PERSISTENT ORGANIC POLLUTANTS (POPs) IN CHICKEN EGGS AND CAMEL MILK FROM SOUTHWESTERN KAZAKHSTAN

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Introduction

This study assessed persistent organic pollutants (POPs) in fatty food products from camels and chickens from southwestern Kazakhstan. POPs are widely distributed throughout the environment; accumulate in animal fats and are toxic to both humans and wildlife[1].The southwestern part of Kazakhstan has large oil and mining industry activities and toxic legacy hot spots from the Soviet era. Camel milk is a significant part of the diet in southwestern Kazakhstan. Contaminated milk from ruminants and poultry eggs can cause significant human body burden as an important part of rural diets and may contain high levels of POPs[2-3]. The most toxic POPs (tetra-to hexachlorinated dioxins and furans, highly chlorinated PCBs, especially congener 126, as well as DDT) are highly transferred to the most consumed food products such as eggs, milk, and to a lesser extent to meat of outdoor reared animals[4-6]. Eggs from contaminated areas can lead to exposures which exceed thresholds for the protection of human health[7-9]. The same applies to milk from ruminants[10-11].

POPs in Kazakhstan: Up to 40 thousand tonnes of pesticides were used annually until the 1990's[12]. This included DDT, HCH and other OCPs. Although DDT was banned in the USSR in 1971 it was used in Kazakhstan until 1990's[12]. PCBs are still widely used in electrical equipment and former producers of capacitors filled with PCBs are located in Ust-Kamenogorsk, Kazakhstan[12]. Kazakhstan carries a PCBs legacy as do other countries (e.g. Slovakia) which have typically high levels of PCBs in environmental samples in general[13]. High levels of PCBs were even measured in free range eggs from the nature reserve Kyzyl Arai[14].

Large volumes of PCBs were used in equipment for military and space exploration infrastructure sites in Kazakhstan in the Soviet era. Most sites were abandoned without safe disposal of PCBs which contaminated large areas afterwards[15-17]. The potential sources of PCB contamination are listed in the basic inventory for a UNDP/GEF project on addressing the issue of obsolete PCB oils in Kazakhstan[18].

This report compiles data from two larger reports[14,19] and for the first time evaluates POPs in both camel milk and free range eggs sampled within the same territory and sites of Kazakhstan. We also build on research of POPs in camel milk carried out in South Kazakhstan[20-22]. More POPs were included in previous research, e.g. BFRs in chicken eggs from Mangystau Region, Kazakhstan[23].

Materials and methods

Two groups of POPs were analysed in eggs and camel milk: a) intentionally produced POPs, listed in Annexes A and B of the Stockholm Convention (SC)[24]: DDT, HCB, 3 isomers of HCH, including γ-HCH, often called lindane, and non-dioxin-like PCBs (ndl PCBs)[25]; b) unintentionally produced POPs listed under Annex C of SC: PCDD/Fs, dioxin-like PCBs (dl PCBs) and HCB in this study.

Sampling: Pooled samples of both camel milk products and free-range hen's eggs were sampled in 2015 - 2016. One sample of raw camel milk was from Kuryk and five *shubat* samples made from camel milk were sampled from 5 communities (Shetpe, Baskuduk, Akshukur, Tauchik, Kyzyl Tube). The eggs and shubat samples were collected at the settlements of Baskuduk, Tauchik, and Shetpe. More information about sampling locations can be found in previous reports[14,19]. The eggs were collected in plastic egg packaging and were boiled for approximately 7 minutes right after sampling. The homogenates from egg whites/yolks and homogenized shubat/milk samples were used for the analyses. The samples were stored and transported in cold conditions. The samples for analysis were mixed from 3 – 4 eggs from the same location. A pooled battery farmed egg sample bought in a convenience store in Karaganda in 2014 is used as reference sample for this study.

