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Assessment of persistant organic pollutants acting as endocrine disruptor chemicals in animal fat, cow milk and lacteous sub-products from Cluj County, Romania

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Abstract. The present study aimed to quantify the levels of largely distributed organochlorine compounds (pesticides and polychlorinated biphenyls) in various biological media of animal origin from Cluj County, Romania. Organochlorine pesticides residues (DDT and its metabolites, aldrin, dieldrin, endrin, hexachlorbenzene, heptachlor, α-hexachlorcyclohexane, β-hexachlorcyclohexane, γ-hexachlorcyclohexane and δ-hexachlorcyclohexane) were detected in 2.3-62.5% cow milk samples, lacteous sub-products and bovine fat tissue samples without exceeding maximum admitted limits according to the 86/363 Directive of the European Council. Polychlorinated biphenyls residues (PCB-28, -52, -101, -138, -153 and -180) were identified in 42.43-60.87% of analyzed samples with mean values similar to those previously reported in products and sub-products of animal origin in other geographical areas from Romania. Hence, the presence of persistent organic pollutants acting as endocrine disruptors in products and sub-products of animal origin from Cluj County is confirmed, thus supporting the passage and bio/accumulation of these compounds across the trophic chain to the man.
Key words: persistent organic pollutants, endocrine disruptors, cow milk, gas chromatography and mass spectrometry.

Rezumat. Studiul de faţă şi-a propus să cuantifice compuşii organoclorurati cu largă răspândire (pesticide şi polidorbifenili) in diferite medii biologice animale în judeţul Cluj, România. Reziduurile de pesticide organoclorurate (DDT şi metaboliţi săi, aldrin, dieldrin, endrin, hexaclorbenzen, heptaclor, α-hexaclorciclohexan, β-hexaclorciclohexan, γ-hexaclorciclohexan şi δ-hexaclorciclohexan) au fost detectate în 2.3-62.5% din probele de lapte de vacă, subproduse lactate şi ţesut gras taurin, fără a depăşi limitele maxim admise, conform Directivei 86/363 a Consiliului Europei. Reziduurile de bifenili policloruraţi (PCB-28, -52, -101, -138, -153 şi -180) au fost identificate în 42.43-60.87% din probele analizate, cu valori medii similare celor comunicate în produse şi subproduse prelevate de la animale din alte zone geografice ale României. Se confirmă, astfel, prezenţa poluanţilor organici cloruraţi persistenţi cu efect de disruptori endocrini în produse şi subproduse animale aflate în consumul curent al populaţiei din judeţul Cluj, fapt ce susţine posibilitatea transmiterii şi acumulării acestor compuşi la nivelul lanţului trofic până la om.
Cuvinte cheie: poluanţi organici persistenţi, disruptori endocrini, lapte de vacă, gaz cromatografie şi spectrometrie de masă.

Introduction. Development of intensive agriculture and industrialization have been accomplished by using large groups of chemical substances, several of them exhibiting adverse effects unknown for a long time. Some of these chemical substances are termed endocrine disruptor chemicals (EDCs) and include pesticides and other man-made synthetic compounds that interfere with the biosynthesis and biological effects of endogenuous hormones.

The EDCs belong to the category of persistent organic pollutants (POP). The persistent organic pollutants (POPs) are chemical substances with lengthy remanence (for

tens of years) in the surrounding environment (water, air, soil) with noxious effects on the human beings and animal organisms, and which exhibit the phenomenon of bio-accumulation. Accordingly, EDs are defined as chemical substances acting as hormone agonists or antagonists in human and wildlife (Georgescu et al 2004, 2006). In 1997, in USA, *The Environmental Protection Agency (EPA)* defined EDs as exogenous agents that may alter endocrine functions, eventually leading to hormonal effects on one individual and its descendents and/or subpopulations.

EDCs include herbicides, fungicides and insecticides, largely used in agriculture but also chemical substances contained by transformers, cables, cleansers, plastic products, dyes, bags and cosmetic products. The main EDCs groups are organochlorine pesticides, some organophosphoric pesticides, polychlorinated biphenyls (PCBs), dioxin and its derivates (Georgescu, 2005). In human, EDCs spread within the food chain, on the following route: soil (water)-plant-animal-food-humans; for the organochlorine compounds (organochlorine pesticides and PCBs), which are lyophilic, the main environments for bio-accumulation are milk and the adipose tissue (Grosvenor et al 2002).

