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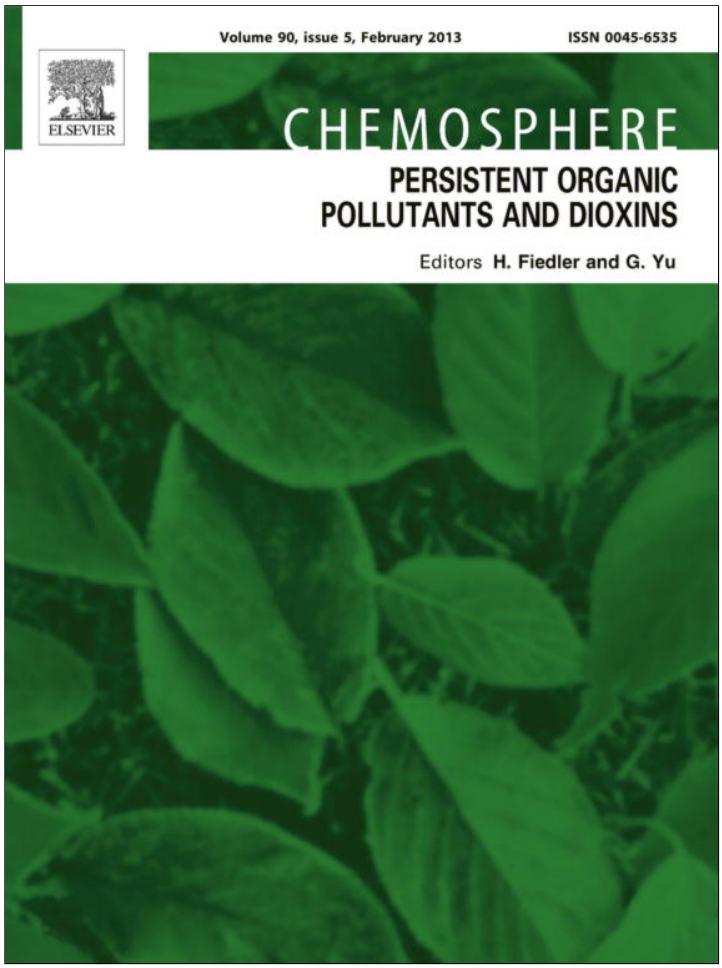

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Chemosphere 90 (2013) 1652–1657

Contents lists available at SciVerse ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Analysis of organochlorine pesticide residues in human and cow's milk in the towns of Asendabo, Serbo and Jimma in South-Western Ethiopia

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HIGHLIGHTS

- High levels of DDT were determined in human and cow milk.
- The ratio of DDT to DDE has revealed the continued use of DDT in the area.
- Transfer of the DDT from mother to child was estimated.
- Infant EDI was higher than the maximum tolerable limit.

ARTICLE INFO

Article history:
Received 19 April 2012
Received in revised form 30 July 2012
Accepted 6 September 2012
Available online 11 October 2012

Keywords:
Ethiopia
Organochlorine pesticides
DDT
Human milk
Cow milk

ABSTRACT

The level of some OCPs in human and cow milk collected from Asendabo, Serbo and Jimma in South-West Ethiopia were analyzed using GC–ECD. Results of the analysis indicated that all samples contained detectable quantities of *p,p′*-DDT and its metabolites, *p,p*-DDE and *p,p*-DDD, but none of the other OCPs analyzed. Mean levels of total DDT in the human and cow milk samples in the three areas were 12.68 and 0.389 μg g^{-1} respectively. The distributions of *p,p*-DDT, *p,p*-DDE and *p,p*-DDD in the human milk samples from the three locations followed the same trend in which the proportion of *p,p*-DDT was the highest in all the three cases, comprising 55–71% of total DDT, followed by *p,p*-DDE, 26–39%, and the least, *p,p*-DDD of 2–5%. The mean ratio of DDT/DDE concentration for the three areas was calculated to be 2.01. This value was much higher than the values reported from other countries in earlier studies and indicates the existence of a higher quantity of DDT from a fresh input in the three study areas. The mean estimated daily intake of DDT by infants from mother's milk in the three locations was found to be 62.17 μg kg^{-1} body weight, which is about three times higher than the acceptable daily intake set by WHO/FAO for total DDT, 20 μg kg^{-1} of body weight. This alarmingly high daily intake value is a cause for concern, since children are highly susceptible to effects from such environmental contaminants. The study has revealed that people in the study areas are facing exposure to DDT from recent use. The observed contamination of mother's milk and the possible transfer of the contaminant from mother to child is an obvious risk associated with breast-feeding in the study areas and possibly in other parts of the country too.

