TRANSFER FACTORS FOR ENDOCRINE DISRUPTING COMPOUNDS FROM FEED TO MILK

Mirela Miclean[1], Cecilia Roman[1], Ioan Stefan Groza[2]

[1]INCDO-INOE 2000, Research Institute for Analytical Instrumentation, Cluj-Napoca, Romania, email: mirela.miclean@icia.ro
[2]University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Cluj-Napoca, Romania

Abstract

Toxic compounds, such as organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) are ubiquitous in the environment, thus indirectly in animals feed. The transfer of these compounds from animal feed to food products contributes to the human exposure, through consumption. OCPs and PCBs are highly persistent, biocumulative and exercise a wide range of toxic effects, including endocrine system disrupting, being considered as a new class of nonsteroidal xeno-estrogens. In this study, the transfer factors (TFs) for 19 OCPs and 7 PCBs have been evaluated for the quantification of their transfer from cows' fodder to milk. The samples (milk and feed from cow's diet) were collected in two villages from Maramures County, Romania. The compounds OCPs and PCBs were analyzed using gas-chromatography coupled with electron capture detection after solvent extraction. Among the OCPs, all the HCH isomers were detected in all the investigated samples, the highest concentration were obtained for α-HCH. All the isomers DDTs (except 2,4'-DDD) were determined, with highest concentration for 4,4'-DDE. The obtained results showed that the TFs ranged between 0.03-0.28, the highest value was recorded for hexachlorobenzene and the lowest for lindane. High values were determined also, for dieldrin (0.25) and for heptachlor (0.23).

Key words: *endocrine disruptors, feed, milk, transfer factor.*

INTRODUCTION

Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) are persistent, bioaccumulative and toxic compounds (Tajkarimi et al., 2008). PCBs have been produced for many applications since 1929 and OCPs have been extensively used in agriculture and public health since the Second World War. Due to their wide range of toxic effects, the use and the production of PCBs and OCPs have been regulated worldwide, but they are still present in the environment (Herceg Romanic and Krauthacker, 2006).
Due to the lipophilic characteristic of organochlorine compounds (OCCs), they tend to accumulate and persist in fat tissues through dietary intake. Ingestion of contaminated food, especially with fat content (dairy products, meat and fish) is the main intake route through which OCPs and PCBs enter the human body (Huang et

al., 2008). Milk-producing animals, such as cows, accumulate residues of OCCs through contaminated feed and inhaled air (Heck et al., 2007).
There are evidences that OCCs exhibit a wide range of toxic effects and pose a serious risk to human health, especially for infants, since their enzymatic and metabolic systems are not fully active (Falandysz et al., 2004). Some OCPs (aldrin, dieldrin, DDT, 4,4-DDE, endosulfan, heptachlor, heptachlor-epoxide) and PCBs may interfere with the endocrine system and produce adverse effects in human and wildlife, these chemicals are referred as endocrine disruptors (Prins, 2008). DDT and its principal metabolites (p,p′-DDE, p,p′-DDT, p,p′-DDD, o,p′-DDT) can be involved also in human reproductive toxicity, cancer development, neurodevelopment and intellectual dysfunction in infants (Wang et al., 2009).
Different parameters can be used to describe the potential of various contaminants to be transferred from feed to milk (eggs, tissue) in order to assess the risk of these contaminants. The most used parameter is Transfer Factor (TF), based on long-term exposure of animals to a particular contaminant, so the contents in the milk (eggs, tissue) reach a constant value over time. The TF is calculated according to equation 1 (MacLachlan, 2011):

$$FT = C_i/C_{feed} \quad (1)$$

where: C_i is the residual concentration of the compound or element in animal product (milk, eggs, tissue),
C_{feed} is the residual concentration of the compound or element in the diet of the animal, including the contribution of soil ingestion (mg/kg dry weight).
In this study, the (TFs) for 19 OCPs and 7 PCBs were evaluated for the quantification of their transfer from cows' fodder to milk.

MATERIALS AND METHODS

Sampling
This study was conducted on animals that were exposed to food (grass/hay) with relatively fixed concentrations of contaminants/day for long periods. It was assumed that the main source of contamination of milk is the feed (grass/hay), eliminating from the study the water intake, inhalation of air and skin exposure. In July 2012, cow milk and grass/hay samples were collected in two villages from Maramures County: Satu Nou de Sus and Ocolis (Table 1). The grass/hay samples were collected from the investigated animals' diet, on the land where they are grazed.

