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Research Article

The Concentrations of Selective Endocrine Disruptors in Milk from Different Lactation Periods of Cows

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Abstract

Milk can be contaminated with organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and polycyclic aromatic hydrocarbons (PAHs), which are known as endocrine disruptors (EDCs). However, the relationship between the lactation period of cows and the residues of EDCs is unknown. The aim of this study was to determine the relationship between the lactation period in cows and EDCs residues such as OCPs, PCBs, PBDEs, and PAHs. Milk collected from cows during each lactation period was analyzed in terms of fat content and EDC residues. One or more contaminants were detected in almost all (98%) of the milk taken in three lactation periods. Three PCBs and 11 PAHs were found together in 1st lactation period, and one more PAH was added to them in other periods. However, the sample rate exceeded the maximum residue limit of 48%, 10%, and 16% in the first, second and third lactation periods, respectively. Also, it was seen that the riskiest period was the first lactation period, followed by the third and second periods. The same ranking is valid for the fat content in milk. Thus, it was concluded that the excretion of lipophilic EDCs in milk is related to the fat content in milk, and the higher the fat content in milk, the more lipophilic EDCs are excreted in milk.

Keywords: Endocrine disruptor, Lactation period, Milking, Persistent organic pollutants, Polycyclic aromatic hydrocarbons, Risk assessment

Introduction

Persistent organic pollutants (POPs) are substances highly stable to chemical and biological degradation, non-degradable for a long time, and are well soluble in lipids [1]. Organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs) are environmental pollutants. OCPs have been widely used in pest control for the plant protection products and protection of public health [2]. PCBs have been used greatly as insulator and cooling liquid, especially in capacitors, transformers, hydraulic pumps, in printing inks and paint production as plasticizer, in carbonless copy papers, in polyvinyl chloride (PVC) coatings of electrical cables to provide durability, etc. [1]. PBDEs are used as flame retardants in diverse products, including building materials, electronic tools, furnishing, motor instruments, airplane, polyurethane foams, and textiles [3].

POPs can lead to various kinds of cancer, anomalies, developmental and reproductive damage, nervous system disturbance, suppression of the immune system, and other harmful effects in humans and animals because of their endocrine-disrupting effects [4]. Thus, since 2001, many countries such as Turkiye, which have evaluated the risks of POPs to the environment and human health, have signed the International Stockholm Convention on POPs to terminate or restrict the production and use of

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OCPs and PCBs [1]. The same precautions began to be implemented in the production and use of PBDEs in 2009 [5]. Polycyclic aromatic hydrocarbons (PAHs) are also common environmental pollutants generated during deficient burning of organic matter and anthropogenic movements [6]. Since PAHs are disintegrated easier than other environmental pollutants, they are not usually accepted as POPs. However, they are candidate substances for POPs due to being highly extensive and lipophilic [7].

The milk and dairy products may be contaminated with contaminants such as endocrine disruptors and therefore may both threaten human health and affect the commercial image by creating a technological risk factor [8-12]. One study showed that carryover rates of OCPs from feed to cow milk can range from 0.10% to 250% [13].

Many studies focused on POP residues in milk and risk assessment, however, it is known that the lactation period is an important factor that significantly affects the excretion of xenobiotics [14]. Lactation in cows is divided into three periods. The first period is the beginning of lactation and includes 10 weeks after birth (approximately 70 days). Body reserves are used for milk production. During this period, milk yield increases rapidly and within 6-8 weeks, milk yield reaches its highest value and the animal's feed consumption cannot meet the energy excreted from the body with milk. Thus, the animal uses the fat in its body to meet its energy needs. The second period is between the 70th and 140th days of lactation, and in this period, milk yield and milk fat ratio begin to decrease. The third period covers the 140th to 305th day of lactation. During this period, the animal's feed consumption begins exceeding its needs and the animal regains its body reserves for the next lactation (21-44 weeks). Milk yield had started to decrease. The animal is pregnant and the needs can be easily met as the consumption exceeds the needs [15]. Here, it can be predicted that more POP residues may be encountered in the lactation period when the amount of fat in the milk increases. Because milk is an indicator of the bioconcentration process of POPs, because of their lipophilic properties, they are first stored in fat-rich tissues and then excreted via milk fat [16]. To test this hypothesis, a farm in Ankara was selected and milk was collected from cows according to the lactation period. Thus, it was aimed to establish a relationship between fat content, milk yield and POP residues in cow's milk collected according to the lactation period.

