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Organochlorine Pesticides in Breast Milk and Other Tissues of Some Egyptian Mothers

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Abstract: Organochlorine pesticides, are organic compounds that, are characterized by their lipophilicity persistence and semi-volatility. These characteristics pre-dispose them to long environmental persistence and to long range transport. Their ability to persist in the environment, to concentrate up in food chain and to bioaccumulate in fatty tissues of animals and humans remain a cause for global concern. The present study aimed to assess the residues levels of organochlorine pesticides in breast milk, the maternal serum (MS), umbilical serum (US) and abdominal adipose tissue (Adp T) of 38 women giving birth by cesarean section at Kasr El-Eini hospital. It also aimed to detect the relations between it's presence and it's secretion in the breast milk and umbilical cord serum, in order to establish a possible correlation between organochlorine contents in these biological compartments and the current status of pesticide burden on child's health. The pesticide residues were determined by gas chromatography, the results obtained from different sample groups were statistically analysed through SPSS version 15. The distribution of the organochlorine pesticides in the four types of tissues (Breast milk, maternal serum (MS), Umbilical serum (US), abdominal adipose tissue (AdpT)), revealed that endrin, DDT and its dervatives followed by dieldrin, were the main contaminants. Abdominal adipose tissue (AdpT) samples showed the higher contamination levels for these main residues, followed by the breast milk samples then the serum samples. Results of correlation analysis indicates positive high correlation between concentrations of DDD and DDE in breast milk and adipose tissue and another positive correlation between concentrations of endrin and dieldrin, in breast milk and maternal serum. This in breast milk may indicate a need for continued monitoring of these residues, for which breast milk is a suitable medium. And we can conclude that accumulation of these pesticide residues may be less during fetal development than during breast feeding. Despite the possibility of harm from these contaminants in breast milk, breastfeeding for long periods should still be recommended as the best infant feeding method, children who were breastfed for more than 20 weeks had better cognitive performance regardless of their in utero exposure to DDT.

Key words: Organochlorine Pesticides · Breast milk · Maternal serum · Umblical serum and Adipose tissue · Maternal and child health

INTRODUCTION

Pesticide use remain a cause of global concern and still raises a number of environmental concerns, as over 98% of sprayed pesticides and 95% of herbicides reach a destination other than their target species, including air, water, bottom sediments and food [1]. Pollution from organochlorine pesticides is more potent than organophosphates, carbamates and others due to their persistence in nature [2].

Persistent organic pollutants (POPs) are organic compounds that, to a varying degree, resist photolytic, biological and chemical degradation. POPs are characterised by low water solubility and high lipid solubility, leading to their bioaccumulation in fatty tissues. They are also semi-volatile, enabling them to move long distances in the atmosphere before deposition occurs. Although many different forms of POPs may exist, both natural and anthropogenic, POPs which are noted for their persistence and bioaccumulative characteristics

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include many of the first generation organochlorine insecticides such as dieldrin, DDT, toxaphene and chlordane and several industrial chemical products or byproducts. Many of these compounds have been or continue to be used in large quantities and, due to their environmental persistence, have the ability to bioaccumulate and biomagnify. Some of these compounds may persist in the environment for periods of years and may bioconcentrate by factors of up to 70,000 fold [3].

Organochlorine pesticides have the ability to concentrate up in the food chain [4], to be detected in breast milk [5] and have the ability to be stored in the adipose tissue of animals and humans [6-9].

The Canadian and American Academies of Pediatrics have published an urging recommendation to return to breast feeding as the best nutrition for infants for the first 6 months of age [10]. With increased interest in breast feeding, the nature and source of contamination by pesticide still remain questionable, as the transfer of pesticides to breast milk depends on their concentration in the serum of mother and their properties [11]. The concentrations of pesticide residues, in human milk, vary considerably from one pesticide to another, from country to country and were influenced by factors such as legalization and the culture of society and diet [5].

Some pesticides; such as DDT and its most prevalent breakdown products; the dichlorodiphenyldichloroethylene (DDE), persists in the environment, concentrates up in the food chain [12] and stores in fatty tissues of animals, fish and humans humans [13, 14], is widely detected in breast milk [15-17].

Some types of organochlorides have been linked to a variety of health problems, including reproductive health, developmental and immune disorders and some cancers [6, 17]. Also, it has significant toxicity to plants or animals, including humans [2].