Table 1. Sampling points and investigated samples' type

Village	Milk	Grass/hay
Satu Nou de Sus, nr. 179	1 sample (raw milk)	1 sample grass/hay
Ocolis, nr. 57	1 sample (raw milk)	1 sample grass/hay

Raw milk was collected in sterilized glass, previously cleaned with nitric acid 1:1 v/v and distilled water. The samples were refrigerated at -20°C until analysis procedure (Heck et al., 2007).

Reagents

For OCCs analysis, the used solvents (n-hexane, acetonitrile and ethanol) were gas-chromatography grade of quality (Merck, Darmstadt, Germany). Anhydrous sodium sulphate, silica gel and Florisil were acquisitioned from Merck (Darmstadt, Germany). Standard solution (NE7550) for OCPs and PCBs was purchased from LGC Promochem (Germany) and contained: α-, β-, γ-, δ-, ε-isomers of hexachloro-cyclohexane (expressed as HCHs), 4,4′-DDE, 2,4′-DDE, 4,4′-TDE, 2,4′-TDE, 2,4′-DDT and 4,4′-DDT (expressed as DDTs), aldrin, dieldrin, heptachlor, heptachlorepoxide (isomer A and B), alfa-endosulfan, beta-endosulfan, hexachlorobenzene (HCB) and the following PCB congeners: tri (28), tetra (52), penta (101), hexa (138, 153), hepta (180) and octa (194), at 10 μg/mL each analyte and working standard solutions were prepared diluting accurate volumes of mix standard solution in dichloromethane.

Instrumentation

In order to separate, detect and quantify the OCCs, gas chromatography electron-capture detection (GC-ECD) was used (Agilent Technologies 6890N GC-μECD) equipped with a DB-608 capillary column, 30 m L×0.32 mm ID×0.50 μm, (J&W). Helium was used as carrier and nitrogen as make up gas. For solvents evaporation was used a rotary evaporator, Laborota 4010 (Heidolph, Germany) coupled with a vacuum pump (Ilmvac, Germany).

Sample preparation

For OCCs analysis, the extraction of milk samples was carried out according to the method described by Ennaceur et al. (2007). The method consists of extraction with n-hexane, acetonitrile and ethanol, clean-up on Florisil column and elution with dichloromethane and n-hexane, evaporation with rotary evaporator and then GC-ECD analysis. The grass/hay samples were analysed according to the method reported by Yenisoy-Karakas (2006), using ultrasonic extraction with dichloromethane, clean-up on Florisil column and elution with cyclohexane–ethyl acetate mixture, evaporation with rotary evaporator and then GC-ECD analysis.

Sample analysis

In order to separate and quantify the organic compounds, subsequently the extraction and evaporation, 1 μL of purified extract was injected in the GC-ECD at 280°C. The oven temperature program consists of 4 stages, from 80°C to 275°C.

Three independent replicates of each sample were measured, and the concentrations were calculated using the average of each value.

RESULTS AND DISCUSSIONS

Organochlorine compounds in milk

The obtained values of the organochlorine compounds for raw milk samples collected from two villages in Maramures County are given in Table 2. Values are expressed in µg/L wet weight (ww) and represent mean ± standard deviation of three replicate determinations.

Table 2. Average concentrations of organochlorine compounds (µg/L ww) with standard deviations, in raw milk samples

Compound	**Milk, Satu Nou de Sus**	**Milk, Ocolis**
Hexachlorbenzene (HCB)	0.125 ± 0.044	<LQ
Aldrin	<LQ	0.030 ± 0.005
Dieldrin	0.362 ± 0.089	0.023 ± 0.007
α-HCH	1.010 ± 0.36	1.410 ± 0.315
β-HCH	0.116 ± 0.012	0.288 ± 0.081
γ-HCH (Lindane)	0.453 ± 0.101	0.173 ± 0.024
δ-HCH	0.128 ± 0.036	0.038 ± 0.004
ε-HCH	<LQ	0.283 ± 0.012
Heptachlor	<LQ	0.282 ± 0.054
Heptachlor epoxide β	<LQ	0.146 ± 0.0038
Heptachlor epoxide α	<LQ	0.035 ± 0.007
Endosulfan α	<LQ	0.012
Endosulfan β	<LQ	<LQ
2,4'-DDE	0.005 ± 0.001	0.012 ± 0.003
4,4'-DDE	0.501 ± 0.121	0.567 ± 0.108
2,4'-DDD	<LQ	<LQ
4,4'-DDD	0.034 ± 0.019	<LQ
2,4'-DDT	0.018 ± 0.008	0.007 ± 0.002
4,4'-DDT	0.093 ± 0.006	<LQ
PCB28	0.125 ± 0.341	<LQ
PCB52	1.010 ± 0.151	1.410 ± 0.76
PCB101	0.116 ± 0.031	0.288 ± 0.069
PCB138	0.453 ± 0.078	0.173 ± 0.008
PCB153	0.128 ± 0.024	0.038 ± 0.007
PCB180	<LQ	0.283 ± 0.086
PCB194	<LQ	0.030 ± 0.005

