Kinetics of polychlorinated biphenyls in bactrian camels

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ABSTRACT

The study aimed to determine the accumulation and depuration of polychlorinated biphenyls (PCBs) in Bactrian camels. Four lactating, two-humped camels (Camelus bactrianus) received 0.8 mg PCBs (1.3 μg/kg body weight) daily for 56 days. Then, the depuration of the animals was monitored for the next 4 months. Milk, blood and hump fat of the camels were sampled every 2 weeks and analyzed. Body weight increased significantly, from approximately 550 to 613 kg, by the end of the study. The fat mass in the humps initially decreased (-2.3 kg, P<0.05) then increased at the end of the depuration period (+2.0 kg, P<0.05). At the end of the exposure period, the concentrations of the indicator PCBs were 1.6 mg/g hump fat, 0.85 mg/g milk fat and 0.56 mg/L blood serum, i.e., ten times over the background level. The concentrations in the hump fat decreased significantly during the depuration period, for congeners 28, 52, 101 and 118, but did not vary appreciably for the heavily chlorinated congeners 138, 153 and 180. The apparently stable concentrations of the heavier congeners may be an artifact of the reduced fat mass in the humps during the first part of the depuration period, combined with fat mobilization, which may mask the reduction of stored PCBs. PCB concentrations in the milk and blood were not significantly reduced during the depuration periods, as they represent the outflow of PCBs from the pool stored in the humps and have a weak affinity for lipophilic compounds, respectively. Therefore, it should be recommended to avoid the consumption of raw fat from camel hump in polluted areas because this organ would easily bioaccumulate organic pollutants during an exposure and store it over an extended period. PCB contaminants in milk would reflect the intensity of the outflow from the stored pool, and it would take a longer time in camels than in other ruminants to obtain safe food after the exposure of the animals to persistent organic pollutants.

Keywords: Bactrian camels; Depuration; Hump; Milk; Polychlorinated biphenyls

INTRODUCTION

Nowadays, increasing attention must be paid to environmental protection. Therefore, there is a real need to integrate this feature into the risk assessment approaches, to ensure the safety of food produced by free-range animals. Especially, persistent organic pollutants (POPs) remain intact in the environment for prolonged periods and are bioaccumulative and toxic. Consequently, their risk of transfer into food needs to be evaluated. Chlorinated compounds, such as polychlorinated biphenyls (PCBs), can accumulate in the soil for decades without losing their toxicity (Creaser et al., 2007). The continued presence of contaminants in the environment leads to pollution of soil, sediment, plants and animals, representing several modes of entry into the food chain (Stockholm Convention on POPs, 2009). Prolonged exposure of humans to POPs causes various diseases, such as reduced fertility, the initiation of chronic diseases and cancer and, therefore affect human and environmental health, globally (Ogura, 2004; Ritter et al., 2014).

In Kazakhstan, the Stockholm Convention was signed on 23 May 2001 and ratified on 7 June 2007. On 7 November 2007, Kazakhstan was included in the list of convention parties and on 8 December 2009, a National Implementation Plan about POPs was approved (Beibitova, 2014). Nevertheless, studies on POPs have not yet received adequate attention in countries like Kazakhstan, where PCB-containing equipment is estimated at 980 tons, and the total mass of waste at 250,000 tons (Ishankulov, 2007). PCB sources, such as transformers and condensers, are out of service and no longer used in the industry, yet remain in the environment due to a lack of proper disposal. Consequently, the risk of inadvertent waste pollution on the territory of...
Kazakhstan has increased (Astanina, 2006). However, the country lacks information about the presence of PCBs in the food chain. In a comparative study of PCBs in fish from several regions of Kazakhstan, the highest PCBs concentrations were measured in vobla fish (Rutilus caspicus) from West Kazakhstan, which contained up to 250 ng/g fat for the total PCBs (sum of 80 individually measured congeners). Moreover, human breast milk samples selected in this region contained 820 ng total PCBs/g fat (Hopper et al., 1997).