Nowadays, several scientific observations incriminate EDCs in the etiology of hormone-dependent cancers such as breast cancer, prostate cancer or testicle cancer (Charlier et al 2003), in abnormalities of sexual differentiation or of fertility reduction in animals and humans as well (Hayes et al 2002; Ormond et al 2009; Roy et al 2009), as in the case of thyroid pathology (Kőhrle et al 2008; Miller et al 2009). Recent researches, in vitro and on animals, have argued a POP with a EDCs function role in the etio-pathogenesis of obesity, the metabolic syndrome and type 2 diabetes as well as in the determinism of the polycystic ovary syndrome (Rylander et al 2005; Tabb et al 2006; Lee et al 2006).

One classical example is that of vaginal adenocarcinoma, induced in female descendents by the estrogen agonist diethylstilbestrol (DES) prescribed to pregnant women to block spontaneous abortion. Moreover, it is speculated that the carcinogenetic effect of DES is transmitted in successive generations (Newbold et al 2000). Certainly, organisms in their developmental phase (from in utero to postpuberty) are more sensitive to the noxious actions of EDCs.

Different from other European countries, in Romania, the monitoring of the POP is restricted to the surrounding environment (air, water, soil) and the researches on the impact of these noxious substances on humans are limited to persons within areas of high exposure (Covaci 2001; Cădariu 2005). On a national level, the researches on the concentration of the compounds taken in studies on animal biological environments or food are emerging (Hura 1999; Mencinicopschi 2006).

With this background, the present study aimed to quantify the level of well-characterized organochlorine compounds –pesticides and PCBs- in cow milk, lacteous sub-products (cheese) and animal (bovine) fat samples originating from different zootechnical exploitation stations and milk processing units within Cluj County.

Materials and Method. Animal products and sub-products were collected between 2007-2008 from zootechnical exploitation stations, private animal farms and milk processing units located in or around cities of Cluj County. In detail, the samples were collected from following cities: Cluj-Napoca, Câmpia Turzii, Turda, Dej, Gherla and Huedin. For comparison, cow milk specimens were obtained from the area of Apuseni Mountains (Măguri-Răcătău village), a location considered to be an unpolluted area, optimal for the production of pollutant-free, ecological cow-milk.

Of the 155 animal samples we collected, 90 (58%) were cow milk samples, 33 (21.2%) were cheese samples and 32 (20.8%) were animal fat specimens. All samples were gained from animals who where either in the first trimester of lactation or in the last trimester of gestation. These periods are characterized by high needs of energy and, hence, lipid stores are intensively mobilized. POPs are lipophilic pollutants with bio-accumulation in

adipose tissues and may be transfered into the blood stream in large quantities during lipid mobilization.

The collection, transport and storage of cow milk samples was done in tightly closed polyethylene containers. Each sample was represented by 250-500 ml milk, collected during afternoon, around 17.00 p.m. To prevent sample damage due to light exposure, each container (sample) was wrapped in aluminium foil.

Both animal fat samples and cheese samples of about 100 g were collected in the same polyethylene containers. Bovine fat was obtained from those locations where slaughter houses or private slaughter points were available.

All samples were handled afterwards in a similar way. Immediately after collection, cow milk and lacteous sub-products samples were introduced in a freezing bag and cold down to +4°C. On the same day the samples were collected, they were stored at -20°C until further assay.

In order to quantify the levels of organochlorine pesticides and PCBs, extraction methods accoding to the european standard EN 1528-1:1996, EN 1528-2:1996 and EN 1528-3:1996 were applied. The standard was adopted by the Romanian Association for Standardization (ASRO) and published with permision from the European Committee for Standardization (CEN). To extract fat from cow milk, the AOAC (Association of Analytical Chemists) protocol was employed. Variable amounts of fat (between 2.50-5.07 g, mean 3.65±1.58 g) resulted from 100 ml cow milk and were further processed. The Soxhlet method was used to extract fat from cheese specimens, whereas the traditional cold fat extraction was used for animal adipose tissue samples.

Fat samples were purified by liquid-liquid partition with acetonitril, followed by additional purification by passage through a Fluorisil column. Concomitantly, to allow the assessment of hexachlorbenzene residues in animal samples, liquid-liquid partition with dimethylformamide was done separately followed by purification on Fluorisil columns.

Eventually, organochlorine compounds residues were determined by gas chomatography with electronic capture detector (GC-ECD) using the gas chromatography-mass spectrometry (GC-MS) equipment (Perkin Elmer, USA).

