**Pesticide residues in breast milk and the associated risk assessment**

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**Abstract:**

The aim of this study was to determine the types and levels of organochlorine pesticides (OCPs) present in the human milk collected from cities in eastern and central Saudi Arabia. This study is part of assessment of various persistent organic pollutants (POPs) in human milk in four cities of eastern and central Saudi Arabia, namely, Riyadh, Al-Kharj, Al-Jobail and Al-Dammam. Milk samples were collected from a total of 50 donors according to the WHO/UNEP protocol for monitoring human milk for POPs. The OCPs in each of the 50 milk samples were analyzed using as triple quadrupole gas chromatography mass spectrometry-mass spectrometry detection (GC-MS/MS). Quality assurance included the analysis of blank, spiked and reference samples. Sixteen different OCPs were identified namely: aldrin, dieldrin, endrin, hexachlorobenzene , alpha, beta, gamma and delta hexachlorocycohexame, pp’-,op’-DDT, pp’-DDE, pp’-DDD, alpha and gamma chlordane, heptachlor, mirex and methoxychlor. The results of the analysis OCPs in human milk samples indicated that the tested positive samples for one or more pesticide at the limits of determination used in this study. As required by the Stockholm Convention on POPs, the levels of certain POPs in human milk will serve as an indicator of the effectiveness of the treaty in eliminating or reducing emissions of selected POPs. This study contributes to that effort by providing seline data on current levels of OPCs in human milk in Saudi Arabia.

**Keywords:** Organochlorine pesticides, Persistent organic pollutants, Human milk, Stockholm Convention and Saudi Arabia.

**1. Introduction**

Human milk is a good medium to evaluate human exposure to persistent lipophilic chemical especially organochlorine pesticides (OCPs). Human milk is considered as one of the best sampling matrices for biomonitoring due to its availability and non‐invasive nature when collecting individual samples. Its high lipid content makes the extraction of OCPs easier and the precision of the measurements higher (UNEP, 2003). While not noted for their acutely toxic, the role of low level exposure to OCPs in disrupting the endocrine systems of animals and possibly humans resulted in the banning of many OCPs for agricultural uses. Based on evidence that OCPs as well as other persistent organic pollutants (POPs) posed a major and increasing threat to human health and the environment, governments adopted the Stockholm Convention on Persistent Organic Pollutants which committed ratifying countries, including Saudi Arabia, to eliminate or reduce POPs emission into the environment. The treaty came into force in May 2004.

Of the initial 12 POPs included in the treaty, 9 were OCPs, including aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, hexachlorobenzene, mirex, and toxaphene . Since that time, 6 additional OCPs have been added to the Stockholm Convention, including chlordecone, endosulfan, alpha hexachlorocyclohexane, beta hexachlorocyclohexane, lindane, and pentachlorobenzene. However, certain OCPs are still widely used by farmers in developing countries because of their effectiveness and low cost (Amoah et al., 2006). Some POPs are industrial by-products and through contamination or improver disposal, environmental pollution by this chemicals can also contaminate food, especially food of animal origin.

Over the last decades, human milk has been used as a medium to measure contamination in humans, and analytical techniques have been well established for most POPs included in the Stockholm Convention. Furthermore, the uptake of these chemicals by the infant via human milk is of high toxicological relevance. The risk‐benefit assessment of breastfed infants represents one of the most challenging aspects of human toxicology, as possible adverse health effects associated with exposure to POPs concur with significant health benefits of breastfeeding. The analysis of human milk fat can provide a simple assessment of the OCPs as it reflects their relative presence in the environment. Because human exposure in almost exclusively through food, such data can also provide an assessment of OCPs in the food supply. Finally, the analysis of human milk can provide an integrated exposure of the mother as well as exposure of the developing fetus and neonate to OCPs and provide a baseline for epidemiological studies of assess possible adverse effects in human. Some studies found associations between prenatal exposures to POPs and altered reproductive function in males and females; increased incidence of breast cancer, abnormal growth patterns and neurodevelopmental delays in children, as well as changes in immune function (WHO/UNEP, 2012).