Many countries have phased out the use of some types of organochlorides such as the US ban on DDT. In Egypt, the use of DDT in agriculture has been officially banned since about 30 years, followed DDT cancellation, the use of other pesticides (e.g., aldrin, dieldrin, chlordane, heptachlor, lindane, parathion, parathion methyl, leptophos) was gradually restricted in our country, most, if not all, of the banned or severely restricted pesticides have been used in Egypt, exposure to pesticides in Egypt may be excessive through ground application of pesticides in cotton fields [18]. And the general population can be exposed to low levels of pesticides in three general ways: vector control for public health and other non-agriculture purposes; environmental residues and food residues [19]. Unfortunately, in Alexandria, tapwater samples contain pesticide residues higher than those taken from plant samples after treatment [20]. River Nile water samples at El-Malek El-Saleh contain many OC pesticides, which accounted to 0.00005 ppm as total organochlorine residues [21]. The latter investigator reported similar trends for OC contaminants in fish samples collected from Great Cairo Governorates [22]. Also, Abou-Arab *et al.* [23] analysed 20 different vegetables and fruits [23] as potato tubers and onion, strawberry specifically have been, heavily contaminated by certain pesticide as DDT, lindane, aldrin+dieldrin above the Maximum Required Level established by FAO/WHO [12].

Persistent DDT and other organochloride residues continue to be found in humans and mammals across the planet many years after production and use have been limited, in Arctic areas, particularly high levels are found in marine mammals [24]. These chemicals concentrate in mammals and are even found in human breast milk [25]. Males typically have far higher levels, as females reduce their concentration by transfer to their offspring through breast feeding.

AIM OF WORK

The present study aimed to assess the residues levels of organochlorine pesticides in breast milk, the maternal serum, umbilical serum and the adipose tissue of some Egyptian mothers. It also aimed to detect the relations between it's presence and it's secretion in the breast milk and blood cord, in order to establish a possible correlation between organochlorine pesticide residues in these biological compartments and the current status of pesticide burden on child's health

MATERIALS AND METHODS

Study Design: The present study was designed to analyze the levels of organochlorine pesticide residues in the maternal serum (MS), umbilical serum (US), abdominal adipose tissue (Adp T) and breast milk of 38 women giving birth by cesarean section at Kasr El-Eini hospital. Milk samples (40-60 g each) were also collected from the donors 10-15 days postpartum. They were manually expressed by donors into solvent- washed screw-capped glass bottles of 100 ml capacity, frozen immediately and stored at -20°C until analysis.

Analytical Methods: The AOAC method [26] was employed to extract fat. The procedure of Tanabe *et al.* [27] was followed for the analyses of organochlorines.

Standards: Pesticide standards of lindane; heptachlor; heptachlorepoxide; dieldrin; endrin; 1, 1, 1-tri-chloro-2, 2 bis(p-chlorophenyl) ethane (p. p'- DDT); 1-(o-chlorophenyl)-1-(p-chlorophenyl)-2, 2, 2-tri-chloroethan (o. p'- DDT); 1-(o-chlorophenyl)-1-(p-chlorophenyl)-2,2-dichloroethylene (o. p'- DDE); 1, 1-di-chloro-2, 2- bis (p-chlorophenyl) ethane (p. p'- DDD); 1-(o-chlorophenyl)-1- (p-chlorophenyl) -2, 2-dichloroethane (o. p'- DDD); were provided by chem. Service, Inc.(West Chester, PA).

Determination of Pesticides Residues: Residues of pesticides were extracted from the different samples, ie Breast Milk, Maternal serum (MS), Umbilical serum (US), Abdominal adipose tissue (Adp T) by different solvents according to the methods of Manual of Analytical Methods [28] and Tanabe *et al.* [27]. Residues in solution were separated from the sample co-extractives on column of Florisil absorbant; eluants of increasing polarity sequentially remove residues from the column by using diethyl ether/petroleum ether at different ratio (6, 15 and 50%). Aliquots of 1-2 ul of extracted samples were injected into a gas chromatography.