QL – quantification limit

The analysis of the obtained results allows the following observations:

All the investigated compounds recorded low concentrations.
In the milk sample collected from Satu Nou de Sus, the following analytes were detected: HCB, dieldrin, α-HCH, β-HCH, γ-HCH (lindane), δ-HCH, endosulfan-beta, 2,4'-DDE, 4,4'-DDE, 4,4'-DDD, 2,4'-DDT, 4,4'-DDT, PCB28, PCB52, PCB101, PCB138, PCB153.
In the milk sample collected from Ocolis, the following analytes were detected: aldrin; dieldrin; α-HCH; β-HCH; γ-HCH; δ-HCH; ε-HCH; heptachlor; heptachlor epoxide β; heptachlor epoxide α; endosulfan alfa; 2,4'-DDE; 4,4'-DDE; 2,4'-DDT; PCB52; PCB101; PCB138; PCB153; PCB180; PCB194.
Among the OCCs, the highest concentrations were determined for α-HCH, both in sample collected from Satu Nou de Sus (1.010 µg/L ww) and Ocolis (1.410 µg/L ww). All HCH isomers were determined in relatively high concentrations, especially in the sample collected from Ocolis (α-HCH – 1.410 µg/L ww; β-HCH – 0.288 µg/L ww, γ-HCH (lindane 0.173 µg/L ww, δ-HCH – 0.038 µg/L ww; ε-HCH – 0.283 µg/L ww; heptachlor – 0.282 µg/L ww). In general, the average concentrations of OCCs were higher in milk samples from Satu Nou de Sus, for: dieldrin (15.74 times); γ-HCH (2.62 times); δ-HCH (3.37 times); 2,4'-DDT (2.57 times); PCB138 (2.62 times) and PCB153 (3.37 times).
For the following compounds the average concentrations recorded in Ocolis were higher than those of Satu Nou de Sus: α-HCH (1.40 times); β-HCH (2.48 times); 2.4'-DDE (2.40 times); 4.4'-DDE (1.13 times); PCB52 (1.40 times); PCB101 (2.48 times). Compared with the sample of Ocolis, only in the sample from Satu Nou the following compounds were determined: HCB (0.125 µg/L ww); 4,4'-DDT (0.093 µg/L ww); PCB28 (0.125 µg/L ww).

Organochlorine compounds in grass

The average concentrations of organochlorine compounds (mg/kg dry weight, dw) in grass samples from both studied villages are shown in Table 3.

Table 3. Average concentrations of organochlorine compounds (mg/kg dw) with standard deviations, in grass samples

Compound	**Satu Nou de Sus**	**Ocoliş**
Hexachlorbenzene (HCB)	0.13 ± 0.08	0.06 ± 0.02
Aldrin	0.05 ± 0.01	0.03 ± 0.01
Dieldrin	0.39 ± 0.12	0.02 ± 0.01
α-HCH	1.09 ± 0.32	1.59 ± 0.08
β-HCH	0.13 ± 0.08	0.32 ± 0.06
γ-HCH (Lindane)	0.51 ± 0.14	0.19 ± 0.05
δ-HCH	0.14 ± 0.07	0.04 ± 0.02
ε-HCH	0.08 ± 0.02	0.31 ± 0.10
Heptachlor	0.09 ± 0.03	0.32 ± 0.07
Heptachlor epoxide β	0.05 ± 0.01	0.16 ± 0.05
Heptachlor epoxide α	0.02 ± 0.01	0.04 ± 0.02

Compound	Satu Nou de Sus	Ocoliş
Endosulfan α	0.07 ± 0.03	0.01 ± 0.005
Endosulfan β	0.06 ± 0.02	0.05 ± 0.02
2,4'-DDE	0.01 ± 0.002	0.01 ± 0.004
4,4'-DDE	0.55 ± 0.07	0.64 ± 0.12
2,4'-DDD	0.06 ± 0.01	0.05 ± 0.02
4,4'-DDD	0.04 ± 0.02	0.04 ± 0.01
2,4'-DDT	0.02 ± 0.01	0.01 ± 0.003
4,4'-DDT	0.10 ± 0.03	0.01 ± 0.005
PCB28	0.14 ± 0.04	0.01 ± 0.003
PCB52	1.14 ± 0.42	1.62 ± 0.68
PCB101	0.12 ± 0.06	0.31 ± 0.06
PCB138	0.51 ± 0.11	0.19 ± 0.07
PCB153	0.14 ± 0.08	0.00 ±
PCB180	0.06 ± 0.02	0.32 ± 0.08
PCB194	0.05 ± 0.01	0.03 ± 0.01

QL – quantification limit

In the grass samples from the two investigated localities all the analysed endocrine disruptors were determined.