Following compounds were assessed:

a). Organochlorine pesticides:

– dichlorodiphenyltrichloroethane (p'p'-DDT, o'p'-DDT) and its metabolites p'p'-DDE and p'p'-DDD, dieldrin, aldrin, endrin. After 1985, the use of these substances was banned (Cădariu 2005), however, these are still present in different media due to their long half-time.

-hexachlorbenzene and heptachlor, used in our country until 1995 (Cădariu 2005)

-hexachlorcyclohexane (HCH) and its isomers (α,β,γ,δ) and in particular γ-HCH, currently used as insecticide and deparasintante in animal husbandry

b). polychlorinated biphenyls mixture, resulting from industry (transformers, condensers, batteries and energetics).

Results and Discussion. All results were expressed in mg/kg (ppm)/product, as mean ± SD. For pesticides, data were compared to the maximum admitted limits approved by the European Union legislation through the 86/363/EEC Directive of the European Council concerning the presence of these residues in food of animal origin (EFSA, Council Directive "86/363/EEC" Pesticides MRLs - Animal origin).

Table 1 presents the levels of organochlorine compounds obtained by GC-ECD analysis in cow milk specimens. It is revealed that DDT and DDT metabolites residues were detected in 48% of cow milk samples but did not reached the maximum admitted limit (figure 1). Similar results were reported in other european countries (Losada et al 1997; Mallatou et al 1997; Cerknenik et al 2000).

With regard to hexachlorcyclohexane isomers, it is shown that they were detectable in about 56% of all cow milk samples; however, in none of samples the HCH levels reached the maximum admitted limit (Figure 1).

As depicted in Figure 1, all other pesticides were present only in part of the analyzed samples and in small amounts (between 8.1-21.2%); heptachlor was identified in highest quantities.

Of the 90 cow milk samples, it was demonstrated that in 50 samples none of the PCB congeners was identified (see Figure 2).

Table 1

Levels of organochlorine pesticides and total PCBs in cow milk from Cluj County, Romania

Compound	*Number of samples*	*Mean level mg/kg (ppm)*
DDT and metabolites	90	0.013
Aldrin	90	0.002
Dieldrin	90	0.002
Endrin	90	0.0003
Hexaclorbenzene	90	0.0084
Heptachlor	90	0.0025
α-HCH	90	0.0021
β-HCH	90	0.0014
γ-HCH (lindane)	90	0.0042
δ-HCH	90	0.0009
PCB mixture	90	0.0001

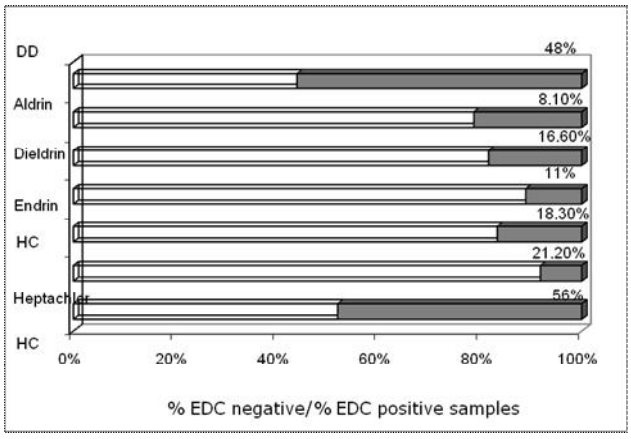

Figure 1. Detection (%) of abovementioned organochlorine pesticides in cow milk samples from Cluj County, Romania.

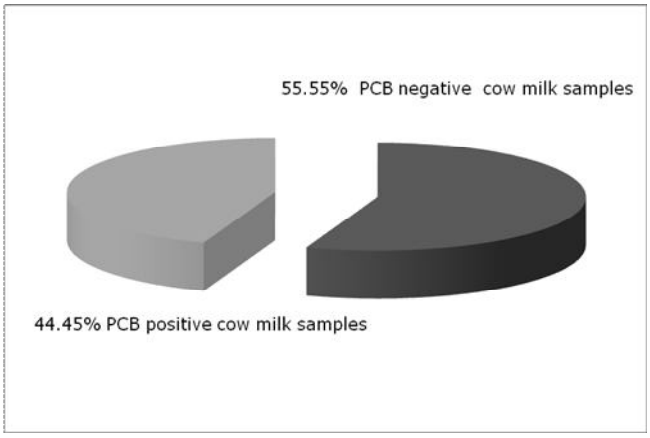

Figure 2. Presentation (%) of PCB (mixture) in cow milk samples in the area of Cluj County, Romania.