Previous studies, predominantly on sheep, showed that contamination by POPs mainly accumulated in different compartments of body fat (Berg et al., 2010; Lerch et al., 2016). Moreover, studies on lactating goats (Costera et al., 2006; Ounnas et al., 2010) reported very high transfer rates for some PCB congeners into milk. In this regard, camels are of particular interest because they possess specialized body fat depots in the humps, representing up to 80% of the fat stored in the camel body (Kamili et al., 2006). The lipid metabolism of camels differs significantly from the other ruminants and is, consequently, worthy to be studied in a separate approach.

In Kazakhstan, despite the environmental context described above, a field study (Konuspayeva et al., 2011a) revealed less than 4 ng dioxin-like PCBs per g milk fat and lower than 18 ng non-dioxin-like PCBs in the milk of Bactrian camels or dromedaries, sampled in four different regions of Kazakhstan. These concentrations were below the maximum value allowed by the European regulation for dairy products (Directive 2002/7/EC). Another field study, focusing on indicator PCBs (iPCBs), showed weak contaminations in camel milk, within the limit of quantification (0.1 mg iPCBs/kg milk (Konuspayeva et al., 2011b), corresponding to below 2 ng iPCBs/g milk fat). In this second study, only one milk sample from South Kazakhstan (Kyzylorda region) showed traces of specific congeners (0.2 and 0.25 mg/kg milk for PCB 52 and PCB 138, respectively).

In Kazakhstan Camel Breeding is one of the most important animal breeding because, 116 million ha of all grasslands (64%) belong to deserts and semi deserts favorable for camels and its gives for development of this field (Davletov, 2015). Camel milk and its fermented product (shuhat) have always been important foods in Kazakhstan, where they are known for their medicinal and dietary properties. Even if recent statistics on the consumption of camel milk food in Kazakhstan are sparse, camel breeding farms have re-emerged and intensified, to meet the increasing demand (Faye, 2015a). Hence, studies of camel products are attracting a certain economic interest (Faye, 2015b).

The low level of iPCBs in the samples described above underlines the question of the link between the identified PCBs in the environment and the low contamination in the milk of camel herds migrating over a wide area. In this way, a high dilution of time-point exposure could lead to low concentrations of pollutants, which the animals are probably exposed to, for only short periods. Elsewhere, the specific intake behavior of camels could make them less exposed to ingestion of pollutants via the main accumulation vectors soil or dust. Moreover, a low efficiency to digest lipids and lipophilic compounds, combined with a considerable storage aptitude in the humps, may modify the excretion in milk in comparison to other farm animals (Nurseitova et al., 2012).

In this context, the study of the camel milk kinetics and the possible storage of PCBs in the hump is of great interest. Moreover, it is necessary to understand how these pollutants can accumulate in these animals and to what extent PCBs would then be excreted in milk, to ensure consumer safety. Based on these perspectives, the present work aimed to assess the transfer of orally administered PCBs into milk collected in Bactrian camels during a chronic exposure. At the end of the exposure period, the animals were followed-up, to identify the depuration time necessary to ensure food safety.

**MATERIALS AND METHODS**

**Location, animals and experimental treatment**

Four lactating Bactrian camels (Camelus bactrianus) from the “Aigene” farm, located in Sozak district (43°53’N; 69°09’E) in South Kazakhstan oblast were used to carry out this experiment. As no ethics approval commission procedures exist in the Republic of Kazakhstan, the study protocol was established according to the European guidelines for experimentations with animals (Directive 2010/63/EU).

The experimental design included two stages, first a controlled exposure of the animals to PCBs (period 1), followed by a depuration (period 2). The latter was divided into depuration under the conditions of fat mobilization (sub-period 2a) and then, depuration with fat storage (sub-period 2b). Period 1 involved a controlled exposure of lactating Bactrian camels to a chronic oral administration of a PCB mixture (Aroclor 1254) at 1.3 µg/kg body weight (BW), for 56 days. This duration aimed to reach stable concentrations (steady-state) in the milk, blood and hump tissues. After the exposure period, a 4-month (120 days) depuration was conducted, split into two sub-periods: depuration with fat mobilization, corresponding to summer, i.e., normally the peak of lactation and dry vegetation, leading to the use of fat reserves stored in
the hump (sub-period 2a), and depuration with hump fat storage, corresponding to the decrease in milk yield, recovering vegetation and restarting the fat storage in the humps (sub-period 2b).

