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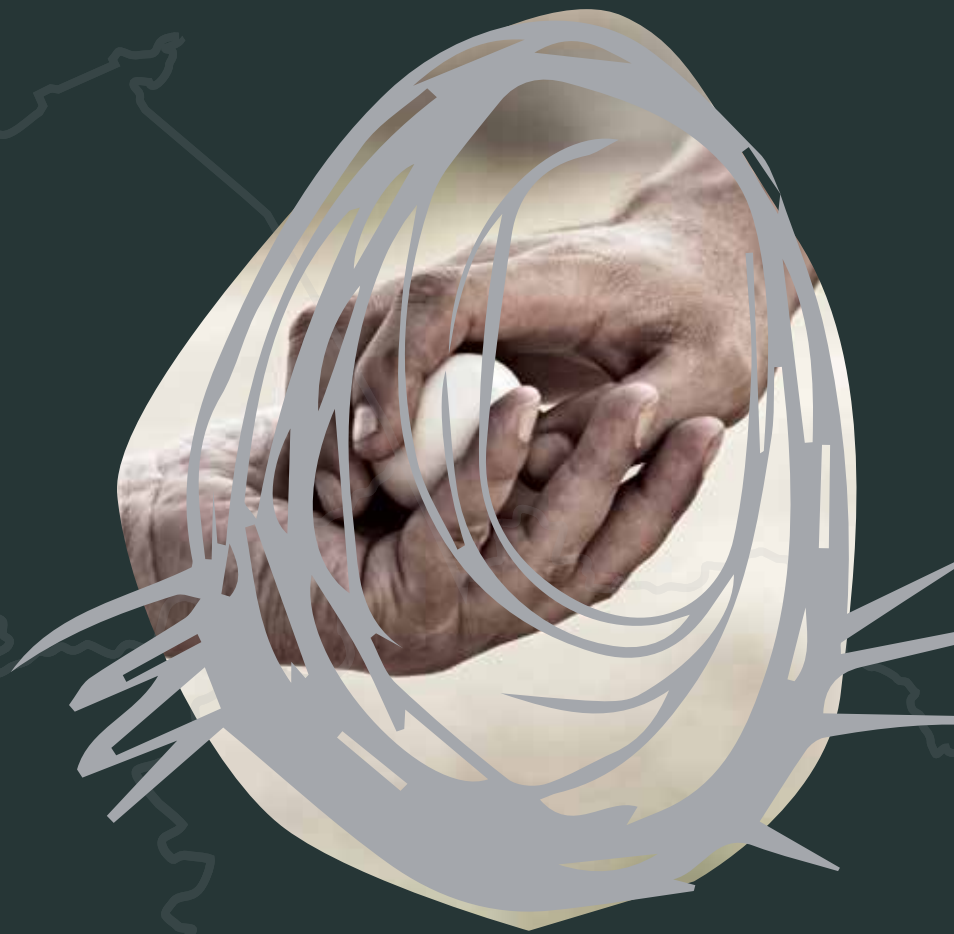


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RESULTS OF SAMPLING CONDUCTED IN 2013—2016

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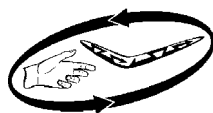
CHICKEN EGGS AS THE INDICATOR OF THE POLLUTION OF ENVIRONMENT IN KAZAKHSTAN

RESULTS OF SAMPLING CONDUCTED IN 2013 – 2016

Prague - Karaganda - Aktau
2016



TRANSITION



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USE OF FREE RANGE CHICKEN EGGS AS THE INDICATOR OF THE POLLUTION IN KAZAKHSTAN RESULTS OF SAMPLING CONDUCTED IN 2013 – 2016

An updated report based on the results of environmental sampling conducted in Kazakhstan in 2015-2016 as a part of the project “Enforcing citizens’ rights and public participation in decision making on environmental issues – practical implementation of Aarhus Convention in Mangystau” financially supported by the European Union and Transition Promotion Programme of the Czech Republic. We would like also acknowledge financial support from the Government of Sweden which made possible part of the chemical analyses and preparation of the report through the grant to IPEN.

This report is published in English and Russian languages.

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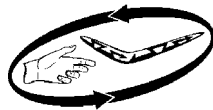
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TRANSITION



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1. INTRODUCTION

In this study, we present the results of the monitoring of the free-range chicken eggs from selected sites in Kazakhstan contamination by persistent organic pollutants (POPs). Free range chicken eggs were used for monitoring levels of contamination by POPs at certain places in many previous studies (Pless-Mulloli, Schilling et al. 2001, Pirard, Focant et al. 2004, DiGangi and Petrlik 2005, Shelepchikov, Revich et al. 2006, Aslan, Kemal Korucu et al. 2010, Arkenbout 2014). Eggs have been found to be sensitive indicators of POP contamination in soils or dust and are an important exposure pathway from soil pollution to humans, and eggs from contaminated areas can readily lead to exposures which exceed thresholds for the protection of human health (Van Eijkeren, Zeilmaker et al. 2006, Hoogenboom, ten Dam et al. 2014, Piskorska-Pliszczynska, Mikolajczyk et al. 2014). Chickens and eggs might therefore be ideal “active samplers” and indicator species for evaluation of the level of contamination of sampled areas by POPs, particularly by dioxins (PCDD/Fs) and PCBs. Based on this assumption, we have chosen sampling of free range chicken eggs and their analyses for selected POPs as one of the monitoring tools within the projects “Empowering the civil society in Kazakhstan in improvement of chemical safety” and “Enforcing citizens’ rights and public participation in decision making on environmental issues - practical implementation of Aarhus Convention in Mangystau” (further information about the project can be found at <http://english.arnika.org/kazakhstan>).

The data and analyses of free range chicken eggs discussed in this report were obtained during the period of the two above mentioned joint projects of Kazakhstani and Czech NGOs. They were obtained during several field visits in 2013 - 2016. The sampled localities are the same or similar as in some previous reports by Dvorská (2015), Šír (2015) and Petrlik et al. (2016). A general description of samples and sampled localities can be found in chapter 2.

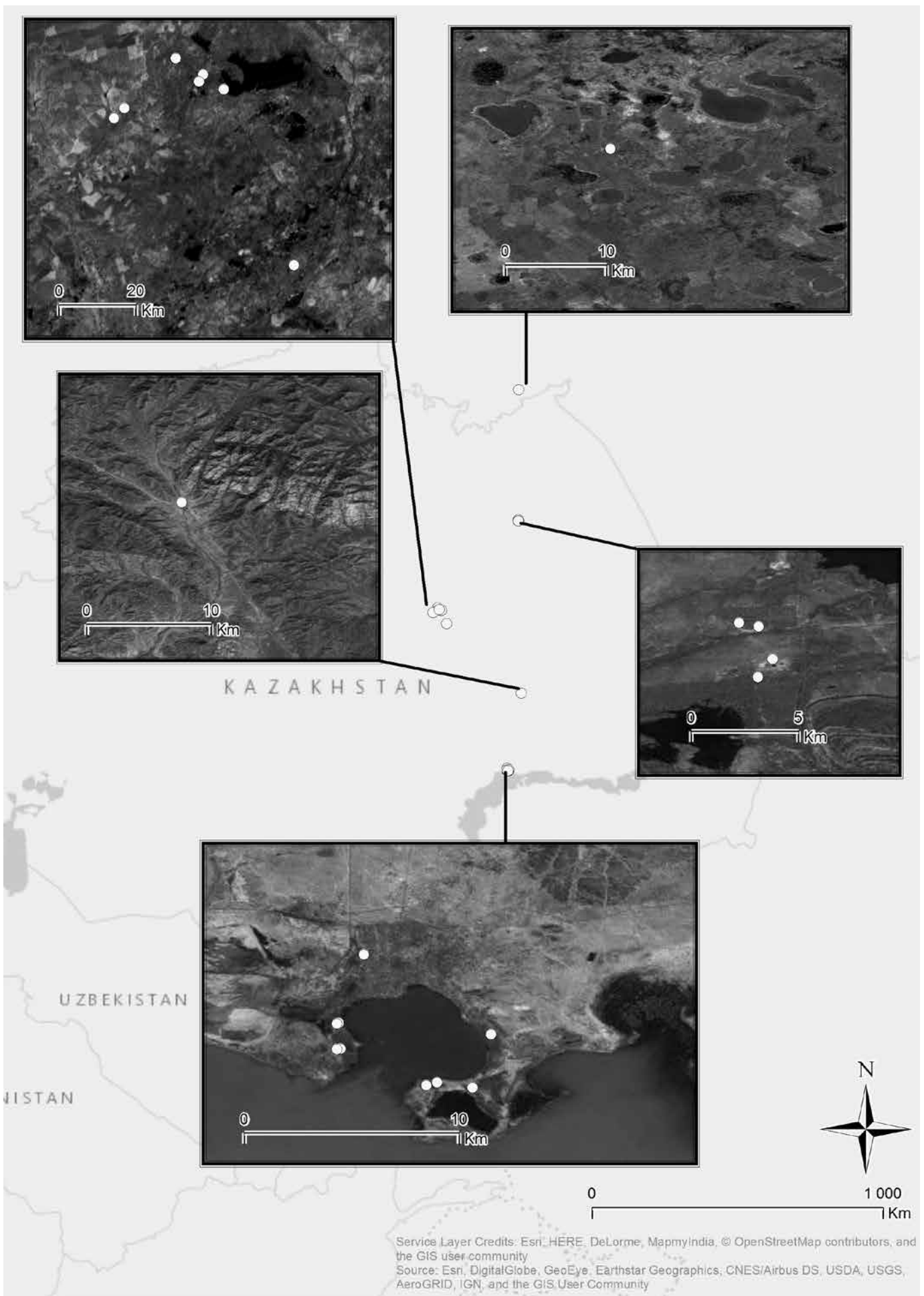
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1.2 ABBREVIATIONS

AAC - Approximately allowed concentrations (Ориентировочно-допустимая концентрация (уровень))	NGO - non-governmental organization (civil society organization)
AMA - Advanced Mercury Analyser	NIP - National Implementation Plan of the Stockholm Convention
BDS - BioDetection Systems (laboratory in Netherlands)	OBIND - octabromotrimethylfenylindane
BGMK - Balkhash Non-Ferrous Metals Processing Plant	OCPs - organochlorinated pesticides
BEQ - bioanalytical toxic equivalent	OCDD - octachlorodibenzo-p-dioxin
CALUX - chemically activated luciferase gene expression	OCDF - octachlorodibenzo-p-furan
CAS - chemical abstracts service registry number (a unique numerical identifier assigned to every chemical substance described in the open scientific literature)	PBDD/Fs - polyfrominated dibenzo-p-dioxins and furans
BTBPE - 1,2-bis(2,4,6-tribromo-fenoxy)ethane	PBEB - pentabromoethylbenzene
DDD - dichlorodiphenyldichloroethane (a metabolite of DDT)	PBT - pentabromotoluen
DDE - dichlorodiphenyldichloroethylene (a chemical compound formed by the loss of hydrogen chloride from DDT)	PCBs - polychlorinated biphenyls
DDT - dichlorodiphenyltrichloroethane (pesticide)	PCDD/Fs - polychlorinated dibenzo-p-dioxins and furans
DI - dietary intake	PCDDs - polychlorinated dibenzo-p-dioxins
DL PCBs - dioxin-like PCBs	PCDFs - polychlorinated furans
d.w. - dry weight	PeCB - pentachlorobenzene
EFSA - European Food Safety Agency	PeCDD - pentachlorodibenzo-p-dioxin
EU - European Union	PeCDF - pentachlorodibenzo-p-furan
f.w. - fresh weight	POPs - persistent organic pollutants
GC - gas chromatography	SC - Stockholm Convention on Persistent Organic Pollutants
GEF - Global Environment Facility	TEQ - toxic equivalent
GoK - Government of Kazakhstan	TCDD - tetrachlorodibenzo-p-dioxin
GPC - gel permeation chromatography	TCDF - tetrachlorodibenzo-p-furan
GPS - global positioning system	TDI - tolerable daily intake
HBB - hexabromobenzene	TEQ - Toxic equivalent
HCB - hexachlorobenzene	TWI - tolerable week intake
HBCD - Hexabromocyclododecane	UHPLC - ultra-high performance liquid chromatography
HCHs - hexachlorocyclohexanes (pesticides and their metabolites)	UNDP - United Nations Development Programme
HpCDD - heptachlorodibenzo-p-dioxin	UNECE - United Nations Economic Commission for Europe
HpCDF - heptachlorodibenzo-p-furan	US EPA - United States Environmental Protection Agency
HRGC-HRMS - high resolution gas chromatography - high resolution mass spectroscopy	WHO - World Health Organization
HxCDD - hexachlorodibenzo-p-dioxin	WHO-TEQ - toxic equivalent defined by WHO experts panel in 2005
HxCDF - hexachlorodibenzo-p-furan	w.w. - wet weight
IPEN - International POPs Elimination Network	
IARC - International Agency for Research on Cancer	
INC - Intergovernmental Negotiating Committee (normaly set up for negotiations of new international convention)	
LOD - limit of detection	
LOQ - limit of quantification	
MAC - maximum acceptable (allowable) concentration	
ML - maximum level	
MRL - maximum residue level	
NA - not analyzed	

Figure 1: Maps with locations where the samples were taken (2014).



