LEVELS OF PCDD/Fs AND PCBs IN CAMEL MILK (CAMELUS BACTRIANUS AND CAMELUS DROMEDARIUS) FROM KAZAKHSTAN

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Introduction

Kazakhstan is a large country (2,717,300 km^2) comprising only around 15.5 10^6 inhabitants and that is made of approximately 80% of desert and steppe. Around 40% of the total Kazakhstan population leaves in rural regions and depends on traditional breeding of livestock. The particularity of Kazakhstan is to breed horses and camels in addition to cows for dairy production. There are however, to our best knowledge, no national statistical data available for horse and camel milk production, although could represent more than 25% of the whole consumed milk in the country. The milk from those two non-conventional dairy animals is generally transformed and consumed in traditional fermented drinks called kumis for horses and shubat for camels. Those liquid preparations can somewhat be compared to the cheese preparation in Western European countries. Certain places in Central Asian Republics are known to be potentially contaminated by toxic compounds like radio nuclides, heavy metals, persistent organic pollutant (POPs), … In terms of POPs, large quantities of organochlorine pesticides have been (are potentially still) used to control parasites and increase the yield of cotton for intensive farming. In addition, industrial pollutants such as polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are also released and accumulate at various levels in the environment and food chain. Although Western European cow milk production is continuously monitored for PCDD/Fs and PCBs, no controls are performed in Kazakhstan and virtually no data are even available concerning levels in foodstuffs of animal origin, especially camels. In this study, a sampling plan was designed to estimate the background levels of 35 PCDD/Fs and PCBs in milk of camels and dromedaries located in various places of Kazakhstan (Almaty, Atyrau, Aralsk, Shymkent).

Materials and Methods

Chemicals, sample collection and storage

All data concerning the quality and potential pre-treatment of the entire chemical used for those analyses are available in a previous report[1]. Quality control (QC) samples consisted in a home made pool of cow milk fortified with the PCDD, PCDF, and DL-PCB congeners at a level below the EU regulation value. All QA/QC criteria are similar to those we routinely use for food control under EU regulation[2]. A total of 127 camel milk samples were collected for the present study. They originated from four regions of Kazakhstan (Almaty, Atyrau, Aralsk and Shymkent) and the milk was collected at two different seasons (spring and fall). Samples came from 57 Kazakh Bactrian (double humped) and 70 dromedaries Arvana breed. All samples were collected at the end of milking, stored at 4°C until they reached the laboratory, then frozen and stored at -18°C. Samples collected at the same season and originating from the same region were pooled. It resulted in 15 composite samples.

Analytical procedure

All samples were processed in series of routinely analyzed samples (one method blank, one instrumental blank, one QC and 10 unknown) in an ISO17025 BELAC accredited laboratory. Sample sizes ranged between 35 and 50 ml. Each pool was liquid-liquid extracted. The fat residue was weighed until constant weight (less than 1.5% variation). This weight was used to determine the lipid content of samples. The first step of sample clean-up was a multilayer

silica gel column in a disposable glass column (5 g of sodium sulfate, 5 g of silicagel, 20 g of 44% sulphuric acid silicagel, and 20 g of 22% sulphuric acid silicagel). Further sample clean-up was achieved using an automated system (Power-Prep™, Fluid Management Systems Inc., Waltham, MA, USA)[3]. Measurements of MO-PCBs and NDL-PCBs were carried out on a MAT95 XL (ThermoFinniganMAT, Bremen, Germany). The GC column was an HT-8 (25 m x 0.22 mm ID x 0.25 µm df) (SGE, Villebon, France). 1.2 µl of the final extract in nonane (95 µl) were injected into a split/splitless injector held at 275°C in splitless mode. Measurements of PCDDs, PCDFs, and NO-PCBs were carried out on an Autospec Ultima (Micromass, Manchester, United Kingdom) The GC column was a VF-5MS (50 m x 0.2 mm ID x 0.33 µm df) (Varian Inc., Sint-Katelijne-Waver, Belgium). 5 µl of the final extract in nonane (10 µl) were injected into programmable temperature vaporization (PTV) injector (Agilent Technologies, Diegem, Belgium). Additional GC and HRMS parameters were described previously[4]. In accordance with EU Commission Directive 2002/70/EC, all TEQ values were based on upper bound data (Commission Directive 2002/70/EC, 2002).