Four adult camels (7–16 years old) were used in the study, identifiable by individual ear tag numbers and with BW at the beginning of the trial ranging from 514–593 kg. They had a similar stage of lactation (2 months after calving) and were still suckling their calves. The calves were separated during the night and had access to their mother twice daily after milking, at around 06:00 and 12:00 h. After 12:00 h, the calves went to the steppe for grazing with their mothers.

We used a commercial mixture of PCBs (Aroclor 1254, Sigma-Aldrich Inc., Saint-Quentin-Fallavier, France), which has a congener composition similar to the Sovol mixtures used in the former USSR. This PCB mixture contains some dioxin-like PCBs (congeners 105, 156 and 157) but mainly indicator congeners 153 (3.1%), 101 (5.6%), 138 (6.5%) and 118 (11.2%), and to a lesser extent the congeners 28 (0.1%), 180 (0.4%) and 52 (0.7%).

The daily exposure dose was 0.8 mg Aroclor 1254/camel/day, corresponding to approximately 1.3 µg/kg BW/day. Moreover, an initial dose of 9.13 mg of the same Aroclor mixture was given on the first day of administration to accelerate reaching the concentration plateau. This procedure enhanced the total exposure dose of 53.9 mg PCBs per camel, during the 56 days of the study. The chemicals were incorporated into icing sugar via gelatin capsules and orally administered to the animals inside of breadcrumbs.

**Measurements and sampling procedures**

The concentrations of pollutants were assessed in the blood, hump fat and milk. Additionally, milk yield, BW, and hump height (H) were evaluated throughout all the experimental periods.

Milk samples were collected during the adaptation duration (before exposure at day 0), at the end of the exposure period (days 44 and 49) and eight times throughout during the depuration period (days 58, 75, 93, 107, 122, 137, 161, 175). In total, there were 11 milk samples during the experiment. Immediately after sampling, the milk samples were kept in an icebox during their transportation, then stored at -20 °C before analysis. At each sampling point, milk yield was estimated, as described by Nurseitova et al. (2014). The ejection of the morning milk was initiated by the presence of the calf. The calf emptied one teat, and the three others were milked simultaneously by the farmer. The yielded milk of the three milked teats was measured in a graduated measuring cup, then divided by 0.75 to evaluate the full milk yield of the entire udder. As this result corresponds only to the milk synthesis during the night (i.e. 12 h), it was multiplied by two to obtain the corresponding 24-h milk synthesis.

Blood was sampled in dry tubes from the mammary vein, at the same time points as the milk. After their transport to the laboratory in an ice box, the samples were centrifuged at 1500 rpm for 30 min. Next, the serum was separated, immediately frozen and stored at -18 °C, until analysis. Hump fat was sampled by biopsy, according to the technique described by Faye et al. (2013), before exposure (initial), twice during the exposure period and five times during the depuration period, i.e., at day 0, then days 43, 49, 58, 75, 107, 137 and 175. Control samples of blood, milk and hump fat were collected on non-exposed animals to derive the local background levels.

Body and hump measurements were made on standing animals, in a corridor with a meter-length ribbon to determine body length (BL), height (H), and small (l) and large (L) diameters of the humps. The BW of the animals was evaluated by a barymetric method, based on the body length (in cm), corresponding to the distance between the shoulder and the hip of the animal (Nurseitova et al., 2015).

Body weight [kg] = 9.01 Body length [cm] - 838.6
(SE= 0.265)

The hump weight was evaluated by first estimating the volume of both humps, as proposed by Kamili et al. (2006): \[ V = \frac{1}{2} \left( \frac{4}{3} \pi r_l^2 r_L^2 H \right) \]

where \( r_l \) is the radius of the small length of the hump; \( r_L \) is the radius of the large length of the hump, and \( H \) is the hump height.

The hump volume was calculated after subtraction of 1 cm from the \( r_l \), \( r_L \), and \( H \) values, corresponding approximately to the skin thickness (personal observation). Then, the mass of both humps was calculated by multiplying the estimated volume by the fat density of 0.84 (Kamili et al., 2006). Finally, the hump mass was multiplied by 0.97, considering fat represents 97% of the hump on a dry matter (DM) basis (Kadim et al., 2002).

