HIGH RESOLUTION ORIBITAL TRAPPING MASS SPECTROMETRY MEASUREMENT OF PERSISTENT ORGANIC POLLUTANTS IN COW's MILK

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Introduction

Polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) are classes of related environmental pollutants produced through diverse sources or industrial use and are known to strongly bio-accumulate and produce multiple toxic endpoints in animals and humans[1,2]. The 2,3,7,8-tetrachlorodibenzobenzo-p-dioxin (TCDD) isomer has been classified as a group 1A human carcinogen by IARC[2]. PCBs with dioxin-like properties are often measured with the PCDD/Fs. These three classes of chemicals are listed as persistent organic pollutants (POPs) by the Stockholm convention designated for world-wide reduction in the environment[1].The primary route of non-occupational human exposure to PCDDs and PCDFs is foods and animal feeds[3-5]. Monitoring programs established are aimed to aid efforts to control contamination in foods and animal feeds[5]. Central to these efforts are confirmatory methods capable of measuring these chemicals at low concentrations in foods. We investigated an orbital trapping mass spectrometer for the measurement of PCDD/Fs and PCBs at low concentrations in whole cow's milk.

Materials and methods

Two calibration curves were constructed. One curve was prepared using Wellington Laboratory mixtures of all 17 native 2,3,7,8-substituted congeners and all 17 $^{13}C_{12}$ labeled standards at concentrations of 0.1, 0.2, 1, 5, 10, 20 and 40 ng/mL for PCDD/F along with PCBs 77, 81, 126 and 169 at 1, 2, 5, 20, 100, 1000 ng/mL used only with Maryland milks. The second curve uses the Cambridge Isotope laboratories (CIL) mixtures, EDF-9999-1-5, diluting them 10 fold resulting in concentrations of 0.25, 1, 5, 20 and 100 ng/mL. PCBs 77, 81, 126 and 169 were added at 0.25, 1, 5, 20 and 100ng/mL. Whole cow's milks from 3 commercial sources in Arkansas, USA were fortified 6 times near the maximum level allowable by EU (2.5 pg PCDD/F TEQ/g fat) and 6 unfortified milk portions (90-100g each) 2 each from the 3 sources were also analyzed. Whole milk samples from 3 commercial sources in Maryland USA, were collected and measured. The Arkansas whole milks were extracted using a Gerhardt Hydrotherm™/Soxtherm™ using 90-100g milk, 20 mL ethanol and 0.5g sodium oxalate and processed with 2M sulfuric acid using a Hydrotherm™/Soxtherm™ system. Fat obtained was removed by GPC followed by multi-layered silica columns. Final PCDD/F separation was performed with HPLC employing a 2-(1-pyrenyl)ethyl silica (PYE) column. The PCDD/Fs fraction was reconstituted in 10 µL of nonane[6]. Whole milks from Maryland were extracted using hexane/diethyl ether after addition of ethanol and sodium oxalate following a previously described method[4]. Whole milk extracts from Arkansas milks were measured using a Micromass AutoSpec Premier high resolution mass spectrometer a double focusing instrument (sector) with an Agilent 7890A gas chromatograph(GC) equipped with a 4 mm ID split/splitless liner with 1µL injections onto a 60 M x 0.25 mm ID DB-5ms column[6]. All milk extracts from Arkansas and Maryland, USA were measured using a TRACE 1300 GC coupled to a Q-Exactive mass spectrometer (QE) (ThermoFisher, San Jose, CA, USA). The GC column was a 40 M x 0.18 mm DB-5ms with a split/splitless injector, 4mm ID Restek sky liner. Two µLs were injected splitless at 300° C. GC was programmed from a starting temperature of 120 ^{0}C which was held for 2 min prior to ramping at 20 °C /min. to 200 °C. It was then

ramped at 5 ºC /min. to 240 ºC and held for 12 min. before ramping at 10º/min. to 280º C with a final hold time of 10 min. The QE was operated in full scan SIM mode with 6 quadrupole filters. Settings used were 285-340, 300-340, 335-354, 335-380, 369-410, 435-480 *m/z*. An offset of -2 V was set between the source and the C-Trap. Other electron ionization source parameters were default including 70 eV, 50 µamps emission current, 1E6 target, AGC. No lock masses were used. Transfer line and source temperatures were 250 ºC and 300 ºC, respectively.

