TRANSFER OF INDICATOR PCBS IN DAIRY CAMELS IN KAZAKHSTAN

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Introduction

Kazakhstan has suffered for many years from chemical pollution of different origin (radionuclides in nuclear test site, heavy metals in metal industry, organochlorine compounds from the massive use of Sovol mixtures and pesticides in cotton fields). A part of these pollutants is concentrated in steppe areas where Bactrian camels are reared (Konuspayeva et al. 2011a). Those animals are used for meat and milk, especially for preparing *shubat*, fermented milk which is a national beverage (Faye and Konuspayeva, 2012). Thus, the risk of contamination of human consumers could be not negligible (Kenesariyev et al., 2008) although a previous field study reported only modest concentrations of polychlorinated biphenyls and pesticides in camel milk (Konuspayeva et al. 2011a and b). This seems amazing in comparison to transfer rates reported in other ruminants. However, the capacity of transfer of those pollutants, as well as pesticides, was not known in camels. Indeed these animals have a very specific lipid metabolism which is submitted to important variations during the yearly changes in climate and roughage offer. Therefore, the objective of the present communication was to assess the risk of excretion of organic pollutants in milk collected in Bactrian camels exposed to PCBs and DDT as well as their capacity to become decontaminate.

Materials and methods

The trial has been carried out in Suzak region of South Kazakhstan, close to the Moyun-Kum desert with approximately 100 mm of yearly rainfall. Three lactating adult multiparous Bactrian Camels (*Camelus bactrianus*) have been enrolled. They had calved between the 28th March and the 3rd April 2013 before starting the trial the 8th of May 2013. Animals were housed in a special barn of the Aigene Farm (43° 53'N and 69° 09' E), allowed to pasture in the steppe but turning back to farm four times daily for drinking and milking (6h, 11h, 17h and 22h). Experimental camels spent the night without their calves in a special barn separated from the herd.
The trial consisted in a 56 days exposure of the animals to a controlled dose of indicator PCBs (**iPCBs**) and DDT followed by a 4 month decontamination period. This latter period corresponded to two very hot summer months followed by September and October were vegetation recovered and body conditions of the animals improved in order to prepare winter.
The daily dose consisted to 0.8 mg of Aroclor 1254® (mixture "late", batch n° 4-8586, Sigma-Aldrich France) and 0.12 mg of DDT (Pestanal analytical standard, batch n° 31041, Fluka Analyticals, France) which were incorporated in a gelatin capsule filled in with icing sugar. Each morning, each camel received one capsule hidden in a small lump of dough directly in the mouth. The first day, a priming dose of 9.13 mg iPCBs and 1.41 mg DDT was injected IM in order to speed the reach of the enrichment plateau (steady state).
Camels were milked four times daily (see above) and all milk was destroyed. The first morning milking was recorded and milk samples were taken. Milk yield for 24h has been determined on an individual scale as described by Nurseitova et al. (2014).
Milk samples were analyzed using a liquid-liquid extraction followed by cleanup on a multi-layer silica gel column, evaporative concentration to 30 µL and analysis on 7890A/5975C TAD TVL GC-MS (Agilent, USA) equipped with Combi-PAL autosampler (CTC Analytics AG, Switzerland). Two µL of sample was injected to

split/splitless inlet heated to 250°C in splitless mode. Separation was done on a DB-5MS 60 m x 0.25 mm, 0.25 µm film column (Agilent, USA) at a constant flow of helium (purity 99.995%, Orenburg-Tehgas, Russia) equal to 1 mL/min. Detection was done in selected ion monitoring (SIM) mode using 6-group program for detection of target ions. PCB209 was used as internal standard spiked to samples at the amount of 300 pg.
Excretion of congeners in milk has been calculated by multiplication of the analyzed concentrations and the 24h milk yield. The amounts of contaminants excreted in milk (Y) have been plotted on a time scale (t) to the model

$$Y = a + b\,(1-e^{-ct})$$

The individual excreted amounts were summarized per period: control samples (blanks of each camel before starting the trial), samples taken on contamination plateau (after 41, 47 and 56 days of the exposure), decontamination period (samples taken 6 and 8 weeks after the stop of exposure) and finally fat storage period (samples taken 10, 12 and 14 weeks after the stop of exposure corresponding to samples taken from mid-September to the end of October). All these points were compared by a simple t-test of Student at signification was declared at $P<0.05$ although tendencies were also given at ($P<0.10$).
After checking steady state conditions, carry-over rates (**COR**) have been calculated at the end of the contamination period according to McLachlan & Richter (1998). COR was used as an ideal parameter to describe contaminant transfer to lactating animals.

Results and discussion
Concentrations in control milks ranged between 60 ng/L (PCB 180) and 600 ng/L (PCB52) corresponding respectively to 1 to 10 ng/g of milk fat. These background levels seemed quite elevated what would reflect a certain natural exposure of these animals to their environment. DDT and its main metabolite DDE has been found at concentrations of 0.15 µg/L (or 2.5 ng/g of milk fat) corresponding to concentrations previously reported in Kazakh camel milk (Konuspayeva et al 2011a).
Steady state conditions were met in all compounds at the end of the 56 days of exposure as expected after the analysis of the study of Costera et al (2006) on goats.
The individual values varied between the three camels what would be mainly attributed to differences in body weight (between 420 to 490 kg) and milk yield (between 2 and 5 kg/d). Nevertheless, the excreted amounts of all analyzed PCB congeners and DDT at the different periods of the trial could be well characterized as shown in table 1.