2. SAMPLING AND ANALYTICAL METHODS

Samples of free range chicken eggs were collected at seven localities in Central Kazakhstan and at three localities in Western Kazakhstan from which one (Shabanbai Bi) was expected to be clean and one was sample from a supermarket in the city of Karaganda, considered as a background sample for Kazakhstan as suggested by Dvorská (2015). Shabanbai Bi village in Kyzyl Arai Natural Protected Area (zakaznik) was originally chosen as a potentially clean background site, while five others in Central Kazakhstan were expected to be contaminated by POPs to a certain level. The cities of Balkhash and Temirtau were expected to be polluted by POPs as cities with large metallurgical plants. This assumption was also based on data from the Kazakhstani National Implementation Plan for the Stockholm Convention (Republic of Kazakhstan 2009). The villages of Rostovka and Chkalovo are located on the Nura River, downstream from abandoned chemical facility Karbid (production of acetaldehyde) located near and on the territory of the city of Temirtau. This facility polluted the river Nura with mercury. The same chemical plant can also be a source of pollution by POPs (e.g. PCBs). The neighbourhood of former electricity substation was sampled in the city of Ekibastuz. This abandoned facility is assumed to be a potential source of contamination by PCBs and partially also by PCDD/Fs as impurities in PCB oils (see also Dvorská (2015)).

There were three locations selected in Mangystau Region, Western Kazakhstan: Shetpe, Tauchik and Baskuduk. All of them were also localities, where camel milk was sampled for chemical analyses evaluated in the report by Petrлік et al. (2016), and levels of POPs observed in chicken eggs can be compared with those found in camel milk samples, although locations of free range chicken eggs sampling are different within these settlements. Some potential sources of contamination were determined in Shetpe and Baskuduk (see subchapter 2.1.). There was no obvious source of chicken eggs contamination observed in Tauchik.

More information about selected sites is in the following subchapter 2.1.

2.1 SAMPLING SITES

Detail description of the sampled sites and information about samples taken are provided in the following text and tables. The location of sampled localities is also shown on the maps of Kazakhstan in Figures 1 and 2.

Figure 2: Maps with locations where the samples were taken (2016).



2.1.1 BALKHASH

Geographical coordinates - 46°32'27" N 74°52'44" E

Balkhash is a city in Karaganda Region (Oblast), located on the northern shore of Lake Balkhash, at the Bay Bertys. Balkhash city (76,000 inhabitants) and its surroundings (30-50,000 inhabitants) are dominated by mining and nonferrous metallurgical enterprises. The major enterprise is Balkhashtsvetmet (the earlier [in Russian] Balkhashski Gorno-Metallurgicheski Kombinat). Further, the Balkhash Non-Ferrous Metals Processing Plant (in Russian Zavod Obrabotki Tsvetnych Metallov) is former part of the Kazakhmys Corporation LLC. Kazakhmys is a UK-registered copper mining company and the largest producer of copper in Kazakhstan. The Balkhashtsvetmet smelter is estimated to be the 22nd largest in the world and is one of only three plants in the world which still use the stationary Vanyukov submerged-tuyere furnaces developed in the former Soviet Union (Schlesinger 2011). In the early 1990s, emission levels were reported to be 280-320 thousand tonnes per year, depositing 76 tonnes of copper, 68 tonnes of zinc and 66 tonnes of lead on the surface of the lake. Since then, emissions have almost doubled (Wikipedia 2015).

Among the largest enterprises, the Balkhashtsvetmet is considered to be the largest atmospheric polluter and contributes about 20% of all pollution in the Republic (UNECE and KAZHYDROMET 2003); in spite of this the city is only ranked 16th on the UNECE priority list. Wikipedia confirms that emissions due to mining and metallurgical processes are a key factor affecting the ecology of the Ili-Balkhash basin and that it is mainly associated with pollution from the Balkhash BGMK operated by Kazakhmys (Wikipedia 2015).

Chemicals unintentionally produced in these industrial processes that are subject to Annex C of the SC (PCDD/Fs, PCBs and HCB) are reported to be one of major subjects of health concerns in Balkhash city.

The waste of the both Balkhash smelters is stored at tailing ponds occupying the 25 km², twice as large as Balkhash city itself.

Figure 3: Sampled eggs locations within area of Balkhash city.



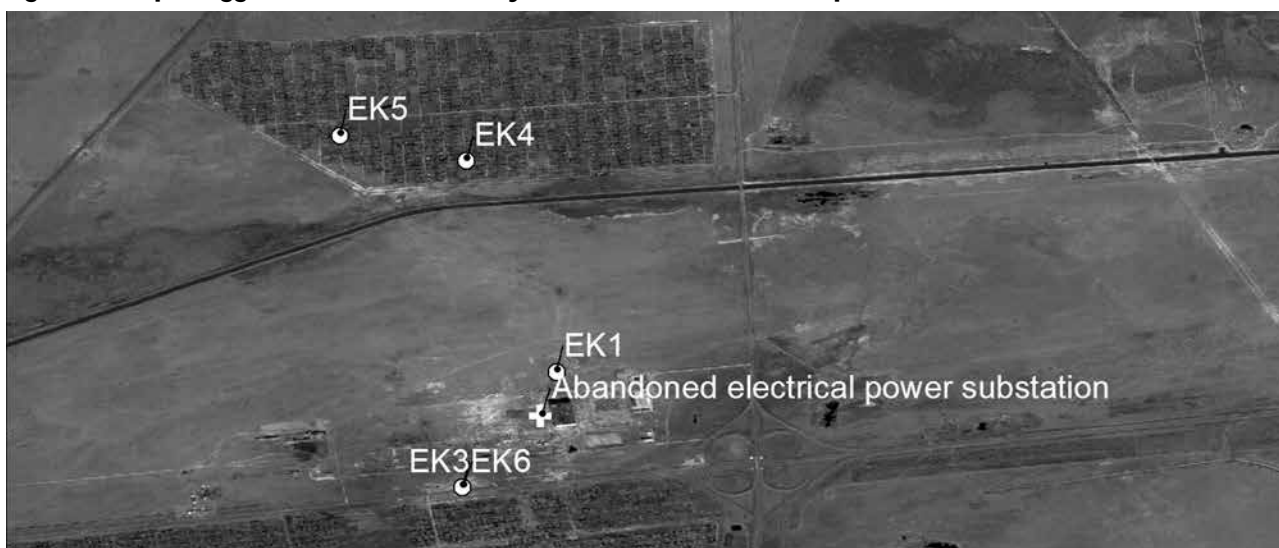
2.1.2 EKIBASTUZ – ABANDONED ELECTRICAL POWER SUBSTATION

Geographical coordinates - 51°48'59.10" N 75°18'46.00" E

The Ekibastuz electrical power substation was constructed for modifying alternating current (AC) to direct current (DC) using 15,000 capacitors placed in two outdoor areas. After the collapse of the Soviet Union the substation was left without an owner or guard. During the economic crisis, local residents illegally dismantled capacitors in order to remove copper scrap and this resulted in PCB leakage into the soil. During emergency clean-up works in 2002 the capacitors were dismantled and "sealed" with foam by the new owner of the area of former substation. Part of the PCB contaminated soil was removed and packed in bags. Capacitors and contaminated soil were removed and placed in underground storage at the former Semipalatinsk nuclear testing site (technical test area Opytnoe Pole).

The substation is on the edge of Ekibastuz city in Pavlodar Oblast with a population of more than 125,000. In the vicinity of the site there are large areas of suburban gardens – "dachas" (minimum distance is 500 m, total area is about 3 km²). In the guarded and fenced area of the facility (300 m from the object) resides a family whose job is to guard the site. The family grows and grazes their livestock on the site – cows, sheep and poultry.

Figure 4: Sampled eggs locations in the vicinity of the abandoned electrical power substation in Ekibastuz.



2.1.3 KARAGANDA CITY

Geographical coordinates - 49°48'40.76" N 73°05'27.37" E

We only sampled eggs from a poultry farm in a supermarket in order to get an idea about background levels of POPs.

2.1.4 NURA (RIVER)

Geographical coordinates - 50°07'45.82" N 72°50'27.62" E

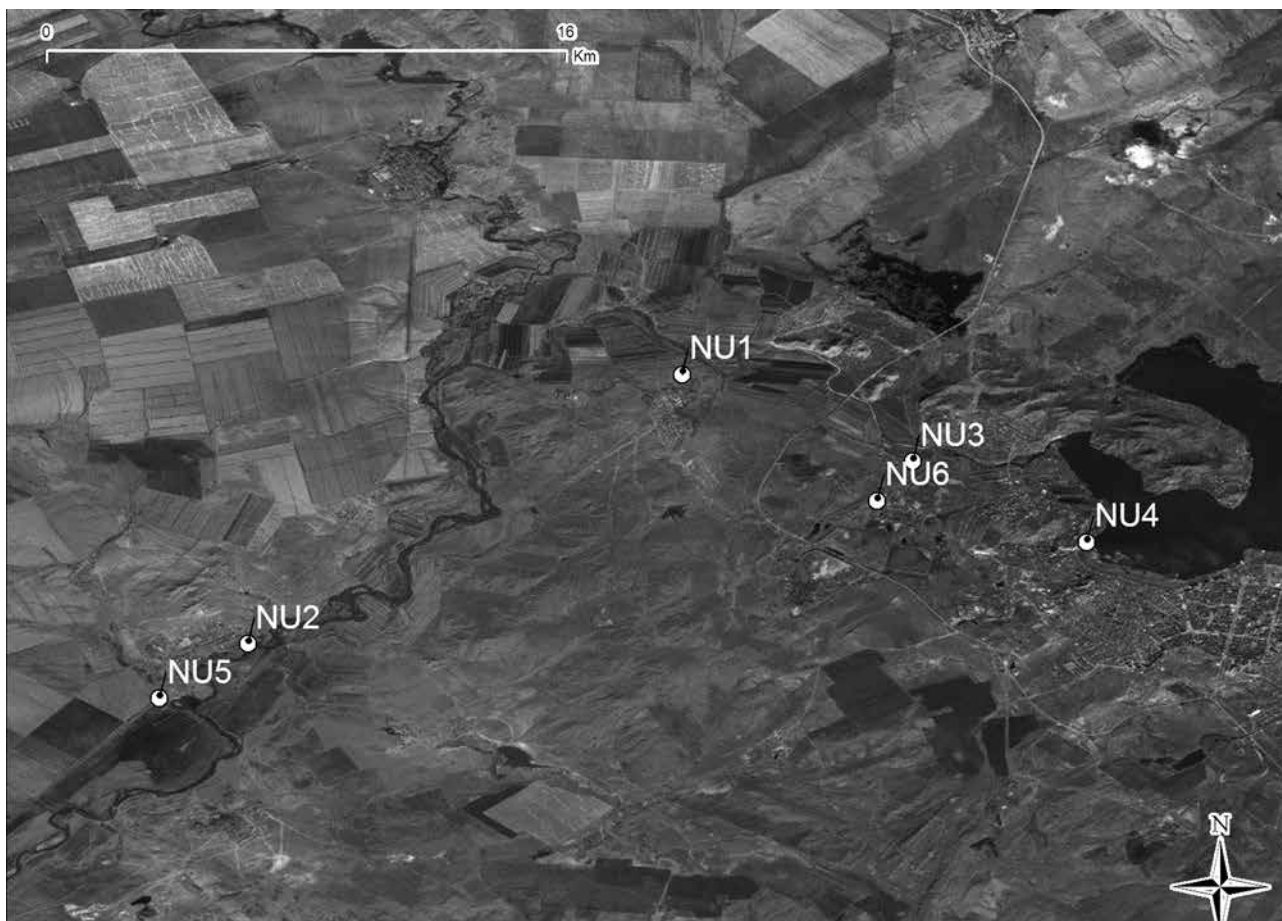
The Nura River is the main river of Central Kazakhstan. The river rises in the Kyzyltas Mountains in the northeast of Karaganda oblast and passes through the heavily industrialized area of Karaganda oblast, and then flows another 260 km to the capital Astana and Korgalzhyn State Nature Reserve, international important as the birds area and included in Sary-Arka UNESCO World Heritage Site. The total length of the river is 978 km. The river is a typical steppe river: 80% of the flow is caused by the spring thaw. Water is widely used for household water supply, irrigation, industrial use and also for recreation and commercial fishing. Nura is also a potential source of drinking water for the capital Astana.

The Nura has received high inputs of mercury since the 1950s, the source being the former Karbid chemical factory in the city of Temirtau near Karaganda. This chemical factory produced acetaldehyde by direct hydration of acetylene in the presence of a catalyst – mercuric sulphate. Development of the project was carried out by the Hiprokauchuk Company. Wastewater from the acetaldehyde factory with a high content of mercury was discharged into the river without treatment

for a period of approximately 25 years. During that time, total mercury concentrations in the effluent are suspected to have reached up to 50 mg/L and the average annual input of mercury to the river between 1950 and 1976 has been estimated as 22-24 tons. Until 1969 sludge containing mercury was discharged into Zhaur swamp. Preliminary investigations of the extent of pollution on the Nura carried out in the 1980s revealed extremely high levels of contamination. This non-statistical based study of mercury in the silt of 33 river profiles showed that the sediments are highly polluted, with average total mercury concentrations in excess of 200 mg kg⁻¹ in the first 9 km downstream of the source. On the basis of the detected concentrations, it was estimated that the total amount of mercury in the bed of the river could be in the order of 140 tons. During the period when the mercury was discharged, up to 5 million tonnes of fly ash was also discharged into the river by a local coal power station. During the spring floods, large amounts of these highly contaminated sediments were transported down the river and dispersed over the floodplain and caused widespread pollution.

