Chemosphere 106 (2014) 70–74

Contents lists available at ScienceDirect

Chemosphere

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

Persistent organochlorine pesticides residues in cow and goat milks collected from different regions of Ethiopia

Habtewold Deti [a], Ariaya Hymete [b], Adnan A. Bekhit [b,c,*], Abdel Maaboud I. Mohamed [b,d], Alaa El-Din A. Bekhit [e]

[a] Department of Pharmaceutical Chemistry, School of Pharmacy, Jimma University, Ethiopia
[b] Department of Pharmaceutical Chemistry, School of Pharmacy, Addis Ababa University, Ethiopia
[c] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Alexandria, Alexandria 21521, Egypt
[d] Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, University of Assuit, Egypt
[e] Food Science Department, University of Otago, Dunedin, New Zealand

HIGHLIGHTS

- Extremely high levels of organochlorines were found in cow and goat milks.
- Regions known for their malaria epidemics were the most contaminated with DDT.
- Environmental contamination prevented distinction of specie effect for contamination.

ARTICLE INFO

Article history:
Received 13 February 2013
Received in revised form 10 February 2014
Accepted 15 February 2014
Available online 12 March 2014

Keywords:
Organochlorines
Milk
Goat
Cow

ABSTRACT

The present study investigated the bioaccumulation of organochlorines in two milk-producing animals (goats and cows) grazed on the same feed to explore the extent of organochlorines availability in milk and any species effect on the bioaccumulation pattern. Six organochlorine pesticides: aldrin, α-endosulfan, β-endosulfan, *p,p′*-DDE, *o,p′*-DDT and *p,p′*-DDT were determined in samples collected from four regions in Ethiopia. Aldrin (11.6 μg kg^{-1}) was detected only in one cow milk sample and α-endosulfan was detected in one goat milk sample at a level of 142.1 μg kg^{-1}, and in one cow milk sample (47.8 μg kg^{-1}) from the same region. *p,p′*-DDE was detected in 40% of the milk samples analyzed while *o,p′*-DDT and *p,p′*-DDT were found in high amounts in almost all samples. The average total DDT (excluding DDD) in the samples was 328.5 μg kg^{-1}. Regions known for their malaria epidemics were the most contaminated with DDT residue. The accumulation pattern in both species was not clear under natural sampling.

1. Introduction

The use of pesticides under intensive agricultural and farming practices is considered to be a viable option to overcome the problems of food shortage, shrinking farm size and food insecurity. Government extension services promote the use of pesticides to improve the productivity of small holder farming (Environmental and Social Assessment International, 2006) despite their known negative impact on the environment (Ciscato et al., 2002). Organochlorine pesticides, polychlorinated biphenyls, dibenzo-*p*-dioxins and dibenzofurans are widely distributed halogenated aromatic compounds which persistently contaminate the environment (Campoy et al., 2001). These compounds are chemically stable with long biological half life which leads to high biomagnification in the food chain across a wide range of trophic levels (Serrano et al., 2008).

Milk-producing animals accumulate pesticides from contaminated feed and by inhaling contaminated air. Organochlorine pesticides due to their lipophilic properties are initially stored in fat-rich tissues and subsequently translocated and excreted in milk. Therefore, the consumption of dairy products together with other contaminated food may expose consumers to unexpected levels of organochlorine pesticides (Waliszewski et al., 2003; Armendariz et al., 2004).

Organochlorine pesticides being endocrine disrupting chemicals (McKinlay et al., 2008) are believed to produce a wide variety of adverse health outcomes including reduced fertility and fecundity, spontaneous abortion, skewed sex ratios within the offspring

* Corresponding author at: Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Alexandria, Alexandria 21521, Egypt. Tel.: +20 3 4871317; fax: +20 3 4873273.
E-mail addresses: adnbekhit@hotmail.com, adnbekhit@alexpharmacy.edu.eg (A.A. Bekhit).

http://dx.doi.org/10.1016/j.chemosphere.2014.02.012
0045-6535/

of exposed communities (Fuortes et al., 1997; Abell et al., 2000; Windham et al., 2005), male and female reproductive tract abnormalities (Bretveld et al., 2008), neurobehavioral disorders, impaired immune function and a wide variety of cancers (Høyer et al., 2000). Infants relying on breast-feeding and/or animal milk are especially vulnerable to exposure to these pollutants because of their higher intestinal permeability and premature detoxification systems. Exposure to these pollutants can induce a wide range of adverse health effects including possible long-term effects on intellectual function in infants and negative effects on central nervous system functioning (Zhao et al., 2007).