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1. Introduction

Pesticides, including the organochlorines (OCPs) are chlorine-containing compounds which are found in the environment as a result of human activities. The compounds were heavily used in agriculture and to control termites and mosquitoes from the mid-1940s to mid-1980s (Brasher and Anthony, 1998). Due to their persistence, tendency to accumulate in soil, sediment, biota, and their harmful effects on wildlife, developed countries have restricted or banned many of these pesticides. The Stockholm Convention on Persistent Organic Pollutants (POPs) has also globally banned the production and use of persistent, bioaccumulative chemicals and, a number of chemicals including DDT are already listed under the Convention (IISD, 2008). Developing countries, however, maintain that they cannot afford, for reasons of cost and/or efficacy, to ban certain of these chemicals. As a result, most of these chemicals have been or continue to be used in large quantities in many developing countries, including sub-Saharan Africa (Ullah et al., 2010).

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http://dx.doi.org/10.1016/j.chemosphere.2012.09.008

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OCPs tend to bioaccumulate in foodstuffs of animal origin, mostly in meat and tissues that contain fat, in milk and dairy products, eggs and fish due to their lipophilic nature (Waliszewski et al., 1997). The intake of contaminated feed and fodder by milch animals is the main source of entry of pesticides into the animal body which ultimately results in the contamination of milk, meat and other food consumed by human beings. Thus the human body also gets contaminated (Nag and Raikwar, 2008). OCPs have a proven detrimental impact on the human body, and children have been found to be especially susceptible (Dekoning and Karmaus, 2000). Special attention should therefore be paid to the presence of OCPs in food designed for infants. A serious phenomenon in this regard is their presence in human milk, which is the only proper food for infants. Cow milk is also a basic component of the human diet including children's diets, which contain a high proportion of milk and milk products, and diets of the elderly, for whom cow milk is considered a perfect natural food. The milk can make an important contribution to the intake of OCPs by all age groups of humans. It is an important medium of OCP accumulation and hence one of the convenient food stuffs for measuring the persistent OCPs recommended by the United Nations Environment Programme (Leslie and deBoer, 2011).

In Ethiopia, from 1950s to about 2000, DDT has been sprayed outdoors (for agricultural use) as well as indoors for malaria control by reducing the density and longevity of vector mosquitoes using IRS (ISD, 2009; Wassie et al., 2012). DDT spraying is commonly conducted during the rainy months, from June to October. Currently, however, the use of DDT is limited to indoor spraying for disease vector control. In addition to this, Ethiopia has one of the largest stockpiles of obsolete pesticides in Africa. FAO estimates that almost 3000 tons of hazardous pesticide waste has been stored at nearly 1000 sites around the country over the past 30 years threatening the health of thousands of people and polluting the environment. The most dangerous pesticides include aldrin, heptachlor, chloradane, DDT, dieldrin, endrin, malathion, pirimiphosmethyl and fenitrothion; these have been banned in most countries and are found in these dumpsites (Hussein, 2007). Most of these obsolete stockpiles in Ethiopia are removed through the support of African Stockpile Project. The level of human exposure due to the long time use of these pesticides in the country and the impact of long time presence of such huge quantities of obsolete pesticides has not been assessed at all. Therefore, the main objective of this research was to determine the levels of 18 OCP residues in human and cow milk samples collected from Jimma zone, Western Oromia, Ethiopia and assess the level of human exposure.

2. Materials and methods

2.1. Study site, and collection of human and cow milk samples

Breast milk samples were collected from mothers accessed through Jimma University Specialized Hospital in Jimma and public clinics at Asendabo and Serbo. The three towns are found in Jimma zone, Western Oromia Regional State, Ethiopia. Jimma is located 350 km south-west of the capital Addis Ababa, and the other two towns, Serbo and Asendabo, are 20 and 55 km east of Jimma respectively. The three selected areas are malarious and annual spraying of DDT for malaria control is common.