The mean PCBs level measured in cow milk in the present study was below 0.0001 mg/kgc and this is in agreement to previous reports on cow milk contamination with PCBs (Cerknenik et al 2000).

As presented in Figure 3, the concentration of organochlorine pesticides in lacteous sub-products (cheese) was below maximum admitted limits. In fact, organochlorine pesticides residues were identified in 2.3-49% of examined samples and the pattern was similar to that from cow milk: DDT and its metabolites were followed by HCH and, thereafter, heptachlor (Table 2). With the exception of HCH residues, organochlorine pesticides residues were not detectable in cow milk and cheese samples originating from the Apuseni Mountains area.

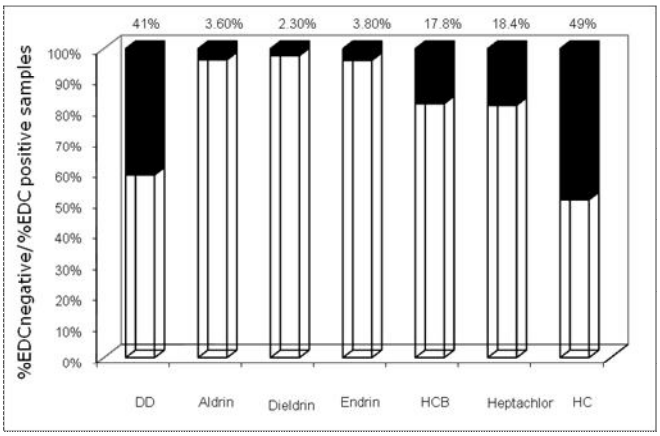

Figure 3. Detection (%) of organochlorine pesticides in lacteous sub-products (cheese) samples from Cluj County, Romania.

Of the 33 cheese samples collected from milk processing units and private farms located in Cluj County, PCBs congeners were identified in 11 samples, as shown in Figure 4.

Table 3 presents the mean values of organochlorine compounds in bovine animal fat as quantified by GC-ECD.

Organochlorine compounds were found in quantities below the maximum admitted limit.

Table 2

Levels of organochlorine pesticides and total PCBs in lacteous sub-products from Cluj County, Romania

Compound	*Number of samples*	*Mean level mg/kg (ppm)*
DDT and metabolites	33	0.0098
Aldrin	33	0.0033
Dieldrin	33	0.0032
Endrin	33	0.0002
Hexachlorbenzene	33	0.0045
Heptachlor	33	0.0014
α-HCH	33	0.0019
β-HCH	33	0.0008
γ-HCH (lindane)	33	0.0062
δ-HCH	33	0.0005
PCB total	33	0.0003

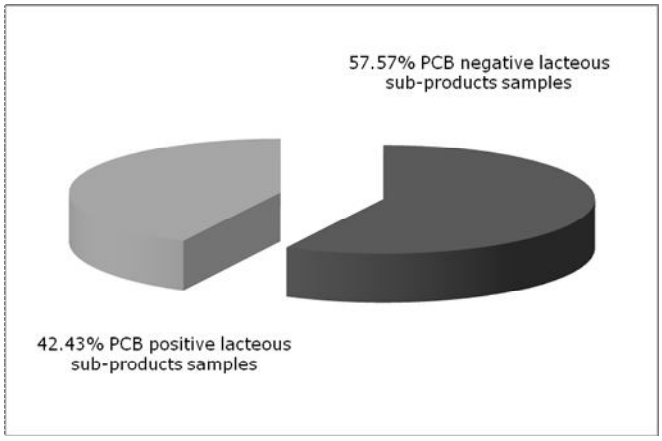

Figure 4. Presentation (%) of PCB (mixture) in lacteous sub-products (cheese) samples in the area of Cluj County, Romania.

Although, residues of DDT, aldrin, dieldrin and HCH were identified in several samples, as shown in figure 5, chromatographic peaks were below maximum admitted limits.

Concerning PCBs, these were detected at a mean concentration of 0.028 mg/kg animal fat, which is comparable to the mean levels of PCBs reported in other geographical areas from our country (Covaci 2001). Under the detection limit levels of PCBs were observed in 9 (28.12%) animal fat samples, of bovine origin (Figure 6).