The project team took samples of soil, sediments, chicken eggs and fish in locations of the river Nura and settlements on this river, however evaluation of these samples is not part of this publication but other output of this project which is called "Contaminated sites and their management" apart free range chicken eggs samples which are listed below. Basic information about other samples can be found in Šir's report (Šir 2015).

Figure 5: Sampled eggs locations in the broader area of Nura river downstream for the abandoned acetaldehyde factory, and by the Samarkand water reservoir in the vicinity of the city Temirtau (see chapter 2.1.6.).



2.1.5 SHABANBAI BI

Geographical coordinates - 48°24'13.76" N 75°23'42.65" E

Shabanbai Bi is a village located in the southern part of the Karaganda Oblast. The village is situated at the foot of Aksoran, the highest peak of the Kyzylarai mountains (nature protected area - "zakaznik"), is one of the places in Central Kazakhstan where ecotourism is developed based on the local community. Tourists are encouraged to lodge in the houses of local inhabitants as an incomparable way of getting acquainted with the simple way of village life, and to sample the traditional Kazakh cuisine (visitkazakhstan.kz 2014). We have chosen this site as a clean background locality, however the results of egg analysis have shown hidden problems, as described further in this publication.

Figure 6: A satellite map of the Shabanbai Bi area with marked location of the eggs sampling site



2.1.6 TEMIRTAU

Geographical coordinates - 50°03'5.77" N 72°57'58.58" E

Temirtau city (170,000 inhabitants) and its surroundings (100,000 - 200,000 inhabitants) are dominated by industries including a coal-fired power station, chemical production plants, foundries, forges and large steelworks belonging to the ArcelorMittal group. The steel mill Arcelor Mittal Temirtau (AMT) is located a distance of 500 m from the nearest houses. According to the Kazakhstani NIP of the Stockholm Convention from 2009, there were 105 transformers filled with Sovtol (commercial PCB mixture marketed in the former USSR) and 1,024 capacitors containing PCBs in use in AMT (Republic of Kazakhstan 2009). The situation was addressed under the UNDP project "Development and implementation of the comprehensive plan on the management of PCBs" in 2014, when the Sovtol liquid was relocated to France. However, EcoMuseum and CINEST, two NGOs based in Karaganda, report some PCB containing electrical equipment to still be in use in AMT.

Industries unintentionally producing PCDD/Fs include coke and foundry productions, both taking place in AMT as the only such enterprise in Kazakhstan. The processes of unloading and coke extinction, when PCDD/Fs can be released, are taking place in open air without a gas trapping and cleaning device. Formation of PCDD/Fs is also possible during limestone burning in shaft kilns. In Kazakhstan, lime is produced in the JS Temirtau Electro Metallurgical Plant (former Temirtau Chemical Metallurgical Plant). The Bashkortastan Republican Scientific Ecological Center carried out the first sampling campaign focused on PCDD/Fs in Kazakhstan in 2005. The PCDD/Fs concentration in indoor air sampled at the AMT sinter machine no. 5 was 42.64 pg m⁻³ (3.77 pg WHO-TEQ m⁻³), in the dust (wall scrapes) 5419.7 pg g⁻¹ (607.7 pg WHO-TEQ g⁻¹). According to the Kazakhstani NIP (2009), wastes produced by these industries may be a source of environmental pollution with POPs.

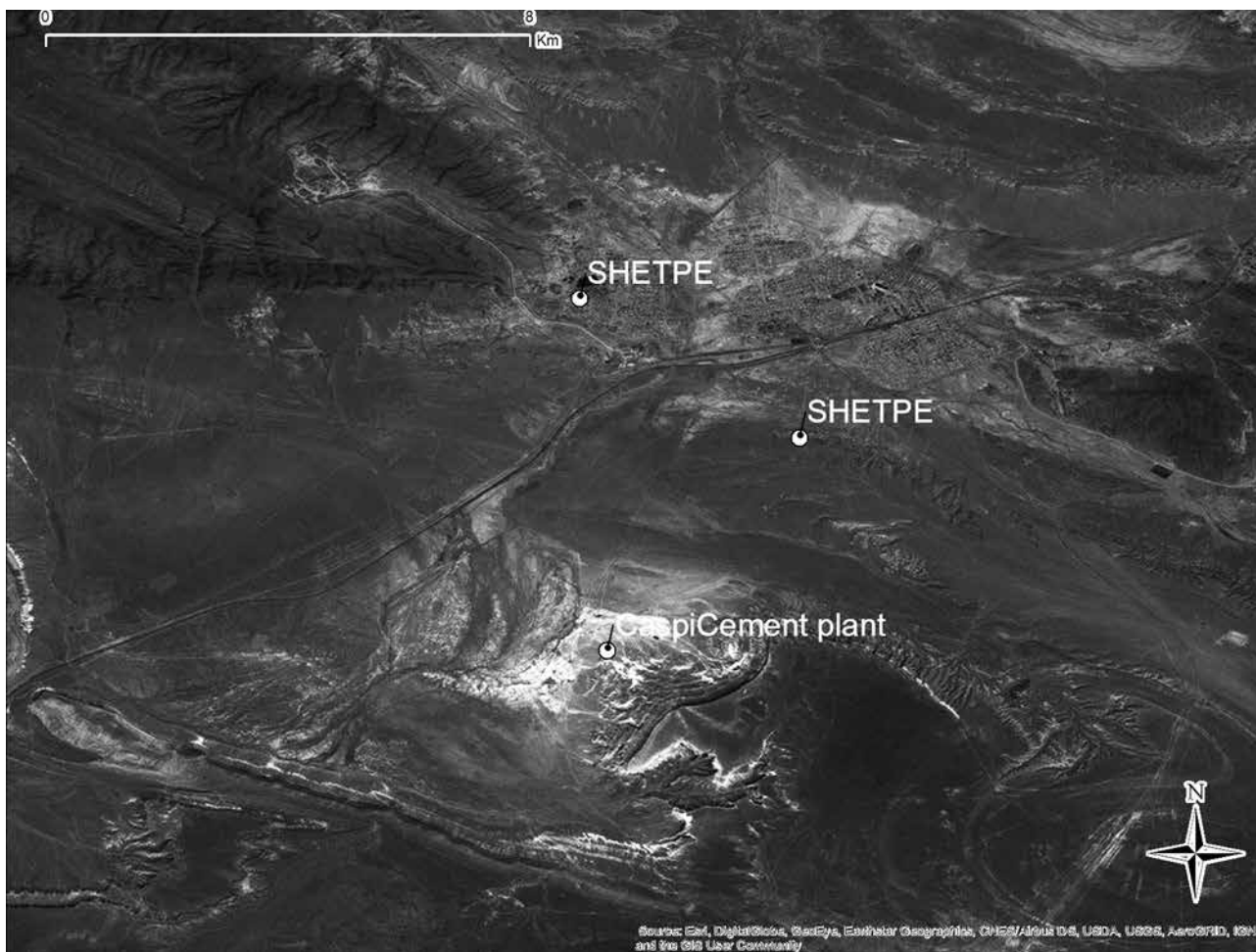
Samples were taken in surrounding of the Samarkand water reservoir on Nura river.

2.1.7 SHETPE

Geographical coordinates - 44°05'21.3" N 52°07'16.0" E

Shetpe is a town with population of 13,364 (according data from 2012)¹ – administrative centre of the Mangystau District of the Mangystau Region, lies 108 km from the city of Aktau, and a settlement at important rail junction. Its economy is based on agriculture and especially cattle breeding, mining and cement production (there are 16 stone quarries surrounding the town). Shetpe is slowly becoming an important starting point of the tourism, as the majority of natural and cultural sites of Mangystau Region can be easily reached from here – such as Ustyurt Plateau, Adai Ata Monument or legendary mountain Sharkala.

Figure 7: Satellite map of Shetpe with marked locations of sampled free range chicken eggs.



Possible source of the pollution might be the CaspiCement plant which is situated 7 kilometers from the centre of Shetpe. CaspiCement is a new plant constructed in the multinational HeidelbergCement corporate group, officially opened in July 2014. It is the only cement production site in Mangystau with production capacity of 800 thousand tons of cement per year. It is one of the first plants in the world using dry chalk for clinker production. The plant is using oil as a fuel. Local people state that the factory often releases uncontrolled exhaust gases during nights and weekends, when the state authorities cannot thoroughly control the facility.

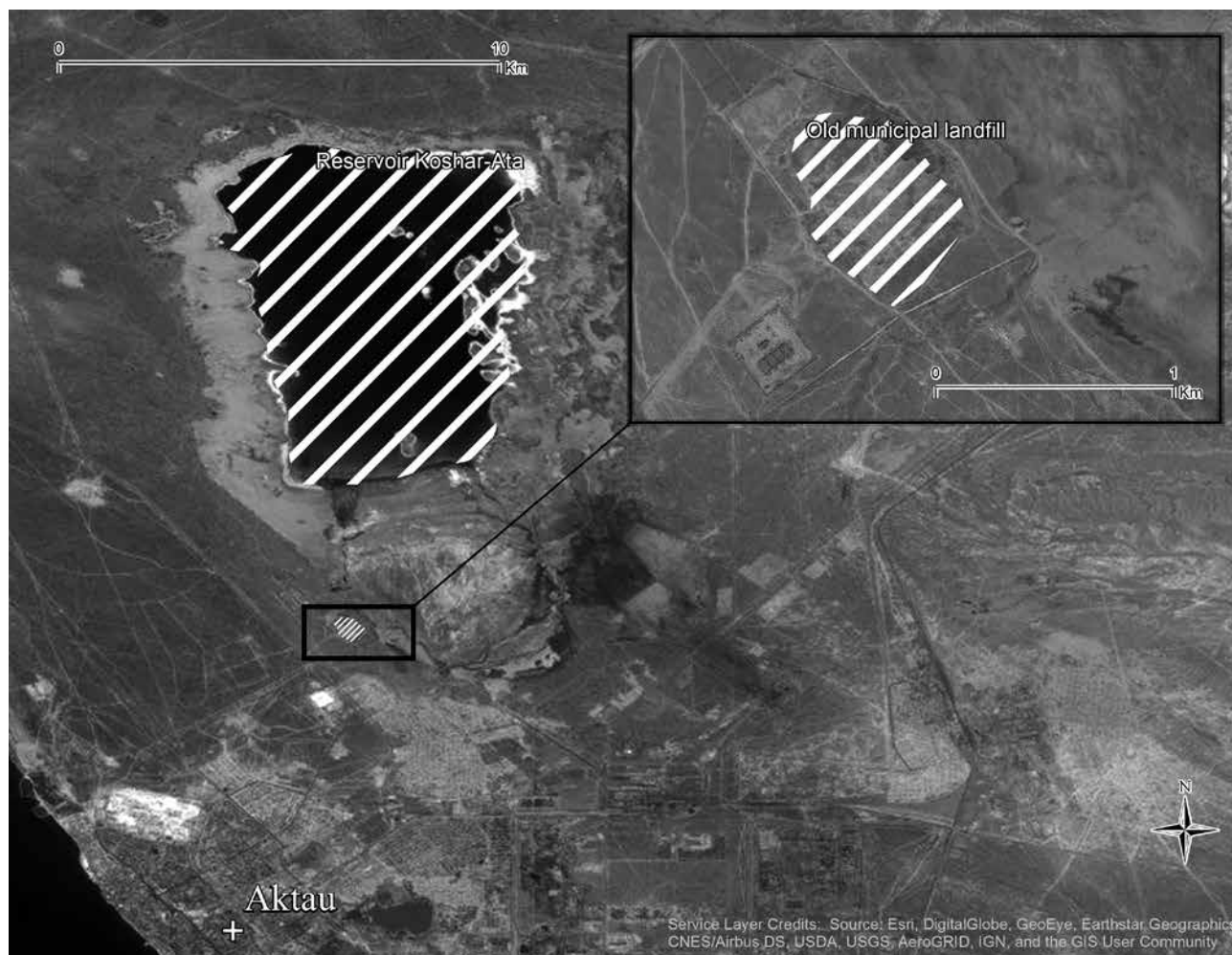
¹ Wikipedia. (2016, 30-03-2016). "Шетпе." Retrieved 31-07-2016, 2016, from <https://ru.wikipedia.org/wiki/%D0%A8%D0%B5%D1%82%D0%BF%D0%B5>.

2.1.8 AKTAU: BASKUDUK

Geographical coordinates - 43°41'50.5" N 51°12'28.4" E

The pooled sample of free range chicken eggs was taken from a north-western part of the Baskuduk, fast growing suburb of Aktau approximately 5 kilometers north of centre of Aktau. The settlement is inhabited mainly by Kazakh minorities repatriated from the surrounding countries (Uzbekistan - Karakalpakstan, Turkmenistan). A substantial part of the newcomers had illegally occupied land on outskirts of the city, because of insufficiency of the state housing programme. The area is located in the northern part of Aktau city, squeezed between an industrial tailing pond Koshkar Ata and a municipal landfill in the north and an industrial zone including chemical industry in the east (see map at Figure 8).

Figure 8: Map of situation in surrounding of sampling site in Baskuduk.