Human milk samples were collected, between March and May of 2010, from a total of 101 mothers (33 from Asendabo, 29 from Serbo and 39 from Jimma) who were either in maternity wards or attending post-natal clinics in the selected areas. Nipples of each donor mother were thoroughly cleaned with tap water and about 10 mL of milk was collected from each mother, by expressing directly into precleaned and labeled glass vials with Teflon-lined caps under supervision of a qualified nurse. A total of 30 cow milk samples (10 from each location) were also collected from randomly selected farmers' cows in the villages around Asendabo, Serbo and Jimma, following the same procedure. All milk samples were immediately transported to the laboratory in an ice-box and frozen at −20 °C in the laboratory until analysis.

2.2. Ethical clearance

Ethical approval to carry out the study was obtained from Jimma University Ethical Review Committee. Before collection of milk samples, the purpose of the study was clearly explained to each of the mothers who fulfilled the selection criteria and were requested to complete an informed consent form. A basic questionnaire was completed to collect information on mothers' age, diet, number of children, longevity in the area and occupational exposure to DDT or other pesticides.

2.3. Exclusion criteria

Those mothers who gave complicated birth or premature delivery, who were smokers or were suffering from serious disease during pregnancy, and those who were not residents of the study area were not included in the study.

2.4. Analysis of organochlorines

All sample preparation and analysis of both the human and cow milk samples was undertaken at the Quality Monitoring and Testing Laboratory of the Ministry of Agriculture. The human or cow milk samples from each of the three sampling locations were pooled and analyzed for 18 OCPs, including: HCH isomers (α-BHC, γ-BHC, β-BHC, δ-BHC), aldrine, heptachlor epoxide, heptachlor, γ-chlordane, α-chlordane, DDT and its metabolites (*p*,*p*-DDT, *p*,*p*-DDE and *p*,*p*-DDD), endosulfan-I, endosulfan-II, dieldrine, endrine, endosulfan sulfate, endrine ketone and methoxychlore.

Extraction and clean-up of OCP residues were performed according to the AOAC official Method 970.52 for fatty substances (AOAC, 2007). The separation, identification and quantification of OCPs were performed using Agilent Model 7890 gas chromatograph equipped with dual ^{63}Ni μ-ECD. A primary analytical column, CLP-Pestcides-2 (30 m length × 0.25 mm id, 0.25 μm film thickness) capillary column (Restek Corp, Bellefonte, PA) was used for the separation of analytes and a second CL-Pesticide column was used as a confirmatory column. The column oven temperature was set at 120 °C for 0.5 min and ramped to 180 °C at a rate of 20 °C min^{-1} and then to 280 °C at a rate of 10 °C min^{-1} with hold time of 10 min and a total acquisition time of 25.5 min. The temperatures of the detector and injector were 300 and 230 °C, respectively.

Identification of the OCP residues was carried out on the basis of retention time and confirmation using analytical column of different phase polarity. Quantification of OCPs was carried out using 1-Bromo-2-Nitrobenzene as the internal standard. The linearity of the chromatographic analysis was checked by and the use of five point external standards (all obtained from Restek Corp, USA) corresponding to each of the target OCPs.

2.5. Quality assurance

Quality control samples consisting of method blank and three spiked blanks were included in every batch of samples analyzed to check for interferences and cross-contamination. No contaminant was detected in the method blank analysis, indicating no contamination from laboratory sources. Results of the analyses of the

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spiked blanks have consistently shown good agreement between the triplicate data sets. Percent recoveries were determined from duplicate spiked cow-milk samples. Analysis of sample extracts was performed in triplicates and a solvent blank was run before a batch of analyzed samples every day. Calibration standards were re-established every day by using continuous calibration verification standards and verified before and after analysis of each batch of samples. All measurements were performed within the range of linearity found for each compound. Repeatability and within-lab reproducibility of the method were evaluated from seven and three spiked cow-milk samples for within a day and inter-day precision studies respectively. The repeatability and reproducibility of the method expressed as RSDs were <10% and 20% respectively in all cases. The limits of detection (LOD) and quantification (LOQ) were determined by analyzing seven spiked blank samples as described in the US EPA Method 507 (FEPA, 1995) and were found to be in the ranges of 0.19–0.61 ng g^{-1} and 0.605–1.942 ng g^{-1} respectively. All reported results were above the limit of quantification. Linearity of the detector response was determined from the regression analysis of a five point calibration curve for each OCP. The linearity of calibration curves was evaluated by calculating their squared correlation coefficient (r^2) values.