To avoid or minimize the adverse health effects of pesticide residues, their level in food should be monitored using appropriate analytical tools. Therefore, the present study aimed to investigate the levels of organochlorines in milk, an essential food item that is regularly fed to infants and children in Ethiopia, and explore whether the accumulation profile is different in cow and goat milks. Aldrin, α-endosulfan, β-endosulfan and DDT were of interest due to their historical wide use and their availability in illegal markets.

2. Materials and methods

2.1. Standards and reagents

Sulfuric acid (96%) was purchased from (Merck, Stockholm, Sweden). Anhydrous sodium sulfate (99% purity) was from Technopharmchem (Bahaduragarh, India). Hexane and reference standards: l,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (*p,p′*-DDT) (99.6% purity); 1,1,1-trichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl) ethane (*o,p′*-DDT) (99.6% purity); l,l-dichloro-2,2-bis(*p*-chlorophenyl) ethylene (*p,p′*-DDE) (99% purity); α-endosulfan (99% purity); β-endosulfan (99% purity); aldrin (98.1% purity); and internal standard triphenyl phosphate (99.5% purity) were purchased from Sigma Aldrich (Germany). All the solvents and reagents used were of analytical grade or higher.

2.2. Apparatus

Centrifuge (Gemmy industrial corp., Taiwan), rotary evaporator (Ika® Labortechnik, Germany), glass chromatograph column (40 × 3 cm) (Sinta Glass, England), gas chromatograph-mass spectrometer (GCMS-QP2010, Shimazdu, Japan), fitted with an Auto sampler (AOC-20i + s) automatic injector and fussed silica capillary column: DB-5MS (95% dimethyl-5% diphenyl polysilphenylene) 30 m × 0.25 mm ID with 0.25 μm film thickness (J and W scientific) were used in the analysis. GC–MS solution release 2.30 version software was used to process the result.

2.3. Sample collections

Cow and goat milk samples were collected from four localities in Ethiopia namely; Lole and Gonde (Arsi) (7057′N and 3907′E), Adami Tulu (East Showa) (709′N and 3807′E), and Asendabo (Jimma) (7040′N and 36050′E). A total of 30 milk samples (20 cow milk and 10 goat milk samples) were collected and frozen at −20 °C until analysis. The places were selected based on information of pesticides use to control pests and malaria.

2.4. Sample preparation and clean-up of the extract

The sample preparation and analysis method employed in the present study were those reported by Armendariz et al. (2004) with slight modifications. Ten milliliters of milk was centrifuged at 3000 rpm for 1 h (Gemmy industrial corp., Taiwan) to separate the fat from the rest of milk components. The fat layer was weighed and then mixed with 30 g anhydrous Na_2SO_4 in a glass beaker to form a solid mass. The obtained solid mass was transferred to a glass chromatograph column (40 × 3 cm) (Sinta Glass, England), and the organochlorine pesticides were eluted twice with 50 mL hexane. The eluted fractions was then concentrated *in vacuo* to 1 mL at a temperature <50 °C. The volume of the concentrate was adjusted to 10 mL using the liquid resulting from rinsing of the evaporator with small aliquots of hexane. Concentrated sulfuric acid (1 mL) was added to the 10 mL extract in a volumetric flask, strongly shaken for 1 min, and left to stand for 2–3 min to allow the separation of the organic phase. A 5 mL of the organic layer was transferred to a small funnel containing a glass bead and 1 g anhydrous Na_2SO_4. The residue on the funnel was then washed with two aliquots of hexane (5 mL) and the filtrate was evaporated to dryness *in vacuo*. The residue was subsequently collected with 1 mL hexane and injected into the gas chromatograph.