In Saudi Arabia, little work has been done to measure the organochlorine compounds in human milk. Levels of OCPs in samples taken from 115 mothers residing in Al-Kharj were reported (Al-Saleh et al., 1998) and a number of OCPs exceeded the maximum tolerable daily intakes of the WHO acceptable daily intakes calculated for a 5-kg infant. In 2003, DDT and its metabolites were measured in 878 milk samples collected from mothers living in Al-Ehssa where DDT spraying for vector control continued until 1995. Compared to DDT levels in samples from mothers living in Riyadh region where no spraying had been conducted, levels in the Al-Ehssa region were significantly higher and exceeded than maximum tolerable intake for a 5 kg infant (Al-Saleh et al, 2003). Both studies recommended the establishment of widespread monitoring programs for food and human milk to better understand sources and trends of exposure to OCPs. The aim of this study was to determine levels of selected organochlorine pesticide residues in human milk samples in Saudi Arabia and to provide baseline assessments in order to monitor trends in the future and identify priorities for further study.

**Meanwhile, Hajjar, and Al-Salam (2017)** reported that the level of organochlorine pesticide (OC) residues in human milk samples collected from donor mothers aged from 18 to 30 years old, from four cities in Eastern district of Saudi Arabia (Al- Hassa, Al- Khobar, Al-Jubail, and Al-Dammam). Pesticides residues were extracted from the samples and analyzed using GC-MS. The results showed that, only pp’DDE and p,pDDD, were found in 82.5% and 70% of analyzed samples respectively, the total DDT were at level of 0.37, 0.32, 0.30 and 0.46 µg/L in the four cities respectively and were far below the MRL of 50 µg/L (FAO/WHO). The estimated daily intake (EDI) of DDT ingested by infant weight 3.5 kg ranged between 0.06 and 0.10 µg/kg, which is less than the ADI issued by (EFSA, 2014).

**2. Materials and Methods**

***2.1 Samples Collection.***

Fifty samples were collected from five hospitals located in central and eastern regions of Saudi Arabia, including two in Riyadh, one each in AL-Kharj, Dammam and Al-Jobail. Milk samples was collected by nurses that were trained for this study. The Ethical Committees at each collaborating hospital reviewed and approved the purposes and protocol of this study, which may not be employed for other purposes. Questionnaires and donor-related records conform to national requirements and international norms concerning confidentiality.

Prospective donors were asked to complete a personal questionnaire modeled after the one that is internationally recommended (UNEP, 2012). Because only 4 women were primiparuos during the sampling period, women with prior children were enrolled in the study, including 19 with 1 previous child, 9 with 2 previous children, 3 with 3 previous children and 3 with 4 previous children. The mothers participating in this study ranged from 26 to 36 years of age with a mean of 31 years. All donors lived at their current locations for at least 8 years. Most donors consumed a mixed diet with 9 indicating ate only fish and vegetables. The mean lipid content of the all samples was 2.57 g lipid/L milk. All the sample donors were healthy, had normal deliveries and were nursing a single infant (no twins). A summary of donor profiles is provided in Table 1.

Table 1. Profile of donors of milk samples in Saudi Arabia

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Location | Number of Donors | Mean Age | Mean g lipid/L Milk | Fish/Vegetarian | Body Mass Index (BMI) |
| Riyadh | 15 | 30.7 | 2.62 | 1 | 29.37 |
| Al-Dammam | 10 | 30.0 | 2.54 | 3 | 31.62 |
| Al-Kharj | 15 | 31.0 | 2.64 | 2 | 32.31 |
| Al-Jubail | 10 | 32.2 | 2.43 | 3 | 26.18 |

A total of 50 human milk samples of about 100 mL each were collected using sterilized glass pumps at times that varied from 2 to 8 weeks post-partum. Each samples was transferred directly to clean dark glass jar with Teflon cap and transported under refrigeration to the analytical laboratory. Samples that were not immediately analyzed were stored under at - 20 °C until the sample could be analyzed

***2.2 Statistical methods***

Statistical analysis was applying to calculate minimum, maximum, mean, standard deviation and confidence levels. All the data was subjected for statistical analysis of variance (ANOVA) to determine the significance of the categorical factors on different OCPs levels. Most of the analyses (96.2%) were above their respective limits of quantification (LOQ), values below the LOQ were treated as ½ of these limits in calculating the means.

