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STUDY OF DIOXIN LEVELS IN RAW MILK OF COWS AND GOATS IN POLAND

BADANIE POZIOMÓW DIOKSYN W SUROWYM MLEKU KRÓW I KÓZ W POLSCE

Abstract: Dioxins due to their toxic properties may pose health risks. These compounds comprise seven congeners of polychlorinated dibenzo-*p*-dioxins (PCDDs), 10 polychlorinated dibenzofurans (PCDFs) and 12 dl-PCBs. Because the main source of human exposure to dioxins is food of animal origin, the goal of the studies was to assess the levels of 29 congeners of dioxins in cow and goat's milk from Poland. High resolution gas chromatography coupled with high resolution mass spectrometry (HRGC-HRMS) were used to analyze more than 120 samples in the period of 2006 to 2011. In 94 samples of raw cow's milk an average concentration of PCDD/PCDFs was 0.84 ± 0.60 pg WHO-TEQ/g fat whereas the sum of 29 congeners was 1.35 ± 0.89 pg WHO-TEQ/g fat. The concentrations of dioxins and dl-PCBs were low (30% of limits for whole milk) and the samples met the requirements of the national and European legislation. Within the period of examinations two samples of cow's milk demonstrated the concentrations of PCDD/PCDFs at the action level (2 pg WHO-TEQ/g fat) whereas only one sample revealed the concentration exceeding the permissible content of PCDD/PCDFs (3 pg WHO-TEQ/g fat). Raw goat's milk contains generally higher concentrations than those found in cow's samples tested (PCDD/PCDFs and dl-PCBs) and the average concentration of PCDD/PCDFs was 1.49 ± 0.97 pg WHO-TEQ/g fat. Of the 33 examined goat's milk samples, 6 demonstrated the concentrations of dioxins at the "action level" whereas in 1 sample the content of dioxins exceeded the acceptable limits. The obtained results allow to conclude that cow and goat's milk contains generally low levels of dioxins. High concentrations of dioxins found could be a result of improper feeding of food producing animals.

Keywords: dioxins, furans, PCBs, milk, cows, goats

Introduction

Dioxin is the name generally given to a class of very toxic chemicals, the chlorinated dioxins and furans, formed as a by-product of the manufacture or burning of organic chemicals and plastics that contain chlorine. These compounds comprise seven congeners of *polychlorinated dibenzo-p-dioxins* (PCDDs), 10 *polychlorinated dibenzofurans* (PCDFs) and 12 *dioxin like PCBs* (dl-PCBs). Dioxins due to their toxic properties may pose health risks. They are everywhere in nature because they get carried by wind to all parts of the globe. Remain on pastures, plants and grains that form part of the food chain for food producing animals. Over 90% of human exposure to dioxin and related compounds occurs via food ingestion, primarily meats, fish and dairy products [1]. Like the other organochlorine compounds dioxin accumulates in the fat cells of the animals, and re-appears in meat and milk.

Food safety in addition to its nutritional value is one of the most important factors affecting human health. Monitoring of food is a fundamental way which enables estimation of care for the population. Since 2006 Polish General Veterinary Inspectorate has

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undertaken a program to monitor the national food supply for dioxin and dioxin-like compounds. Because the results of initial milk goat testing suggested higher dioxin levels in subsequent years the number of goat milk samples was increased [2].

This paper reports the survey for PCDD, PCDF and dl-PCB in raw cow and goat's milk collected from 2006 to 2011. The purpose of this survey was to assess the levels and congener profiles of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and dioxin-like polychlorinated biphenyls (dl-PCBs) in the general milk supply.

Materials and methods

Sample collections

For the official food control program raw cow and goat's milk samples were collected by veterinary inspection and sent to the National Veterinary Research Institute in Pulawy, Poland for chemical analysis. Number of yearly tested samples was set according to the Commission Recommendation 2006/794/EC [3]. Milk samples were collected also by national export dairies. Sample collections followed the requirements of the official food control standards [4]. From 2006 to 2011, 127 of raw milk samples were analyzed: 94 cow milk samples and 33 goat milk samples.