Material and Methods

Ethical Approval

Since only milking was performed in this study, there is no requirement for ethics committee approval according to national legislation. Additionally, informed consent was obtained from the farm owners

Sampling Strategy

A herd of approximately 500 cows in Ankara was selected to test our hypothesis. There were Holstein and Holstein's crossbred cows aged 3-8 years on the farm where milking was done automatically and good veterinary practices were implemented. The lactation period of the cows is approximately 305 days and it has been stated that the milk yield of each cow is between 5.000 and 7.000 liters per year. The cows selected for sampling were in the same environment. During each lactation period, 100 mL-milk, approximately 25 mL from each udder lobe, was milked from 50 cows and mixed. To ensure the results, morning milking was performed on days 49, 50, and 51 in the first lactation period, on days 99, 100 and 101 in the second lactation period, on days 199, 200, and 201 in the third lactation period. All milk samples were taken in a glass jar, brought to the laboratory within 2–3 hours under cold chain, and kept at -20°C until analysis. The analyses were performed no later than 5-7 days after all the samples were collected. Results representing each lactation period were given as the mean of 3 days of milk. Thus, 450 milk samples were analyzed in terms of milk fat content and POP residue. Calculations were made based on the results from 150 cows.

Chemicals and Reagents

Analytical standards for OCPs, PCBs and PAHs were purchased from Dr. Ehrenstorfer (Augsburg, Germany), while those for PBDEs were obtained from Wellington Laboratories (Guelph, Canada). Solvents of analytical grade, sodium chloride (NaCl), and anhydrous sodium sulfate ($MgSO_4$) were procured from Sigma (Steinheim, Germany), while primary-secondary amine (PSA) and C18 Solid Phase Extraction (SPE) adsorbent were obtained from Agilent (Santa Clara, USA). All stock and working solutions were made ready in acetonitrile.

The SPE column was designed by adding 0.2 g C18, 0.4 g PSA, and 0.2 g $MgSO_4$ to the glass Pasteur pipette with glass cotton at the bottom, respectively [1].

Extraction

To extract target EDCs from milk, we used a modified method developed by Simsek et al.[1]. Two g of milk sample was weighed separately in screwed glass tubes with glass beads in it, and was homogenized for 15 min by adding 5-mL acetonitrile, and kept in an ultrasonic bath at room temperature for 10 min. One g NaCl was put in the specimens moved away from the bath and vortexed for 5 min. It was centrifuged at 1968 × g for 10 min at +4°C. The supernatant was taken and transferred to a glass tube, and 3-mL acetonitrile was added to the precipitate left in the tube and shaken in vortex for another 10 min. One g $MgSO_4$ was added to the tube containing the supernatants

and shaken manually for 30 sec and kept in the freezer at -20°C for 12 h. The next day, it was removed from the freezer and centrifuged at 1968 × g for another 10 min. The supernatant was removed and evaporated under nitrogen at 35°C until 1 mL. It was passed through the SPE column previously conditioned with 4×1 mL acetonitrile. The filtrate was collected with 4×1 mL acetonitrile and completely evaporated under nitrogen. The dry residue was collected with 90 µL acetonitrile, and applied to the gas chromatography-mass spectrometry (GC-MS) device by adding 10 µL of PCB30 solution (1‰ w/v). PCB30 is used as an injection internal standard.

Gas Chromatographic Analyses

Analysis of target analytes was done by GC-MS (Thermo Finnigan, CA, USA). The instrument was employed in a splitless mode. Electronic ionization, external ion source, interface and injector port temperatures were set to 70 eV, 250°C, 270°C and 280°C, respectively. In this work, 2 µL sample was applied to the autoinjector. The separation was performed with a HP-5MS capillary column (30 m × 0.25 mm diameter, 0.25 µm film thickness) (Agilent Technologies, CA, USA). Helium (99.995% purity) was used as the carrier gas at a running flow rate of 1 mL/min. A separate furnace work schedule was applied for OCPs, PCBs and PBDEs, and PAHs. The furnace program used for each group was determined according to the method of Simsek et al.[1]. Mass spectrometric evaluation was performed in the selected ion monitoring (SIM) mode using 2 or 3 characteristic fragment ions for each compound (1 target ion and 1 or 2 qualifier ions) *(Table 1)*.

Quality Assurance and Quality Control

The method validation was performed by SANTE guideline [17]. Milk samples without target analytes were used to validation the method. For validation, linearity, limit of detection (LOD), limit of quantification (LOQ), selectivity, precision and recovery parameters were assessed.

The milk fat content was measured by the Gerber method with the help of a butyrometer [18].