Analysis: Egg samples were extracted by a mixture of organic solvents hexane:dichloromethane (1:1) and further purified and fractionated on an activated carbon column. All samples were analyzed for their content of individual PCDD/Fs and dl PCBs by GC/HRMS in an ISO 17025 accredited laboratory at the State Veterinary Institute in Prague, Czech Republic, with a resolution >10,000 using ^{13}C isotope labelled standards. PCDD/F and dl-PCB analysis followed the methods of analysis for the control of levels of PCDD/Fs and dl-PCBs in foodstuffs according to the EU regulation[26]. The samples were also analyzed for their content of indicator congeners of PCBs (ndl PCBs), HCB and other OCPs in laboratory at Department of Food Chemistry and

Analysis of University of Chemistry and Technology, Prague. Levels of PCDD/Fs and dl PCBs are expressed in total WHO-TEQ set by WHO experts panel in 2005[27].

Results and discussion
The results of analysis for POPs in the eggs and camel milk samples from the Mangystau Region are summarized in Tables 1 and 3. Table 1 shows data for PCDD/Fs, dl PCBs and ndl PCBs while levels of, HCB, DDT and HCH are summarized in previous reports[14,19].

The PCDD/Fs and dl PBCs were measured in all camel milk samples but only in two free range egg samples out of four. In both analysed eggs samples there were higher concentrations of total PCDD/F + dl PCBs than the EU limit for chicken eggs (5.0 pg WHO-TEQ g^{-1} fat). The PCDD/Fs content in all samples complied the EU regulation limit as well as with Kazakhstani and Russian hygienic limits set for chicken eggs and milk (see Table 1). The background levels for PCDD/Fs and dl PCBs were measured as 0.90 and 0.0003 pg WHO-TEQ g^{-1} fat respectively in eggs from a store in Karaganda. Six indicator PCB congeners representing ndl PCBs had the highest level of 44 ng g^{-1} fat in the shubat sample from Tauchik which also exceeded the EU limit for ndl PCBs.

Table 1. Results of analyses for PCDD/Fs, dl PCB and ndl PCBs for eggs and shubat/milk samples from Mangystau Region

Matrix	Year	Sample	Eggs/ camels[a]	Fat	PCDD/Fs	dl PCB	PCDD/F + dl PCBs	ndl PCBs
		Locality / ID	No in pool	%	pg WHO TEQ g^{-1} fat			ng g^{-1} fat
Eggs	2016	Baskuduk / BAS 02	3	15.6	2.05	11.48	13.5	27
	2016	Shetpe / SHE-E-1	4	15.4	0.92	5.34	6.26	11
	2014	Karaganda / KAR-SUP	6	14.0	0.90	0.0003	0.90	0.99
		EU limits[28]			2.5	-	5.0	40
		Russian limits[29]			3	-	-	-
Camel milk products	2015	Shetpe / KZ-M-15-1*	3	0.62	0.45	3.02	3.47	15.7
	2015	Baskuduk / KZ-M-15-2*	<200	0.85	1.33	14.9	16.3	22.2
	2015	Akshukur / KZ-M-15-4*	<200	2.21	0.01	2.07	2.08	3.5
	2015	Tauchik / KZ-M-15-5*	1	1.06	0.31	47.3	47.6	44.6
	2016	Kyzyl Tube / KZ-M-16-6*	25	1.63	0.24	3.24	3.48	0.82
	2015	Kuryk / KZ-M-15-3**	3	3.13	1.3	5.25	6.55	8.0
		EU limits***			2.5	-	5.5	40
		Kazakhstani/Russian limits***[29, 30]			3	-	-	-

*shubat **raw camel milk ***limits set for milk [a] number of individual camels from which milk was collected

The toxicity of dl PCBs is higher than PCDD/F congeners toxicity in all camel milk as well as egg samples in this study. The sample from a lactating camel from Tauchik showed the highest level of dl PCBs (47.3 pg WHO-TEQ g^{-1} fat). It is almost 23-times more than the lowest level of dl PCBs (2.07 pg WHO-TEQ g^{-1} fat) observed in the sample from Akshukur accompanied also by lowest level of PCDD/Fs (0.01 pg WHO-TEQ g^{-1} fat). One of six samples of shubat from Baskuduk, contained high levels of dl PCBs, accompanied by the highest level of 1.33 pg WHO-TEQ g^{-1} fat PCDD/Fs. Total WHO-TEQ levels of PCDD/Fs and dl PCBs in samples collected for this study are much higher than in those collected by Konuspayeva et al[20] as noted in Table 2.