Chemical and instrumental analysis

Analyses were carried out in an accredited laboratory. The concentration of seventeen 2,3,7,8-substituted PCDDs and PCDFs congeners and 12 individual congeners dl-PCBs (non-*ortho* PCBs 77, 81, 126, 169 and mono-*ortho* PCBs 105, 114, 118, 123, 156, 157, 167, 189) were determined using gas chromatography coupled with high resolution mass spectrometry [4].

Briefly, chemical analysis based on a modified AOAC and EPA 1613 method. Two hundred and fifty milliliter of sample was freeze and lyophilized. Amount of 5 g dry milk was fortified with all of interest ^{13}C labeled standards and extracted by *accelerated solvent extraction* (ASE 300), with a solvent mixture of n-hexane/dichloromethane/methanol (5/2/1, v/v/v) under pressure of 10 MPa and temperature of 100°C. After removal of solvents the lipid content of each sample was determined gravimetrically. The samples were further cleaned up by column chromatography utilizing multi-layer acid silica column (22 and 44%). PCBs were separated from the dioxins/furans using Florisil column. Carbon/Florisil column was used for purification and separation mono-*ortho* from non-*ortho* PCB and carbon column was used for purification of dioxins/furans fraction. Collected fractions were concentrated to small volume and fortified by recovery/syringe standards before instrumental analysis. All compounds were analyzed using the isotope dilution method with GC connected with MAT 95XP (Thermo Scientific, Germany) operated in *electron impact* ionization (EI) mode. The chromatographic separation was achieved by splitless injection on a capillary column with length of 60 m, i.d. 0.25 mm and 0.1 µm thickness stationary phase film (Agilent J&W Scientific, USA). The MS was used in the SIM mode with the two most intensive ions of the molecular ion cluster monitored in specific windows. *Limits of Quantitation* (LOQs) and *Limits of Detection* (LODs) were

estimated during validation process (Tables 1 and 2). Laboratory procedures for measuring dioxins and dioxin-like compounds in animal fat matrices developed in our laboratory can be found in the paper of Lizak at al [2].

Analytical procedures yielded three synthetic figures per sample: the concentrations of PCDD/PCDFs, dl-PCBs and the total TEQ concentration (the sum of PCDD/PCDFs and dl-PCBs) using WHO-TEF_{1998}. Results were expressed in pg WHO-TEQ/g fat and interpreted according to the thresholds recommended by the European Union regulations [5].

Table 1

PCDD/PCDFs. Limit of detection (LOD) and quantitation (LOQ) in raw milk

Congener	**LOD [pg/g]**	**LOQ [pg/g]**
2378-TCDD	0.05	0.13
12378-PeCDD	0.05	0.13
123478-HxCDD	0.06	0.15
123678-HxCDD	0.06	0.15
123789-HxCDD	0.06	0.15
1234678-HpCDD	0.20	0.50
OCDD	0.50	1.25
2378-TCDF	0.05	0.13
12378-PeCDF	0.05	0.13
23478-PeCDF	0.05	0.13
123478-HxCDF	0.06	0.15
123678-HxCDF	0.06	0.15
234678-HxCDF	0.06	0.15
123789-HxCDF	0.06	0.15
1234678-HpCDF	0.10	0.25
1234789-HpCDF	0.10	0.25
OCDF	0.50	1.25