2.6. Statistical analyses

Statistical analysis was performed using Microsoft Exel 2007 software. One factor Analysis of Variance (ANOVA) and *t*-test were used to evaluate the significance of concentration differences between OCP residues. In all cases, differences were considered to be significant at probabilistic values of $p < 0.05$.

3. Results and discussion

3.1. Participants profile

More than 70% of the participant mothers were residents of rural areas around the three towns: Asendabo, Serbo and Jimma, and the remaining 30% reside in the towns. No socioeconomic circumstances of the mothers who donated breast milk sample such as employment in firms related to pesticide production, distribution or application, or residential area are expected to be a cause for additional exposure to the investigated OCPs. The only causes of exposure are expected to be activities related to malaria control or background levels. There were also no obvious factors that would have resulted in a major variation in exposure to DDT for the mothers in the three locations.

3.2. Analysis of OCPs in human and cow milk samples

The % recoveries of the OCPs were found to be acceptable ranging from 75% for γ-BHC to 119% for DDT. The limits of detection and quantification respectively expressed on fat basis for the OCPs studied were: α-BHC (0.461, 1.467 ng g^{-1} fat), γ-BHC (0.61, 1.942 ng g^{-1} fat), β-BHC (0.19, 0.605 ng g^{-1} fat), Aldrin (0.497, 1.584 ng g^{-1} fat), Heptachlor epoxide (0.406, 1.293 ng g^{-1} fat), γ-Chlordane (0.514, 1.638 ng g^{-1} fat), α-Chlordane (0.491, 1.563 ng g^{-1} fat), *p*,*p*-DDT (0.599, 1.907 ng g^{-1} fat), *p*,*p*-DDE (0.562, 1.789 ng g^{-1} fat), *p*,*p*-DDD (0.445, 1.416 ng g^{-1} fat) and. Endosulfan I (0.437, 1.393 ng g^{-1} fat).

3.2.1. Concentrations of OCPs in human milk

The lipid contents of the human milk samples collected from the three areas in this study ranged 3.12–3.24%. Of the total OCPs analyzed, *p*,*p*-DDT and its metabolites (*p*,*p*-DDE and *p*,*p*-DDD) were the only species detected in the human milk samples. Relative to the values reported in the literature, the total DDT (*p*,*p*-DDT, *p*,*p*-DDE and *p*,*p*-DDD) determined in the human milk samples from the three areas in our study were noted to be alarmingly high ranging 6.42–17.17 mg kg^{-1} fat. Table 1 shows the concentrations of the total DDT determined in the pooled breast milk samples of each of the three areas. The means and standard deviations of the mean (SD) are presented on fat weight basis.

Table 1
Average concentrations of OCP residues in human milk samples from Asendabo, Serbo and Jimma areas. Values in bracket correspond to percent from total.

DDT species	μg g^{-1} milk fat (mean ± SD), and % from total		
	Asendabo (n = 33)	Jimma (n = 39)	Serbo (n = 29)
p,*p*-DDE	4.58 ± 0.28 (26.67%)	4.76 ± 0.60 (32.92%)	2.52 ± 0.50 (39.25%)
p,*p*-DDD	0.39 ± 0.07 (2.27%)	0.32 ± 0.04 (2.21%)	0.35 ± 0.11 (5.45%)
p,*p*-DDT	12.20 ± 0.80 (71.05%)	9.38 ± 1.24 (64.87%)	3.55 ± 0.40 (55.30%)
Total DDT	17.17	14.46	6.42