Risk Assessment

The Environmental Protection Agency (USEPA) guidelines were followed in calculating the risks to human health of contaminants detected in milk [11]. Risks were determined for both children and adults. Risk assessment was performed based on both the average concentration and the maximum concentration. For this, the estimated daily milk intake (EDI) was calculated first. The EDI depends on both the concentration of each compound and the daily consumption of the food. The following formula was used to calculate the EDI;

$$EDI\ (\mu g/kg/day) = CR \times C/body\ weight$$

where "CR" is the daily milk consumption rate (kg/day), "C" is the concentration of the pollutant measured in milk (µg/kg). According to the Turkiye Nutrition and Health Survey [19], the average daily consumption of milk and dairy products for adults over the age of 15 is 0.19 kg and the body weight is 75 kg. Since milk consumption and body weights are not given to 10-15-year-old children in this guide, a study by Bıyıklı and Akman [20] was used. Accordingly, the average milk consumption of 10-15-year-old children in Türkiye was calculated as 0.3 kg/day and body weight as 33 kg. According to USEPA standards, the risk of non-carcinogenic effects is expressed by comparing the exposure dose with the dose considered to have no effect [21]. This rate is called the target hazardous quotient (THQ) and is calculated as follows, based on the acceptable daily intake (ADI) of each compound.

$$THQ=EDI/ADI$$

If the THQ value is less than 1, exposed individuals do not show significant toxic effects. If one exceeds and the THQ value increases, significant toxic effects can be experienced.

The Hazard Index (HI) is a measure of the risk of potential adverse health effects from the mixture of chemicals in milk [22]. The sum of the THQ values gives the hazard index (HI), which is the sum of the contaminants in the milk samples, and is calculated as follows [9].

$$HI= THQ_1+THQ_2+\ldots. THQ_n$$

The Toxic Equivalency Quotients (TEQ)

We assessed TEQ concentrations of 16 target PAHs with the following formula. The toxic equivalency factors (TEFs) advanced by Nisbet and Lagoy [23] were employed to assess the TEQ_{BaP} value.

$$TEQ_{BaP}=C_i \times TEF_i$$

where, "C_i" is a PAH congener, "i" is the sample concentration (µg/kg), "TEF_i"is the BaP value relative to the potency value published for each individual PAH.

Statistical Analysis

Descriptive statistics were performed on all samples accumulated from different lactation periods. The concentrations of compounds in milk were shown by arithmetic means with minimum and maximum values and standard error (SEM). All statistical analyses were carried out by Stata 12/MP4 statistical software (StataCorp LP, College Station, TX, USA). Duncan multi-scope test was used to compare mean of pollutants different lactation periods. Non detected data were presumed to be half the method limit of quantification for calculation of P value. A significant level of 0.05 was performed for all analyses.

Table 1. *Retention times and selected ion monitoring (SIM) ions for targeted organochlorine pesticides, polychlorinated biphenyls, polybrominated biphenyl ethers and polycyclic aromatic hydrocarbons*

Compounds	Retention Time (min)	SIM Ions	Compounds	Retention Time (min)	SIM Ions
Organochlorine pesticides (OCPs)			***Polycyclic aromatic hydrocarbons (PAHs)***		
α- HCH	12.17	181* 183 219	Naphthalene	5.97	128* 129 102
HCB	12.31	284* 286 282	Acenaphthylene	8.64	150* 153 152
Lindane	13.00	183* 181 219	Acenaphtene	9.07	154* 153
β- HCH	13.24	183* 181 219	Fluorene	10.58	166* 165 163
Heptachlor	15.76	272* 270 274	Phenanthrene	13.85	178* 152 176
Aldrin	17.58	263* 293	Anthracene	14.07	178* 176 152
α-Endosulfan	20.16	251* 253 183	Fluoranthene	19.86	202* 200 203
Dieldrin	22.45	239* 237 235	Pyrene	21.38	202* 200 201
p,p′-DDE	23.04	246* 248 318	Benzo(a)anthracene	29.34	228* 226 229
p,p′-DDD	25.20	235* 165 237	Chrysene	29.34	228* 226
o,p'- DDT	25.47	235* 165 237	Benzo(b)fluoranthene	34.44	252* 253
p,p′-DDT	27.34	235* 165 237	Benzo(k)fluoranthene	34.60	252* 253
Methoxychlor	29.68	227* 274	Benzo(a)pyrene	35.96	252* 253
Polychlorinated Biphenyls (PCBs)			Indeno(1,2,3,c,d)pyrene	41.20	276* 277
PCB28	15.43	186* 258 256	Dibenz(a,h)anthracene	41.47	278* 279
PCB52	16.64	220* 257 292	Benzo(g,h,i)perylene	42.29	276* 277
PCB101	21.36	254* 326 328	***Internal Standards for PAHs***		
PCB118	24.82	326* 256 254	Naphthalene d_8	5.92	108* 136
PCB153	25.84	290* 360 288	Acenaphthene d_{10}	9.02	164* 162
PCB138	27.01	290* 360 288	Phenanthrene d_{10}	13.75	188* 189
PCB180	30.13	324* 396 394	Chrysene d_{12}	29.19	240* 236
Polybrominated Diphenyl Ethers (PBDEs)			Perylene d_{12}	36.24	264* 260
PBDE 28	25.17	246* 248 406			
PBDE 47	30.36	486* 488 484			
PBDE 66	31.11	326* 486 484			
PBDE 85	36.63	404* 406 566			
PBDE 99	34.67	404* 406 566			
PBDE 100	33.58	404* 406 566			
PBDE 153	38.52	484* 486 482			
PBDE 154	37.26	484* 486 482			
PBDE 183	42.95	564* 562 566			
Internal Standards for OCPs, PCBs and PBDEs					
PCB30[a]	13.12	186* 258 256			
PCB153-C13	25.84	372* 302 374			
PCB209	36.10	498* 428 500			