Table 2

Dioxin like PCBs. Limit of detection (LOD) and quantitation (LOQ) in raw milk

Congener	**LOD [pg/g]**	**LOQ [pg/g]**
3,3’,4.4’-TeCB (77)	1.00	2.50
3,4,4’,5-TeCB (81)	0.50	1.25
3.3’,4,4’,5-PeCB (126)	1.00	2.50
3,3’4,4’,5,5’-HxCB (169)	1.00	2.50
2,3,3’,4,4-PeCB (105)	0.20	0.50
2,3,4,4’,5-PeCB (114)	0.05	0.13
2,3’,4,4’,5-PeCB (118)	0.40	1.00
2’,3,4’,5-PeCB (123)	0.05	0.13
2,3,3’,4,4’,5-HxCB (156)	0.05	0.13
2,3,3’,4,4’,5’-HxCB (157)	0.05	0.13
2,3’,4,4’,5,5’-HxCB (167)	0.05	0.13
2,3,3’,4,4’,5,5’-HpCB (189)	0.05	0.13

Quality assurance (QA) and quality control (QC)

QA/QC was performed through the analysis of procedural blanks, a duplicate sample and a *standard reference material* (CRM) for each set of samples. Additionally, the method performance was assessed through *interlaboratory studies* (PT study). Expanded uncertainty was estimated at the level of interest and was established below 20% for both WHO-PCDD/PCDFs and WHO-PCDD/PCDF/dl-PCB. Concentrations of PCDD/PCDFs and dl-PCBs are expressed as pg WHO-TEQ/g on a lipid basis.

Results and discussion

Dioxin concentration from this study is shown in Tables 3 and 4. For all results, the lipid-adjusted concentrations were converted to the 2, 3, 7, 8-TCDD *toxic equivalence* (TEQ) using the WHO *Toxic Equivalence Factor* (WHO-TEF_{1998}). The results were calculated using upper-bound concentration, *ie* all analytes under the limit of determination would be equal to that limit [4].

Table 3

Dioxin levels in cow and goat's milk

Milk samples	pg WHO-TEQ/g fat				
	PCDD/PCDF ($x_{Av.}$ ± std dev.)	**Limit**	**dl-PCB** ($x_{Av.}$± std dev.)	**PCDD/PCDF/ dl-PCB** ($x_{Av.}$ ± std dev.)	**Limit**
Cow n = 94 Range	0.84 ± 0.60 0.37÷4.88	**3**	0.51 ± 0.35 0.12÷3.05	1.35 ± 0.89 0.67÷7.93	**6**
Goat n = 33 Range	1.49 ± 0.97 0.51÷4.64		0,87 ± 0,47 0.32÷2.07	2.35 ± 1.27 0.88÷5.08	

Table 3 displays the average concentration PCDD/PCDFs, dl-PCBs, average sum of PCDD/PCDF/dl-PCB in cow and goat's milk samples and the concentration range. The samples with higher level of tested compounds are presented in Table 4. The European Union (EU) has established maximum limits for these undesirable substances in food including milk, as well in feed aiming to ensure that food is safe for consumer. As specified in EC Regulation 1881/2006 the maximum permissible milk levels for human consumption are of 3 and 6 pg/g toxic equivalents (WHO-TEQ), for PCDD/PCDFs and PCDD/PCDFs plus dioxin-like PCBs compounds, respectively. The data here showed that the concentrations of both group of contaminants in most samples tested were below the legal limits. There were only two samples exceeding legal permit level (sample no. 049/2008 and 026/2008) and eight samples close to the action level (Table 4).

Dioxin and furans congeners were the dominant chemicals in milk tested (over 60% of the PCDD/PCDFs and 40% of dl-PCBs). The percentage of dominating congeners are presented in Figure 1. In goat and cow milk samples, highest contribution to the toxicity of the matrix resulted PCB-126 about 31%, 2,3,4,7,8-PeCDF - 21%, 1,2,3,7,8-PeCDD - 13%, and 2,3,7,8-TCDD - 11%.