***2.3 Analytical methods***

A total of 16 OCPs were analyzed including: 1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane and 1,1,1-trichloro-2-(o-chlorophenyl)-2-(p-chlorophenyl) ethane (p,p’- o,p’-DDT), 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (p,p’-DDE) and 1,1-dichloro-2,2-bis(p-chlorophenyl) ethane (p,p’-DDD), hexachlorobenzene (HCB), alpha-, beta-, gamma- and delta-hexachlorocyclohexane, (α-, β-, γ-, and δ-HCH), alpha- and gamma-chlordane (α- and γ-chlordane), heptachlor epoxide , mirex and methoxychlor. The chemical analysis has previously been described (Eik Anda et al.,2007).

Briefly, each sample was homogenized and diluted by 1:1 with phosphate buffered saline and lipid fraction separated by centrifuge. The lipid fraction was extracted with ASE 350 technology. The same ASE 350 procedures was applied for equivalent samples spiked with internal standards of 3 13C12 labelled 3 OCPs and blank samples. The concentrated extracts were analyzed using the Thermo Scientific TMQ 8000™ triple quadrupole GC-MS/MS system equipped with TR™ TR-Pesticide 30 m 0.25 mm IDx0.25 um. The limits of quantification (LOQ) for the pesticides ranged from 10 16 pg/Lipid

***2.4 Quality assurance***

Duplicate samples, blanks and spiked samples and/or Certified Reference Material CRM were processed and analyzed with each batch (1-10 samples) of samples. The limit of detection (LOD) and Limit of Quantification (LOQ), repeatability, reproducibility, accuracy and precession also were determined for each compound. Calibration curves were constructed for each OCP using a series of concentrations (0.001- 1000 ng/g lipid) for each compound that covered the dynamic range in which the targeted compounds are expected to be present. Quality assurance were maintain throughout the course of the project using standard quality assurance procedures and documentation. For external assessment, random analyzed samples were sent to reference accredited laboratories with the high resolution gas chromatography and mass spectrometry (HRGC/HRMS). These results were used for estimating methodology uncertainty and performance.

**3. Results and Discussion**

***3.1 Levels of OCP residues in human milk***

The maximum, minimum and mean levels of OCPs in human milk, expressed as ng/g lipid, from the 48 samples collected in four cities in Saudi Arabia are presented in Table 2.

**Table 2. Levels of organochlorine pesticide residues in human milk from Saudi Arabia\* (ng/g lipid) (n = 50)**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **HCB** | **alpha-HCH** | **beta-HCH** | **gamma-HCH** | **delta-HCH** | **Ʃpp, op DDT**  | **Ʃpp, op DDE** | **Ʃpp, op DDD**  | **alpha-Chlordane** | **gamma-Chlordane** | **Aldrin** | **Dieldrin** | **Endrin**  | **Heptachlor**  | **Mirex** | **Methoxychlor**  |
| Max | 248 | 100 | 146 | 24 | 184 | 71.4 | 135 | 31.8 | 9.71 | 4.39 | 19.7 | 9.66 | 18.6 | 13.8 | 9.28 | 10.5 |
| Min | 57.4 | 21.6 | 35.8 | 9.22 | 75.9 | 0.04 | 17.2 | 0.04 | 1.11 | 0.04 | 4.68 | 1.03 | 2.36 | 1.89 | 0.48 | 0.04 |
| Mean | 117 | 40.4 | 70.2 | 14.0 | 104 | 28.1 | 79.9 | 12.4 | 3.52 | 1.77 | 11.2 | 5.07 | 9.46 | 4.48 | 4.03 | 3.05 |
| SD | 46.9 | 24.0 | 30.0 | 3.88 | 30.1 | 16.0 | 26.3 | 8.04 | 2.51 | 1.25 | 3.78 | 2.43 | 4.03 | 2.99 | 2.55 | 3.05 |
| CV | 0.40 | 0.59 | 0.43 | 0.28 | 0.29 | 0.57 | 0.33 | 0.65 | 0.71 | 0.70 | 0.34 | 0.48 | 0.43 | 0.67 | 0.63 | 1.00 |