The potential source of pollution is a tailing pond **Koshkar Ata** (77 km²). Its southern border is located about 7 kilometers from the sampling point. During the Soviet era the Koshkar Ata depression was chosen as a convenient location to accumulate liquid industrial waste from Aktau industrial zone. After dozens of years of the wastewater discharge, a large artificial lake was formed. Lake is considered to be the most hazardous object in Mangystau Region (Kadyrzhanov, Kuterbekov et al. 2002), although some more recent studies concluded that *"The negative impact is registered only in the immediate vicinity of the perimeter of the tailing lake in the first hundreds of metres"* (Zhanpeissova, Kuterbekov et al. 2005). The lake allegedly contains almost 360 millions tons of radioactive waste by weight (Akhmetov, Kadyrzhanov et al. 1999).

One of majors operators in the industrial zone was a uranium processing plant. Another large factory located in the area was the Aktau Plastics Plant (Aktau Zavod Plastmass produced polystyrene, styrene, ethylene, ethylbenzene, polysulfones, polyethersulfones). There are also a number of other factories, mainly from the chemical sector emitting various toxic pollutants. Nowadays, the wastewater from industrial zone is still discharged to the lake by open canal. Sewage water is also discharged into the lake because the city failed to finish the construction of wastewater treatment plant for its northern part including new districts being under construction nowadays (2016). Surrounding of Koshkar Ata is widely used as an illegal

dumping ground for industrial and household waste. Dumpsite contains a diverse mixture of waste: used furniture, animal carcasses, fluorescent tubes, food leftovers, glass bottles, oil sludge, etc. The animals such as sheep, horses and camels come here to drink as the Mangystau land is short on water. The highest concentration of contaminants, solid waste and high levels of radioactivity are concentrated in the southern part of the lake.

More information about broader area can be found in previous report on contaminants in camel milk by Petrlík et al. (2016).

Municipal waste dump located right on the edge of Baskuduk may also contribute to overall pollution of the site. During our visit we found also an old leaking transformer by the entrance to the landfill (see Photo 1 in Chapter 9).

2.1.9 TAUCHIK

Geographical coordinates - 44°20'54.1" N 51°21'11.2" E

The sampling site is located within the Tauchik village, which is a settlement in the Tuparkagan district, 100 km north from Aktau. The population of Tauchik is about 2,600 people as of the 2009 census.² It was founded at the beginning of the 30s of the 20th century near the deep coal reserves in vicinity. In the period 1932 - 39 the coal mining flourished, and later on it produced coal for supply in the World War II but it stopped operation after the 1950 - 60s period.³

The area is also associated with oil extraction and transport, which could be another pollution source. Nearby oil fields are called Karazhanbas, Karakuduk, Buzachi. About 4 km from Tauchik to the South, there is a small settlement with the KazMunaiGaz Gas station at the junction of the road Tauchik-Zhyngyldy and a larger road connecting Aktau and Kiyakty and the Khalamkhas oil field, operating on the shelf of the Caspian sea, owned by the MangystauMunaiGaz company and operating together with the KazMunaiGaz at the northern extraction site. A pipeline Khalamkhas-Karazhanbas-Aktau stretches along the road.

2.2 SAMPLING AND ANALYTICAL METHODS

Pooled samples of more individual egg samples were collected at each of the selected sampling sites in order to get more a representative sample. Table 1 summarizes the basic data about size of samples and measured levels of fat content in each of the pool samples. Twenty pool samples of free range chicken eggs were taken in total plus one sample was taken in 2015 in Karaganda, where we bought chicken eggs in a supermarket as suggested by Dvorská (2015). The last one of above mentioned samples is used to exhibit background levels of POPs because a remote location in Shabanbai Bi didn't prove to be clean, as explained further. Eleven samples were taken in 2013 and ten in following year 2014. Another sample in Shabanbai Bi was taken in 2015 in order to re-examine high levels of POPs found in this location, and four samples in Mangystau Region were taken during field visit in September 2016.

Free range chicken eggs from the first field visit determined for the analysis of PCDD/Fs and dioxin-like PCBs using the DR CALUX® method were sent to a Dutch ISO 17025 accredited laboratory (BioDetection Systems B.V., Amsterdam). The procedure for the BDS DR CALUX bioassay has previously been described in detail (Besselink H 2004) but, briefly, H4IIE cells stably transfected with an AhR-controlled luciferase reporter gene construct were cultured in α -MEM culture medium supplemented with 10% (v/v) FCS under standard conditions (37°C, 5% CO₂, 100% humidity). Cells were exposed in triplicate on 96-well microtiter plates containing the standard 2,3,7,8-TCDD calibration range, a reference material (tested by GC-HRMS), a procedure blank and a DMSO blank according EC/589/2014 guideline (European Commission 2014). Following a 24 hour incubation period, cells were lysed, a luciferin containing solution (Illuminate Mix) was added and the luminescence was measured using a luminometer (Berthold Centro XS3).

² Wikipedia. (2015, 08-09-2015). "Таушық." Retrieved 31-07-2016, 2015, from <https://ru.wikipedia.org/wiki/%D0%A2%D0%B0%D1%83%D1%88%D1%8B%D0%BA>.

³ Wikipedia. (2015, 17-05-2015). "Таушық." Retrieved 31-07-2016, 2015, from <https://kk.wikipedia.org/wiki/%D0%A2%D0%B0%D1%83%D1%88%D1%8B%D2%9B>.

Table 1: Overview of free range chicken egg samples from selected sites in Kazakhstan.

No	Sample	Locality	Year of sampling	Number of eggs in pooled samples	Fat content
1	BAL-EGG-14-1	Balkhash - southwest	2014	6	12.45
2	BAL-EGG-14-2	Balkhash - southwest	2014	6	9.95
3	BAL-EGG-14-3	Balkhash - Rembaza	2014	6	10.15
4	BAL-EGG-14-4	Balkhash - Rembaza	2014	4	11.35
5	B 1	Balkhash - Rembaza	2013	6	13.2
6	B 2	Balkhash - Rembaza	2013	6	16.4
7	B 3	Balkhash - southwest	2013	6	18.6
8	B 4	Balkhash - north	2013	4	13.5
9	B 5	Balkhash - southwest	2013	10	14.9
10	EKI egg 1	Eikbastuz - substation	2013	4	10.5
11	EKI egg 2	Eikbastuz - Soyuz	2013	4	16.4
12	EKI-14-1-egg and EKI-27-egg	Ekibastuz - substation	2014	6	12.4
13	EKI-14-2-egg	Ekibastuz - Soyuz	2014	6	11.7
14	EKI-14-3-EGG	Ekibastuz - Soyuz	2014	6	13.3
15	NUR-EGG-14/2	Chkalovo; Nura	2014	6	13.7
16	NUR egg 24-2	Chkalovo; Nura	2013	6	12.5
17	KAR-SU	Karaganda - supermarket	2015	6	14.0
18	NUR-EGG-14/1	Rostovka; Nura	2014	6	15.0
19	NUR egg 24-1	Rostovka; Nura	2013	6	16.2
20	NUR egg 1	Samarkand village; Nura	2013	6	18.0
21	NUR egg dam	Temirtau; Samarkand dam	2013	6	24.1
22	ARAI EGG	Shabanbai Bi	2014	6	10.15
23	Shabanbai Bi-2	Shabanbai Bi	2015	6	28.8
24	BAS 02	Baskuduk	2016	3	15.6
25	TA E-1	Tauchik	2016	2	16.2
26	SH E-1/1-2	Shetpe	2016	4	15.4
27	SH E-2	Shetpe	2016	3	12.5

The DR CALUX bioassay method is proven for screening analyses which can give a good figure about the level of pollution⁴; however, for confirmation it is necessary to go for more specific PCDD/Fs and DL PCBs congener analyses, which also allows examination of finger prints of dioxins (PCDD/F congener patterns), specific for different sources of pollution. Most of the samples from the second and third sampling period (September 2014 and September 2016) were analyzed for content of individual PCDD/Fs and an extended list of PCB congeners by HRGC-HRMS at the accredited laboratory of the State Veterinary Institute in Prague, Czech Republic. PAHs were analyzed by the HPLC-FLD method in the same laboratory. Some of the samples were from at least the same area as those from the first field sampling.

Egg samples were also analyzed for content of non-dioxin-like PCBs, OCPs and BFRs in a Czech certified laboratory (Institute of Chemical Technology, Department of Food Chemistry and Analysis). The analytes were extracted by a mixture of organic solvents hexane:dichloromethane (1:1). The extracts were cleaned by means of gel permeation chromatography (GPC). The identification and quantification of the analyte was conducted by gas chromatography coupled with tandem mass spectrometry detection in electron ionization mode. The method of ultra-high-performance liquid chromatography coupled with tandem mass spectrometry detection (UHPLC-MS/MS) was chosen for the analysis of isomers of HBCD. The other BFRs were analyzed via previously mentioned technique.

The mercury content in the samples was analyzed with atomic absorption spectrometry in an Advanced Mercury Analyser (AMA 254, Altec) using standard operating procedure SOP 70.4 (AAS-AMA) at the State Veterinary Institute, Prague.

4 *“Bioanalytical methods” means methods based on the use of biological principles like cell-based assays, receptor assays or immunoassays. They do not give results at the congener level but merely an indication of the TEQ level, expressed in Bioanalytical Equivalents (BEQ) to acknowledge the fact that not all compounds present in a sample extract that produce a response in the test may obey all requirements of the TEQ-principle. European Commission (2014). Commission Regulation (EU) No 589/2014 of 2 June 2014 laying down methods of sampling and analysis for the control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs and repealing Regulation (EU) No 252/2012 (Text with EEA relevance). European Commission. Official Journal of the European Union. EC 589/2014: 18-40.*

3. THE KAZAKHSTANI, EU, AND OTHER LIMITS FOR POPs IN EGGS

Chicken eggs are a quite common part of the diet in Kazakhstan and it is also common that people in Kazakhstan raise their own chicken and partly sell left over chicken eggs at markets as raw eggs or as food in cafes. One should expect that for such a common food there would be limits for the content of certain contaminants; however, we didn't find any limit values set up for chicken eggs in Kazakhstani legislation for any POPs. We had to compare the results of analyses for POPs with other than Kazakhstani national legislation limit values for contaminants in food. Those we used for free range chicken eggs are summarized in Table 2. There is a Kazakhstani limit value for the content of mercury in chicken eggs (see Table 2).

Table 2: Limit concentration values for OCPs, mercury, PCBs and PCDD/Fs TEQs in chicken eggs

	Hen eggs				
	Kazakhstani MAC ⁷	Russian MAC ¹	Russian MAC ⁴	EU ML ²	EU MRL ³
Unit	ng g ⁻¹ *	pg g ⁻¹ fat	ng g ⁻¹ *	pg g ⁻¹ fat	ng g ⁻¹ fat
WHO-PCDD/Fs TEQ		3.0		2.5	
WHO-PCDD/Fs-dl-PCB TEQ				5.0	
PCBs ⁵				40	
DDT total ⁶					50
p,p'-DDT			100		
γ-HCH (lindane)			100		10
α-, β-HCH			100		20, 10**
HCB					20
Mercury	20				

¹ Current Russian СанПиН 2.3.2. 2401-08 Hygienic safety and nutrition value requirements for food. Sanitary-epidemiologic rule and normatives (СанПиН 2.3.2. 2401-08 Гигиенические требования безопасности и пищевой ценности пищевых продуктов Санитарно-эпидемиологические правила и нормативы)

² EU Regulation (EC) №1259/2011

³ Regulation (EC) №149/2008. Maximum residue level (MRL) means the upper legal level of a concentration for a pesticide residue in or on food or feed set in accordance with the Regulation, based on good agricultural practice and the lowest consumer exposure necessary to protect vulnerable consumers.

⁴ Russian Federation GN 1.2.2701-10 Hygienic normatives (standards) pesticides concentration in environmental media (ГН 1.2.2701-10 "Гигиенические нормативы содержания пестицидов в объектах окружающей среды")

⁵ sum of PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180

⁶ sum of p,p'-DDT, o,p'-DDT, p,p'-DDE and p,p'-DDD

⁷ Kazakhstan SanPin Hygienic safety requirement and nutrition value for food since 11.06.2003.

* not clear whether calculated for fat content or not

** for each isomer is MRL set separately

4. RESULTS

The results of the analyses by using DR CALUX are summarized in Table 3 and the graph in Figure 9. The results of the analyses for other POPs and congener analyses by using HRGC-HRMS are summarized in Table 4. There are also few results for analyses of mercury content in selected samples of eggs in Table 4. The graph in Figure 10 compares the results of the analyses for 6 PCB indicator congeners. The graph also shows a comparison with EU limit value for PCBs content in chicken eggs. Free range chicken eggs from China and Belarus were also analyzed by using the same methods. So we can compare data from Kazakhstani hot spots with similar locations in other countries as well. The results for samples from China and Belarus are presented in Tables 3 and 4 together with the results for samples from Kazakhstan.

The results for OCPs on fresh weight basis are summarized in Table 7 and compared with respective EU limit values.

4.1 DIOXINS (PCDD/Fs) AND DIOXIN-LIKE PCBs MEASURED BY DR CALUX

All samples of chicken eggs collected at Kazakhstani hot spots during a field visit in 2013 were screened for dioxins and dioxin-like PCBs by using the DR CALUX method in BDS laboratory, Amsterdam. Two samples from a second sampling period in 2014 were also analyzed there. The results are summarized in Table 3 together with selected results for the eggs from China and Belarus.

When PCDD/Fs and dl-PCB levels determined by the DR CALUX method are discussed, the following has to be considered. This cell based reporter gene assay is a validated method for screening for PCDD/Fs and dl-PCBs contents in food according to EU Commission Regulation EC/589/2014 (European Commission 2014). Screening methodologies are usually used to exempt

Table 3: Results of DR CALUX bioassay analyses for both PCDD/Fs and DL PCBs for samples from Kazakhstan, China and Belarus. Data are in pg BEQ g⁻¹ fat.