Results and Discussion

Table 1 provides the TEQs measured by the sector and QE for PCDD/Fs, PCB-81, 77, 126 and 169 in Arkansas milks. The TEQs for the PCBs agree closely with a range of 0.05-0.08 pg/g fat for the HRMS-sector and 0.05-0.07 pg/g fat for the QE. However, the PCDD/F TEQs were lower with the QE (0.06-0.22 pg/g fat upper bound) than the sector (0.19-0.38 pg/g fat). The sector identified PeCDD and 2,3,4,7,8-PeCDF in all milks, while QE found only 2 of 6 milks extracts with these congeners identified (ion ratios within ±15% of theoretical). The QE did not detect any signals for TCDD, TCDF or 1,2,3,7,8-PeCDF in the unfortified milk unlike the sector which measured these congeners in nearly all milks (data not shown). Five of the six Arkansas milks by QE failed the upper bound/lower bound criteria[7] of ±30% for the PCDD/F TEQ. For milks collected in Maryland, USA, PCDD/Fs and PCBs were measured using the QE only (**Table 2**). PCDD/F TEQs were 0.19-0.54 pg/g fat the QE (**Table 2**) in a similar range to Arkansas milks measured the by the sector (**Table 1).** All the PeCDD/Fs and HxCDD/Fs were measurable in all Maryland milk by the QE with isotope ratios ±15%, except 1,2,3,7,8,9-HxCDF(not detected). All these milks passed the upper bound/lower bound criteria. All milk PCDD/F TEQs from the Arkansas or Maryland were 5-12 times below the maximum limit set by the EU. The results from the two instruments were comparable for the milks fortified near the current EU maximum limit for PCDD/Fs (2.5 pg/g fat TEQ). Mean congener amounts were between 76-116% of theoretical with 1-28 relative standard deviations (RSDs) using the QE while the sector was 99-112% and 1.5-13 RSDs. TCDD, TCDF 1,2,3,7,8-PeCDF amounts by the QE were lower due to evaporation losses during and after multiple injections in the sector and APGC (**Table 3**). The mean amounts of PCDD/F TEQ measured in the fortified milks were nearly the same using either instrument (9.3 and 9.9 pg) (**Table 3**). The milk cleanup aided QE measurements in the fortified milks by maintaining low noise and interference in the TIC. Nevertheless, HpCDD was observed co-eluting with an unknown compound that produced a 0.8 minute wide response in the TIC of most milk samples. The peak was 1000 times larger than the labeled standard with a fragment ion at *m/z* 423.17 close to the native HpCDD mass of 423.7767. The presence of this compound may be responsible for a lower than expected result for one fortified HpCDD measurement increasing that congener's RSD.

References:

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Table 1. Duplicate measurements for milks collected from three commercial sources (1, 2 and 3) in Arkansas (Ar), USA. Toxic equivalency (TEQ) expressed in pg/g fat for PCDD/F (D/F) and PCB-TEQ shown separately. TEQs are upper bound, split extracts.