A Hewlet- Packard 5890 A gas chromatograph with ^{63}Ni electron capture detector (ECD) was used- capillary column, HP-101(methyl silcon gum fluid), 25 meter length, 0.20 mm diameter and 0.2 um film thickness. Programmed oven temperatures were started at 160°C, increased to 220°C at rate 2°C/min. Injection and detector temperatures were 220°C and 300°C, respectively. Carrier gas, nitrogen; column head pressure, 10 psi; make-up gas, nitrogen 50ml/min.

For identification and quantification the external standard method was used. The compounds were identified by their retention times as compared to known standards. Quantification was done by linear regression in which areas of identified peaks were regressed against amount/area ratio of the corresponding peak in external standard mixture. The recoveries from the different samples fortified at 0.1 μ/g ranged from 83.05 to 90.74%. Limit of quantification (Lg) which defines by FDA, as the Lowest level of residue that can be quantified by a given method was calculated for different compounds which ranged between 0.003 to 0.017 μ/g.

Statistical Analysis: The results obtained from different sample groups were statistically analyzed through SPSS version 15. The frequencies, mean, median and range were calculated for each of the detected organochlorine pesticides in the four types of samples (breast milk, maternal serum, adipose tissue and cord serum). Pearson correlation coefficients were also used in the analysis of the relationships.

RESULTS

Tables 1-4 show the distribution and the concentration of Organochlorine pesticides in four types of tissues breast milk, maternal serum (MS), umbilical serum (US), abdominal adipose tissue (Adp T) of 38 women giving birth by cesarean section at Kasr El-Eini hospital.

Table 1: Distribution and concentration levels of organochlorine pesticide residues (ng/g) detected in breast milk samples collected from Egyptian mothers

	Frequency* n (%) (N=38)	Mean	Median	Minimum	Maximum
Lindane	4 (15.2)	0.0018	0.002	0.001	0.003
heptachlor	4 (15.2)	23.70950	23.710	21.403	26.016
heptachlor epoxide	7 (18.4)	43.62333	6.232	0.935	123.703
Endrin	19 (50.0)	2.20438	1.068	0.034	7.686
Dieldrin	12 (31.6)	2.64700	3.230	0.123	5.256
o. p'- DDE	12 (31.6)	24.71860	3.451	0.369	107.57
o. p'- DDD	10 (26.3)	5.14000	4.741	0.031	11.048
p. p'- DDD	14 (36.8)	1.68100	0.906	0.226	4.689
o. p'- DDT	9 (23.7)	0.22825	0.190	0.168	0.366
p. p'- DDT	1 (2.6)	0.002	0.002	0.002	

Positive sample

Table 2: Distribution and concentration levels of organochlorine pesticide residues (ng/g) detected in maternal serum samples collected from Egyptian mothers.

	Frequency* n (%) (N=38)	Mean	Median	Minimum	Maximum
Lindane	ND	-	-	-	-
heptachlor	ND	-	-	-	-
heptachlor epoxide	6 (15.8)	0.5133	0.563	0.41	0.569
Endrin	10 (26.3)	3.1490	0.776	0.23	12.83
Dieldrin	4 (10.5)	0.2785	0.278	0.16	0.396
o. p'- DDE	8 (21.1)	1.7445	1.179	0.34	4.276
o. p'- DDD	12 (31.6)	5.9795	4.076	0.164	18.339
p. p'- DDD	4 (10.5)	0.4050	0.405	0.35	0.462
o. p'- DDT	1(2.6)	0.12	0.12	0.12	
p. p'- DDT	10 (26.3)	1.2970	0.477	0.19	4.633

ND: not detected, Below the detection limits

Table 3: Distribution and concentration levels of organochlorine pesticide residues (ng/g) detected in umbilical cord serum samples from Egyptian mothers

	Frequency* n (%) (N=38)	Mean	Median	Minimum	Maximum
Lindane	ND	-	-	-	-
heptachlor	ND	-	-	-	-
heptachlor epoxide	4 (10.5)	3.4800	3.480	0.49	6.469
Endrin	16 (41.2)	2.8440	1.105	0.22	9.941
Dieldrin	4 (10.5)	0.3270	0.327	0.33	0.328
o. p'- DDE	1 (2.6)	0.537	0.537	0.537	
o. p'- DDD	9 (23.6)	3.2870	2.347	0.60	7.856
p. p'- DDD	9 (23.6)	0.4423	.446	0.35	0.525
o. p'- DDT	4 (10.5)	2.0195	2.020	0.16	3.881
p. p'- DDT	ND	-	-	-	-