Table 4

Samples exceeding permit levels. Results are presented as a mean X ± U (uncertainty)
*2006/88 EC, ** 1881/2006/ EC

	Material	Year	Sample No.	REGION	X ± U			Action level (A)* Maximum limit (ML)** [pg WHO-TEQ/g fat]		
					PCDD/F	dl-PCB	PCDD/F/ dl-PCB	PCDD/F	dl-PCB	PCDD/F/ dl-PCB
1	Cow milk	2007	076/2007	Podlaskie	3.09 ± 0.49	1.15 ± 0.26	4.24 ± 0.96	A - 2 ML - 3	A - 2	A - 4 ML - 6
2		2008	049/2008	Mazowieckie	**4.87 ± 0.78**	**3.05 ± 0.69**	**7.93 ± 1.8**			
3		2009	048/2009		2.88 ± 0.46	0.87 ± 0.20	3.75 ± 0.85			
4	Goat milk	2008	026/2008	Lubelskie	**4.64 ± 0.74**	**0.44 ± 0.10**	**5.08 ± 1.15**			
5		2009	067/2009		2.75 ± 0.44	0.67 ± 0.15	3.43 ± 0.78			
6		2010	012/2010	Malopolskie	2.86 ± 0.46	1.78 ± 0.40	4.64 ± 1.05			
7		2010	035/2010		2.67 ± 0.43	1.46 ± 0.33	4.14 ± 0.94			
8		2010	036/2010		2.64 ± 0.42	1.49 ± 0.34	4.13 ± 0.94			
9		2010	066/2010	Lubelskie	2.61 ± 0.42	0.93 ± 0.21	3.54 ± 0.80			
10		2011	036/2011		3.01 ± 0.48	2.07 ± 0.47	5.08 ± 1.15			

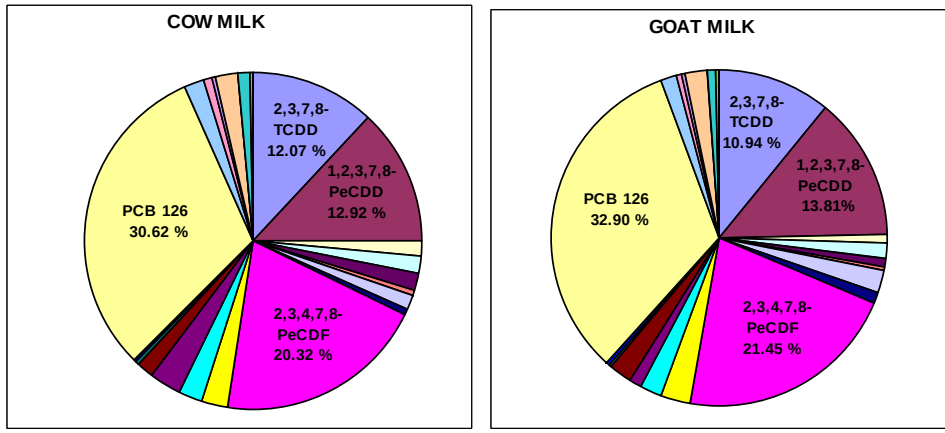

Fig. 1. Percentage of PCDD/F/dl-PCB congeners contribution in matrix toxicity. Cow and goat's milk

Milk fat and dairy foods contribute from 5.2 to 80% to the total daily exposure of general population, depending on the eating habits [6-8]. In this study it was found that dioxins, furans and dl-PCBs levels were very low, and only two milk samples were non-compliant with EC legislation. The higher values of dioxin in milk could be due to an increase in the burning of various materials, in particular chlorine-containing plastic wastes, burnt illegally near the pastures where the cows and goats graze as well as contaminated mixed feed or accidentally contamination. The milk levels were in agreement with those reported in other countries in areas with similar background levels of exposure. Congener levels and profiles of PCDDs, PCDFs, and PCBs in feedstuffs and milk samples that were compared describe congener-specific transfer behavior [9-17]. In 2010 EFSA has shown in Scientific Report "Results of the monitoring of dioxin levels in food and feed", that the

level of PCDD/PCDFs and dl-PCBs, found within national cow's milk surveys in Europe is about 1.05 pg WHO-PCDD/F-TEQ/g fat and 2.42 pg WHO-PCDD/F-PCB-TEQ/g fat [18].

