PRESENCE OF DIOXINS IN MILK MARKETED IN MONTERREY, NUEVO LEON, MEXICO

Naccha L[1], Alanis G[2], Torres A[1], Abad E[3], Ábalos M[3], Rivera J[3], Heyer L[4], Waksman N[1]

[1]Universidad Autónoma de Nuevo León. Facultad de Medicina-Departamento de Química Analítica. Avenida Madero y Dr. Aguirre Pequeño S/N Col. Mitras Centro, Monterrey, Nuevo León, México; [2]Facultad de Biología. Cd. Universitaria. Av. Pedro de Alba s/n. San Nicolás de los Garza, Nuevo León, México; [3] Laboratory of Dioxins, Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Jordi Girona Salgado 18-26, 08034 Barcelona, Spain; [4]Unidad Académica Multidisciplinaria Agronomía y Ciencias, Centro Universitario Victoria, Cd. Victoria Tamaulipas, Mexico.

Introduction

The polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are commonly known as "dioxins". Dioxins are persistent polychlorinated global pollutants, widely distributed in the environment, which tend to accumulate in fatty foods. They have been considered the most toxic man-made compounds. The most toxic is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD).[1,2]

Depending on the exposure, different effects result, including chloracne, as illustrated by the known case of Ukraine president Yushchenko in 2004, metabolic and hormonal changes, alteration of the reproductive system, fetal effects, immune and hepatic damages, teratogenicity, and carcinogenicity.[3] PCDDs and PCDFs are found in environmental and biological matrices as complex mixtures.

Human exposure to dioxins can happen through different ways: air inhalation, dermal absorption, and food intake. WHO estimates that 90% of current human exposure to dioxins is through their diet, mainly from meat, fish, and dairy products.[4]

Special efforts have been made to analyze these compounds in foods as well as in the environment; however, because of the complexity and high costs of these determinations, only some industrialized countries can afford to develop and apply the appropriate methods in samples of different origins.

In contrast to the industrialized countries, information in Mexico about dioxin contamination is scarce. Jiménez *et al*. (2005) reported the presence of organochloride compounds (including PCDDs/PCDFs) in birds from Baja California.[5] Cañedo and Macias (2007) reported the presence of PCDDs/PCDFs in fish from four different regions in México.[6] Naccha *et al.* (2010) reported the presence of dioxins in beef samples from Mexico.[7] However, until now, no reports about the presence of dioxins and furans in milk had been available. Moreover, no analytical method had been established to determine dioxin levels in the aforementioned matrix.

The objective of the present research was to develop an analytical method using gas chromatography/low-resolution mass spectrometry (GC/LRMS) to quantify dioxin levels in milk marketed in Monterrey, Nuevo Leon, Mexico.

Materials and methods

Samples

Samples were taken at random from different supermarkets in the Metropolitan Area of Monterrey. It acquired 10 retail milk samples from different batches of the largest consumer brand.

Analysis

The analyses were performed according to the minimum requirements described in the USEPA 1613 method, after some minor modifications being applied.[8,9,10,11] Briefly, the method consists of an extraction step and a cleanup step that comprises purification and fractionation. Prior to sample extraction, fat was extracted from milk, for which weighed 200 mL of milk and transferred to a separatory funnel, 2000 ml, were added 2 g of sodium oxalate to break

the emulsion and facilitate the release of fat, stirred for 20 seconds, then were added 200 mL of methanol and agitated for 10 seconds. Then were added 200 mL of diethyl ether and the samples were spiked with known amounts of mixtures of $^{13}C_{12}$-PCDD/Fs (5 µL EPA-1613LCS, Wellington Laboratories Inc., Guelph, Ontario, Canada) and shaken for 30 seconds, at this stage begins the separation of fat. Finally were added 200 mL of petroleum ether and stirred for 60 seconds. It let stand for 24 hours. The organic phase was received in a flask. There was a second extraction into the aqueous phase but only from the extraction step with diethyl ether. The filtrate was concentrated and then the fat was weighed. Concentrated sulfuric acid was used to remove interferences. Details of cleanup step process and quality control are given in previous Naccha *et al*. work. [7]

Extracts were analyzed with an Agilent Technologies (Wilmington, DE) Model 6890N gas chromatograph coupled with a 5973N mass selective detector (MSD) using an electron impact source and quadrupole mass analyzer.

Chromatographic separation was achieved in a HP-5ms (Agilent Technologies) capillary column, (60 m × 0.25 mm i.d. × 0.25 µm), using helium as the carrier gas (3.4 mL min^{-1}) in splitless injection mode (2 µL).

The temperature program was: 180 °C (1 min), increasing to 200 °C at 25 °C min^{-1}, then to 270 °C at 3 °C min^{-1} (44 min). The injector temperature was 300 °C, ion source temperature, 200 °C, and the interface and quadrupole temperatures, 270 °C and 120 °C, respectively, with ionization energy 70 eV. The resolution power was 1000, and the mass interval was 304–472 m/z. Data was acquired in SIM mode.