ND: not detected

Below the detection limits

Table 4: Distribution and concentration levels of organochlorine pesticide residues (ng/g) detected in abdominal adipose tissue specimens collected from Egyptian mothers

	Frequency* n (%) (N=38)	Mean	Median	Minimum	Maximum
Lindane	1 (2.6)	0.029	0.029	0.029	
heptachlor	1 (2.6)	4.028	4.028	4.028	
heptachlor epoxide	ND	-	-	-	-
Endrin	22 (57.9)	5.1121	3.963	0.06	18.811
Dieldrin	9 (23.7)	16.0070	18.240	7.68	22.097
o. p'- DDE	6 (15.8)	7.9045	7.905	4.18	11.631
o. p'- DDD	6 (15.8)	5.5940	5.594	3.53	7.663
p. p'- DDD	ND	-	-	-	-
o. p'- DDT	19 (50.0)	1.2757	1.066	0.01	2.835
p. p'- DDT	ND	-	-	-	-

ND: not detected

Below the detection limits

Table 5: Correlation between levels of OCPs in breast milk with maternal serum and adipose tissue in the studied mothers

	Breast milk-maternal Serum		Breast milk-Adipose tissue	
	r	p-value	r	p-value
Heptachlor epoxide	-0.16	NS	-	-
Endrin	0.99	0.000**	-0.32	NS
Dieldrin	0.65	0.001**	0.84	0.000**
o. p'- DDE	-0.10	NS	0.97	0.000**
o. p'- DDD	-0.33	NS	0.90	0.000**
p. p'- DDD	-0.28	NS	-	-
o. p'- DDT	-	-	0.48	NS

** significant at p=0.001 level NS: non-significant

N.B. Heptachlor epoxide; p. p'- DDD and p.p'- DDT were not detected in the adipose tissues

Table 6: Correlation between levels of OCPs in umbilical cord serum with maternal serum and adipose tissue of the studied mothers

	Umbilical cord-maternal Serum		Umbilical cord-Adipose tissue	
	r	p-value	r	p-value
heptachlor epoxide	-0.15	NS	-	-
Endrin	0.07	NS	-0.41	NS
Dieldrin	-0.15	NS	-0.50	NS
o. p'- DDD	0.09	NS	-0.51	NS
p. p'- DDD	-0.21	NS	-	-
o. p'- DDT	-	-	-0.33	NS

NS: non-significant

N.B. Lindane, heptachlor and p. p'- DDT were not detected in the umbilical cord serum.

The most frequent detectable pesticide residues in breast milk of the studied mothers was endrin (50%), followed by p. p'- DDD (36.8%) then o. p'- DDE and dieldrin (31.6%). The highest median level found among the most frequent detectable pesticide residues was that of o. p'- DDE (most prevalent breakdown products of DDTs), while the heptachlor, followed by its epoxide residues had contaminated the breast milk samples with the highest concentrations [Table 1].

The most frequent detectable pesticide in maternal serum of the studied mothers was o. p'- DDD (31.6%) and followed by endrin and p. p'- DDT (both 26.3%) then o. p'- DDE (21%). The o. p'- DDD (DDT metabolite) detected in maternal serum of the studied mothers, was the most frequent pesticide residue with the highest median of contamination (4.076 mg/g fat) [Table 2].

The most frequent detectable pesticide in umbilical cord serum of the studied mothers was endrin (41.2%) then DDD. DDD and endrin were detected with higher median of contamination, in addition to the heptachlor epoxide and the DDT (Table 3).

The most frequent detectable pesticide in adipose tissue of the studied mothers was endrin (57.9%) and o. p'- DDT (50%). Dieldrin, DDD, DDE then endrin showed the highest median of contamination in adipose tissue of the studied mothers (Table 4).