In conclusion, this survey shows a low concentration of PCDD/PCDFs and dl-PCBs in cow and goat's milk in Poland. Higher concentrations of dioxins found could be also a result of improper feeding of food producing animals (contaminated feed). As food contamination is directly related to feed contamination the integrated approach must be adopted. Persistent, bioaccumulative and toxic pollutants, including dioxins, bioaccumulate through the food chain and ultimately result in low-level contamination in most animal fats. It is important to understand the levels in milk, as milk fat may be one of the highest dietary sources of dioxin exposure.

Monitoring of dioxins in milk could provide information for contamination of milk itself or other associated food (milk products). Analysis of milk also allows the opportunity to investigate geographic variability, relations between environment and area were milk is produced and distributed. It is essential to reduce dioxin and dioxin like compounds in food and feed.

References

[1] US EPA 1994 Estimating Exposure to Dioxin-Like Compounds. Exposure Assessment Group, Office of Health and Environmental Assessment, Office of Research and Development. EPA/600/6-88/005Ca-c. Review Draft. June; 1994.

[2] Lizak R, Maszewski S, Piskorska-Pliszczynska J. Occurrence and profile of polychlorinated dibenzo-p-dioxins, dibenzofurans, and dioxin-like polychlorinated biphenyls in Polish farm milk. Bull Vet Inst Pulawy. 2009;53(4):833-838.

[3] Commission Recommendation 2006/794/EC of 16 November 2006 OJ L 322/2.

[4] Commission Regulation 1883/2006 of 19 December 2006 OJ L 364/3.

[5] Commission Regulation 1881/2006 of 19 December 2006 OJ L 364/5.

[6] Djien Liem AK, Furst P, Rappe Ch. Exposure of populations to dioxins and related compounds. Food Addit Contam. 2000;17(4):241-259. DOI: 10.1080/026520300283324.

[7] Montaña MJ, Bescós L, Martí R, Ferrer C, Yusà V, Díaz-Ferrero J. Results from food monitoring program for dioxins in Comunitat Valenciana (Spain):2004-2005. Organohalogen Compd. 2006;1874-1877.

[8] Windal I, Vandevijvere S, Maleki M, Goscinny S, Vinkx C, Focant JF, Eppe G, Hanot V, Van Loco J. Dietary intake of PCDD/F and dioxin-like PCB for the Belgian population. Chemosphere. 2010;79:665-668. DOI: 10.1016/j.chemosphere.2010.01.031.

[9] Piskorska-Pliszczyńska J, Maszewski S, Lizak R, Warenik-Bany M, Wijaszka T. PCDD, PCDF and dl-PCB national food survey in Poland. Organohalogen Compd. 2010;72:142-145.

[10] Chovancova J, Kocan A, Jursa S. PCDDs, PCDFs and dioxin-like PCBs in food of animal origin (Slovakia). Chemosphere. 2005;61:1305-1311. DOI:10.1016/j.chemosphere.2005.03.057.

[11] Fernández MA, Gomara B, Bordajandi LR, Herreroa L, Abad E, Abalos M, Rivera J, González MJ. Dietary intakes of polychlorinated dibenzo-*p*-dioxins,dibenzofurans and dioxin-like polychlorinated biphenyls in Spain. Food Addit Contam. 2004;21:983-991. DOI: 10.1080/02652030400007286.

[12] Schuda L, Schaum J, Lorber M, Ferrario J, Sears R. Evaluation of dioxin in U.S. cow's milk. Organohalogen Compd. 2005;66:1928-1933.

[13] Windal I, Vandevijvere S, Maleki M, Goscinny S, Vinkx C, Focant JF, Eppe G, Hanot V, Van Loco J. Dietary intake of PCDD/Fs and dioxin-like PCBs of the Belgian population. Chemosphere. 2010;79:334-340. DOI: 10.1016/j.chemosphere.2010.01.031.