Table 1. Excretion of indicator PCBs in milk (µg /d) at background level and the experimental periods exposure plateau, after 2 month of decontamination and during mobilization of body fat.

Congener	Control milk	End of the contamination period	After two months of decontamination	Period of fat storage
PCB 28	1.4 b	1.5 b	1.1 b	2.8 a
PCB 52	2.8 b	2.2 b c	1.7 c	3.6 b
PCB 101	0.6 c	1.2 a B	0.7 c	2.1 a A
PCB 118	0.7 d	8.1 a	1.9 c	3.4 b
PCB 138	1.0 b	5.4 a	1.7 b B	3.2 a A
PCB 153	0.4 b B	3.3 a	0.9 b A	2.5 a
PCB 180	0.3 c	3.3 a	0.6 c	2.1 b
DDT	0.45 b	0.86 ab A	0.64 b B	2.0 a

a,b means in the same line with different minuscule letters indicated a difference at the signification threshold of P<0.05.
A, B means within the same line with different capital letters indicated a difference at the signification threshold of P<0.10.

The daily excreted amounts of tri- or tetrachlorinated congeners (PCB28, PCB52) were not statistically increased during the exposure period in comparison to the previously measured background levels. Nevertheless, the much lower excretion amounts after the decontamination period let suppose an overestimated background level, possibly linked to environmental presence to these congeners. At the end, the daily excreted amounts of these low chlorinated congeners increased again in autumn when animals reconstructed fat reserves in humps for winter. This would suggest an intermediate storage of fat (and lipophilic compounds as POPs) in another tissues (for ex. visceral or perirenal fat which could represent up to 30% of the fat storage in camel; Faye et al., 2001)

before being reinserted in blood circulation what would allow to transport them to humps but also to the udder and therefore increase their excretion in milk.
Penta- (PCBs 101 and 118), hexa- (PCBs 138 and 153) and heptochlorinated (PCB 180) congeners increased significantly during the exposure period (table 1). Then the excretion decreased significantly during two months without reaching again completely the numeric values of the background levels of daily excretion (table 1). The excretion of these congeners also rose on, right up from September in the same time as fat storage in humps grew on. To our knowledge such phenomenon has not been reported in other mammals but Konuspayeva et al (2011a) reported amazingly low or no contamination of iPCBs in camel milk of areas where at least background contamination was likely. This point needs to be more precisely examined in further work.
The calculated CORs based on plateau excretion at the end of the exposure period are shown in table 2.

Table 2. Carry over rate (%) of iPCBs and DDT in camel milk in comparison to milk of other species in the literature.

	Our results	McLachlan (1993)	Thomas et al (1999)	Costera et al (2006)	Ounnas et al (2010)
specie	3 Bactrian camels	1 cow	5 cows	3 goats	3 goats
Duration of exposure (d)	56	permanent	permanent	70	45
PCB 28	nd	nd	nd	25	nd
PCB 52	6	nd	< 2	10	nd
PCB 101	2	nd	4	5	nd
PCB 118	19	33	94	85	59
PCB 138	14	63	69	41	36
PCB 153	13	78	75	45	nd
PCB 180	71	63	63	55	nd
DDT (DDE included)	3.6	4			

Although hierarchic order between the CORs of congeners seems to be the same, the general transfer rate is clearly lower than in other studies (table 2). *Non-coplanar*, low chlorinated PCBs (i.e. 52 and 101) were weakly transferred (<10%) into camel milk as previously reported in cows and goats. Contrarily, the only *mono-ortho* congener (PCB 118) was transferred into camel milk to a much lesser extent (19%) than in goats and cows (table 2). The same tendency has been observed for hexachlorinated iPCBs: transfer rates seem clearly lower than in other ruminants and finally, the heptachlorinated PCB 180 has been transferred at a rate at least as high as in other ruminants. At the end, the transfer rate of DDT in our camels seems low but consistent to this reported by McLachlan (1993).
That means that low chlorinated compounds but also heptachlorinated PCB 180 have similar transfer patterns in milk from camels in comparison to other ruminants coplanar PCB 118 and hexachlorinated congeners are less transferred. This difference could be partially due to the difference in exposure dose between the studies. Indeed, we used much higher exposure doses (2.2 μg/kg BW & day) in our camels in comparison to studies on goats using only 0.03 μg/kg BW & day (Costera et al, 2006; Ounnas et al, 2010) during a similar duration of exposure (56 days for our camels in comparison to 45 to 70 days in goat studies). We cannot exclude that some transfer mechanisms, especially for highly transferred compounds, reached a saturation of absorption what would reduce mathematically the transfer rates.

Conclusions
Higher chlorinated congeners, i.e. penta- to heptachlorinated, have been excreted in milk approximately 10 times more at the end of the exposure period in comparison to excretion under background conditions. This enrichment turned down, close to background levels after 2 months of decontamination. Finally, the strong increase of iPCB excretion in milk during the fat storage period in autumn consist a surprise what have to be studied on further work. DDT followed the same kinetics as iPCBs over the trial periods but to a much lesser extent.
Camels will transfer indicator PCBs into milk whether in the same extent as other ruminants (low chlorinated congeners) although hexachlorinated and coplanar congeners seemed to be transferred to a lesser rate. DDT and

its metabolite DDE have been excreted in milk to less than 5% of the daily ingested dose. In the context of this experiment, the large camelids have shown a special metabolism of xenobiotics with a more efficient excretion,
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