** Quantifier ion; [a] Internal injection standard;* ***α-HCH:*** *alpha- Hexachlorocyclohexane;* ***HCB:*** *hexachlorobenzene;* ***β-HCH:*** *beta-hexachlorocyclohexane;* ***p,p′-DDE:*** *p,p′-dichlorodiphenyl dichloroethylen;* ***p,p′-DDD:*** *p,p′-dichlorodiphenyl dichloroethan;* ***o,p'- DDT:*** *o,p'-dichlorodiphenyl trichlorethane; p,p′-DDT: p,p′-dichlorodiphenyl trichlorethane*

Results

The average milk yield of 305 days in the cows included in the study was measured as 6146.32 kg/cow. While the milk fat content in the first period of lactation was 5.32%, it was 4.13% and 4.68% in the second and third periods, respectively. While average daily milk yield was 21.62 kg in the first period of lactation, it increased in the second period (average 26.11 kg) and decreased in the third period (average 25.46 kg).

The validation data of the method used to determine the contaminants in milk are shown in *Table 2* and *Table 3*. Mean recovery values were found to be between 88.7-105.4%, 90.8-96.9%, 86.5-96.2%, and 88.7-106.8% for OCPs, PCBs, PBDEs and PAHs, respectively, and repeatability and intermediate precision (RSD%) <13.2%

for all analytes. The LOQ values of the method in milk were determined as 0.82-2.28 µg/kg, 1.01-1.98 µg/kg, 0.37-0.92 µg/kg and 0.33-0.63 µg/kg for OCPs, PCBs, PBDEs and PAHs, respectively.

One or more contaminants were found in almost all milk samples (98%) during all lactation periods *(Table 4)*. For example, PCB28, PCB153, PCB180, *p,p'*-DDE, phenanthrene, anthracene, fluoranthene, pyrene, benz(a)anthracene, benzo(b)fluoranthene, benzo(k) fluoranthene, benzo(a)pyrene, indeno(1,2,3,c,d) pyrene, dibenz(a,h)anthracene and benzo(g,h,i)perylene were found together in the first lactation period, and acenaphthene was added to them in the second and third lactation periods.

PAH4 and benzo(a)pyrene were predominantly detected during the second lactation period. PAH4 was detected the least during the first lactation period, whereas benzo(a) pyrene was detected the least during the third lactation period. PAH16 and PAH8 groups are the most common compounds in all lactation periods with 98% and 74%, respectively *(Table 5)*.

Table 2. *Validation data of targeted organochlorine pesticides, polychlorinated biphenyls and polybrominated biphenyl ethers in cow milk*