Sample	Locality	Country	PCDD/Fs and DL PCBs (DR CALUX)	PCDD/Fs (DR CALUX)
EKI-14-3-EGG	Ekibastuz - Soyuz	Kazakhstan	3.8	1.3
eki egg 2	Eikbastuz - dachas South	Kazakhstan	4.8	NA
eki egg 1	Eikbastuz - substation	Kazakhstan	6.4	NA
NUR egg 1	Samarkand village; Nura	Kazakhstan	9.2	NA
NUR egg 24-1	Rostovka; Nura	Kazakhstan	9.4	NA
B 2	Balkhash - Rembaza	Kazakhstan	12	NA
B 3	Balkhash - southwest	Kazakhstan	15	NA
ARAI EGG	Shabanbai Bi	Kazakhstan	16	7.6
NUR egg 24-2	Chkalovo; Nura	Kazakhstan	18	NA
B 1	Balkhash - Rembaza	Kazakhstan	24	NA
NUR egg dam	Temirtau; Samarkand dam	Kazakhstan	28	NA
B 5	Balkhash - southwest	Kazakhstan	33	NA
B 4	Balkhash - north	Kazakhstan	101	NA
LN 321/14	Beijing - supermarket	China	1.2	NA
LN 272/14	Gatovo	Belarus	8.1	5.2
Beihai 3 and 4	Beihai II	China	8.9	7.4
Likeng	Likeng	China	17	NA
Beihai 5 and 6	Beihai III	China	24	20
Beihai 1 and 2	Beihai I	China	37	30
LN 273/14	Wuhan 1	China	35	31

those samples that are below the maximum permitted limit (i.e. are compliant with the limit) and that can, therefore, be released to the market. In addition, one needs to select those samples that require confirmation (i.e. are suspected to be non-compliant) of their PCDD/F/dl-PCB-TEQ levels.

Thirteen pool egg samples were analyzed using the DR CALUX method for determination of dioxin/DL PCB-TEQ activity in total. Among those only two samples from Ekibastuz were below limits set up by the EU also used for consideration of results obtained by DR CALUX analyses. All other eleven samples were above 5 pg BEQ g⁻¹ fat level for total PCDD/Fs and DL PCBs content, as it is shown by the graph in Figure 9. The highest level of 101 was measured in chicken eggs from Balkhash city, north from the metallurgical plant. The sample was taken in 2013 at the site where we were not able to repeat sampling in 2014. All other samples from Balkhash city were also high, between 12 and 33 pg BEQ g⁻¹ fat. These levels are comparable to those obtained for free range chicken eggs from Beihai, a similar hot spot with a metallurgical plant in China (8.9 – 37 pg BEQ g⁻¹ fat). Samples for Temirtau broader area show slightly lower levels, between 9.2 and 28 pg BEQ g⁻¹ fat, however not significantly lower than those from Balkhash. The sample from Shabanbai Bi which was considered to be a clean area and sampled as such in 2014 has shown high content of dioxins and DL PCBs at a total level of 16 pg BEQ g⁻¹ fat, although the results suggest higher levels of DL-PCBs rather than PCDD/Fs, which is the complete opposite compared to the locality of, for example, a waste incinerator in Wuhan, China and/or metallurgical site of Beihai, China (see results for PCDD/Fs share on total BEQ in Table 3). The sample from Shabanbai Bi was also analyzed by GC-HRMS for specific congeners and results have shown an even higher total TEQ content of PCDD/Fs plus DL PCBs and confirmed that the PCB share is higher than PCDD/Fs. This difference is in agreement with findings by Gasparini et al. (2011) who suggested that: “From a preliminary evaluation of the contamination profile, it should seem that the major differences between screening and confirmation results, are present in those sample where the contribution to total TEQ is due overall to dl-PCBs rather than to PCDDs and PCDFs.”

PCDD/Fs and DL PCB (DR CALUX)

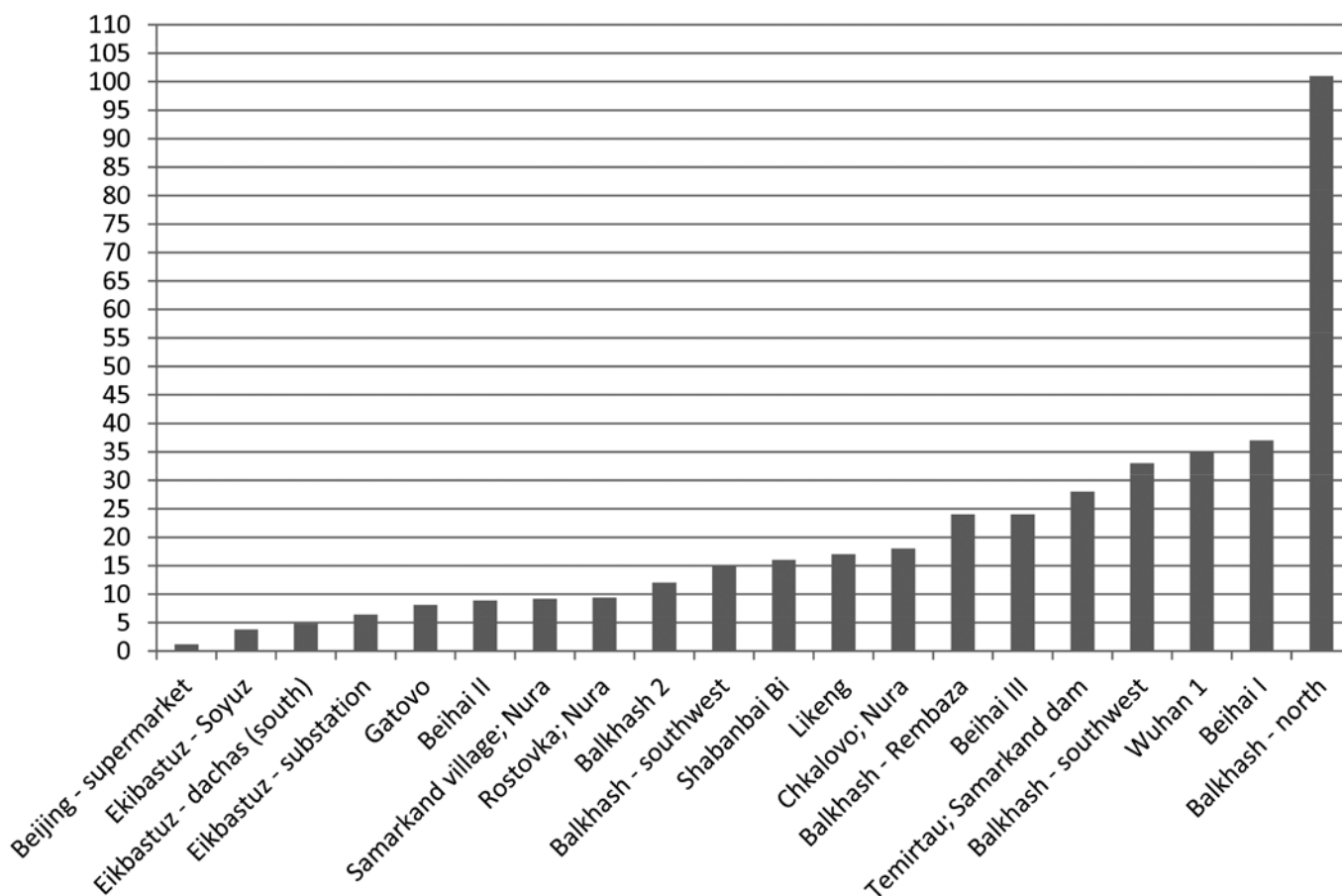


Figure 9: Graph showing comparison of total PCDD/Fs and DL PCBs in pg BEQ g⁻¹ fat for different pooled chicken eggs samples from Kazakhstan, China and Belarus (full set of results is in Table 3).

4.2 DIOXINS (PCDD/Fs), PCBs AND OTHER POPs MEASURED BY GAS CHROMATOGRAPHY METHODS

GCMS-HRMS analyses were chosen for confirmation of contamination by dioxins and dioxin-like PCBs of chicken eggs at the same localities where they have been shown to be suspected for potentially high levels of these POPs after the first year of sampling. The same samples were also analyzed for other POPs, group of OCPs: hexachlorobenzene (HCB), hexachlorocyclohexanes (HCHs) and DDT and its metabolites. HCB is also considered to be unintentionally produced POP (U-POP) in the same processes as dioxins and DL PCBs (Stockholm Convention on POPs 2008), although it is commonly measured together with other OCPs. Also two other U-POPs, pentachlorobenzene (PeCB) and hexachlorobutadiene (HCBd) were analyzed in some samples. Thirteen pooled samples of eggs were analyzed for PCDD/Fs and DL PCBs in total and sixteen samples for other POPs. A few samples were also measured for mercury content. The results are summarized in Table 4 (see pages 22-23).

4.2.1 DIOXINS (PCDD/Fs) AND DIOXIN-LIKE PCBs (DL PCBs)

Dioxins belong to a group of 75 polychlorinated dibenzo-p-dioxin (PCDD) congeners and 135 polychlorinated dibenzofuran (PCDF) congeners, of which 17 are of toxicological concern. Polychlorinated biphenyls (PCBs) are a group of 209 different congeners which can be divided into two groups according to their toxicological properties: 12 congeners exhibit toxicological properties similar to dioxins and are therefore often referred to as 'dioxin-like PCBs' (DL PCBs). The other PCBs do not exhibit dioxin-like toxicity but have a different toxicological profile and are referred to as 'non dioxin-like PCB' (NDL PCBs) (European Commission 2011). Levels of PCDD/Fs and DL PCBs are expressed in total WHO-TEQ calculated according toxic equivalency factors (TEFs) set by WHO experts panel in 2005 (Van den Berg, Birnbaum et al. 2006). These new TEFs were used to evaluate dioxin-like toxicity in thirteen pooled samples of chicken eggs from Kazakhstan.

Eight out of thirteen and seven out of thirteen samples from Kazakhstan exceeded EU and Russian MAC of PCDD/Fs congeners in chicken eggs, respectively (compare Tables 4 and 2) and ten out of total thirteen samples exceeded EU limit value for both PCDD/Fs and DL PCBs in chicken eggs (European Commission 2011). The background levels for PCDD/Fs and DL PCBs measured in chicken eggs from a supermarket in Karaganda were 0.90 and 0.00 pg WHO-TEQ g⁻¹ fat, respectively (see also discussion about background levels further in text). The highest level of dioxins (9.81 pg WHO-TEQ g⁻¹ fat) was measured in eggs from Balkhash - southwest (BAL-EGG-14-2) and almost the same level was measured in the sample from Shabanbai Bi, which had the second highest level of total WHO-TEQ (37.88 pg WHO-TEQ g⁻¹ fat), and toxicity of DL PCBs has prevailed in that sample (see graph in Figure 11). The other sample from Shabanbai Bi taken one year later contained even higher concentration of DL PCBs and showed the highest total TEQ level among all samples in this study (155.27 pg WHO-TEQ g⁻¹ fat). Most of the egg samples showing high levels of total WHO-TEQ had a prevalence of DL PCBs share over PCDD/Fs on total WHO-TEQ, as shown by the graph in Figure 11. It shows how big the impact is of potential PCBs sources of pollution such as for example obsolete transformers with PCBs containing oils or their creation in industrial processes such as metallurgical plants. Only in two egg samples from Balkhash - southwest was the balance between PCDD/Fs and PCBs in total WHO-TEQ opposite, and PCDD/Fs prevailed.

Total WHO-TEQ levels of PCDD/Fs and DL PCBs in samples from Kazakhstani hot spots are higher than in selected samples from Chinese and Belorussian hot spots; however, higher levels of PCDD/Fs were observed in eggs from the surroundings of the Wuhan waste incinerator in China. Samples from Shabanbai Bi, Rostovka, Chkalovo and Balkhash - Rembaza would belong to those with rather high levels of dioxins and DL PCBs, also in comparison with collection of samples from IPEN's The Egg Report from 2005, and comparable with samples from Dandora, Kenya (mixed waste dumpsite), Lucknow, India (medical waste incinerator site) or Mbeubeuss, Senegal (mixed waste dumpsite) (DiGangi and Petrlik 2005, IPEN Dioxin PCBs and Waste Working Group, Envilead et al. 2005, IPEN Dioxin PCBs and Waste Working Group, Pesticide Action Network (PAN) Africa et al. 2005, IPEN Dioxin PCBs and Waste Working Group, Toxics Link et al. 2005). Putative sources of dioxin and dioxin-like PCBs contamination at selected Kazakhstani hot spots are considered in the discussion part below.

4.2.2 POLYCHLORINATED BIPHENYLS (PCBs)

Six out of a total of fifteen free range chicken eggs samples from Kazakhstani hot spots exceeded the EU limit for 6 PCB indicator congeners in hen eggs. An extremely high level of 1976 ng g⁻¹ fat was observed in eggs from Shabanbai Bi sampled

in 2014 and exceeded the EU limit by almost 50 fold. Six PCB congener levels in the samples from Rostovka and Chkalovo on river Nura exceeded the EU limit by seven and nine fold, respectively. Also more recent sample of free range chicken eggs from Shabanbai Bi from the same farm exceeded the EU limit by five fold. All these samples can be considered as highly contaminated by PCBs and potential sources of contamination should be found. Also, dioxin-like PCBs were high in all these three samples and were major contributors to the overall WHO-TEQ level in these pooled egg samples (see the graph in Figure 11). The highest level of 6 PCB indicator congeners (26.85 ng g⁻¹ fat) among samples from Mangystau Region was found in free range chicken eggs sampled nearby the landfill site in Baskuduk, although it didn't exceeded the EU limit.