Quantification was carried out by the isotopic dilution method using a mixture of $^{13}C_{12}$-labeled and unlabeled standards.[12] Relative response factors were calculated for each individual congener by analysis of five different mixtures of labeled and unlabeled standards. Results are reported in pg WHO-TEQg^{-1} fat weight.

Complementary analysis was also achieved by high resolution mass spectrometry coupled to high resolution mass spectrometry (HRGC/HRMS). Instrumental analysis has been extensively documented in the literature.[9,12]

Results and discussion:

In this paper, all results come from 10 samples of different batches of the same brand. The concentrations of individual congeners ranged from not detected (ND) to 9.82 pg / g lipid weight basis. Table 1 shows the average contents and the range of each of the congeners found in 10 samples expressed in pg / g fat. All values were adjusted for fat content of the sample. To calculate the average of each congener was also taken into account the samples which were not detected by assigning zero value. The LOD of individual congeners ranged from 0.14 to 0.71 pg / g fat.

Figure 1 shows the maximum values reported for each congener. This also was similar to that reported in industrialized countries Netherland and Belgium.[13,14] The 2,3,7,8-TCDD was not detected. The 1,2,3,6,7,8-HxCDD was the most abundant, with a range of 2.01 to 9.82 pg / g fat, with an average of 2.39 this value is greater than that reported by Liem *et al.* (1990) with an average of 2 pg / g fat.[13]

Using the concentrations obtained for each congener and their TEF values, pg WHO-TEQ g-1 fat values were calculated. Levels found are between 0.16 and 2.81 pg WHO-TEQ g-1 fat, with a mean value of 1.95, see Table 1. These values are below the maximum limit allowed by the EU of 3 pg WHO-TEQ PCDDs/PCDFs /g milk fat,[15] therefore any sample is considered contaminated.

Some of the processed samples were complementarily analyzed by high resolution gas chromatography coupled with high resolution mass spectrometry (HRGC/HRMS). With this equipment reported values between 0.32 and 0.98 pg WHO-TEQ of PCDDs / PCDFs / g fat, while in our laboratory has reported values of 1.59 to 2.69 for the same samples. Despite that we obtained higher concentrations than in the reference laboratory, in both cases the samples are not contaminated as established by the EU. Some congeners detected in HRGC / HRMS in very small concentrations were not detected in GC / LRMS, but this does not alter the outcome.

HRGC/HRMS has the advantage of high selectivity, sensitivity, specificity, and simplicity, without the need for excessive purification, because it can discriminate between interferences with similar masses. On the other hand, the specificity of an analytical method based on LRMS can be improved by the selection of appropriate purification steps. In the present case, the technique used for purification and fractionation was selective on account of the use of different adsorbents. Therefore, good results could be obtained to identify contaminated samples.[11]

Table 1. Mean of PCDDs/PCDFs levels (pg/g fat weight) and WHO-TEQ (pg/g fat weight) in milk samples

Congeners	Mean	S.D.	Range	Positives
2,3,7,8-TCDF	0,35	1,10	ND-3,49	1
1,2,3,7,8-PCDF	-	-	ND	0
2,3,4,7,8-PCDF	1,16	1,46	ND-3,40	5
1,2,3,4,7,8-HxCDF	0,68	1,70	ND-5,34	2
1,2,3,6,7,8,-HxCDF	-	-	ND	0
2,3,4,6,7,8-HxCDF	0,26	0,54	ND-1,28	2
1,2,3,7,8,9-HxCDF	-	-	ND	0
1,2,3,4,6,7,8-HpCDF	1,51	1,54	ND-5,11	8
1,2,3,4,7,8,9-HpCDF	-	-	ND	0
OCDF	-	-	ND	0
2,3,7,8-TCCD	-	-	ND	0
1,2,3,7,8-PCCD	0,88	1,16	ND-2,80	4
1,2,3,4,7,8-HxCDD	0,83	1,20	ND-3,47	4
1,2,3,6,7,8-HxCDD	2,39	3,21	ND-9,82	5
1,2,3,7,8,9-HxCDD	0,13	0,21	ND-0,46	3
1,2,3,4,6,7,8-HpCDD	0,53	0,81	ND-2,60	5
OCDD	0,15	0,47	ND-1,50	1
pg WHO-TEQ/g grasa	1,95	0,79	0,16-2,81	-

(n=10). ND= No detected

The results here presented, demonstrate that GC/LRMS can be used for the screening of dioxins in milk. As far as we know, this is the first report of dioxins analysis in milk in Mexico. This method could be used for monitoring these contaminants in other dairy products.

Acknowledgements:
We wish to thank SAGARPA – CONACYT 2002-C01-1140, CONCYTEC, and PAICYT CA440 – 04 for financial support.