Compound	Linearity (µg/kg)	Correlation Coefficient (r^2)	LOD (µg/kg)	LOQ (µg/kg)	Mean Recovery (%)	Repeatability (RSD%)	Intermediate Precision (RSD%)
Organochlorine pesticides (OCP)							
α- HCH	1-100	0.990	0.73	2.2	93.2±10.1	3.0	8.1
β- HCH	1-100	0.995	0.63	1.89	101.3±11.4	9.4	6.3
Lindane	1-100	0.991	0.68	2.05	105.4±9.5	7.7	9.7
HCB	1-100	0.997	0.51	1.54	95.6±7.8	2.3	8.6
HEP	1-100	0.995	0.45	1.36	96,4±9.4	10.1	7.3
α-Endosulfan	1-100	0.997	0.51	1.53	89.8±9.9	10.8	5.1
Aldrin	1-100	0.995	0.77	2.3	92.4±11.2	7.6	10.4
Dieldrin	1-100	0.995	0.76	2.28	88.7±10.1	11.0	9.9
p,p'-DDD	1-100	0.995	0.43	1.29	106.8±5.7	8.3	9.3
p,p'-DDE	1-100	0.998	0.31	0.94	97.9±6.5	2.1	6.8
o,p'- DDT	1-100	0.995	0.43	1.29	93.6±6.5	6.7	8.4
p,p'-DDT	1-100	0.999	0.27	0.82	95.4±9.2	7.2	6.8
Methoxychlor	1-100	0.998	0.54	1.62	97.4±8.4	9.9	7.3
Polychlorinated Biphenyls (PCBs)							
PCB28	1-100	0.993	0.44	1.33	93.6±6.5	8.6	2.2
PCB52	1-100	0.992	0.43	1.28	95.4±9.2	7.3	10.3
PCB101	1-100	0.994	0.48	1.45	95.6±7.7	5.1	9.7
PCB118	1-100	0.993	0.49	1.47	93.9±7.1	4.9	4.8
PCB153	1-100	0.997	0.34	1.01	90.8±8.2	2.2	13.2
PCB138	1-100	0.995	0.36	1.07	95.0±5.9	10.1	3.6
PCB180	1-100	0.991	0.66	1.98	96.9±9.3	6.8	5.3
Polybrominated Diphenyl Ethers (PBDEs)							
PBDE 28	1-100	0.993	0.12	0.37	93.3±3.8	4.9	8.1
PBDE 47	1-100	0.997	0.18	0.53	93.8±5.8	5.5	6.3
PBDE 66	1-100	0.994	0,20	0.61	96.2±6.9	7.3	9.7
PBDE 99	1-100	0.995	0.20	0.59	91.2±7.2	6.2	8.6
PBDE 85	1-100	0.993	0.16	0.47	92.5±3.9	4.8	7.3
PBDE 100	1-100	0,994	0.16	0.49	90.1±8.4	3.6	5.1
PBDE 153	1-100	0.991	0.17	0.52	92.5±3.9	5.3	4.9
PBDE 154	1-100	0.992	0.30	0.89	91.1±1.7	2.2	2.2
PBDE 183	1-100	0.990	0.31	0.92	86.5±8.3	10.1	10.1

Table 3. *Validation data of targeted polycyclic aromatic hydrocarbons in cow milk*

Compound	Linearity (µg/kg)	Correlation Coefficient (r^2)	LOD (µg/kg)	LOQ (µg/kg)	Mean Recovery (%)	Repeatability (RSD%)	Intermediate Precision (RSD%)
Naphthalene	1-100	0.988	0.21	0.63	88.7±10.1	3.0	9.4
Acenaphthylene	1-100	0.992	0.17	0.51	106.8±5.7	9.4	10.5
Acenaphthene	1-100	0.991	0.19	0.57	97.9±6.5	7.7	12.3
Fluorene	1-100	0.995	0.14	0.42	90.9±8.2	2.3	3.7
Phenanthrene	1-100	0.995	0.19	0.57	95.0±5.9	10.1	5.8
Anthracene	1-100	0.994	0.17	0.51	95.1±6.9	10.8	5.8
Fluoranthene	1-100	0.996	0.18	0.54	91.2±7.2	7.6	10.1
Pyrene	1-100	0.996	0.15	0.45	92.3±6.4	11.0	12.9
Benzo(a)anthracene	1-100	0.994	0.11	0.33	97.0±6.3	8.3	7.6
Chrysene	1-100	0.993	0.12	0.36	93.0±6.7	2.1	3.2
Benzo(b)fluoranthene	1-100	0.996	0.15	0.45	90.5±7.2	6.7	4.8
Benzo(k)fluoranthene	1-100	0.997	0.15	0.45	95.1±6.9	7.2	4.8
Benzo(a)pyrene	1-100	0.997	0.12	0.36	91.2±7.2	9.9	3.6
Indeno(1,2,3,c,d)pyrene	1-100	0.994	0.15	0.45	95.1±6.9	2.3	5.3
Dibenz(a,h)anthracene	1-100	0.992	0.13	0.39	91.2±7.2	4.9	2.2
Benzo(g,h,i)perylene	1-100	0.994	0.17	0.51	92.3±6.4	4.9	8.1

Table 4. *Frequency of milks with and without detected endocrine disruptor residues, and milks containing residues above maximum residue level (MRL) for milks collected during different lactation periods*

Lactation Period	No Residue Detected Sample Rate (%)	Sample Rate <MRL (%)	Sample rate >MRL (%)
First	2	50	48
Second	2	70	28
Third	2	60	38

Table 5*. Detected endocrine disruptors residues in milks collected during different lactation periods*