The concentrations of the DDT species in the breast milk samples of all the three sampling areas were found to follow the same order: *p*,*p*-DDT > *p*,*p*-DDE > *p*,*p*-DDD. The concentration of *p*,*p*-DDD was found to be significantly smaller than those of *p*,*p*-DDE and *p*,*p*-DDT in all the breast milk samples ($p < 0.01$). The differences between *p*,*p*-DDE and *p*,*p*-DDT were also significant in the Jimma and Asendabo samples ($p < 0.001$) but not in that of Serbo ($p > 0.05$). The mean concentrations of *p*,*p*-DDD in the breast milk samples of the three locations were not significantly different ($p > 0.05$). The mean concentrations of *p*,*p*-DDE and *p*,*p*-DDT in the breast milk of Serbo mothers were, however, significantly lower than in those of Asendabo and Jimma mothers' milk ($p < 0.05$).

A comparison of the mean concentrations of *p*,*p*-DDT and its metabolites (*p*,*p*-DDE and *p*,*p*-DDD) determined in breast milk samples in this study with results of studies conducted in some other countries is given in Table 2. The mean concentration of total DDT (*p*,*p*-DDT, *p*,*p*-DDE and *p*,*p*-DDD) for the three areas in our study, 12683 μg kg^{-1} fat is 10 times higher than the results from Egypt; 4, 11 and 15 times higher than the values from Jozini, Mkuzi and Kwaliweni respectively, in South Africa; 259 and 275 times higher than from Almeria and Grenada respectively, in Spain; 30 times higher than from New Delhi in India; 20, 12, 10 and 13 times higher than from Jakarta, Bogor, Purwakarta and Lampung respectively, in Indonesia; three times higher than Tunesia; four times higher than from Noushahr in Iran; 75 times higher than from Philippines, five times higher than from Turkey, 15 times higher than from the Star Mountains region in Papua New Guinea; 200 times higher than from South Bachka in Yugoslavia; 53 times higher than from Germany; and, 27 times higher than from Nairobi. The mean DDT/DDE ratio of the three locations in our study, 2 (Table 3, column 7) was also found to be much higher than in those of other countries listed in Table 2.

3.2.2. Estimated infant daily intake

To evaluate the toxicological significance to the infants in the study areas, we calculated the estimated daily intake (EDI) of DDT by infants based on the assumption that the average breast milk consumption of a 5 kg infant is 700 mL d^{-1} (or 700 g d^{-1}) (Azeredo et al., 2008; Okonkwo et al., 2008) and using the equation suggested by Minh et al. (2004).

$$\mathrm{EDI} = \frac{C_{\mathrm{milk}} \times 700\ \mathrm{g} \times C_{\mathrm{lipid}}}{5}$$

where EDI is estimated daily intake (μg kg^{-1} body wt d^{-1}); C_{milk}: concentration of the chemical in breast milk (μg g^{-1} lipid mass); C_{lipid}: lipid content in breast milk (%).

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Table 2
A comparison between levels of pesticides (μg kg^{-1} milk fat) in human milk samples from different countries and the three areas (Asendabo, Serbo and Jimma) considered in the present study.

Country	Place and/or year of study	*p,p*-DDT	*p,p*-DDE	*p,p*-DDD	ΣDDT	DDT:DDE	References
Ethiopia	Asendabo, 2010	12200	4580	390	17170[a]	2.66	This study
	Jimma, 2010	9380	4760	320	14460[a]	1.97	
	Serbo, 2010	3550	2520	350	6420[a]	1.41	
Egypt	ng	ng	ng	ng	1315[b]	-	Abd Al-Rahman (2010)
	Jozini	1396	1866	97	3358[a]	0.75	Bouwman et al. (2006)
	Mkuzi	307	833	16	1156[a]	0.37	
	Kwaliweni	247	568	61	875[a]	0.44	
Ghana	Accra	3.1	23	ng	26[c]	0.13	Tutu et al. (2011)
Kenya	Nairobi, 1991	152	306	29	473[d]	0.48	Kinyamu et al. (1998)
Tunisia	Bizerte and Beja states, 2002–2003	1015	2421	279	3863[e]	0.42	Ennaceur et al. (2007)
	Almeria	4.7	37	6.4	49[d]	0.07	Campoy et al. (2001)
	Grenada	1.1	30	14	46[d]	0.24	
India	New Delhi, 2005–2006	ng	ng	ng	430[d]	–	Devanathan et al. (2009)
	Jakarta, 2003	33	600	1.4	640[d]	0.06	Sudaryanto et al. (2006)
	Bogor, 2003	32	1100	1.2	1100[d]	0.03	
	Purwakarta, 2003	180	1100	2.8	1300[d]	0.16	
	Lampung, 2003	75	920	2.5	1000[d]	0.08	
Iran	Noushahr, 2006	ng	ng	ng	3563[d]	–	Behrooz and Sari (2009)
Philippines	2004	ng	ng	ng	170[a]	–	Malarvannan and Kunisue (2009)
Turkey	1995–1996	ng	ng	ng	2400[b]	–	Cok et al. (1997)
Papua N.G	Star mountain, 1990	430	450	40	870[a]	0.96	Spicer and Kereu (1993)
Canada	National Capital Region	4.9	29	ng	34[c]	0.17	Mes et al. (1984)
Mexico	1997–1998	ng	ng	ng	4700[d]	–	Waliszewski et al. (2001)
Yugoslavia	Yugoslavia, 1984/1985	9.9	53	ng	63[b]	0.19	Galetin-Smith et al. (1990)
Germany	1995–1997	ng	ng	ng	240[b]	–	Schade and Heinzow (1998)