Results and Discussion

Lipid contents

The mean value for all samples was 4.7 ± 1.2 % (median 4.3 %, range 3.3-7.4 %), similar to literature data (5.2%)[5]. No significant differences were observed between camel species or in relation with seasonal sampling. The lower and higher lipid contents were observed for dromedary in spring (mean 4.2 ± 0.5 %, median 4.2 %), and Bactrian in spring (mean 5.3 ± 1.3 %, median 5.0 %), respectively.

NDL-PCB levels

The mean NDL-PCB level, based on the sum of PCB 28, PCB 52, PCB 101, PCB 138, PCB 153, and PCB 180 for all samples was 6.3 ± 2.7 ng/g fat, lower than the value of 9.2 ± 3.4 ng/g fat value reported in Europe for cow milk[6], and well below the maximum level of 40 ng/g fat foreseen by Europe[7]. Bactrian NDL-PCB levels (mean 7.2 ± 3.2 ng/g fat) were higher than dromedary levels (mean 5.1 ± 2.0 ng/g fat). PCB 52 and PCB 101 surprisingly appeared to contribute to more than 60% to the sum of NDL-PCB concentration although limited to less than 10% for European dairies where this is the sum of PCB 138 and PCB 153 that typically account for more than 60% to the sum of NDL-PCB concentration. Because such an important difference in congener specific bio-accumulation or metabolization is unlikely to exist between camels and cows, such a difference in NDL-PCB profiles tends to indicate a possible difference in the route of exposure, such as ambient air and soil, in Kazakhstan, compared to European countries. One cannot exclude the possibility that the extensive use of trichlorobiphenyl mixtures by large capacitor plants until the beginning of the 1990's could somehow be related to this uncommon pattern. Based on the present set of data, no significant differences were observed in terms of seasonal variation for NDL-PCB levels. Data from the Aralsk region were located in the upper part of the range, especially for PCB 52 and PCB 101. Additionally, because camel milk could be used as a human milk substitute and because intakes during the nursing period are known to be a couple of orders of magnitude higher than for adults, the unusual PCB 52 and PCB 101 pattern should be elucidated as the lower chlorinated congeners are known to cause DNA damages.

NO-PCB and MO-PCB levels

The mean NO-PCB and MO-PCB level, based on the sum of PCB 77, PCB 81, PCB 126, PCB 169, PCB 105, PCB 114, PCB 118, PCB 123, PCB 156, PCB 157, PCB 167, and PCB 189 for all samples was 1.7 ± 0.7 ng/g fat (median 1.5 ng/g fat, range 0.3 – 4.2 ng/g fat). In terms of profiles, the DL-PCB congener distribution for camel milk look similar to the one of Belgian cows, except for PCB 105 and PCB118 that are present at significantly higher mean levels for camel milk samples. PCB 105, PCB 118, and PCB 156 are the major congeners for DL-PCBs, accounting for 92% of the sum of concentrations of DL-PCBs (88% for Belgian cows). In terms of DL-PCB (sum of 4 NO-PCBs and 12 MO-PCBs) TEQ concentrations, the mean values for all camel milk samples were 2.18 ± 1.27 pg $TEQWHO_{05}$/g fat (median 1.66 pg TEQWHO05/g fat, range 0.77 – 5.53 pg TEQWHO05/g fat). From a recent EFSA report[8], the mean value for DL-PCB TEQ concentrations were 0.95 ± 1.10 pg $TEQWHO_{05}$/g fat (median 0.45 pg $TEQWHO_{05}$/g fat). As for cow milk, PCB 126 and PCB 118 are the major contributors and represent 80% and 14% of the DL-PCB $TEQWHO_{05}$ concentrations. The respective contributions of the NO-PCBs and MO-PCBs to the DL-PCB TEQ concentrations are 83% and 17% of the DL-PCB $TEQWHO_{05}$ concentrations. No significant inter-racial

or geographical trends were observed for DL-PCB profiles. However, levels of all DL-PCBs appeared to be significantly higher for samples collected in Atyrau region (Figure 1 a and b). Because of the lack of laws and regulations on safe management of PCB-containing equipment and materials in Kazakhstan, it is difficult to estimate if the use of capacitors and transformers by the power industry is significantly larger in that area. Additionally, one should also consider the fact that the Atyrau region hosts the field of Tengiz the largest producing oil field of the country, and associated gas flaring. In their study of Kazakhstan human milk, Hooper et al.[9] also reported higher DL-PCB levels for the region of Atyrau. Seasonal differences were observed for DL-PCB levels. The congener pattern were the same, but the levels were higher for spring samples (2.1 ± 0.8 ng/g fat, 2.51 ± 1.50 pg $TEQWHO_{05}$/g fat), compared to fall samples (1.3 ± 0.6 ng/g fat, 1.80 ± 0.97 pg $TEQWHO_{05}$/g fat).