2.5. Identification of organochlorines by GCMS

A gas chromatograph-mass spectrometer (GCMS-QP2010, Shimazdu, Japan), fitted with an auto sampler (AOC-20i + s) automatic injector and fussed silica capillary column: DB-5MS (95% dimethyl-5% diphenyl polysilphenylene) 30 m × 0.25 mm ID with 0.25 μm film thickness (J and W scientific, Agilent Technologies) was used for the analysis. The chromatographic conditions were: the injector was in a splitless mode; the injection port was at 260 °C; sample volume was 1 μL; the carrier gas was helium at a pressure of 79.5 kPa and a flow-rate of 1 mL min^{-1}; the furnace temperature was initially at 50 °C for 3 min and then the temperature was increased at a rate of 30 °C min^{-1} to 180 °C, followed by 1 min stabilization, then the temperature was increased at a rate of 6 °C min^{-1} ramp to 220 °C, followed by 11 min stabilization and then the temperature increased at a rate of 5 °C min^{-1} to 250 °C and followed by10 min stabilization. The mass spectrometer conditions were: ion source temperature of 230 °C; interface temperature 230 °C; detector gain was in absolute mode; detector voltage at 1.0 kV; acquisition was in SIM mode; scan interval 0.2 s; solvent cut, 13 min. GC–MS solution software (release version 2.30) was used to identify and process the data. The organochlorines concentrations are reported as μg kg^{-1} fat basis. Spiking recoveries were determined by adding the pesticides to milk samples at the fortification level indicated and recovery assays were done in triplicate (Table 1). Limits of detection for pesticides in milk samples were established by calculating the concentration of each compound that corresponds to three times the background noise level (Åkerblom, 1995). The detection limits were low and ranged between 0.01 and 0 μg kg^{-1} milk fat basis. The limits of quantification (LOQ), were 0.01 μg kg^{-1} for *p,p′*-DDT, aldrin, α-Endosulfan, β-Endosulfan and 0.03 μg kg^{-1} for *p,p′*-DDE and 0.04 μg kg^{-1} for *o,p′*-DDT.

Table 1
Mean percent recovery ± RSD of six pesticides in milk at fortification level indicated ($n = 3$).

Pesticide	Fortification level (ng mL^{-1})	Mean recovery (ng mL^{-1})	% Recovery ± RSD
Aldrin	300	272.8	90.9 ± 3.5
α-Endosulfan	400	367.2	91.8 ± 3.6
β-Endosulfan	200	193.2	96.6 ± 5.5
p,p′-DDE	300	285.3	95.1 ± 3.0
o,p′-DDT	133.3	129.2	96.9 ± 3.8
p,p′-DDT	400	378.3	94.6 ± 1.8

3. Results and discussion

Aldrin (11.6 μg kg^{-1} fat basis) was detected in one of the cow milk samples from Adami Tulu (Table 2). This level of aldrin is higher than the maximum residue level (MRL, 6 μg kg^{-1}) recommended by European Union and FAO/WHO for aldrin and dieldrin (FAO/WHO, 2008; European Pesticides Database, 2010). Although aldrin is currently banned in Ethiopia, the pesticide was used extensively in the past and the amount detected in one of the milk samples indicates its persistence in the environment. Aldrin was detected in raw cow milk from Egyptian markets at concentration of 3.93–50 μg kg^{-1} milk fat (Ahmed and Zaki, 2009; Abou Donia et al., 2010); Ugandan markets at concentration of 9 μg kg^{-1} milk fat (Kampire et al., 2011). The occurrence and concentration of aldrin in milk was reported to be influenced by the animal species. For example, aldrin was about 30% higher in buffalo milk compared to cow milk (Abou Donia et al., 2010) and higher incidence was found in buffalo milk compared with cow milk (33.3% and 26.7%, respectively). Low positive detection % of aldrin was reported in goat milk (25%) compared to buffalo, cow, sheep and donkey (62.5–73.3%) (Ibrahim et al., 1994). While these results suggest a potential species-dependent accumulation pattern, this suggestion could not be confirmed in the present study since only one cow milk sample was positive for aldrin.

α-Endosulfan was detected in three samples from two areas (Table 2) at 47.8 μg kg^{-1} and 77.6 μg kg^{-1} in cow milk samples from Adami Tulu and Gonde respectively, and 142.1 μg kg^{-1} in one goat milk sample from Adami Tulu. This was not expected as the daily feed intake for cows is much higher than goats, and other routes (e.g. inhalation or milk production:feed ratio relationship) may be implemented in the observed higher α-endosulfan in goat milk. β-Endosulfan was detected in only one goat milk sample from Assendabo (87.0 μg kg^{-1}). α and β-Endosulfan are known to have short half life in the environment (30–70 d) (Tadeo, 2008) and relatively short life in animals bodies with the excretion of endosulfan residue in goat milk reach normal background in ≈45 d (Nag et al., 2007). These fast degradation rates may be contributed to the lower detection levels observed in the present study. The residue level of endosulfan in the present study was much higher than the MRL recommended by the EU and FAO/WHO (50 μg kg^{-1}). In particular, the goat milk sample from Adami Tulu was about three times the MRL which can pose risk to milk consumers in that region and more monitoring to milk produced in this area is needed.