**Analyses and calculations**

Milk composition, including the fat content (FC), non-fat solids (NFS) and milk density (De), was determined at each sampling date, using a mid-infrared spectrophotometer (Lactan 1-4 MINI®, Sibagropribor, Krasnoobsk, Russia). The total DM of milk corresponds to the sum of the FC and NFS. Sample preparation for chromatographic analyses of milk and serum was achieved by applying a liquid-liquid
and solid-phase (for fat) hexane extraction, followed by cleanup on a multi-layer silica gel column, with evaporative concentration to 20 µL. Seven PCBs congeners (28, 52, 101, 118, 138, 153, 180) were analyzed in the milk, blood serum and hump fat, based on the method reported by Costera et al. (2006). PCB 209 was used as the internal standard, spiked into all samples. Afterward, the analysis was carried out using a gas chromatograph-mass spectrometer (7890A/5975C, GC-MS, Agilent, USA), equipped with a purge and trap sample concentrator, for liquid and solid samples (Velocity XPT, Agilent). Two µL of each sample was injected into a split/splitless inlet heated to 250 °C in splitless mode. Separation was accomplished using a DB-5MS column (60 m × 0.25 mm, 0.25 µm film thickness; Agilent, USA) at a constant 1 mL/min. The flow rate of helium (purity 99.995%, Orenburg-Tehgas, Russia). Detection was done in selected ion monitoring mode (SIM) using a 6-group program for detection of target ions.

Statistical analysis
The concentrations of the different congeners in the hump, milk fat and blood serum were analyzed by a one-way analysis of variance (ANOVA), using the following model:

\[ Y = \alpha (\text{camel}) + \beta (\text{period}) + \varepsilon (\text{residual error}) \]

The tested factors were the camels (n=4), and the number of periods (n=3). The means were compared using a multiple t-test (Tukey’s correction). Significance was declared at \( P<0.05 \) but tendencies (i.e., \( P<0.10 \)) were also mentioned. All data analyses were performed in R software (version 3.2.5).

RESULTS AND DISCUSSION

Experimental camels yielded around 3.3 kg milk/day, but individual variations and the weak number of animals meant that differences in milk yield would not reach statistical thresholds (\( P>0.10 \)). The peak of lactation classically reported in summer was not observed in our experimental camels. A more detailed analysis of the milk yields in this herd was given by Nurseitova et al. (2014). The estimated BW of the experimental camels was close to 550 kg at the start of the trial and increased significantly during the last two months to over 610 kg. The exposure period coincided with spring (May and June) in South Kazakhstan and the fat stored in humps was estimated at nearly 13 kg per animal during the first phase of the experiment. This mass decreased significantly during the sub-period 2a, by up to 10.6 kg, as the depuration period started during the hot summer. This season is characterized by dry vegetation, and the animals can just maintain their BW, without developing the lactation peak and reducing their fat reserves in the humps. From September, vegetation progressively recovers, thereby improving the nutritional supply. Therefore, BW increased and fat reserves in the humps could be restored, resulting in the corresponding significant increases observed compared to the initial masses (Table 1). The observed variations in stored fat mass, but also body mass, confirmed the partition of the depuration (period 2) into two separate sub-periods: 2a “fat mobilization” and 2b “fat storage.” These body mass and fat reserve kinetics would enhance the concentration and dilution effects on the observed concentrations of PCBs in the studied samples.

Concentrations of PCBs in the tissues
As expected, the amounts of the different PCB congeners varied considerably among the time points, but also among the animals. Therefore, the results regarding the analyzed PCB congeners in the samples were expressed as raw means of the control samples for comparison, as well as least square means of the three main periods of the experiment (Table 1).

