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 $\mu g\ kg^{-1}$) was detected only in one cow milk sample and α-endosulfan was detected in one goat milk sample at a level of 142.1 $\mu g\ kg^{-1}$, and in one cow milk sample (47.8 $\mu g\ kg^{-1}$) 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 $\mu g\ kg^{-1}$. 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 $\text{min}^{-1}$; the furnace temperature was initially at 50 °C for 3 min and then the temperature was increased at a rate of 30 °C $\text{min}^{-1}$ to 180 °C, followed by 1 min stabilization, then the temperature was increased at a rate of 6 °C $\text{min}^{-1}$ ramp to 220 °C, followed by 11 min stabilization and then the temperature increased at a rate of 5 °C $\text{min}^{-1}$ 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 $\text{kg}^{-1}$ 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 $\text{kg}^{-1}$ milk fat basis. The limits of quantification (LOQ), were 0.01 μg $\text{kg}^{-1}$ for *p,p′*-DDT, aldrin, α-Endosulfan, β-Endosulfan and 0.03 μg $\text{kg}^{-1}$ for *p,p′*-DDE and 0.04 μg $\text{kg}^{-1}$ 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 $\text{mL}^{-1}$) | Mean recovery (ng $\text{mL}^{-1}$) | % 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 |