\* Maximum (Max), Minimum (Min), Mean, Standard Deviation (SD) and coefficient of variation (CV) where CV = SD/Ave

**3*.2 Discussion***

*Comparison with other countries*

The highest mean concentration in human milk was observed for hexachlorobenzene (117 ng/g lipid). This value is comparable to those reported in Moldova (2009), Ukraine (2001) and Spain (2002) of 153, 72 and 70 ng/g lipid, respectively. However, this level is well over the mean reported for most countries, which was less than 20 ng/g lipid. (WHO/UNEP, 2013) The mean level of alpha-hexachlorocyclohexane of 40.4 ng/g lipid is also high compared to levels in human milk in other countries. For example, the levels in India, Ukraine, Tajikistan and Moldova were reported to be 10.5, 5.5, 4.2 and 3.5 ng/g lipid, respectively. (WHO/UNEP, 2013) The level of beta-hexachlorocyclohexane of 72.2 ng/g lipid was more comparable with other countries of which, six had levels that exceeded 200 ng/g lipid. However, the level for gamma-hexachlorocyclohexane of 14.0 ng/g lipid was only exceeded by Senegal with 16.0 ng/g lipid among the 75 countries for which data are available. (WHO/UNEP, 2013) Although delta-hexachlorocyclohexane comprises 6-10% of technical grade lindane, little information is available on levels of this OCP in human milk. A national survey of human milk in China reported a lower bound of 2.2 ng/g lipid and an upper bound of 3.8 ng/g lipid. (Zhou et al., 2011) Thus, the level determined in this study of 104 ng/g lipid must be viewed with some concern given the toxicity of the isomers in this group. The level of endrin of 9.46 ng/g lipid is higher that most countries, which have discontinued the use of the pesticide for some time. In China, the lower bound is reported to be 0.6 ng/g lipid, while the upper bound is 3.5 ng/g lipd. (Zhou et al., 2011) However, it is low compared to India where the mean level is reported to be 107 ng/g lipid. (Bedi et al., 2013) The mean level of heptachlor, including its epoxide, of 4.48 ng/g lipid is only exceeded by those reported in Bulgaria (2001) and Luxembourg (2001) of 12.0 and 8.2 ng/g lipid, respectively. However, it is similar to levels reported in Belgium (2010) and the USA (2006) of 4.5 and 4.4 ng/g lipid, respectively. (WHO/UNEP, 2013) The levels of DDT, DDE and DDD is close to the median of levels reported by other countries. The ration of DDE to DDT of 2.8 indicates that exposure may have been recent. (WHO, 2011). Very few studies have examined mirex in human milk. Mirex was detected (mean detection limit 3 pg/g lipid) in 62% of 412 breast milk samples collected from women in all Canadian provinces (Mes et al., 1993). The mean, median, and maximum mirex concentrations detected for milk fat were 4.2, 2.3, and 124.5 ng/g, respectively. More recently, mirex was reported at lower and upper bound levels of 1.0 and 3.5 ng/g lipid in China. (Zhou et al., 2011). These levels are comparable the level found in this study 4.03 ng/g lipid. Methoxychlor levels in human milk lipid were reported to be 0.07 and 0.1 ng/g in Denmark and Finland, respectively. (Shena et al., 2007) This study found a mean level of 3.05 ng/g lipid.