PCDD and PCDF levels

The abundance of PCDD/F congeners for all samples, based on the sum of 7 PCDDs and 10 PCDFs, is reported and compared to Belgian cow milk in Figure 2 a and b. Except for 1,2,3,6,7,8-HexaCDD, all PCDD/F congeners were present at higher concentrations in camel samples, although the same pattern is observed. A limited comparison can be done between 2,3,7,8-TCDD level reported in the present study (mean 0.08 ± 0.07 pg/g fat) and levels of a previous study were one sample from state farm and one sample from rural area were reported to be 0.89 pg/g fat and <0.1 pg/g fat, respectively[10]. In terms of PCDD/F TEQ concentrations, the mean values for all camel milk samples were 0.80 ± 0.15 pg $TEQWHO_{05}$/g fat (median 0.73 pg $TEQWHO_{05}$/g fat, range 0.53 – 1.49 pg $TEQWHO_{05}$/g fat). Those camel PCDD/F TEQ levels are similar than the EU cow levels and well below the EU legislation maximum level[11]. 2,3,7,8-TCDD (13% $TEQWHO_{05}$), 1,2,3,7,8-PeCDD (26% $TEQWHO_{05}$), 2,3,4,7,8-PeCDF (27% $TEQWHO_{05}$) contributed to 66% of the $TEQWHO_{05}$. The sum of PCDD/F TEQ and DL-PCB TEQ for all camel milk samples were 2.98 ± 1.28 pg $TEQWHO_{05}$/g fat (median 2.48 pg $TEQWHO_{05}$/g fat, range 1.31 – 6.88 pg $TEQWHO_{05}$/g fat). Those camel total TEQ levels are higher than the EU cow levels (1.61 ± 1.94 pg $TEQWHO_{05}$/g fat, median 0.90 pg $TEQWHO_{05}$/g fat), but well below the EU legislation maximum level[11]. Among camel samples, two pools exceeded the EU legislation maximum level. Both samples, one from Bactrian (6.4 pg TEQWHO05/g fat) and one from dromedary (6.9 pg TEQWHO05/g fat), were issued from Atyrau and were collected in spring. Fall samples from this area were also significantly higher than the mean values, indicating that the geographical localization is most probably the reason for those elevated levels. For both DL-PCB and PCDD/F data, all the detected of the 29 congeners expressed higher concentration levels, although NDL-PCB levels were similar to the other localizations. Further dedicated specific sampling should be carried out to further investigate the situation, especially in the proximity of the field of Tengiz. The relative contributions of PCDFs, PCDDs, NO-PCBs, and MO-PCBs to the total TEQ value for Kazakh camels were 13%, 14%, 60%, and 13%, respectively, and were similar to Belgian cows.

Conclusions

Despite the concern for the potential presence of POPs in camel product, this is the first report on the levels of selected NDL-PCBs, DL-PCBs, and PCDD/Fs in camel milk collected in the Republic of Kazakhstan. Although the number of samples was relatively limited, this study is representative of general Kazakhstan levels because of the fact that those samples were issued from pooling of a large number of specimen collected in different regions, during different seasons, and for two different camel species. In general, it was pointed that levels were higher in the region of Atyrau. This suggests that the human exposure in the Caspian Sea region of Atyrau is expected to be higher than in the other regions studied here. This point could be linked to the importance of oil extraction in the area which is considered as the most risk for air and soil pollution in steppe regions around. Further measurement campaigns are needed to confirm those observations and potentially locate specifically contaminated rural area that might have been 'diluted' in the pooling procedure, especially in the Atyrau region. Because of the high consumption of camel milk based products, this is of prime interest to implement environmental quality control of dairy cattle to reduce risks of human exposure, especially in the eventuality where camel milk would be promoted and used as a complement or a substitute to mother milk for toddler feeding.