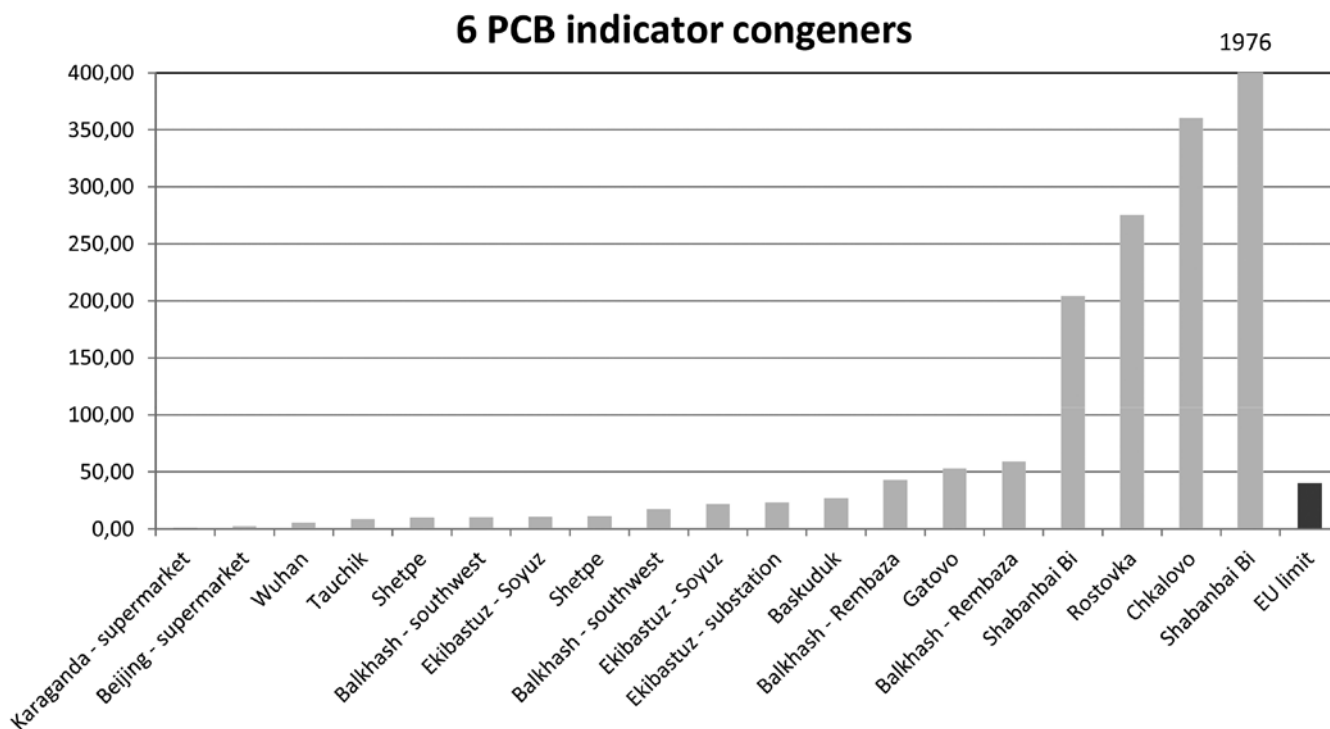


Figure 10: Graph comparing 6 PCB congener levels in different pooled chicken eggs samples from Kazakhstan, China and Belarus (full set of results is in Table 3).

Two samples from Balkhash - Rembaza also exceeded the EU limit value for 6 PCB congeners, although not so significantly. Also in this case DL PCBs were major contributors to total TEQ in eggs (see graph at Figure 11). Prevalence of PCBs upon PCDD/Fs in total TEQ was also measured in samples from Ekibastuz, where an electricity substation contaminated by PCBs is a putative source of egg contamination by U-POPs.

Table 4: Summarized results of analyses for POPs and mercury for ten pooled free range chicken eggs samples from the second year of sampling (2014), plus a background sample from a supermarket in Karaganda sampled in 2015. There are also results for selected samples from China and Belarus, and EU limit values for comparison.

Locality	Balkhash - south-west	Balkhash - south-west	Balkhash - Rembaza	Balkhash - Rembaza	Ekibastuz - substitution	Ekibastuz - Soyuz	Ekibastuz - Soyuz	Rostovka	Chkalovo	Shabanbai Bi	Karaganda - supermarket
Sample	BAL-EGG-14-1	BAL-EGG-14-2	BAL-EGG-14-3	BAL-EGG-14-4	EKI 14-1- EGG/EKI-27-EGG	EKI-14-2-EGG	EKI-14-3-EGG	NUR-EGG-14-1	NUR-EGG-14-2	ARAI-EGG	KAR-SUP
Fat content	12.45	9.95	10.15	11.35	12.4	11.7	13.3	15	13.7	10.15	14.0
PCDD/Fs (pg WHO TEQ g ⁻¹ fat)	7.69	9.81	7.73	4.25	5.73	1.57	NA	2.79	1.82	9.26	0.90
DL PCBs (pg WHO TEQ g ⁻¹ fat)	7.66	2.88	22.33	13.70	6.45	2.89	NA	26.51	25.94	28.62	0.00
Total PCDD/F + DL PCBs (pg WHO TEQ g ⁻¹ fat)	15.35	12.70	30.06	17.96	12.18	4.46	NA	29.30	27.76	37.88	0.90
PCDD/Fs and DL PCB (DR CALUX); (pg BEQ g ⁻¹ fat)	NA	NA	NA	NA	NA	NA	3.8	NA	NA	16	NA
PCDD/Fs (DR CALUX); (pg BEQ g ⁻¹ fat)	NA	NA	NA	NA	NA	NA	1.3	NA	NA	7.6	NA
HCB (ng g ⁻¹ fat)	1.68	2.62	4.39	2.13	5.40	1.28	1.58	2.33	5.04	6.25	1.09
PeCB (ng g ⁻¹ fat)	<0.27	3.2	<0.34	<0.36	NA	NA	NA	NA	NA	NA	2.86
HCBD (ng g ⁻¹ fat)	<0.27	<0.34	<0.34	<0.36	NA	NA	NA	NA	NA	NA	<0.36
7 PCB (ng g ⁻¹ fat)	23.37	11.54	76.64	52.19	27.18	29.49	13.76	319.40	395.18	2001.87	0.99
6 PCB (ng g ⁻¹ fat)	17.47	10.12	58.84	42.74	22.98	21.71	10.45	275.47	360.44	1975.97	0.99
Sum of HCH (ng g ⁻¹ fat)	6.84	114.14	20.71	34.40	11.05	13.25	33.76	45.67	15.33	860.80	0.36
Sum of DDT (ng g ⁻¹ fat)	10.10	34.05	318.87	1057.80	14.52	168.72	126.09	136.40	111.24	287.03	1.03
Hg (ng g ⁻¹)	NA	NA	NA	NA	NA	NA	NA	10.00	NA	1.00	NA

Locality	Shabanbai Bi	Baskuduk	Tauchik	Shetpe	Shetpe	Beijing -supermarket (China)	Wuhan (China)	Wuhan (China)	Gatovo (Belarus)	EU standard
Sample	Shabanbai Bi-2	BAS 02	TA E-1	SH-E-1/1-2	SH-E-2	LN 321/14	LN 324/14	LN 273/14	LN 272/14	
Fat content	28.8	15.6	16.2	15.4	12.5	10.14	12.46	15.5	15.4	
PCDD/Fs (pg WHO TEQ g ⁻¹ fat)	4.90	2.16	NA	1.07	NA	0.20	8.59	12.17	4.25	2.50
DL PCBs (pg WHO TEQ g ⁻¹ fat)	150.37	11.48	NA	5.34	NA	0.28	4.70	3.79	11.33	
Total PCDD/F + DL PCBs (pg WHO TEQ g ⁻¹ fat)	155.27	13.64	NA	6.41	NA	0.48	13.29	15.96	15.58	5.00
PCDD/Fs and DL PCB (DR CALUX); (pg BEQ g ⁻¹ fat)	NA	NA	NA	NA	NA	1.2	8.8	35	8.1	5.00
PCDD/Fs (DR CALUX); (pg BEQ g ⁻¹ fat)	NA	NA	NA	NA	NA	NA	5.8	31	5.2	2.50
HCB (ng g ⁻¹ fat)	1.35	2.94	2.75	6.29	1.30	3.52	28.90	480.65	2.86	20.00
PeCB (ng g ⁻¹ fat)	2.92	0.34	0.39	34.53	0.67	NA	NA	NA	NA	
HCBD (ng g ⁻¹ fat)	<0.03	<0.32	<0.31	<0.32	<0.4	NA	NA	NA	NA	
7 PCB (ng g ⁻¹ fat)	374.00	33.87	11.07	14.20	11.74	2.10	5.29	1.03	66.36	-
6 PCB (ng g ⁻¹ fat)	204.00	26.85	8.67	11.35	9.73	2.10	5.29	1.03	52.92	40.00
Sum of HCH (ng g ⁻¹ fat)	276.32	52.64	759.53	1451.19	503.00	1.34	5.23	2.32	4.94	-
Sum of DDT (ng g ⁻¹ fat)	19.17	155.69	229.00	59.29	45.19	0.21	26.25	33.23	230.65	-
Hg (ng g ⁻¹)	NA	NA	NA	NA	NA	NA	NA	NA	2.00	-

WHO 2005 - TEQ

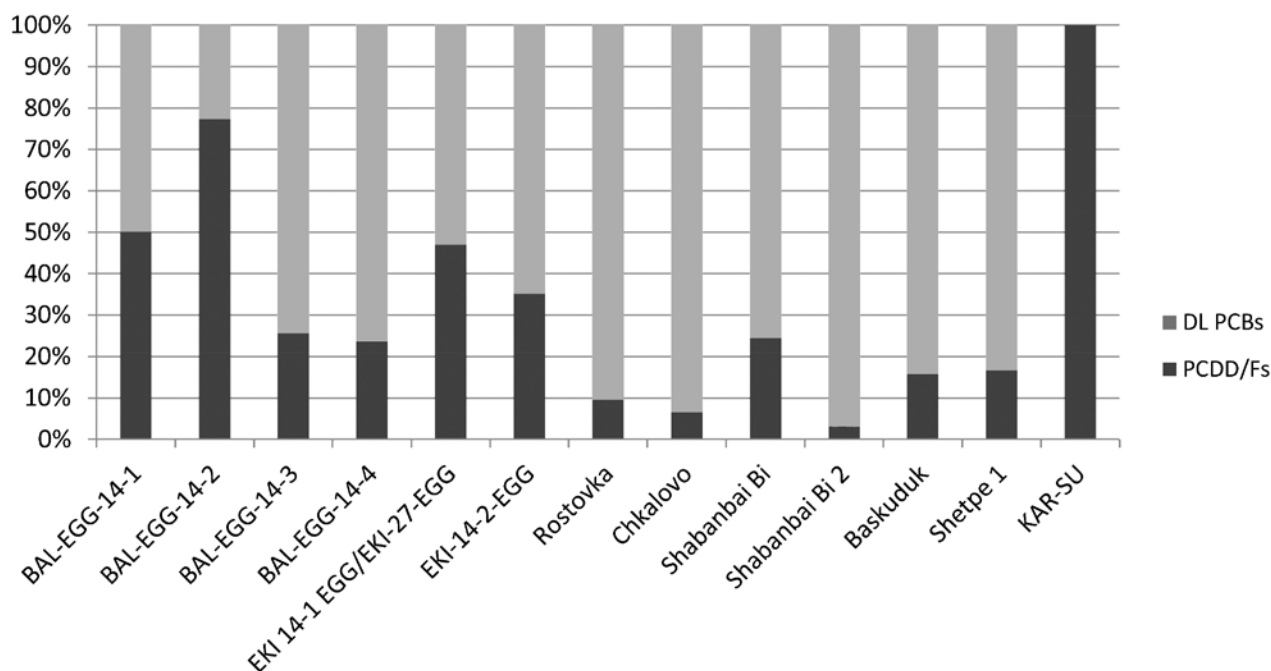


Figure 11: Balance between PCDD/Fs and DL PCBs on total WHO-TEQ levels in chicken egg samples from Kazakhstan.

4.2.3 OTHER U-POPs: HEXACHLOROBENZENE (HCB), PENTACHLOROBENZENE (PeCB) AND HEXACHLOROBUTADIENE (HCBD)

Hexachlorobenzene and pentachlorobenzene were listed as U-POPs in Annex C to the Stockholm Convention (Stockholm Convention 2010). Their unintentional formation in chemical and combustion processes is similar to that described for PCDD/Fs and DL PCBs. The highest levels of HCB were observed in samples from Shetpe, a sample closer to the cement kiln, and Shabanbai Bi (in sample taken in 2014). The sample from Shetpe (SH-E/1/1-2) contained also the highest level of PeCB (34.5 ng g⁻¹ fat).

Hexachlorobutadiene (HCBD) is considered to be a U-POP as well, although it was listed in Annex C to the Stockholm Convention yet. None of the free range chicken eggs samples analyzed for this chemical from Kazakhstani hot spots had level above LOQ of HCBD (see Table 4).