***3.3 Risk assessments***

Because of the similarities in the toxicological profiles, certain OCPs are reported as the sum of their isomers and metabolites. For example, when conducting a risk assessment of DDT, the sum of the isomers of DDT (pp’- and op’-DDT) and its metabolite (op’-DDE and op’-DDD) is used to compare with the health-based guidance value. Similarly, levels of alpha- and beta-chlordane and aldrin and dieldrin are combined to toxicological reasons. Table 3 provides the summation of levels for these toxicological groups,

Table 3. Total mean levels of organochlorine pesticide residue assessed groups in human milk samples in Saudi Arabia (ng/g lipid)

|  |  |
| --- | --- |
| Group Assessed | Total Means (ng/g lipid)=ppb |
| Σ DDT, DDE & DDE | 120 |
| Σ alpha - & beta-Chlordane | 5.29 |
| Σ Aldrin & Dieldrin | 16.3 |

The sum of DDT isomers and metabolites is often referred to the DDT Complex. The World Health Organization has established a Provisional Tolerable Daily Intake for DDT Complex of 10 μg/kg body weight based on developmental toxicity in the rat. (WHO, 2001) Assuming an infant consumes 125 g milk/kg body weight and lipid content of human milk from this study (2.57 % lipid), the tolerable level in human milk lipid is 3.1 ng/g lipid. This is significantly exceed by the mean level in this study by a factor of 39. Furthermore, the levels of DDT Complex in a woman's first lactation are normally higher because the pollutant concentrations are known to decrease significantly with subsequent births and lactations. (Smith, 1999) Consequently, if this study was carried only in primiparuos mother, the level of DDT Complex is expected to be higher.

Chlordane is a mixture of isomers, mainly alpha- and beta-chlordane. Technical chlordane contains 60–75% chlordane isomers and at least 25 other compounds, including heptachlor and nonachlor. Based on an earlier evaluation, WHO established a Provisional Tolerable Daily Intake for chlordane in 1994 of 0.5 μg/kg of body weight. (WHO, 1995) By applying the same assumption as above, the tolerable level in human milk is 0.16 ng/g lipid. The tolerable level is exceed by a factor of 33.

The residue definition for dieldrin usually specifies dieldrin “singly or combined with aldrin, expressed as dieldrin”. In regard to aldrin and dieldrin. This definition reflects the close chemical and toxicological relationship between these two cyclodiene insecticides. In 1994, WHO established a Provisional Tolerable Daily Intake of 0.1 μg/kg body weight. (WHO, 1995) By applying the same assumption as above, the tolerable level in human milk is 32 pg/g lipid. Therefore, the tolerable levels is exceed by a factor of more than 500 and should be considered a priority for further study and intervention.

***3.4 Uncertainty***

Like many countries, Saudi Arabia has many areas where there is no clear demarcation between industrial, agricultural and urban zones. Under such conditions, careful sampling is required to produce a representative mean value. Similarly, the use of an OCP for vector control has been conducted in Saudi Arabia to control leishmaniasis, but only in specific regions, which can skew results. One of the main difficulties is that no comprehensive study of OCPs has been previously carried out and trends in levels over time cannot be assessed. In this study, only 50 milk samples were collected because of the cost and time and conclusions about the entire population cannot be drawn at this time. Further complexities and uncertainties may arise when taking into account the effects of multiple interactions among all OCPs and other POPs measured in human milk. Therefore, the results of this study are only indicative of levels of OCPs in human milk in Saudi Arabia.

**4. Conclusions**

The study was primarily aimed at identifying OCP contaminants in human milk in Saudi Arabia and to provide a baseline for those chemicals for which such information was previously not available. This will allow evaluation of trends in the future. The quantitative differences observed in this study compared to other countries may provide a suitable basis for possible source‐directed measures to further reduce levels of specific OCPs. The levels of some OCPs observed in human milk have been compared with the WHO safety standards with the conclusion that in many chemicals are still within those associated with these standards. However, based on the uncertainty of this study, it can be concluded that the demonstrated benefits of breastfeeding outweigh the disadvantages. While the beneficiary effects of breastfeeding are likely to persist into later life, most OCP-related negative effects are mostly transient in nature and less clinically relevant than *in utero* exposures. Nevertheless, some worrisome adverse health effects were observed, which may persist in later life. Follow-up studies with a large number of participants should be given a high priority as well as total diet studies to determine which foods are contributing to OCP body burden in Saudi Arabia.

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**Conflict of interest**

The authors have no conflicts of interest to declare.

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