For the concentrations of PCBs in the hump fat, the control sample showed notable background contamination, with 154 ng/g hump fat, primarily due to lightly chlorinated congeners. Nevertheless, the concentrations of each congener increased strongly during the exposure period, except PCB 180. After 56 days of exposure, the sum of the seven iPCBs reached 1.6 mg/g hump fat, which is much higher than the milk fat and blood serum samples (Table 2). The levels of the various PCBs decreased during the two depuration periods but with congener-depending slopes. The lightly chlorinated PCBs 28, 52 and 101 halved their concentrations in the hump fat during the depuration with fat mobilization (sub-period 2a), whereas, those of the penta- to heptachlorinated congeners did not notably vary. During the final depuration (i.e. sub-period 2b, increase of the fat mass), the concentrations in the hump fat tended to decrease (\( P<0.10 \)) for PCBs 118 and 153 and remained stable for the other five congeners (Table 2). However, the high variability among the camels reduced the statistical power such that the decrease during the 4-month depuration was not significant. Nevertheless, all congeners in the hump fat rapidly reached high concentrations that were at least five-fold more than the background level and decreased very slowly after administration of the Aroclor mixture ceased.

Regarding the concentrations of the PCBs in milk, analytical problems meant that PCBs 28 and 52 had not been determined in the control milk. Nevertheless, the five other congeners studied were present at approximately 65 ng/g milk fat, being substantially higher than in previously cited field studies (Konuspayeva et al., 2011a, 2011b), thereby
illustrating a relatively high background exposure in these animals. After the administration of Aroclor for 56 days, the sum of the seven congeners in the experimental milk samples reached a concentration of nearly 850 ng/g milk fat, and all congeners increased at least five-fold over the background level (Table 2). These levels in camel milk were considerably higher than those reported in the milk fat of goats by Costera et al. (2006) (26 ng/kg BW) and Ounnas et al. (2010) (7 ng/kg BW). The lower daily exposure doses in these goat trials, relative to our experiment, may explain their relatively lower concentrations in milk (<10 ng iPCBs/g milk fat) for 70 days of exposure.

Marked individual variations among the animals were noted for all congeners in milk, thereby reducing the statistical power of the comparisons. Consequently, only the changes in PCB 153 could be confirmed statistically, demonstrating a tendency ($P<0.10$) to decrease during depuration with fat mobilization but re-increase (not significantly) when depuration occurred with fat storage. Numerically, the concentrations of the lightly chlorinated congeners (PCBs 28, 52, 101) remained similar, but those of the higher chlorinated congeners were on average halved during the first part of the depuration period (with fat mobilization) (Table 2).

The daily excretion via milk (about 0.1 mg/day for the seven iPCBs) was remarkably low in comparison to the stored amount in the humps (15.6 mg iPCBs). The absence of significant variations over the studied time gaps in the milk means that the flux “out of the camel” was somewhat stable.

In blood serum, the control sample showed background concentrations around 40 ng iPCBs/L, and around 50% was contributed by the lightest congeners 28 and 52 (Table 2). After the 56 days of exposure, the concentrations in the blood serum increased by a factor of at least six (PCB 180) but typically more for the less chlorinated congeners PCB 28 and 52. Nonetheless,
the levels remained statistically similar during the two depuration phases to those attained at the end of the exposure period (Table 2). These concentrations in the blood serum were lower relative to the analyzed fats (milk and hump) reflecting its function as a hydrophilic transportation fluid within the organism, with a weak affinity for lipophilic compounds.

Kinetics of PCB fluxes

Similar PCB concentrations were detected in the hump and milk fat despite the different mechanisms involved in their synthesis. During the 56 days of exposure, approximately 12.9 kg of the pollutants accumulated in the hump fat and 9.7 kg was excreted in the milk fat (175 g milk fat excreted per day). When the concentrations were compared at the end of the exposure period, the levels in the hump fat were 1.5 (PCB 138) to 2.7 (PCB 28) times higher than in milk fat, except PCB 180, with a hump fat content representing only half of that detected in the milk fat. The higher affinity of these very lipophilic compounds for hump fat in comparison to fat of camel milk can be linked to the Elevated proportions of polyunsaturated fatty acids in the latter, reported by Konuspayeva et al. (2008).

A quantitative approach to compare the available fat masses emphasizes the bioaccumulation of absorbed lipophilic contaminants in the humps of camels. As observed, except for the less represented congener 180, two-thirds of the PCB amounts detected were recovered from the hump fat, and the remaining third corresponded to the excretion via milk fat, following 56 days of exposure. The PCBs circulating in the blood represented less than 0.1% of the sum of the congeners. While this simplified approach neglects the PCBs deposited in other tissues and hence, does not completely reflect the body burden, the predominance of the absorbed PCBs in the hump is evident. Thus, the contamination and depuration process for lipophilic compounds in camels would be notably decelerated and variations are smoothed by the intermediate storage in the organism, with a weak affinity for lipophilic compounds.