Compound	MRL (µg/kg)	1. Lactation Period		2. Lactation Period		3. Lactation Period		References for MRL
		D.R. (%)	>MRL (%)	D.R. (%)	>MRL (%)	D.R. (%)	>MRL (%)	
Total indicator PCBs*	40	56	10	56	8	56	12	[24,25]
Benzo(a)pyrene	1	18^{b}	4	22^{a}	0	16^{bc}	0	[24,25]
PAH4	1	50^{bc}	30	54^{a}	18	52^{ab}	22	[24,25]
PAH8	n.a.	74	n.a	74	n.a.	74	n.a.	-
PAH16	n.a.	98	n.a.	98	n.a.	98	n.a.	-
p,p'-DDE	20	28	4	28	2	28	4	[26]

** Total indicator PCBs are PCB28, PCB138, PCB153, and PCB180;* ***D.R.:*** *Detection rate;* ***n.a.:*** *Not applicable*
^{abc} *Differences between means with different letters in the same row are significant (P<0.05)*
PAH4: *Benzo(a)piren, benzo(a)anthrasen, benzo(b)fluoranthene and chrysene;* ***PAH8:*** *PAH4 + Benzo(k)fluoranthene, Benzo(g,h,i)perylene, Dibenz(a,h)anthracene and Indeno(1,2,3,c,d) pyrene.*

The highest total indicator PCBs and *p,p*'-DDE concentrations were observed in the first lactation period (19.91±10.1 and 2.74±1.32 µg/kg, respectively), followed by the third (17.93±8.9 and 2.35±1.6 µg/kg, respectively) and second lactation periods (15.79±7.93 and 2.2±1.3 µg/kg, respectively). Whereas, the highest total PAH concentrations (PAH8 and PAH16) were seen in the third lactation period (5.60±3.2 and 13.01±7.3 µg/kg, respectively), followed by the first (1.22±0.8 and 0.93±0.1 µg/kg, respectively) and second lactation periods

Table 6. *Mean concentrations of detected endocrine disruptors in milks collected during different lactation periods. (µg/kg) (Mean±SEM)*

Compound	1. Lactation Period		2. Lactation Period		3. Lactation Period	
	Range	Mean	Range	Mean	Range	Mean
Milk fat content	5.16-5.56	5.32%[a]	3.99-4.29	4.13%[bc]	4.52-4.87	4.68%[b]
Total indicator PCBs	<LOQ-125.63	19.91±10.1[a]	<LOQ-98.26	15.79±7.93[bc]	<LOQ-112.27	17.93±8.9[ab]
Benzo(a)pyrene	<LOQ-1.12	0.22±0.12	<LOQ-0.75	0.21±0.1	<LOQ-0.85	0.20±0.1
PAH4	<LOQ-8.24	1.22±0.8[a]	<LOQ-2.73	0.93±0.1[b]	<LOQ-3.18	0.93±0.1[b]
PAH8	<LOQ-11.41	3.43±1.54[b]	<LOQ-7.74	2.73±1.1[c]	<LOQ-21.85	5.60±3.2[a]
PAH16	<LOQ-29.76	11.19±7.6[b]	<LOQ-24.73	9.81±3.9[c]	<LOQ-32.11	13.01±7.3[a]
p,p'-DDE	<LOQ-32.03	2.74±1.3[a]	<LOQ-24.36	2.2±1.3[bc]	<LOQ-26.35	2.35±1.6b

LOQ: *Limit of quantification;* [abc] *Differences between means with different letters in the same row are significant (P<0.05)*

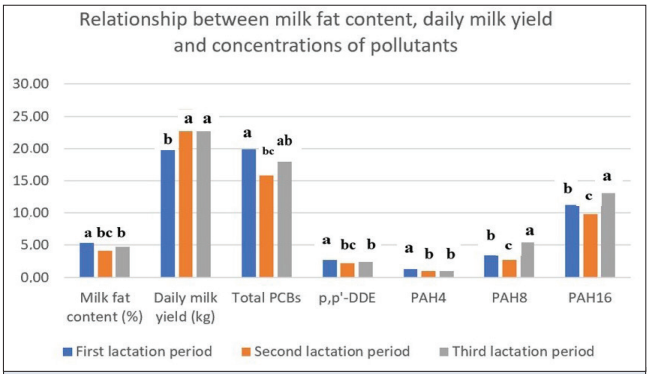


Fig 1. Relationship between milk fat content, daily milk yield and mean concentrations of endocrine disruptors (P>0.05)

(2.73±1.1 and 9.81±3.9 µg/kg, respectively) *(Table 6)*. However, the highest concentrations of total PCB, PAH4, and *p,p'*-DDE were detected in the first period, whereas the highest concentrations of PAH8 and PAH16 were found in the third period (P<0.05).

The first lactation period is seen as the period during which more lipophilic contaminants are most concentrated *(Ü, Fig. 1)*.