DDT and its metabolites (*p,p′*-DDE, *o,p′*-DDT and *p,p′*-DDT) were detected in considerable number of samples (more than 40% of the studied samples). *p,p′*-DDE was detected in samples from all areas except those from Lole, while *o,p′*-DDT and *p,p′*-DDT were detected in samples collected from all the studied areas. The high residue le-

Table 2
Pesticides residue level (μg kg^{-1} fat basis) in cow and goat milk samples collected from 4 regions in Ethiopia.

Region	Aldrin	α-Endosulfan	β-Endosulfan	*p,p′*-DDE	*o,p′*-DDT	*p,p′*-DDT	Total DDT
Adami Tulu							
Cow milk							
Sample 1	ND	ND	ND	57.3	165.7	220.0	443.0
Sample 2	ND	47.8	ND	ND	192.0	244.5	436.4
Sample 3	ND	ND	ND	62.2	125.8	218.1	406.2
Sample 4	11.6	ND	ND	ND	482.0	748.0	1230.0
Sample 5	ND	ND	ND	54.2	ND	205.3	259.5
Goat milk							
Sample 1	ND	ND	ND	ND	65.3	158.6	223.9
Sample 2	ND	ND	ND	ND	ND	120.8	120.8
Sample 3	ND	ND	ND	ND	ND	105.4	105.4
Sample 4	ND	142.1	ND	18.3	300.9	555.4	874.4
Sample 5	ND	ND	ND	82.7	ND	ND	82.7
Aseendabo							
Cow milk							
Sample 1	ND	ND	ND	22.9	146.5	230.9	400.4
Sample 2	ND	ND	ND	50.5	ND	ND	50.5
Sample 3	ND	ND	ND	58.5	ND	ND	58.5
Sample 4	ND	ND	ND	57.1	134.5	194.2	385.7
Sample 5	ND	ND	ND	27.8	154.1	239.0	420.8
Goat milk							
Sample 1	ND	ND	ND	ND	129.5	179.7	309.2
Sample 2	ND	ND	ND	40.4	131.2	153.7	325.2
Sample 3	ND	ND	ND	ND	ND	ND	ND
Sample 4	ND	ND	ND	ND	126.5	176.3	302.1
Sample 5	ND	ND	87.0	25.5	148.5	166.2	340.2
Gonde							
Cow milk							
Sample 1	ND	ND	ND	ND	ND	ND	ND
Sample 2	ND	ND	ND	91.9	ND	141.7	233.6
Sample 3	ND	ND	ND	ND	ND	ND	ND
Sample 4	ND	77.6	ND	ND	109.7	160.1	269.7
Sample 5	ND	ND	ND	ND	80.3	163.6	243.8
Lole							
Cow milk							
Sample 1	ND	ND	ND	ND	ND	ND	ND
Sample 2	ND	ND	ND	ND	ND	ND	ND
Sample 3	ND	ND	ND	ND	ND	106.2	106.2
Sample 4	ND	ND	ND	ND	94.0	162.4	256.4
Sample 5	ND	ND	ND	ND	ND	ND	ND

ND = Not detected.

vel for DDT and its metabolite DDE is believed to be due to past and current use of the pesticide for malaria vector control.

Detectable levels of *p,p′*-DDE was found in samples from all areas except those from Lole. *p,p′*-DDE is likely to be generated from the degradation of DDT in the environment. Cow milk had an average concentration of 57.9 μg kg^{-1} and 43.4 μg kg^{-1} in the samples from Adami Tulu and Assendabo, respectively. These regions are known for their high incidence of malaria cases and the use of DDT as an effective agent to combat malaria vector is present till now. Thus the residual level for DDT and consequently DDE are expected to be relatively high. DDE was also detected in one cow milk sample (91.9 μg kg^{-1}) from Gonde. The concentration of *p,p′*-DDE in cow milk was reported to be very high compared with buffalo milk (4 and 28 μg kg^{-1} fat basis, respectively) but the incidence of detection was similar (Abou Donia et al., 2010). While the incidence of positive detection of DDE in milk samples in the present study was lower than those reported for several countries (summarized by Ahmed and Zaki, 2009; Salem et al., 2009; Abou Donia et al., 2010), the concentration was much higher than those reported in these studies, with the exception of the results reported by Nag et al. (2007) for goats grown in Jhansi, India.