Table 7: Correlation between levels of OCPs in maternal serum and adipose tissue of the studied mothers

	Maternal Serum-Adipose tissue	
	r	p-value
Endrin	0.60	NS
Dieldrin	-0.33	NS
o. p'- DDE	-0.33	NS
o. p'- DDD	-0.57	NS

NS: non-significant

N.B. Lindane and heptachlor were not detected in the maternal serum.
Heptachlor epioxide; p. p'- DDD and p. p'- DDT were not detected in the adipose tissues

In breast milk, there were significant positive correlations (p = 0.001) between the levels of endrin and dieldrin and their corresponding levels in the matched maternal serum. There were also significant positive correlations (p = 0.001) between the levels of dieldrin, o. p'- DDE and p. p'- DDD and their corresponding levels in the matched adipose tissue (Table 5).

However, the detectable pesticide residues in umbilical cord serum showed no correlation with the corresponding levels in the matched maternal serum and adipose tissue (Table 6).

Also there were no correlation between the levels of detectable pesticides residues in the maternal serum and the corresponding levels in the matched adipose tissue (Table 7).

DISCUSSION

Introduced in the 1940s, organochlorine pesticides (OCPs) were widely used in agriculture and pest control until research and public concern regarding the hazards of their use led to government restrictions and bans. Despite restrictions and bans on the use of many organochlorine pesticides in the 1970s and 1980s, they continue to persist in the environment today. In fact, in many ways, human have become reservoirs for these substances.

Breast milk is an ideal medium for assessing exposures to OCPs [17], where they sequester in adipose tissue, serum and breast milk and equilibrate at similar levels on a fat basis [29], so we analyzed the chemical contamination status mainly on a lipid basis because the liposolubility rate is thought to be a major factor influenced by rates of accumulation and elimination from tissues and organs [30] and because the existing differences depend principally on lipid content of the tissues [31]

In the present study, the ages of the included females were in the range 19-37 years with mean of 24.3±4.5 years. Assessment of the distribution of the residues of the organochlorines pesticides (OCPs) revealed that, the most predominant residue found in the breast milk was endrin (50%) followed by dieldrin (31.6%) and among DDTs, the most predominant residues found were p.p'-DDD (36.8%) followed by o.p'-DDE (31.6%) then o.p'-DDD (26.3%). Our findings are consistent with those reported in multiple studies[6, 16, 32, 33, 34, 35] in that, the overall OCPs for most of milk samples was dominated by the degradation product of DDTs, DEE and DDD, while they differ from the Australian study done by Harden *et al.* [32], in being milk samples dominated by beta-HCH and not endrin and dieldrin as well as they differ from those of Jaraczewska *et al.* [6], where they found p,p'-DDT and its major metabolite, p.p'-DDE, together with HCB, were detected in all milk samples studied in Poland. In addition, Saleh *et al.* [35], found that DDE and lindane were the main detected OCPs, but, aldrin and endrin were not detected in most of milk samples.

We investigated the distribution of the above mentioned organochlorine pesticides in three other types of tissues maternal serum (MS), Umbilical serum (US), abdominal adipose tissue (AdpT), we found that endrin, DDT and its dervatives followed by dieldrin, were also the main contaminants. Abdominal adipose tissue (AdpT) samples showed the higher contamination levels for these main residues, followed by the breast milk samples then the serum samples and the concentrations in US were often lower than in MS. In past studies, chemical concentrations were higher in adipose tissues than in serum [36, 37] and in other studies, concentrations were higher in serum lipid than in breast tissues [38]. Moreover, as suggested by Pauwels *et al.* [37], the concentration levels of persistent pesticides varied dramatically depending on the tissues. One of the reasons for the confusion may be the pharmacokinetics of chemicals in blood. Mohammed *et al.* [39] and Norén *et al.* [40] reported that chemicals in blood are bound to lipoproteins and albumin rather than being dissolved in lipid and the distribution in plasma vary according to the chemicals. It is possible that a free form of chemicals is distributed by simple equilibrium, but distribution or transport of bound form of chemicals to protein in blood is more complicated. So the chemical concentration in US might be lower than in MS.

In our study we found that endrin, dieldrin followed by DDTs, were the main contaminants found in all types of samples and this could be explained by the constant finding reported by many authors, that 96.3% of human exposure to organochlorine pesticides is due to ingested food [41], specifically to Egypt, where Abou-Arab *et al.* [22] detected (DDE; endrin; lindane; heptachlor; malathion) at higher frequency among imported fish samples(sardines and mackerel). Also, Abou-Arab *et al.* [23] determined pesticides residues in 20 different vegetables and fruits, where lindane; dieldrin; DDT and its derivatives and malathion predominated in the samples analysed, specifically, potato tubers, spirmint, onion, cantaloupe, strawberry and peach were considered heavily contaminated by certain pesticides (lindane, aldrin+dieldrin). The above mentioned plants contain residues above the MRL established by FAO/WHO [12].