Finally, the risks of dietary exposure to pollutants detected in milk according to the lactation period in children and adults were evaluated. The calculated mean and maximum EDI, THQ and HI levels are shown in *Table 7*.

Discussion

According to studies conducted in Turkiye, milk yield of these cows is higher than some [27] and lower than some

Table 7. *Estimated daily intake and potential health risk of target endocrine disruptors via milk*

Individuals	Indicator	1. Lactation Period			2. Lactation Period			3. Lactation Period		
		PCBs	*p,p'*-DDE	PAH16	PCBs	*p,p'*-DDE	PAH16	PCBs	*p,p'*-DDE	PAH16
	ADI[a]	0.01	0.5	0.5	0.01	0.5	0.5	0.01	0.5	0.5
Adults	aveEDI[b]	0.0504	0.0069	0.0026	0.04	0.0056	0.0023	0.0454	0.006	0.0029
	aveTHQ[c]	5.04	0.0138	0.0052	4.0	0.0112	0.0046	4.54	0.012	0.0058
	aveHI[d]	5.06			4.02			4.53		
Children	aveEDI	0.181	0.0249	0.0092	0.1435	0.002	0.0083	0.163	0.0214	0.0105
	aveTHQ	18.1	0.0498	0.0184	14.35	0.004	0.0166	16.3	0.0428	0.0210
	aveHI	18.17			14.37			16.364		
Adults	maxEDI[e]	0.318	0.0807	0.0069	0.2489	0.0620	0.0058	0.2843	0.0673	0.00715
	maxTHQ[f]	31.8	0.1632	0.0138	24.89	0.1240	0.0116	28.427	0.1345	0.01430
	maxHI[g]	31.98			25.03			28.58		
Children	maxEDI	1.1420	0.2911	0.0245	0.8929	0,0022	0.0209	1.0206	0.2399	0.0259
	maxTHQ	114.202	0.5822	0.0489	89.292	0.0044	0.0417	102.062	0.4799	0.0518
	maxHI	114.83			89.34			102.59		

Note: *The risk assessment was done for PCB28, PCB153, and PCB180 among PCBs, and for phenanthrene, anthracene, fluoranthene, pyrene, benz(a)anthracene, benzo(b) fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3,c,d)pyrene, dibenz(a,h)anthracene, benzo(g,h,i)perylene, and acenaphthene among PAHs.*
[a] *Acceptable Daily Intake (µg/kg/day),* [b] *Average Estimated Daily Intake (µg/kg/day),* [c] *Average Target Hazard Quotients,* [d] *Average Hazard Index,* [e] *Maximum Estimated Daily Intake (µg/kg/day),* [f] *Maximum Target Hazard Quotients,* [g] *Maximum Hazard Index*

other studies [28]. According to the report of the World Holstein Federation in 2020, the milk yield of the same breed of cows in Turkiye is lower than in some European countries, Canada, USA and Japan (6.790-12.431 kg). However, it is higher than the milk yield of cows in New Zealand (average 4.492 kg) [29].

While the amount of milk increased, the fat content in the milk decreased. Bedö et al.[30] reported that there is an inverse relationship between milk yield and the fat content in milk, and that as the yield increases, the fat content decreases.

The analytical method recovery levels met the requirements of the SANTE guidelines [17]. According to the SANTE guideline, if the target analytes are at the ppb level, the recovery should be in the range of 50-120% and the RSD values should be less than 15%.

The proportion of the sample exceeding the maximum residue limit (MRL) was 48%, 10%, and 16% in the first, second, and third lactation periods, respectively *(Table 4)*. However, Aslam et al.[8] that total DDTs were detected in 85% of the samples collected from India. This present study shows that the occurrence of EDCs in cow's milk collected in Turkiye is less frequent compared to that in India. In total indicator PCBs (PCB28, PCB138, PCB153, and PCB180), the proportion of the milk sample exceeding the MRLs reported in the European Union (EU) and Turkish Food Codex (TFC) was 10%, 8%, and 12% in the first, second and third lactation periods, respectively. The MRL determined for milk and dairy products in the EU and TFC is 40 µg/kg lipid weight [24,25].

The PAH concentrations detected in the milk indicate the continued presence of PAH sources in the environment. This situation is similar to other studies; it was reported that PAH16 [12] and chrysene [10] were detected in all milk collected from Nigeria and Turkiye. Because PAHs can be found widely in the atmosphere as well as in aquatic and terrestrial systems [31]. PAH4 concentrations were found to be 1.22-0.8, 0.93-0.1 and 0.93-0.1 µg/kg in the first, second and third lactation periods, respectively, which are much higher than the levels found by Kaçmaz in raw milk (0.10-0.06 µg/kg) [10]. However, it is lower than the concentrations detected for PAH16 in free range cow milk collected from Nigeria.