ng = Not given.
[a] *p,p*-DDT + *p,p*-DDE + *p,p*-DDD.
[b] *p,p*-DDT + *p,p*-DDE.
[c] *p,p*-DDT + *p,p*-DDE (calculated by the authors of the present study).
[d] *p,p*-DDT + *o,p*-DDT *p,p*-DDE + *p,p*-DDD.
[e] *p,p*-DDT + *o,p*-DDT + *p,p*-DDE + *p,p*-DDE + *p,p*-DDD.

Table 3
Average concentrations of OCPs in cow milk samples from Asendabo, Serbo and Jimma areas.

DDT species	μg g^{-1} milk fat (mean ± SD) and% from total		
	Asendabo (n = 10)	Jimma (n = 10)	Serbo (n = 10)
p,p-DDE	0.118 ± 0.001 (43.87%)	0.164 ± 0.001 (34.38%)	0.186 ± 0.003 (44.18%)
p,p-DDD	0.015 ± 0.001 (5.58%)	0.174 ± 0.003 (36.48%)	0.016 ± 0.003 (3.80%)
p,p-DDT	0.136 ± 0.001 (50.56%)	0.139 ± 0.003 (29.14%)	0.219 ± 0.004 (52.02%)
Total DDT	0.269	0.477	0.421

The calculated mean intake of total DDT for infants in the three locations was found to be 62 μg kg^{-1}. The mean EDI calculated for the three locations in our study is more than three times greater than the Acceptable Daily Intake (ADI) set by WHO/FAO for total DDT, 20 μg kg^{-1} body weight (FAO/WHO, 2008). This is an alarmingly high daily intake value that calls for concern since children are highly susceptible to effects from such environmental contaminants. In the study areas, as in most other areas in Ethiopia, breast-feeding of infants is a common phenomenon and mothers breastfeed their children for at least one and a half years postpartum. The exceptionally high level of DDT residues added to the longer duration of breast feeding may undoubtedly cause breast fed children in the study areas to receive DDT in their mothers' milk far more than the limit considered 'safe' by the WHO and the FAO.