4.2.4 POLYCYCLIC AROMATIC HYDROCARBONS (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) were analyzed only in free range chicken eggs from Mangystau Region however we have results of analyses also in chicken eggs from Thailand and one sample from China for comparison. These results are summarized in Table 5. We don't have results for analysis of PAHs in chicken eggs from supermarket in Kazakhstan we only have it for chicken eggs from supermarket in Bangkok which cannot be considered as background level for Kazakhstan. Samples from Mangystau hot spots don't show high levels of PAHs in comparison within presented collection of pooled free range chicken egg samples. The highest level of 6 PAHs (385 ng g⁻¹ fat) among four samples from Mangystau was found in Tauchik. It is also slightly elevated in comparison with sample from supermarket in Bangkok (233 ng g⁻¹ fat), however it is still much lower in comparison with highest concentrations among presented collection of samples (above 1000 ng g⁻¹ fat).

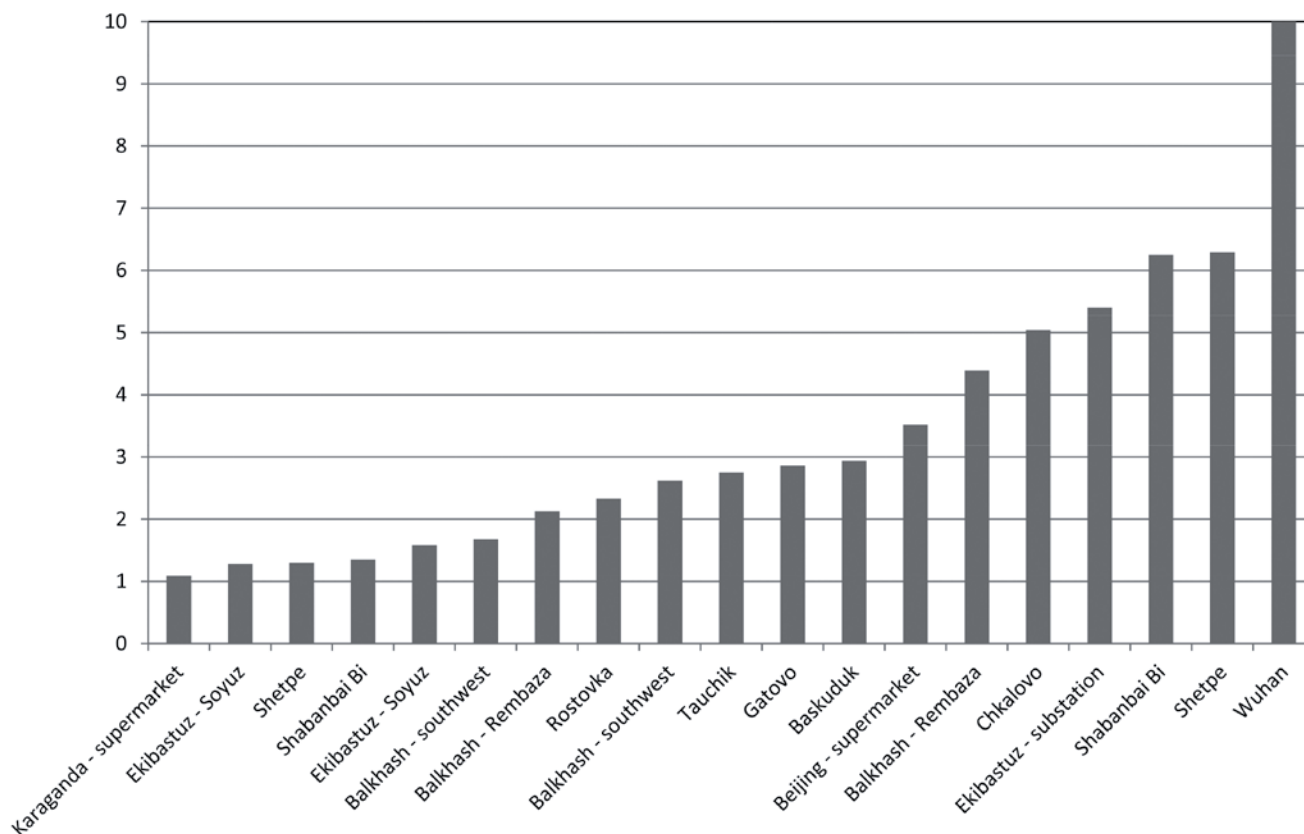


Figure 12: Hexachlorobenzene levels in different pooled samples of free range chicken eggs from Kazakhstani hot spots in comparison with selected sites in China (Wuhan) and Belarus (Gatovo).

Table 5: Summarized results of analyses for 6 PAHs in 4 free range chicken eggs samples from Mangystau Region collected in 2016 in comparison with some samples from Thailand and China collected in 2015–2016 (in ng g⁻¹ fat).

Sample	BAS 02	TA-E-1	SH-E-1/1-2	SH-E-2	SMS2-13	SAR1	MTP2-18	MTP2-19	MTP1-11	Control group, supermarket	Samui 01	Likeng
	Baskuduk	Tauchik	Shetpe	Shetpe	Samut Sakhon (Thailand)	Saraburi (Thailand)	Map Tha Put (Thailand)	Map Tha Put (Thailand)	Map Tha Put (Thailand)	Bangkok (Thailand)	Koh Samui (Thailand)	Likeng (China)
Naphthalene	97	322	107	116	690	1300	170	110	86	140	220	430
Phenanthrene	124	59	54	31	1200	150	110	88	700	93	160	250
Benzo[a]anthracene	1.1	1	<0.4	<0.5	< 30	< 40	< 30	< 30	< 20	< 30	110	< 3
Chrysene	1.6	2.6	<0.4	<0.5	< 30	< 40	< 30	< 30	< 20	< 30	110	3
Benzo[b]fluoranthene	<0.9	<0.8	<0.9	<1.1	< 30	< 40	< 30	< 30	< 20	< 30	110	3
Benzo[a]pyrene	0.5	0.9	0.6	0.9	< 30	< 40	< 30	< 30	< 20	< 30	94	< 3

4.2.5 BFRs IN EGGS

Broad family of brominated flame retardants including those used as alternatives to older ones such as polybrominated diphenyl ethers (PBDEs) and/or hexabromocyclododecane (HBCD) was analyzed in free range chicken eggs from Balkhash city, Baskuduk, Shetpe, Tauchik and sample from supermarket in Karaganda. Results for all measured BFRs in these samples and samples from Thailand and China for comparison are summarized in Table 6.

Table 6: Summarized results of analyses for different BFRs in chicken eggs samples from Central Kazakhstan in comparison with selected samples from Thailand and China (in ng g⁻¹ fat). The levels below LOQ were counted as "0" for calculation of sums of the groups of BFRs.

Locality	Balkhash - south-west	Balkhash - south-west	Balkhash - Rem-baza	Balkhash - Rem-baza	Karaganda - superm.	Baskuduk	Shetpe	Shetpe	Tauchik	Map Tha Put (Thailand)	Koh Samui (Thailand)	Bangkok - super-market (Thailand)	Qihua (China)
Sample	BAL-EGG-14-1	BAL-EGG-14-2	BAL-EGG-14-3	BAL-EGG-14-4	KAR-SUP	BAS 02	SH-E-1/1-2	SH-E-2	TA-E-1	MTP 2-19	SAMUI 01	SUPERM	QIHUA 2
HBB	<0.16*	<0.20*	<0.20*	0.22	<0.21*	<0.33*	<0.31*	<0.01*	<0.01*	<0.16*	<0.21*	<0.26*	<0.17*
Sum of PBDEs**	3.51	25.78	234.56	16.04	9.50	28.10	0.00	0.00	3.03	3.30	0.92	3.10	1.94
PBEB	<0.16*	<0.20*	<0.20*	<0.22*	<0.21*	<0.33*	<0.31*	<0.01*	<0.01*	<0.16*	<0.21*	<0.26*	<0.17*
α-HBCD	<0.69*	197.28	<0.88*	225.40	1035.71	179.08	18320.75	430.11	143.47	165.41	<0.92*	<1.12*	139.44
β-HBCD	<0.69*	<0.88*	<0.88*	<0.94*	<0.93*	<1.63*	<1.57*	<0.025*	<0.025*	<0.70*	<0.92*	<1.12*	<0.72*
γ-HBCD	<0.69*	<0.88*	<0.88*	<0.94*	<0.93*	7.25	<1.57*	<0.025*	<0.025*	<0.70*	<0.92*	<1.12*	<0.72*
Sum HBCD	0.00	197.28	0.00	225.40	1035.71	187.97	18320.75	430.11	143.47	165.41	0.00	0.00	139.44
BTBPE	0.16	<0.20*	<0.20*	<0.22*	0.29	3.46	<0.31*	<0.01*	1.07	0.27	<0.21*	<0.26*	<0.17*
OBIND	<1.33*	<1.70*	<1.70*	<1.80*	<1.79*	<3.27*	<3.27*	<0.1*	<0.1*	<1.35*	<1.77*	<2.16*	<1.39*
DBDPE	<2.66*	<3.40*	<3.40*	<3.60*	<3.57*	<6.54*	<6.29*	<10.00*	<10.00*	<2.70*	<3.55*	<4.31*	<2.78*
Sum - new BFRs	0.16	0.00	0.00	0.00	0.29	3.46	0.00	0.00	1.07	0.27	0.00	0.00	0.00

* below LOQ

** Following congeners were analyzed: BDE 28, BDE 47, BDE 49, BDE 66, BDE 85, BDE 99, BDE 100, BDE 153, BDE 154, BDE 183, BDE 196, BDE 197, BDE 203, BDE 206, BDE 207 and BDE 209

Surprisingly high levels of α-HBCD isomer of 18320.75 respectively 1036 ng g⁻¹ fat were found in chicken eggs obtained from Shetpe respectively supermarket in Karaganda. Level above 18 ppm is the highest level of HBCD ever measured in fat of chicken eggs. Also level in chicken eggs from supermarket is one of the highest concentrations of HBCD found in chicken eggs. Both are higher than those observed in free range chicken eggs from different hot spots sampled by IPEN in 2005 (Blake 2005). Pool sample from supermarket contained lower level of HBCD than high concentration of 2000 ng g⁻¹ fat found in German free range chicken eggs in 2007 (Hiebl and Vetter 2007). Pool sample from Shetpe nine times exceeded that level. It is not very clear how this contamination got into eggs from the large poultry farm, where chicken mostly don't have access to open space. Potential contamination route might be some use of polystyrene somewhere on the place accessible to chicken at the farm and/or contaminated feed for chicken. The sample from supermarket in Karaganda cannot be considered as one showing background level for BFRs in chicken eggs from Kazakhstan. Repeated sampling and monitoring of BFRs in chicken eggs from supermarkets in Karaganda can help to address the source of serious pollution of food chain by HBCD. Polystyrene or car upholstery treated by HBCD as flame retardant can be most likely sources of contamination of free range chicken eggs sampled in Shetpe. It has to be noted that in Shetpe these eggs were sampled near cement kiln.

Significant levels of HBCD were also observed in other pooled eggs samples BAL-EGG-14-4 from Balkhash - Rembaza (225 ng g⁻¹ fat), BAL-EGG-14-2 from Balkhash - Southwest (197 ng g⁻¹ fat), SH-E-2 from Shetpe, further distance from cement kiln

(430 ng g⁻¹ fat), and BAS 02 from Baskuduk (188 ng g⁻¹ fat). In IPEN report focused on different hot spots the maximum level of HBCD (160 ng ng g⁻¹ fat) in free range chicken eggs was observed in sample from Dandora landfill in Nairobi. Highest level of PBDEs (107 ng ng g⁻¹ fat) in that report was observed in sample from the vicinity of hazardous waste incinerator in Turkey. Much higher level, exceeding that one more than twice was found in sample BAL-EGG-14-3 from Balkhash - Rembaza (235 ng ng g⁻¹ fat). Four results with respective seven times higher levels were observed in poultry eggs in the vicinity of Chinese municipal solid waste incinerator (Petrлік 2015) respective e-waste recycling site in Eastern China (Labunska, Harrad et al. 2013).

We looked for BFRs also in sediments of Balkhash Lake during our research in 2014. PBDEs and HBCD isomers were not found in levels above LOQ in four analyzed pooled sediment samples taken near the tailing pond (VŠCHT - Ústav analýzy potravin a výživy 2014).

4.2.6 ORGANOCHLORINATED PESTICIDES (OCPs)

EU limits for pesticide residues, including OCPs in chicken eggs, are set per fresh weight of egg. A comparison of OCPs in eleven samples of chicken eggs from Kazakhstan is in Table 7. EU limits were exceeded only in two samples: the limit for the sum of DDT was exceeded in eggs from Balkhash - Rembaza (BAL-EGG-14-4) by more than two fold, and the limit for β -HCH was

Table 7: Summarized results of analyses for OCPs for ten pooled free range chicken egg samples from the second year of sampling (2014) plus a background sample from a supermarket in Karaganda sampled in 2015. There are also EU limit values (European Commission 2008) for comparison. These results are expressed in ng g⁻¹ fresh weight because EU limits are set for fresh weight for OCPs.