There is no established MRL for PAHs in milk in the EU and TFC. However, the MRL values determined for PAHs, including infant formula and follow-on formula (including infant milk and follow-on milk), are 1 µg/kg lipid weight for benzo(a)piren and PAH4 [benzo(a)piren, benzo(a)anthrasen, benzo(b)fluoranthene and chrysene] [24,25]. When evaluated in terms of these limits, 4% of the milk taken in the first lactation period exceeded the MRLs determined for benzo(a)piren (1.05 and 1.12 µg/kg), while the proportion of the milk sample above the MRLs for PAH4 was 30%, 18%, and 22% for the first, second and third lactation periods, respectively. This may be because the legally established MRL for PAHs is quite low. The proportion of samples exceeding the MRL for PAH4 and benzo(a)pyrene was highest in the first period. This suggests that cows accumulate PAHs during the dry period and gradually eliminate them from their bodies.

There is no MRL in the EU and TFC for DDT. In Codex Alimentarius, the MRL for total DDTs in milk (sum of *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE and *p,p'*-DDD) was determined as 20 µg/kg [26]. Accordingly, the proportion of the sample exceeding the MRL for DDT was determined as 4%, 2%, and 4% for the first, second and third lactation periods, respectively *(Table 5)*.

Normally, the highest concentrations of all target EDCs would be expected during the first lactation period when the fat content is highest. However, the highest PAH concentration was detected in the third lactation period, when the fat content was lower than in the first lactation period. This can be explained by the octanol-water partition coefficient ($logK_{ow}$) of the compounds. As it is known, the higher the $logK_{ow}$ coefficient, the higher the lipophilicity of a substance. Lighter and more hydrophilic PAHs with lower $logK_{ow}$ values (<6) are more water soluble. Among the 16 PAHs, 10 compounds (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthen, pyrene, benzo(a)anthracene, chrysene) have a $logK_{ow}$ value below 6, whereas 6 heavy compounds have a logKow value above 6. The $logK_{ow}$ values for PCB28, PCB138, PCB153, PCB180 and *p,p'*-DDE are 5.69, 7.62, 7.62, 8.27 and 6.0, respectively [32].

The results indicate that the first lactation period is when lipophilic pollutants are most concentrated. This is clearly related to the fat content of the milk. As can be seen, as the fat content in milk increases, the rate of lipophilic EDCs also increases These results are in agreement with the study that found that OCPs detected in human milk colostrum were higher than those collected in other periods [31].

Commercial DDT is a pesticide consisting of a mixture of several isomers and is one of the most important metabolites of *p,p'*-DDE, formed by environmental degradation [33]. In this study, *p,p'*-DDE was found in 28% of the milks in each lactation period. According to the Agency for Toxic Substances and Disease Registry [34], *p,p'*-DDE is the main stable metabolite of DDT. In a study conducted among healthy Chinese mothers, it was reported that the half-life of *p,p'*-DDE in breast milk is 8 months [35]. While the half-life of DDT in soil is 2-15 years, it is more than 150 years in the aquatic habitat [34].

When considered in terms of risk assessment, it is seen that the first lactation period is the riskiest period, followed by

the third and second lactation periods. Additionally, it is seen that the EDI levels calculated for PCBs in the third lactation periods exceed the ADI values determined by the European Food Safety Authority. However, EDI levels remained below the ADI values for PAHs and OCPs. As mentioned before, THQ and HI levels above "1" are indicators of health risk. EDI, THQ, and HI levels are higher than those of adults because children consume more milk than adults and have lower body weight. Thus, in the study conducted with targeted EDCs, it is seen that there is no significant change in the elimination of EDCs in milk during the lactation period, as in aflatoxins, and it is excretion at almost the same rates in each period. Contaminated milk consumption poses a higher risk of children compared to outcomes.

It is seen that endocrine disrupting compounds are excreted more with milk in the first lactation period, followed by the third and second lactation periods. In the evaluation, it was concluded that the elimination was directly proportional to the amount of fat in the milk, and the concentration of endocrine disrupting compounds increased as the amount of fat in the milk increased.

Declarations

Availability of Data and Materials: All data generated or analyzed during this study are included in this published article.

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Conflict of Interest: The authors declared that there is no conflict of interest.

Ethical Statement: This study does not require ethics committee approval.

Author Contributions: Conceptualization, Funding acquisition, Methodology, Investigation, Formal analysis, Visualization: OK, IS, BYD, UTS, AF; collection of samples, UGB, MT, MAH, REH, AF; performing analysis, OK, UGB, MT, MAH, REH, AF; critical reviews of the manuscript, edition, and provision of important intellectual content and final version approval, all authors.

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