3.2.3. Concentration of OCPs in cow milk samples

As in the human milk samples, none of the OCPs analyzed except *p,p*-DDT, *p,p*-DDE and *p,p*-DDD, were detected in the cow milk samples. The mean lipid contents of the cow milk samples in the three locations in our study ranged 3.8–4.3%. The concentrations of the detected DDT species in cow milk samples of the three study areas are given in Table 3. Total DDT concentrations ranging between 0.015 μg g^{-1} and 0.219 μg g^{-1} milk fat were recorded. The principal contributor to total DDT in the Asendabo and Serbo cow milk samples was *p,p*-DDT, (51% and 52% respectively) followed by *p,p*-DDE (44% in both) and the least contributor was *p,p*-DDD (6% and 4% respectively). The level of *p,p*-DDD in each of the cow milk samples from Asendabo or Serbo were noted to be significantly lower than the levels of both *p,p*-DDE and *p,p*-DDT ($p < 0.001$). However, the levels of *p,p*-DDE and *p,p*-DDT were not significantly different in the samples from both areas ($p > 0.05$). In the milk samples from Jimma *p,p*-DDD was the principal contributor to total DDT (36%) followed by *p,p*-DDE (34%) and *p,p*-DDT (29%). The differences however, were not statistically significant ($p > 0.05$). A higher level of *p,p*-DDD than *p,p*-DDE is not common in breast or cow milk samples. However, the results of one study which investigated the level of OCP residues in the breast milk and blood samples of four groups of women in Anupgarh, India, shows a higher level of *p,p*-DDD than *p,p*-DDE in the breast milk samples of one of the four groups which were categorized as 'the general group' (Kumar et al., 2006).

Comparison of the levels of each of the DDT species determined in the cow milk samples from the three areas indicated that the level of *p,p*-DDE in the Serbo and Jimma samples was significantly greater than that of Asendabo ($p < 0.05$). However, those of Serbo and Jimma were not significantly different in their *p,p*-DDE levels ($p > 0.05$). The *p,p*-DDD level in the cow milk samples from Jimma was significantly greater than those of Asendabo and Serbo ($p < 0.01$) while those of Asendabo and Serbo were not significantly different in their *p,p*-DDD levels ($p > 0.05$).

The results obtained in other monitoring studies on OCP residues in cow milk conducted in other countries were compared in Table 4 with that of the present study. Similar to the case of the

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Table 4
Comparison of OC levels (mg kg^{-1} lipid) in cow milk from different countries and EDI (μg kg^{-1} body wt).

Country	Place and/or year of study	*p,p′*-DDT	*p,p′*-DDE	*p,p′*-DDD	Tot DDT	EDI	References
Ethiopia	Asendabo	0.136	0.118	0.015	0.269[a]		This study
	Jimma	0.139	0.164	0.174	0.477[a]		
	Serbo	0.219	0.186	0.016	0.421[a]	0.079	
Poland	ng	ng	ng	ng	0.045[a]	0.009	Radzymińska et al. (2008)
Mexico	ng	ng	ng	ng	0.159[b]	0.033	Waliszewski et al. (2003)
China	Beijing	ng	ng	ng	0.046[b]	0.009	Zhong et al. (2003)
India	ng	0.055	0.036	0.022	0.1724[c]	0.035	Nag and Raikwar (2008)
Spain	Leon, 1987–1988	ng	ng	ng	0.021[a]	0.004	de la Riva and Anadon (1991)
Egypt	2008–2009	0.032	0.028	0.07	0.223[b]	0.046	Abou Donia et al. (2010)
Egypt	Giza, 2009	0.002	0.024	0.007	0.033[a]	0.007	Ahmed and Zaki (2009)
Iran	Ahwaz, 2009	0.015	0.017	0.028	0.125[b]	0.026	Ashnagar et al. (2009)
Romania	Cluj county	ng	ng	ng	0.013[b]	0.003	Georgescu et al. (2011)

ng = Not given.
[a] *p,p*-DDT + *p,p*-DDE + *p,p*-DDD (calculated by the authors of the present study).
[b] *o,p*-DDT + *p,p*-DDT + *p,p*-DDE + *p,p*-DDD.
[c] *o,p*-DDD + *p,p*-DDD + *o,p*-DDE + *p,p*-DDE + *o,p*-DDT + *p,p*-DDT.

human milk samples the level of total DDT determined in our study was found to be higher than all the reported values from the countries given in Table 4. The mean concentration of total DDT in cow milk for the three areas in our study (0.39 mg kg^{-1} fat) was found to be greater (2–30 times) than all the values reported from other countries in Table. The mean value of total DDT obtained in our study is 2, 12, 2, 2, 3, 9, 8, 18 and 30 times higher than the values reported from Egypt (2010 and 2009 respectively), India, Mexico, Iran, Poland, China, Spain and Romania.

