/kg	Average mg/kg	Range mg/kg	Average mg/kg
	HCB	ND	ND	0.167-0.198	0.180± 0.02	0.177-0.200	0.189± 0.02
	Chlorpyrifos	1.870-3.514	3.01±1.0	ND	ND	ND	ND
	Malathion	0.709-0.989	0.90± 0.01	ND	ND	ND	ND

N, total number of samples from local vendors; ND, non detected; n, number of samples/each area

### Dairy farms

The frequency distribution of pesticide residues detected in the analyzed buffalo milk samples obtained from dairy farms is shown in Table 2. Dieldrin, HCB, and methoxychlor were detected in 100% of the analyzed milk samples with average concentrations (mg/kg) of 0.012±0.001(north), 0.008±0.001(middle), and 0.01±0.002(south); 0.030±0.001(north), 0.030±0.001(middle), and 0.026±0.001(south); and 0.142±0.02(north), 0.178±0.03(middle), and 0.140±0.02(south), respectively. Lindane (0.165±0.02mg/kg [north] and 0.097±0.001mg/kg [south]) and parathion-methyl (0.002±0.0001mg/kg [north] and 0.001±0.0001mg/kg [south]) were present in 66% of the

examined samples. Malathion and alachlor were present in 33% of the examined samples at average concentrations of 0.195±0.02 and 0.001±0.0001mg/kg, respectively.

### Dairy shops

We detected dieldrin, HCB, and methoxychlor in 100% of the analyzed raw buffalo milk samples obtained from dairy shops, and they were present at average concentrations (mg/kg) of 0.014±0.001(north), 0.01±0.001(middle), and 0.012±0.001(south); 0.022±0.002(north), 0.025±0.001(middle), and 0.027±0.002(south); and 0.135±0.01(north), 0.198± 0.01(middle) and 0.187±0.02(south),

respectively. Lindane, chlorpyrifos, and malathion were detected in 66% of the examined samples at average concentrations (mg/kg) of 0.155±0.02(north) and 0.187±0.02(south); 0.350±0.02(north) and 0.289±0.02(south); and 0.197±0.01(north) and 0.190±0.02(south) (Table 3).

### Comparison with MRLs

The levels of alachlor, dieldrin, and parathion-methyl residue in the milk samples were below the MRL values for these pesticides set by the European Commission (EC).<sup>19</sup> However, the residue levels of lindane and malathion exceeded the permissible limit in 44% of

samples, and levels of chlorpyrifos, methoxychlor, and HCB residue exceeded MRLs in 33%, 66%, and 88% of the examined samples, respectively (Table 4).

### Frequency distribution of pesticide residues

The frequency distribution of pesticide residues from various sources and locations showed that dieldrin and methoxychlor pesticides were detected in 66% of the examined samples. Lindane and malathion were present in 44% of the examined samples. HCB, chlorpyrifos, parathion-methyl, and alachlor were detected in 88%, 33%, 22% and 11% of the examined samples, respectively (Table 4).

**Table 2** Levels of the pesticide residues in raw buffalo milk from dairy farms in various areas in Assiut city

N	Pesticide	Area					
		North n=5		Middle n=5		South n=5	
15		Range mg/kg	Average mg/kg	Range mg/kg	Average mg/kg	Range mg/kg	Average mg/kg
	Alachlor	ND	ND	ND	ND	0.000 - 0.001	0.001±0.0001
	Dieldrin	0.010- 0.015	0.012±0.001	0.006-0.009	0.008±0.001	0.08-0.012	0.01±0.002
	HCB	0.027-0.033	0.030±0.001	0.028-0.032	0.030±0.001	0.024-0.030	0.026±0.001
	Lindane	0.158-0.176	0.165±0.02	ND	ND	0.090-0.099	0.097±0.001
	Methoxychlor	0.137-0.180	0.142±0.02	0.167-0.185	0.178±0.03	0.137-0.145	0.140±0.02
	Malathion	0.188-0.203	0.195±0.02	ND	ND	ND	ND
	Parathion-methyl	0.001-0.003	0.002±0.0001	ND	ND	0.000-0.001	0.001±0.0001

N, total number of samples from local vendors; ND, non detected; n, number of samples/each area

**Table 3** Levels of the pesticide residues in raw buffalo milk from dairy shops in various areas in Assiut city

N	Pesticide	Area					
		North n=5		Middle n=5		South n=5	
15		Range mg/kg	Average mg/kg	Range mg/kg	Average mg/kg	Range mg/kg	Average mg/kg
	Dieldrin	0.010-0.015	0.014±0.001	0.08-0.012	0.01±0.001	42292	0.012±0.001
	HCB	0.019-0.023	0.022±0.002	0.020-0.028	0.025±0.002	0.023-0.029	0.027±0.002
	Lindane	0.150-0.156	0.155±0.02	ND	ND	0.180-0.192	0.187±0.02
	Methoxychlor	0.130-0.137	0.135±0.01	0.188-0.200	0.198±0.01	0.158-0.165	0.16±0.02
	Chlorpyrifos	0.347-0.360	0.350±0.02	ND	ND	0.282-0.290	0.289± 0.02
	Malathion	0.188-0.200	0.197±0.01	ND	ND	0.185-0.190	0.19±0.02