References:

1. Huwe JK. (2002); *J. Agric. Food. Chem.* 50:1739-1750.
2. World Health Organization (WHO), International Agency for Research on Cancer. IARC monographs on the evaluation of carcinogenic risk to human. Polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans, vol 69. Lyon 1997.
3. Lundqvist C, Zuurbier M, Leij M, Johansson C, Ceccatelli S, Saunders M, Schoeters G, Tusscher GT, Koppe J.(2006*); Acta Pediatrica.* 95:55-64.

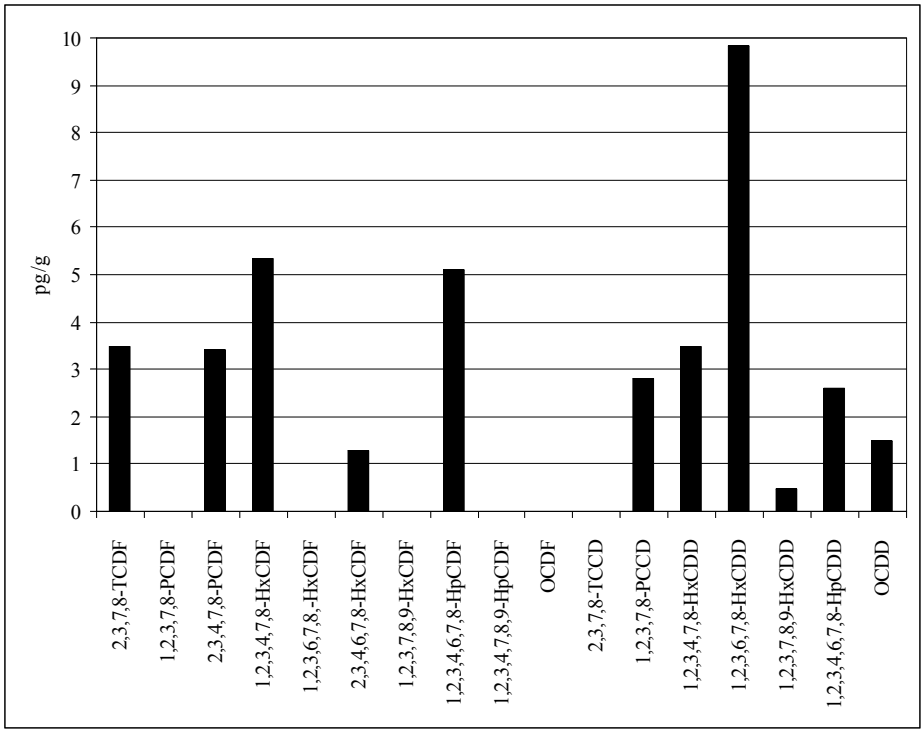

Figure 2. PCDDs/PCDFs congeners distribution in milk samples (data expressed in pg/ g fat).

4. World Health Organization (WHO). (1998); Environmental Health Criteria Series (EHCS). No. 205. Polybrominated dibenzo-p-dioxins and dibenzofurans. Assessment of the health risk of dioxins: re-evaluation of the Tolerable Daily Intake (TDI). Geneva, Switzerland.
5. Jiménez B, Merino R, Rodríguez-Estrella R, Gómez G, Rivera L, Gonzáles JM., Abad E, Rivera J. (2005); *Environ. Pollut.* 133:139-146.
6. Cañedo-López Y and Macias-Zamora JV. (2007); *Ciencias Marinas*. 33: 217-227.
7. Naccha L, Alanis G, Torres A, Abad E, Ábalos M, Rivera J, Heyer L, Morales A, Waksman N. (2010); *Food Add Contam. Part B* 34(1):64-72
8. United States Environmental Protection Agency (USEPA). (1994); Method 1613: Tetra-through Octa-Chlorinated Dioxins and Furans by isotopic dilution HRGC/HRMS. Washington, DC.
9. Abad E, Caixach J, Rivera J. (1997); *J. Chromatogr. A.* 786:125-134.
10. Abad E, Sauló J, Caixach J, Rivera J. (2000); *Journal of Chromatography A*. 893:383-391.
11. Eljarrat E, Casanovas J, Muro R, Huguet X, Caixach J, Rivera J. (1995); *Química Analítica.* 14:89-95.
12. Ábalos M, Parera J, Abad E, Rivera J. (2008); *Chemosphere.* 71:1115-1126.
13. Liem AKD, Fürst P, Rappe C. (2000); *Food Add Contam*. 17:241-259.
14. Focant J.-F, Eppe G, Pirard C, Massart A.-C, André J.-E, De Pauw E. (2002); *Chemosphere.* 48:167-179.
15. Commission Regulation (EC) N° 199/2006 of 3 February 2006 amending Regulation (EC) N° 466/2001 setting maximum levels for certain contaminants in foodstuffs as regards dioxin and dioxin-like PCBs. Official Journal of the European Union L 32:37.