It has to be pointed out that when examining the relationship between POP concentration and the origin of polluted samples, it was observed that highest contaminated samples were found around the cities Cluj-Turda, for cow milk, cheese and animal fat. The important level of pollution of these urban areas may explain the presence of PCBs in over 61% of the fat samples analyzed, originating from the cities of Cluj-Napoca and Turda. In comparison to reports from other regions in Romania, it can be concluded that in the area of Cluj County the level of persistent organic pollutants is between limits recorded nationally.

Table 3

Levels of organochlorine pesticides and total PCB in bovine fat from Cluj County, Romania

Compound	*Number of samples*	*Mean level mg/kg (ppm)*
DDT and metabolites	32	0.091
Aldrin	32	0.073
Dieldrin	32	0.061
Endrin	32	0.002
Hexachlorbenzene	32	0.059
Heptachlor	32	0.063
α-HCH	32	0.055
β-HCH	32	0.012
γ-HCH (lindane)	32	0.108
δ-HCH	32	0.009
PCB total	32	0.028

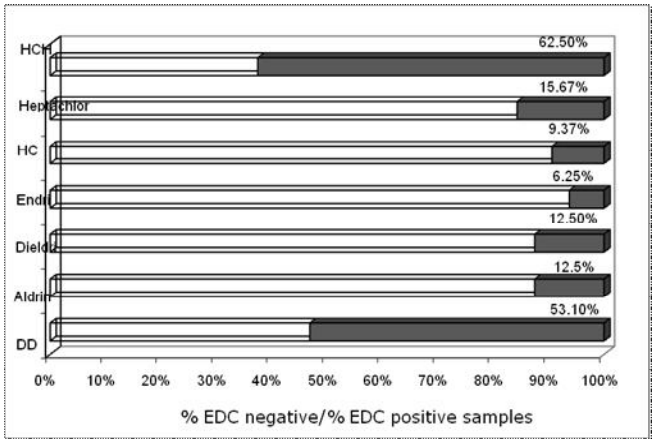

Figure 5. Detection (%) of organochlorine pesticides in bovine fat samples in the area of Cluj County, Romania.

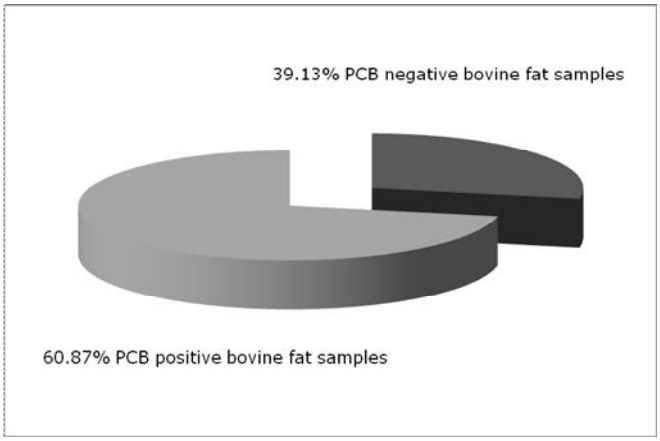

Figure 6. Presentation (%) of PCB (mixture) in bovine fat in the area of Cluj County, Romania.

On the other hand, identification of POPs with endocrine disrupting activities in animal products and sub-products supports the hypothesis of the bioaccumulation process of these products along the food chain and merits further attention through research projects that should establish potential involvement of EDC in the pathogenesis of human diseases.

Conclusions. Organochlorine pesticides residues (DDT and its metabolites, aldrin, dieldrin, endrin, hexachlorbenzene, heptachlor and HCH isomers) were detected in 2.3-62.5% of cow milk samples, lacteous sub-products (cheese) and animal (bovine) fat, without exceeding maximum admitted limits, according to the 86/363 Directive of the European Council. Total polychlorinated biphenyls residues (PCB-28, -52, -101, -138, -153 and -180) were identified in 42.43-60.87% of analyzed samples. The mean PCB concentration in animal products and milk was in agreement to previous reports from other areas in Romania.

Thus, detection of POPs acting as EDCs in animal products is confirmed. By entering the food chain, these compounds suffer a bioaccumulation process until reaching the highest level, the man.

The organochlorine level in cow milk, cheese and fat samples we found is comparable to the levels reported in other regions from Romania. They follow a descending pattern, due to the European Union legislation, adopted by our country, in which the use of organochlorine compounds in agriculture is banned.

The study results support further research in the field to establish the role of these EDC in the pathogenesis of diseases such as obesity, metabolic syndrome, insulin resistance, diabetes mellitus and ovary polycystic disease.

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