In addition, Mansour, [18] said that, even Wadi-el-Rayan which is a natural protectorate situated in the western desert of Egypt, contains two virgin lakes, their major components (water, sediment, fish) seemed to be slightly contaminated by few pesticides. Since about 30 years, the use of DDT and many other organochlorine pesticides in Egyptian agriculture has been banned, However, these long persistent compounds are still detectable in many different types of environmental samples (water, fish, sediment, vegetables, fruits, milk, foodstuffs) [18]. DDT has also been detected in virtually all organochlorine monitoring programs and is generally believed to be ubiquitous throughout the global environment. DDT and its metabolites have been detected in food from all over the world and this route is likely the greatest source of exposure for the general population and DDE was the second most frequently found residue [3].

There were significant positive correlations between the levels of p.p`-DDE (r= 0.97), o.p`- DDD (r= 0.9) and dieldrin (r= 0.84) in the matched adipose tissue and their corresponding levels in breast milk and non significant for the endrin according to the same relation. Our results are similar to that reported by Dorea,*et al.* [42] regarding the DDE and Waliszewski *et al.* [33] showed the same relation but principally for DDTs. Meanwhile a low correlation was found between levels of the cyclodiene pesticides (heptachlor, chlordane and dieldrin) in breast milk and adipose tissue, but levels of DDT and HCB were closely correlated as reported by Stevens *et al.* [43].

In our study we did not confirm the finding of Jimenez Torres *et al.* [14], who observed an association in p.p'-DDE and p. p'-DDD content between adipose tissue and umbilical serum and in p.p-DDT content between adipose tissue and maternal serum [14]. Also we didn't find the highly significant correlation between the adipose tissue and the maternal serum for the DDTs as that reported in the Spanish study [44].

The results of correlation analysis in our study, between maternal serum and breast milk were highly significant for endrin ($r>0.99$), significant for dieldrin ($r> 0.65$), but this correlation analysis for DDTs and heptachlorepoxide was not confirmed in our results, however Jaraczewska *et al.* [6] found a weaker correlation for p,p'-DDT and p,p'-DDE, between maternal serum and breast milk

Several studies have reported that the organochlorine persistent residues showed association between maternal serum and umbilical serum [6, 45, 46] in addition to Jaraczewska *et al.* [6], who found a good correlation (Spearman $r>0.75$, $p<0.001$) for p,p'-DDT and p,p'-DDE, between maternal serum and umbilical cord serum. In our study, when we examined the relation between MS and US for some organochlorine pesticides, we didn't confirm the relation previously observed. Similarly Fukuta *et al.* [47] found no correlation between MS and US of pregnant women living in the cities of Chiba and Yamanashi, near Tokyo, Japan, for some organochlorine pesticides ($r<0.46$). Meanwhile between MS versus CS, HCB, HCHs, heptachlor epoxide, showed a relatively high correlation coefficient ($r > 0.7$) and this tendency was not also confirmed in our study for heptachlor epoxide ($r = 0.15$).

In summary, exposure varies significantly, based on current local use patterns of pesticide and whether these chemicals have been banned or otherwise regulated and on dietary habits, as diet is a major factor that influences breast milk levels of persistent organic pollutants [5]. International efforts to eliminate persistent organic pollutants may help to address some of the areas where levels remain high. Breast milk contamination is an important indicator of potential future public health and environmental problems. The widespread distribution of DDT and its metabolite, endrin, dieldrin, heptachlorepoxide,their marked persistence in our environment, its potential for human toxicity and the positive correlation between concentrations of the above mentioned residues in breast milk and adipose tissue and in breast milk and the maternal serum, may indicate a need for continued monitoring of them, for which breast milk is a suitable medium. So we can conclude that accumulation of these pesticide residues may be less during fetal development than during breast feeding. Despite the possibility of harm from environmental contaminants in breast milk, as breastfeeding for long periods should still be recommended as the best infant feeding method, children who were breastfed for more than 20 weeks had better cognitive performance regardless of their in utero exposure to DDT [48].

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