Table 2: Comparison of PCDD/Fs, dl PCBs and 6 indicator PCBs (= ndl PCBs) measured in samples of camel milk/shubat from this study with previous studies by Konuspayeva, et al.[20]

Group of chemicals	PCDD/Fs (pg WHO-TEQ g^{-1} fat)		dl PCBs (pg WHO-TEQ g^{-1} fat)	
Statistical value	Mean ± st. deviation	Min – Max	Mean ± st. Deviation	Min – Max
Konuspayeva, et al.[20]	0.80 ± 0.15	0.53–1.49	2.18 ± 1.27	0.77–5.53
This report	0.61 ± 0.57	0.01–1.33	12.64 ± 17.63	2.07–47.30
Group of chemicals	PCDD/F + dl PCBs (pg WHO-TEQ g^{-1} fat)		6 indicator PCB cong. (ng g^{-1} fat)	
Statistical value	Mean ± st. deviation	Min – Max	Mean ± st. Deviation	Min – Max
Konuspayeva, et al.[20]	2.98 ± 1.28	1.31–6.88	6.3 ± 2.7	0.6–17.4
This report	13.24 ± 17.61	2.08–47.61	15.81 ± 16.17	0.82–44.61

The highest level of dl PCBs expressed in WHO-TEQ is more than 8.5-fold higher than maximum level (5.33 pg WHO-TEQ g^{-1} fat) reported in previous studies[20], while levels of PCDD/Fs were comparable or slightly higher than results in this study. It was noted by Konuspayeva, et al.[20] that, in general, levels of PCBs were higher in the region of Atyrau and this could be linked to oil extraction in the area. Oil extraction presents the highest risk for air and soil pollution in steppe regions around Atyrau. Our findings are in agreement with that conclusion also for Mangystau as we observed high levels of dl PCBs in particular.

PCDD/Fs and dl PCBs had higher level in pooled eggs sample from Baskuduk compared to samples from Shetpe. This observation is the same as for the levels of PCDD/Fs and dl PCBs in shubat samples from the same locations. PCDD/Fs and dl PCBs in eggs from Shetpe were slightly above the EU limit for eggs, while levels were almost three times higher in eggs from Baskuduk. PCDD/Fs in eggs from Shetpe were at the same level as the reference sample from the store in Karaganda, but for dl PCBs it was 4 magnitudes higher. A recent study has shown that level of PCDD/Fs and dl PCBs found in free range eggs from Baskuduk is similar to the levels measured in eggs from a dumpsite in Yaoundé, Cameroon, a waste incinerator in Wuhan, China, a community cooker using plastic waste as fuel in Nairobi or from a plastic waste yard in Bangun, Indonesia[31]. A large waste dumpsite and electric equipment with PCBs could be the sources of food contamination in Baskuduk[14,19,31].

Organochlorine pesticides: In the OCPs chemical group high levels of HCHs were observed in three of four samples of chicken eggs from Mangystau region. Concentrations of β-HCH in samples SH-E-1 from Shetpe and from Tauchik of 209 and 122 ng g^{-1} fw respectively exceeded an EU limit (10.0 ng g^{-1} fw)[32] more than twenty and twelve times respectively. They also exceeded the Russian limit value (MAC 100.0 ng g^{-1} fw)[33]. Potential sources of this contamination can be either feed or water provided to chickens. The other analyzed OCPs in eggs (HCB, α-HCH,γ-HCH and sum-4DDT) were below the EU and Russian limits[32-33] in all eggs samples. High levels of currently banned POPs pesticides, such as DDT or HCHs, in samples from Shetpe and Tauchik, show a potential ongoing use of these obsolete pesticides or their presence in unknown stockpiles, either at the sampled sites or at the location of origin of the hen's feed.