N, total number of samples from local vendors; ND, non detected; n, number of samples/each area

**Table 4** Total frequency of pesticide residues studied in this work and the incidence of their levels exceeding the legalized maximum residual limits

Pesticide	Number analyzed	Frequency	MRL*	<MRL%
		N- (%)	mg/kg	
Alachlor	45	5 - (11)	0.01	----
Dieldrin	45	30 - (66.6)	0.06	----
HCB	45	40 - (88.8)	0.01	88
Lindane	45	20 - (44.4)	0.01	44
Methoxychlor	45	30 - (66.6)	0.01	66
Chlorpyrifos	45	15 - (33.3)	0.01	33
Malathion	45	20 - (44.5)	0.02	44
Parathion-methyl	45	10 - (22.2)	0.01	----

\*Pesticides for which European Union (EC Commission Regulation) No 149/2008, legislation has established maximum residue level (MRL) in milk and dairy products

N, number of positive contaminated samples

## Discussion

To aid in the protection of consumer health and elimination of unsafe food, as well as to help increase compliance with the requirements of the international food trade, we undertook this study to determine the levels of several OC and OP pesticide residues in raw buffalo milk samples collected from various parts (north, middle, and south) of the city of Assiut in upper Egypt. We detected the OC pesticide residues alachlor, dieldrin, HCB, lindane, and methoxychlor in these samples from local vendors, dairy farms, and shops. The total frequency distributions for these pesticides were 11%, 66%, 88%, 44% and 66%, respectively. Dieldrin was detected in 100% of the samples obtained from the dairy farms and shops at mean concentrations (mg/kg) of 0.012±0.001(north), 0.008±0.001 (middle), and 0.01±0.002(south) and 0.014±0.001(north), 0.01±0.001(middle), and 0.012±0.001(south), respectively. These values are lower than the MRL(0.06mg/kg) set by the EC19 and lower than levels measured in Ghana by Darko & Acquah,<sup>20</sup> who detected an average concentration of 1.32±0.20mg/kg in all milk samples analyzed.

Furthermore, the levels measured in our study were lower than those reported in a study conducted in Giza, Egypt, in which the mean concentration of dieldrin in raw milk was 2.966±0.135mg/kg and the frequency was 55.5%.<sup>21</sup> On the contrary, Salem et al.,<sup>22</sup> detected no dieldrin residues in milk sampled in Jordan. However, the levels of dieldrin in our samples were higher than those detected in raw fresh milk sampled in Kampala markets in Uganda (0.007mg/kg).<sup>1</sup>

In the present study, the mean concentrations (mg/kg) of HCB in milk samples from local vendors was 0.180±0.02mg/kg(middle and south); 0.030±0.001(north), 0.030±0.001(middle), and 0.026±0.001(south) in dairy farm samples; and 0.022±0.002(north), 0.025±0.001(middle), and 0.027±0.002(south) in samples from dairy shops. The total distribution was 88%. The levels in our samples, especially those in local vendor samples, were higher than levels detected by Abu Donia et al.,<sup>23</sup> who reported mean concentrations of 0.162mg/kg and a frequency of 41% in raw buffalo milk. The concentrations detected in our samples were also higher than those

detected by Waliszewski et al.,<sup>24</sup> who reported that the frequency of positive samples was 84.3% with a mean concentration of 0.014mg/kg fat. However, the frequency of HCB-positive samples in the current investigation was lower than those detected by Campoy et al.,<sup>25</sup> Heck et al.,<sup>26</sup> who reported positive frequencies of 94-100% and 100%, respectively, with a mean concentration of 0.003mg/kg.

In the current study, the mean concentrations (mg/kg) of lindane were 0.165±0.02(north) and 0.097±0.001(south) in samples from dairy farms and 0.155±0.02(north) and 0.187±0.02(south) in samples from dairy shops. These levels were higher than the MRL (0.01) set by the EC<sup>19</sup> and higher than those measured by Aman & Bluthgen<sup>27</sup> in Egyptian milk and those reported by Waliszewski et al.,<sup>24</sup> Heck et al.,<sup>26</sup> Darko and Acquah,<sup>20</sup> and Abu Donia et al.,<sup>23</sup> who detected mean concentrations of 0.009mg/kg, 0.030mg/kg, 0.005mg/kg, concentrations below the limit of detection, and 0.046mg/kg, respectively. However, our values resembled those measured by Battu et al.<sup>28</sup> Furthermore, the frequency of lindane-positive samples in our study was lower than those reported by Waliszewski et al.,<sup>24</sup> Battu et al.,<sup>28</sup> and Abu Donia et al.<sup>23</sup> (80%, 53.3%, and 50%, respectively).