PCB patterns in the various samples

In contrast to their presence in the original Aroclor 1254, in which the seven indicator congeners were evenly distributed, the profile in the hump fat was dominated by PCBs 118 (33–40%) and 28 (21–27%), while congeners 101 and 138 were under-represented. Moreover, the highly chlorinated congeners 153 and 180 presented minor proportions in the hump fat (Table 3). During the depuration with fat mobilization, a decreasing tendency in the proportions of lower chlorinated compounds (PCBs 28 and 52) and increasing trend in the amounts of highly chlorinated congeners in the profile of the hump fat was indicated (Table 3).

The PCB pattern in milk fat differed dramatically from that of Aroclor 1254 but was relatively comparable to that observed in the hump fat. The less chlorinated congeners (PCBs 28 and 52) were over-represented relative to the Aroclor mixture, and logically, the highly chlorinated congeners (PCBs 153 and 180, and to a lesser extent, PCB 138, but also PCB 101) presented comparatively lower proportions in the milk fat profile (Table 3). PCB 118 stood out among the monitored PCBs, displaying a strongly increased presence in the milk fat profile after the exposure period and a substantial decrease during the decontaminations.

The pattern in blood serum is dominated by PCBs 28 (60%) and 52 (25%), while the other five congeners had very low proportions (Table 3). The differences in lipophilicity can explain the hierarchy in the congener profiles. For instance, the less lipophilic PCBs 28 and 52 (log $K_{ow} < 6$) represent 85% of PCBs in blood serum but less than 50% in milk and hump fat. Among the congeners, those displaying more lipophilicity had higher proportions in the profiles of the fats. Hump fat seems to have a higher affinity for PCB 118, but milk fat tended to favor the very lipophilic PCB 180 (log $K_{ow} > 7$). The contents of PCBs 101, 138 and 153 were reasonably similar in both fat profiles.

Practical implications

Significantly different pharmacokinetics in camels compared to cattle and horses have been documented for phenylbutazone (Wasfi et al., 1997) and diphenhydramine (Wasfi et al., 2003). Our results showed that the depuration of PCB-contaminated camels could be expected to occur over a more prolonged duration than in other animals as they can store greater amounts in the comparable sizeable fat pool of their humps, which has a high affinity for these very lipophilic compounds.

Therefore, in polluted areas, the consumption of raw fat from camel hump, as is practiced traditionally in some countries, should be avoided. This organ seems particularly prone to accumulate organic pollutants during exposure and to store it over an extended period. Conversely, it can be supposed that camel meat would be less predisposed to POP accumulation, as it has been shown to be leaner than meat from other ruminants (Kadim et al., 2008). The PCB concentrations in milk, reflect the intensity of the outflow from the stored pool, mainly in humps. Thus, the full-fat milk from heavily contaminated camels can represent a real risk to consumer health, even if the fat fraction is separated and the use of skinned milk can reduce this exposure risk.
CONCLUSIONS

Chronic exposure of Bactrian camels to PCBs would lead to consequent contamination of the animals, especially in the humps. The kinetics of PCB contamination and depuration were smoothed by the resultant storage of these compounds in the humps, which buffered these processes, even with repeated exposure. As a consequence, the depuration of these animals after withdrawal from contaminated areas would need more time relative to other ruminants before the safety of the animal-derived products can be assured. Therefore, the traditional consumption of raw fat from camel humps should be avoided in polluted areas. The contamination of milk reflects the intensity of the outflow from the pool of contaminants stored in the body and its consumption appears risky, particularly when the animals mobilize their fat reserves, even if skimming of milk can reduce the risk to the consumer.

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Author’s contribution

S.J. was the project leader, did statistical analysis and wrote the manuscript. M.N. carried out field experiments, performed chemical analyses and was involved in the literature collection. Zh.T. contributed to the research planning and logistical supplies for the study. G.K. contributed to the research planning and was involved in manuscript preparation. B.F. was engaged in the literature collection, statistical analysis and manuscript preparation.

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