There were low concentrations of OCPs in camel milk samples from Mangystau Region, contrary to the findings in eggs [14]. Levels of lindane (γ-HCH) in a camel milk sample from Kuryk (0.244 ng g^{-1} fw) reached a quarter of the EU limit value (1 ng g^{-1} fw)[32]. Lindane showed the highest levels among all individual OCPs analyzed in samples in general. Significant levels of β-HCH was also observed in some samples of animal products (butter) and human tissue samples from the region around the Aral Sea in Uzbekistan[34]. More information about OCPs in samples from the Mangystau Region can be found in two previous studies[14,19].

Body burden: Shubat, fermented camel milk is a very popular special drink in south-western Kazakhstan. The consumption of camel milk reaches 240 liters per person per year[35] (=677 g per person per day), and consumption of eggs was estimated to reach about 28 g per person per day in 2017[14].

Recent meta-analysis of transfer of POPs into food of animal origin observed, *"Lipophilic POPs are not only highly transferred to edible products such as milk, eggs, meat and offal, but even more, these food products present higher POPs exposure sources than the animal feed carrying them"* [4]. Therefore we estimated daily intake of PCDD/Fs and dl PCBs for a combination of samples from Baskuduk and Shetpe, two locations where we have a full set of data available. The calculation of daily intake levels was performed by using the following formula: $DI_{adult} = \frac{\left(\left(\frac{C \cdot F}{100}\right) \cdot ADC\right)}{70}$

where DI = daily intake (pg kg^{-1} bw); C = POPs concentration (pg WHO TEQ g^{-1} fat), and F = fat content in the sample (%); ADC = average daily consumption of the food type; we count 70 kg as bw. Results are summarized and compared with TDI established by EFSA in 2019 at a level of 0.25 pg WHO-TEQ kg^{-1} bw[36] in Table 3.

Table 3: Daily intake of PCDD/Fs/dl PCBs from consumption of shubat and eggs in Baskuduk and Shetpe.

Locality	PCDD/Fs + dl PCBs					
	In pg g^{-1} fresh weight (fw)		Daily intake in pg WHO-TEQ kg^{-1} body weight (bw)			
	shubat	Eggs	shubat	Eggs	shubat + eggs	TDI (EFSA)[36]
Baskuduk	0.14	2.12	1.34	0.85	2.19	0.25
Shetpe	0.022	0.99	0.21	0.39	0.60	0.25

Although Shetpe seems to be less contaminated with PCDD/Fs and dl PCBs, compared to Baskuduk, in Shepte was the highest ever measured level of hexabromocyclododecane (HBCD) in chicken eggs globally[23,31]. We didn't analyse meat for POPs content which is the limitation of this study but even consumption of the estimated daily portion of eggs and camel milk from open space reared animals can lead to intake of PCDD/Fs and dl PCBs in Shetpe and Baskuduk by 2- to almost 5-fold higher than the TDI suggested by EFSA[36] respectively. We used chicken eggs as they are a good indicator of potential contamination of the food chain. Some other studies demonstrated simultaneous contamination of chicken eggs and meat from contaminated sites[37,38].

Conclusions

High levels of dl PCBs were found in free range chicken eggs and camel milk and shubat samples from Mangystau Region in the southwestern part of Kazakhstan. Non dioxin-like PCBs were found in relatively high levels, and in one case of shubat sample from Tauchik exceeding the EU limit. Some abandoned POPs pesticides residues like HCH or DDT were observed in high levels in free range chicken eggs from the researched region. Estimated daily portion of eggs and camel milk from open space reared animals can lead to intake of PCDD/Fs and dl PCBs in Shetpe and Baskuduk by 2- to almost 5-fold higher than TDI suggested by EFSA. Large waste dumpsite and old electric equipment, most likely with PCBs, can be the source of contamination. Our results underline the need to focus on safe remediation of remaining obsolete PCB stockpiles and sites contaminated with POPs in Kazakhstan. Examples of technologies and techniques to remediate these contaminated sites without generating further UPOPs are available[16]. Without remediation such sites will continue to contaminate the food chain as demonstrated in this and other studies from Kazakhstan.

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