The technical grade DDT used to control malaria vector is a mixture of *o,p′*-DDT and *p,p′*-DDT (at ratio of 2:7). The concentration of *o,p′*-DDT detected in the milk samples compared to *p,p′*-DDT do not reflect this ratio and thus the potential of direct contamination is unlikely. *o,p′*-DDT was detected in cow milk samples collected from Adami Tulu, Assendabo and Gonde at average concentrations of 241.4 μg kg^{-1}, 145.0 μg kg^{-1} and 95.0 μg kg^{-1}, respectively. Only one cow milk sample from Lole had *o,p′*-DDT at a concentration of 94.0 μg kg^{-1}. The average concentration of *o,p′*-DDT detected in goat milk from Adami Tulu and Assendabo was 183.1 μg kg^{-1} and 133.9 μg kg^{-1}, respectively. The concentrations of *o,p′*-DDT in the present study were much higher than those reported for milk from different species and countries (Martinez et al., 1997; Salem et al., 2009; Abou Donia et al., 2010) and such concentrations in the milk samples indicate the high contamination of these areas.

p,p′-DDT was found in high amounts in both cow and goat milk samples from all sampling areas. The mean highest concentration detected was 325.2 μg kg^{-1}and the lowest was 134.3 μg kg^{-1} in cow milk samples from Adami Tulu and Lole, respectively. The high level of DDT in the milk samples shows that the environment is contaminated at an alarming level. One cow milk sample from Adami Tulu was found to contain 748.00 μg kg^{-1} of *p,p′*-DDT which is more than 37 times the FAO/WHO MRL (20 μg kg^{-1}). This exceptionally high concentration may be related to the presence of Adami Tulu Pesticides Processing Share Company which is formulating DDT, endosulfan, malathion, diazinon and other pesticides for local consumption in that region. The high *p,p′*-DDT form indicates that this contamination is present in the environment and was not from the milk. Kampire et al. (2011) summarized several studies where higher *p,p′*-DDT content were found compared with its metabolized forms which were mainly from countries with known history for heavy pesticides use.

Total DDT is the sum of *p,p′*-DDT, *o,p′*-DDT and *p,p′*-DDE and *p,p′*-DDD. The amount of these components (with the exception of DDD which was not determined) detected during this study were too high indicating a very high degree of contamination that needs due and timely attention. Values observed for total DDT (ranged 50.5–1230 μg kg^{-1}) are very high compared to EU MRL (40 μg kg^{-1}for total DDT). Average total DDT observed in this study was 328.5 μg kg^{-1}, which is more than eight times the FAO/WHO and EU MRLs for DDT. Had DDD been included in the total DDT value, the figure would have been much higher. The 1230 μg kg^{-1} total DDT detected in one milk sample was more than 30 times the value set by the EU MRL (40 μg kg^{-1}).

Despite the ban on the use of many organochlorines in Ethiopia, the data for inventory and the level of pesticides, including those reported in the present study, show increasing trend over the last 20 years (Haylamicheal and Dalvie, 2009). Despite the efforts of international and governmental organizations in disposing obsolete pesticides, several sources of contamination (e.g. empty pesticide containers, ineffective management of pesticide storage, discussed in detail in Haylamicheal and Dalvie, 2009) still cause new environmental contamination. This was evident from the positive detection of endosulfan which is known to have short life in environment and in animals. It was expected that lower levels of contamination may be present in goat milk given the lower feed intake, however the process is far more complicated and it will involve factors such as the length and intensity level of exposure to contaminants and biochemical factors regulating the metabolism and accumulation in the animals.

4. Conclusion

All pesticides were detected in one or more milk samples indicating their presence and risk in the environment. Aldrin and endosulfan were not present in significant amounts, while *o,p′*-DDT and *p,p′*-DDT were found in high amounts especially in samples from Adami Tulu and Assendabo. α-Endosulfan was also observed in milk samples from Adami Tulu and Gonde. All pesticide residues found above the limit of quantification in this study were also above the EU MRLs. About 80% of the milk samples were found to contain total DDT residue above EU MRL. The use of pesticides needs to be monitored by the concerned bodies and efforts should be made to decrease their level in the environment. The results in the present study indicate that random collection of milk from the natural population to estimate the accumulation profile in different milk producing species is not a suitable strategy and a controlled experimental design where different milk producing animals are fed defined levels of pesticides would be more appropriate to elucidate this phenomenon.

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