We calculated the estimated daily intake (EDI) of total DDT for all the reported values given in Table 4 assuming that a person of 60 kg body weight consumes two glasses (350 mL) of cow milk per day, and an average lipid content of 3.5% (Ahmed and Zaki, 2009). The results (Table 4, column 7) indicate that the EDI of DDT in none of the studies including ours, exceeded the limit recommended by the FAO/WHO, 20 μg kg^{-1} body weight (FAO/WHO, 2008). However, the average EDI calculated for the three locations in our study was found to be 2–31 times higher than those of the other countries. None of the cow or breast milk samples in our study were found to contain detectable quantities of OCPs other than DDT and its metabolites.

4. Discussion

The present study is the first report on OCPs contamination in human and cow's milk from Ethiopia. Among the various OCPs we analyzed, only DDT and its metabolites were detected in both the human and cow milk samples collected from the three areas. However, the concentrations of the DDT residues determined were found to be significantly higher than the values reported from some African, Asian, European and American countries and raise cause for concern. Reports from the various countries whose results are compared with our results revealed that the level of DDT in the environment is gradually decreasing over the years. In our case, however, we cannot tell whether the level is decreasing or increasing since there are no previous reports with which we can compare our findings.

In biological systems DDT is rapidly transformed to its major metabolite, *p,p*-DDE, which tends to persist much longer in the body and is of more concern with regard to bioaccumulation (Jaga and Dharmani, 2003). In countries where DDT is still being used the ratio of *p,p*-DDT to *p,p*-DDE is higher than in countries where the use of DDT was banned long ago. The higher *p,p*-DDT to *p,p*-DDE ratio in human milk in our study has revealed the continued use of DDT in the areas of sampling. In Ethiopia, DDT is currently limited to use for indoor spraying for disease vector control (Hussein, 2007). In this case, DDT is sprayed on the walls and other surfaces inside and around dwellings where female *Anopheles* mosquitoes land and rest before or after a blood meal. During such application, materials that are used to serve food for humans and animals might be contaminated with the chemical. According to the Federal Environmental Protection Authority (FEPA) of Ethiopia: lack of awareness, use of pesticides for unintended purposes and use of pesticide containers for domestic uses are among the major causes of pesticides' impact on the environment in Ethiopia (FEPA, 2004a,b). These practices are common virtually among the entire rural population and many urban residents who have no idea about the toxic effects and routes of exposure of the pesticides. These facts indicate that the relatively high levels of DDT residues determined in human and cow milk in the study areas might have originated from the DDT that entered the environment from its continued use for malaria control and compounded due to the misuse and carefree handling of the chemical resulting from lack of awareness by the society.

Analysis cost has limited us from carrying out analysis of the OCPs on individual milk samples. The use of composite samples from each sampling area might be taken as a limitation that has obscured the range of exposure, but it has no effect on our analytical results. Individual milk sample analysis would have revealed the highest level of exposure which would have been additional useful information to indicate the severity of the exposure.

5. Conclusion

Findings of this study highlight the need for further monitoring studies of the levels of OCPs in biological and food samples, as well as determining the principal routes of exposure to the general population. The observed contamination of breast milk and the possible transfer of the contaminant from mother to child is an obvious risk associated with breast-feeding in the areas considered in this study, and possibly in other parts of the country too. However, breast-feeding should not be discouraged due to the fact that breast milk consists lots of resistant factors which provide immunological protection to the infant and the emotional bonding it offers between mother and child. Instead, better practice of the use of DDT should be introduced within the study areas and other areas in the country. It is also advisable to carry out similar studies in other parts of the country to evaluate the breadth of the exposure. In general, our findings call for urgent action to reduce the level of OCPs' exposure and their effects on wildlife and human health. In addition, the following points are recommended:

1. Provision of information and education to the public in order to minimize the exposure to possibly harmful substances, such as DDT.

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2. Food items, especially those containing high level of lipid such as milk, meat, eggs and fish, should be routinely monitored for their concentrations of OCPs.
3. Current risk assessment methods that include consideration of the potential risks posed to infants and children by exposure to chemical residues in breast milk need to be established.

Acknowledgements

The authors are grateful to the Ethiopian Federal Ministry of Agriculture for allowing the analysis to be carried out in its Central Laboratory for Quality Control and for providing us chemicals when we were under short supply. We are also grateful to Mr. Alexander Hayward for proofreading the manuscript.

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