In the current study, the frequency distribution of alachlor was 11% of the samples examined, and the mean concentration was 0.001±0.0001mg/kg in samples from dairy farms. These values were lower than the MRL (0.01) set by the EC.<sup>19</sup> Methoxychlor residues were detected in 66% of all samples examined, with mean concentrations (mg/kg) of 0.142±0.02(north), 0.178±0.03(middle), and 0.140±0.02(south) in samples from dairy farms, and 0.135±0.01(north), 0.198± 0.01(middle) and 0.187±0.02(south) in samples from dairy shops. These values were higher than the MRL (0.01) set by the EC.<sup>19</sup> To our knowledge, no additional data for these two pesticides in milk are available.

The OP pesticide residues detected in raw buffalo milk sampled from local vendors, dairy farms, and shops in the present study were chlorpyrifos, malathion, and parathion-methyl. The total frequency distributions for these pesticides were 33%, 44%, and 22%, respectively. The mean concentration of chlorpyrifos detected was 3.01mg/kg in the north area from local vendors and 0.350±0.02mg/kg(north) and 0.289±0.02mg/kg(south) from dairy shops. The levels of chlorpyrifos in the tested samples were higher than the MRL (0.01) set by the EC<sup>19</sup> for this pesticide.

In Italy, the main pollutants detected in samples of raw milk collected from four dairy plants in one study were acephate and chlorpyrifos. In every positive sample found, OP pesticide contamination was lower than the EC MRLs.<sup>29</sup> A recent study of raw milk from nine Italian dairy plants found only traces of pesticide residues in approximately 4.4% of the samples analyzed. The main pollutant was chlorpyrifos.<sup>30</sup> In Mexican milk samples, the average concentrations of 13 OP pesticide residues, including chlorpyrifos, were below established MRLs and ranged between 0.0051 and 0.0203ppm.<sup>31</sup>

In the present study, the mean concentration of malathion was 0.9±0.01mg/kg in milk samples from local vendors in the north area, 0.195±0.02mg/kg in dairy farm samples in the north area, and 0.197±0.0mg/kg(north) and 0.190±0.02mg/kg(south) in samples from dairy shops. Our results were higher than those obtained by Fagnani et al.,<sup>5</sup> who detected malathion in milk at a mean concentration of 0.02mg/kg and a positive frequency of 8.33%. The levels of malathion in the samples analyzed in this study were higher than the MRL (0.02) set by the EC.<sup>19</sup>

In the current study, parathion-methyl was present in 66% of the examined samples from dairy farms at mean concentrations of  $0.002 \pm 0.0001$  mg/kg (north) and  $0.001 \pm 0.0001$  mg/kg (south). These residues levels were traces compared with the MRL (0.01) set by the EC.<sup>19</sup> In Spain, the residues of seven OP pesticides were detected in raw milk, and the percentage incidence measured was 0.83% for parathion-methyl with a range of 0.005-0.220 mg/kg.<sup>32</sup> Our results showed that the lowest number of detected pesticides was found in milk samples obtained from local vendors, dairy farms, and shops in the middle area, perhaps because this area is far from the agricultural zones in the north and south areas in which pesticides are applied in fields near grazing lactating animals.

According to Johansen et al.,<sup>6</sup> the main sources of pesticide residues in milk are contaminated feed, grass or corn silage, and direct application of pesticides on dairy cattle in barns or dairy plants. Regardless of area, the lowest number of detected pesticides was found in samples from local vendors, but these samples had high mean values of residues chlorpyrifos, malathion, and HCB in particular. This finding may be attributed to the replacement of OC pesticides by OP pesticides in cattle dips using acaricide.<sup>33</sup> In addition, HCB is found as an impurity in some industrial chlorination processes and is a by-product of combustion processes as well, and these are probably the main sources of environmental contamination.<sup>22</sup> These facts may explain the high frequency of HCB-positive samples in the present study, as well as the high mean levels of residues in the samples from local vendors, where products are exposed extensively to combustion processes in the open streets where they are transferred or prepared. Several OC and OP pesticide residues were detected in raw buffalo milk samples collected from various sources and areas of the city of Assiut. The residue levels of lindane and malathion exceeded EC MRLs<sup>19</sup> in 44% of the samples. In addition, the residue levels of chlorpyrifos, methoxychlor, and HCB exceeded EC MRLs<sup>19</sup> in 33%, 66%, and 88% of the examined samples, respectively. The results of this survey demonstrate the need to establish pesticide residue monitoring programs in milk for human consumption to improve food safety and decrease exposure risks for consumers.

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None.

## Conflict of interest

Author declares that there is no conflict of interest.

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