	Sector			QE		
Milk IDs	**D/F-TEQ**	**PCB-TEQ**	**Total TEQ**	**D/F-TEQ**	**PCB-TEQ**	**Total TEQ**
Ar-S1	0.19	0.08	0.28	0.10	0.07	0.16
Ar-S1	0.22	0.09	0.31	0.09	0.07	0.13
Ar-S2	0.32	0.05	0.37	0.19	0.05	0.21
Ar-S2	0.32	0.05	0.37	0.17	0.05	0.20
Ar-S3	0.32	0.06	0.39	0.22	0.06	0.28
Ar-S3	0.38	0.06	0.44	0.16	0.05	0.19
Means	0.29	0.07	0.36	0.15	0.06	0.20

Table 2. Repeat measurements from whole milk collected from three commercial sources (A, B and C) in Maryland (MD), USA. Toxic equivalency (TEQ) expressed in pg/g fat for PCDD/F (D/F) and PCB-TEQ shown separately. PeCDD/Fs and HxCDD/Fs were measured in all milks with ion ratios ±15% of theoretical. TCDD/Fs were measurable in 4 of 7 milks with ion ratios ±15% of theoretical.

	QE		
Milk IDs	**D/F-TEQ**	**PCB-TEQ**	**Total TEQ**
MD-A1	0.36	0.22	0.57
MD-A2	0.33	0.10	0.43
MD-B1	0.40	0.17	0.57
MD-B2	0.33	0.09	0.39
MD-B3	0.19	0.07	0.24
MD-C1	0.34	0.10	0.43
MD-C2	0.54	0.17	0.71
Means	0.36	0.13	0.48

Table 3. Mean measurements for whole milk fortifications in pg, relative standard deviations (RSD) and % of theoretical spike (n=6) whole cow's milk from Arkansas, USA (90-100 g portions) fortified with 0.8, 4 or 8 pg/sample depending on the specific congener (see below) as measured by QE and Sector. For TCDD/F, PCB-77, 81 n=3 and 1,2,3,7,8-PeCDF n=4 with QE. Split extracts

	QE			**Sector**			
	Mean*	**RSD**	**% Theor.**	**Mean***	**RSD**	**% Theor.**	**Fortified Amount(pg)**
2,3,7,8-TCDF	0.61^	1.0	76	0.83	7.1	103	0.8
1,2,3,7,8-PeCDF	3.5^	17	86	4.3	2.2	108	4.0
2,3,4,7,8-PeCDF	4.3	4.4	109	4.2	2.1	105	4.0
1,2,3,4,7,8-HxCDF	3.9	3.1	97	4.3	2.3	108	4.0
1,2,3,6,7,8-HxCDF	3.9	3.4	97	4.2	3.8	104	4.0
2,3,4,6,7,8-HxCDF	4.2	1.7	104	4.3	2.7	109	4.0
1,2,3,7,8,9-HxCDF	3.8	5.0	95	4.1	2.8	102	4.0
1,2,3,4,6,7,8-HpCDF	4.0	11	100	4.2	2.2	104	4.0
1,2,3,4,7,8,9-HpCDF	4.0	2.5	99	4.4	1.5	110	4.0
OCDF	6.7	4.6	84	9.1	6.7	114	8.0
2,3,7,8-TCDD	0.64^	7.7	80	0.86	3.4	108	4.0
1,2,3,7,8-PeCDD	4.3	5.4	109	4.4	5.3	109	4.0
1,2,3,4,7,8-HxCDD	4.2	5.9	104	4.3	3.8	107	4.0
1,2,3,6,7,8-HxCDD	3.7	15	92	4.2	5.9	106	4.0
1,2,3,7,8,9-HxCDD	4.1	7.4	104	4.5	5.1	112	4.0
HpCDD	4.6	22	116	4.0	3.8	99	4.0
OCDD	7.7	28	96	8.5	7.7	106	8.0
TEQ pg spike	**9.3**			**9.9**			**9.1**
PCB-81	4.1^	21	102	4.6	4.3	114	4.0
PCB-77	4.0^	8.3	105	4.2	13	104	4.0
PCB-126	4.1	9.3	101	4.6	4.0	116	4.0
PCB-169	4.2	2.1	108	4.4	3.3	110	4.0

*Corrected for unfortified milk

^ n=4 1,2,3,7,8-PeCDF; n=3 for TCDD/F, PCB-81, 77