The highest concentrations were recorded for α-HCH (1.09 mg/kg dw in Satu Nou de Sus and 1.59 mg/kg dw in Ocolis) and PCB52 (1.14 mg/kg dw in Satu Nou de Sus and 1.62 mg/kg dw in Ocolis).

Dieldrin concentration recorded in Satu Nou de Sus is 16.04 times higher than in sample taken in the Ocolis. Similar behaviour was recorded also for 4,4 '-DDT (of 10.95 times higher in Satu Nou de Sus) and PCB28 (by 17.50 times higher in Satu Nou de Sus). Also, the following OCCs: alpha-endosulfan (5.30), beta-endosulfan (1.20), PCB138 (2.69) and PCB194 (1.49) present the same type of evolution, but with a lower ratio values was observed.

Transfer factors of organochlorine compounds

The transfer factors for organochlorine compounds from grass to milk are shown in Figure 3.

Regarding the obtained results, the followings can be concluded:

The grass-milk TFs ranged between 0.03 and 0.29, the highest value was recorded for HCB and the lowest for lindane (δ-HCH). High values were also obtained for dieldrin (0.25) and heptachlor (Σ(heptachlor+epoxide) (0.23).

Depending on the obtained values, the Transfer Factors increased in the following order: lindane>δ-HCH>ΣPCBs>DDT (Σ(DDTs+DDEs+DDDs))> heptachlor (Σ(heptachlor+epoxide)>dieldrin>HCB.

Compared to lindane, considered as reference, the TFs of the OCCs recorded the following valus: ΣPCBs were with 433% higher; DDT (Σ(DDTs+DDEs+DDDs)) with 633% higher, heptachlor (Σ(heptachlor+epoxide) with 766% higher; dieldrin with 833% higher, and HCB 966% higher.

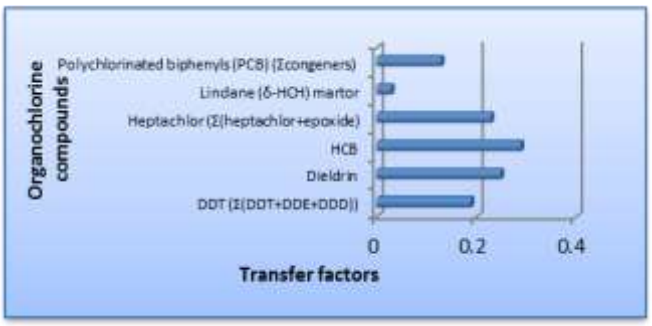

Figure 3. Transfer factors for organochlorine compounds

The grass-milk TFs ranged between 0.76 and 0.94, the highest values were recorded for Co (Satu Nou de Sus) and Pb (Ocolis) and the lowest, for Zn (Satu Nou de Sus) and Cu (Ocolis). Relatively high values were also obtained for all the investigated elements. The TFs for Ocolis samples were higher than TFs for Satu Nou de Sus, for all the elements.
The obtained values are comparable with those reported by MacLachlan (2011) with some exceptions, taking into consideration the particularities and the specificities of the investigated areas.
In the risk assessment it is important to determine the transfer factors of contaminants compounds, taking into consideration also the differences in animal physiology and the growth rates.

CONCLUSIONS

All the investigated compounds recorded low concentrations. Among the OCCs, the highest concentrations were determined for α-HCH, in both samples. HCH isomers were determined in relatively high concentrations, especially in the sample collected from Ocolis. In general, the average concentrations of OCCs were higher in milk samples from Satu Nou de Sus, for: dieldrin, γ-HCH, δ-HCH, 2,4'-DDT, PCB138 and PCB153.
In the grass samples from the two investigated localities, all the analysed endocrine disruptors were determined. The highest concentrations were recorded for α-HCH and PCB52.
The grass-milk TFs ranged between 0.03 and 0.29, the highest value was recorded for HCB and the lowest for lindane (δ-HCH).

Depending on the obtained values, the Transfer Factors increased in the following order: lindane>δ-HCH>ΣPCBs>DDT (Σ(DDTs+DDEs+DDDs))> heptachlor (Σ(heptachlor+epoxide)>dieldrin>HCB.

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