[14] Kim MK, Kim DG, Yun SJ, Son SW. Relationship of PCDD/Fs congener profiles between beef and raw milk in South Korea. Chemosphere. 2008;70:1563-1567. DOI: 10.1016/j.chemosphere.2007.08.060.

[15] Esposito M, Serpe FP, Cavallo S, Pellicanò R, Gallo P, Colarusso G, D'ambrosio R, Baldi L, Iovane G, Serpe L. A survey of dioxins (PCDDs and PCDFs) and dioxin-like PCBs in sheep and goat milk from Campania, Italy. Food Addit Contam Part B. 2010;3(1):58-63. DOI: 10.1080/19440040903527350.

[16] Durand B, Dufour B, Fraisse D, Defour S, Duhem K, Le-Barillec K. Levels of PCDDs, PCDFs and dioxin-like PCBs in raw cow's milk collected in France in 2006. Chemosphere. 2008;70:689-693. DOI: 10.1016/j.chemosphere.2007.06.057.
[17] Kiviranta H, Hallikainen A, Ovaskainen ML, Kumpulainen J, Vartiainen T. Dietary intakes of polychlorinated dibenzo- p-dioxins, dibenzofurans and polychlorinated biphenyls in Finland. Food Addit Contam. 2001;18(11):945-953. DOI: 10.1080/02652030110057134.
[18] Scientific Report of EFSA Results of the monitoring of dioxin levels in food and feed. EFSA Journal, 2010; 8(3),1385. DOI: 10.2903/j.efsa.2010.1385.

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Abstrakt: Dioksyny ze względu na właściwości toksyczne mogą stanowić zagrożenie dla zdrowia. Do grupy tej należy 7 kongenerów *polichlorowanych dibenzo-p-dioksyn* (PCDD), 10 *polichlorowanych dibenzofuranów* (PCDF) oraz 12 dl-PCB. Ponieważ głównym źródłem narażenia ludzi na dioksyny jest żywność pochodzenia zwierzęcego, celem pracy była ocena poziomów 29 kongenerów dioksyn w mleku krowim i kozim pochodzącym z terenu kraju. Metodą wysokorozdzielczej chromatografii gazowej sprzężonej z wysokorozdzielczą spektrometrią mas (HRGC-HRMS) analizowano w latach 2006-2011 ponad 120 próbek. W 94 próbkach surowego mleka krowiego średnie stężenie PCDD/PCDF wynosiło 0,84 ± 0,60 pg WHO-TEQ/g tł., zaś suma 29 kongenerów 1,35 ± 0,89 pg WHO-TEQ/g tł. Zawartość dioksyn i dl-PCB była niska (do 30% dopuszczalnych limitów dla mleka pełnego) i próbki spełniały wymagania przepisów krajowych i unijnych. W badanym okresie jedynie w dwóch próbkach mleka krowiego stwierdzono stężenie na poziomie progu podejmowania działań (2 pg WHO-TEQ/g tłuszczu) oraz w 1 próbce stężenie przekraczające dopuszczalną zawartość PCDD/PCDF (3 pg WHO-TEQ/g tłuszczu). Surowe mleko kozie generalnie zawierało wyższe niż krowie stężenia badanych związków (zarówno PCDD/PCDF, jak i dl-PCB) i średnia dla PCDD/PCDF wynosiła 1,49 ± 0,97 pg WHO-TEQ/g tłuszczu. W 6 spośród 33 badanych próbek mleka koziego stężenia dioksyn znajdowały się na poziomie „action level", zaś w jednej próbce zawartość dioksyn przekroczyła dopuszczalne limity. Uzyskane wyniki pozwalają na stwierdzenie, że mleko zawiera niskie poziomy dioksyn, jakkolwiek zdarzały się incydenty wynikające najprawdopodobniej z błędów żywieniowych zwierząt hodowlanych (karma zawierająca dioksyny).

Słowa kluczowe: dioksyny, furany, PCB, mleko, krowy, kozy