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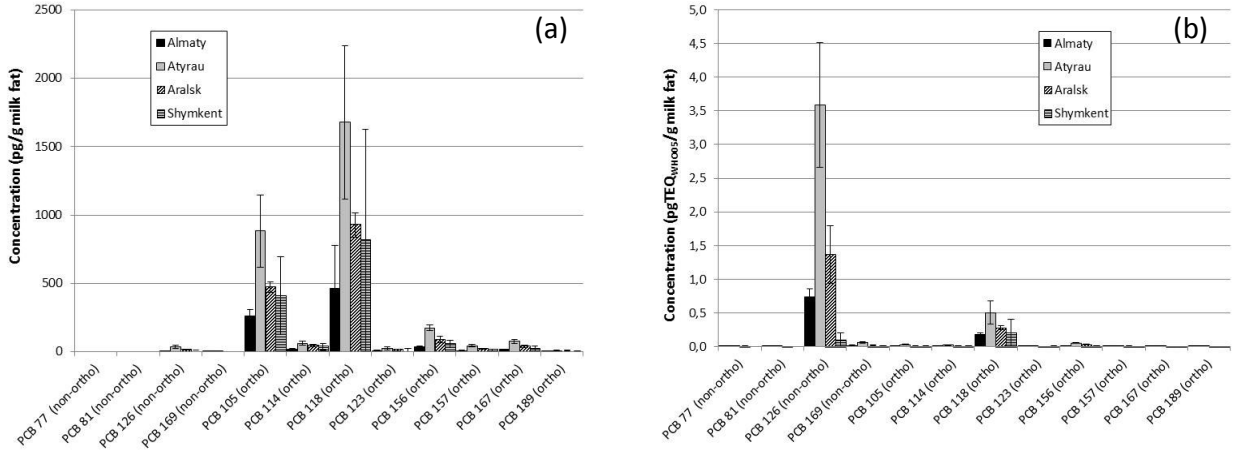

Figure 1: Levels of 12 DL-PCBs of the camel milk samples by geographical region in concentration (a), and in $TEQWHO_{05}$ (b).

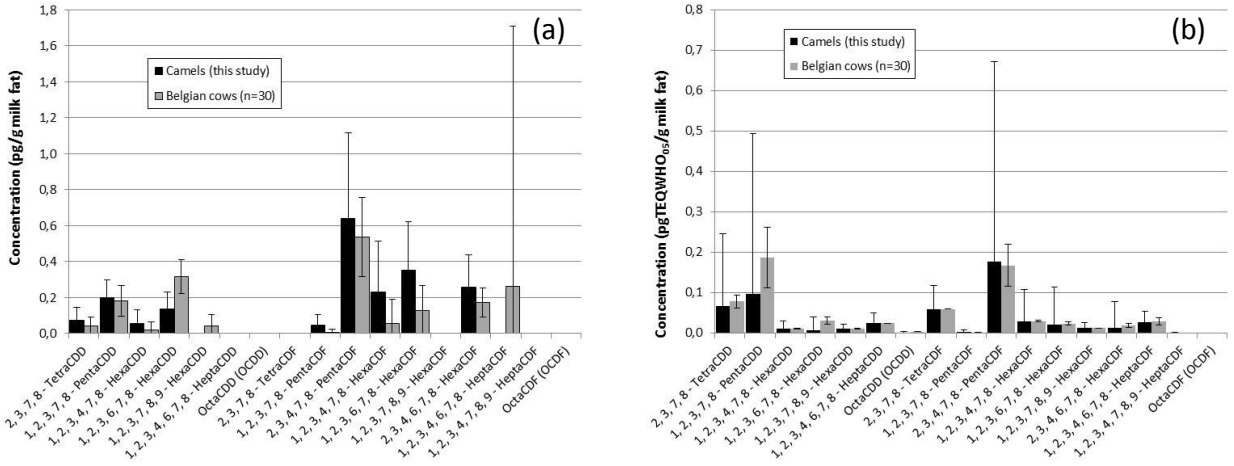

Figure 2: Levels of the 17 PCDD/Fs of the camel milk with comparison with cow milk in concentration (a), and in $TEQWHO_{05}$ (b).