Locality	Ekibastuz - substation	Ekibastuz - Soyuz	Ekibastuz - Soyuz	Shabanbai Bi	Shabanbai Bi	Rostovka	Chkalovo	Karaganda - superm.	EU stand.
Sample	EKI-14-1-EGG; EKI-27-EGG	EKI-14-2-EGG	EKI-14-3-EGG	ARAI-EGG	Shabanbai Bi 2	NUR-EGG-14-1	NUR-EGG-14-2	KAR-SUP	
HCB	0.67	0.15	0.21	0.63	0.39	0.35	0.69	0.15	20.00
α -HCH	0.07	0.16	0.75	18.47	3.62	0.48	0.14	0.01	20.00
γ -HCH	0.01	0.45	0.41	2.52	0.96	0.34	0.16	0.05	10.00
β -HCH	1.30	0.94	3.33	66.38	75.00	6.03	1.80	0.01	10.00
sum-4DDT (EU)	1.82	19.68	16.77	29.02	5.52	20.46	15.24	0.17	50.00

Locality	Balkhash - southwest	Balkhash - southwest	Balkhash - Rembaza	Balkhash - Rembaza	Baskuduk	Tauchik	Shetpe	Shetpe	EU stand.
Sample	BAL-EGG-14-1	BAL-EGG-14-2	BAL-EGG-14-3	BAL-EGG-14-4	BAS 02	TA E - 1	SH-E-1/1 - 2	SH-E-2	
HCB	0.21	0.26	0.45	0.24	0.46	0.45	0.97	0.16	20.00
α -HCH	0.13	1.30	0.26	0.80	0.20	0.79	12.5	1.56	20.00
γ -HCH	0.00	0.71	0.28	0.51	0.13	0.15	1.95	0.31	10.00
β -HCH	0.72	9.34	1.56	2.60	7.88	122	209	61.0	10.00
sum-4DDT (EU)	1.28	3.39	32.30	119.83	24.11	36.78	8.82	5.33	50.00

exceeded in the sample from Shabanbai Bi by more than six fold. Levels of p,p'-DDT were 124 and 441 ng g⁻¹ fat respectively in two samples from Balkhash – Rembaza, which is over MAC according Russian legislation (see Table 2).

All free range chicken egg samples exceeded the background level of DDT in the eggs bought in the supermarket. Significantly higher levels of DDT were in the sample from Balkhash – Rembaza (BAL-EGG-14-3), the sample from Shabanbai Bi, two samples from Ekibastuz – Soyuz, and in samples from Rostovka, Chkalovo, Baskuduk and Tauchik. A comparable level of DDT was also found in free range chicken eggs from Gatovo, Belarus.

High levels of HCHs were observed in two samples from Shabanbai Bi, and in three of total four samples from Mangystau Region. Concentration of β -HCH in sample SH-E-1/1-2 from Shetpe exceeded an EU limit more than twenty times (see Tables 2 and 7) as well as it exceeded Russian limit value twice (see Tables 2 and 7). An elevated level of β -HCH near to the EU limit value was also found in eggs from Balkhash – southwest (BAL-EGG-14-2). β -HCH is an isomer of pesticide lindane. Potential source of this contamination can be either feed or water provided to chicken. High levels of β -HCH were observed for example in dairy milk in Saco Valey, Italy where fodder provided to cattle was found to be a source of contamination (Sala, Caminiti et al. 2012). Contrary to the findings in free range chicken eggs there were very low concentrations of OCPs in camel milk samples from Mangystau Region (Petrlik, Kalmykov et al. 2016). Significant level of β -HCH was also observed in some samples of animal products (butter) and human tissues samples from the surrounding of Aral Sea in Uzbekistan (Ataniyazova, Baumann et al. 2001).

4.3 MERCURY

Mercury levels in the two analyzed pooled samples of eggs were below the Kazakhstani limit. The level in eggs from Rostovka on the river Nura was 10-times higher than in the sample from Shabanbai Bi.

5. DISCUSSION

5.1 BACKGROUND LEVELS OF POPs IN EGGS

The locality of Shabanbai Bi as a remote area in Kazakhstan has shown high levels of certain POPs in free range chicken eggs and therefore cannot be considered as a background locality for POPs. As this was discovered at a very late stage in our research; we sampled chicken eggs from a supermarket in Karaganda from chickens raised in a large farm without access to open air space in April 2015 in order to obtain information about background levels of POPs in chicken eggs from Kazakhstan. The results of the analyses for this sample are in Tables 4 and 7. The levels of POPs in this sample were similar for PCDD/Fs and DL PCBs (DiGangi and Petrlik 2005) or lower, e.g. for HCB, non-ortho PCBs or DDT (DiGangi and Petrlik 2005, IPEN Pesticides Working Group 2009), compared to those observed in the background samples from other studies of POPs in chicken eggs. For PeCB the level of almost 3 ng g⁻¹ fat was measured in sample from supermarket in Karaganda of chicken eggs. Lower levels were analyzed in some other samples (see Table 4). HCBd was found at level below LOQ (<0.36 ng g⁻¹ fat) in this sample.

While for above mentioned POPs sample from supermarket can be considered as showing background levels it is not possible to say for BFRs as the second highest level of HBCD among all samples collected in Kazakhstan for this study was measured in this sample (see Chapter 4.2.5, Table 6).

5.2 DIOXIN CONGENER PATTERNS AND PUTATIVE SOURCES

We can compare dioxin congener patterns in free range chicken eggs with their typical patterns for certain types of pollution sources in order to get closer to discovery of their sources at the studied sites. The graph in Figure 13 shows the balance between PCDDs and PCDFs in egg samples and two non-egg samples (air emissions from a lead smelter and the content of PCDD/Fs in asbestos cement fibre plates). There are egg samples from a previous IPEN report where the most likely dioxin sources were identified. The division between PCDD and PCDF congeners in toxic equivalents is used as one of the criteria for a basic classification of potential sources (Sam-Cwan 2003, Yoon-Seok 2003). However, it can be only used as basic information; further analysis of the dioxin congener pattern is needed.

Balance of PCDD/PCDF in WHO-TEQ values

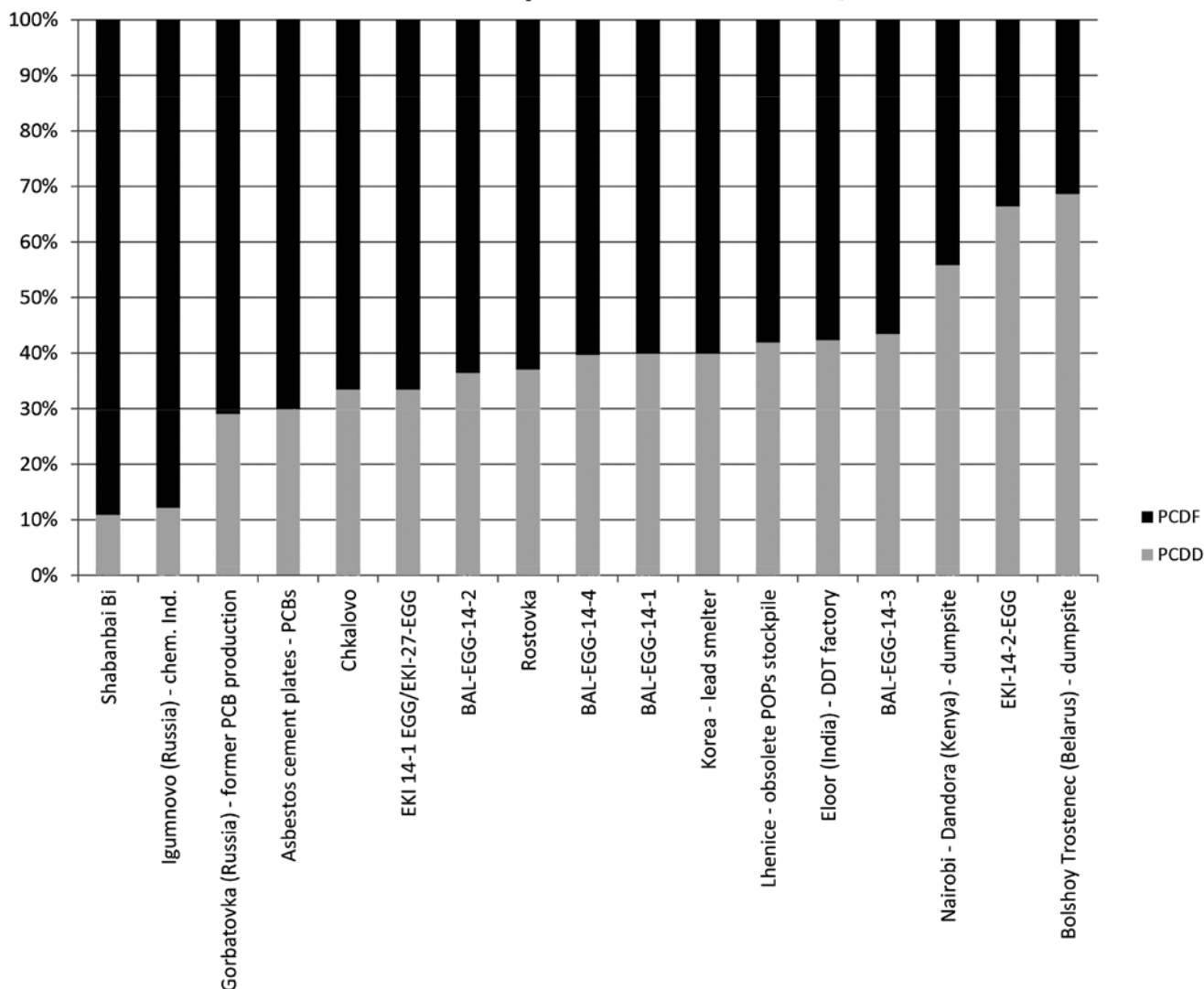


Figure 13: Share of PCDD and PCDF congeners on total WHO-TEQ values in different free range chicken eggs (in darker colours) and two other matrix samples (bars in less intense colour). Sources of information: for data on eggs (IPEN Dioxin PCBs and Waste Working Group, Eco-SPES et al. 2005, IPEN Dioxin PCBs and Waste Working Group, Envilead et al. 2005, IPEN Dioxin PCBs and Waste Working Group, Foundation for Realization of Ideas et al. 2005, IPEN Dioxin PCBs and Waste Working Group, Periyar Malineekarana Virudha Samithi - PMVS et al. 2005); for other data (Sam-Cwan 2003, Winkler 2015).

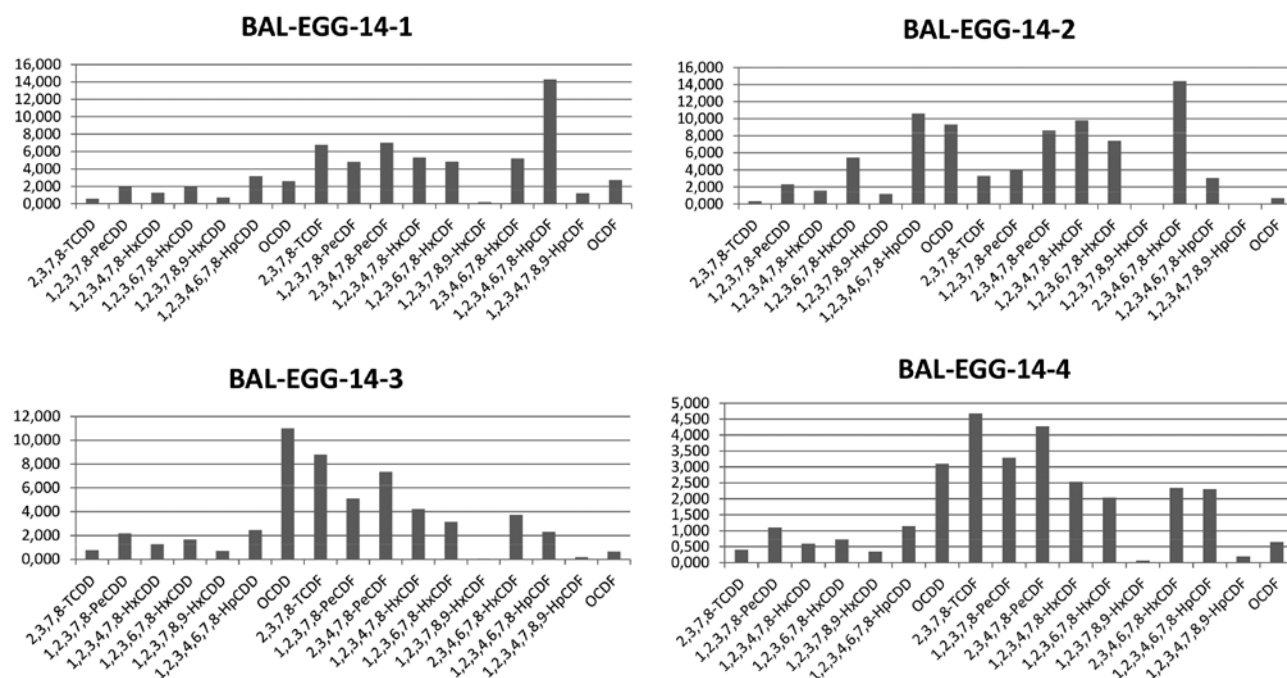
5.2.1 BALKHASH

It is clear that there is not only one single source of contamination of free range chicken eggs in Balkhash city when we compare dioxin congener patterns for these samples (see graphs in Figures 14-17) although they are close to each other from Balkhash - southwest and Balkhash - Rembaza. A similar dioxin congener pattern to sample BAL-EGG-14-4 can be found in the eggs collected in Lhenice, Czech Republic (see graph in Figure 18), where the putative source of contamination is an old environmental burden, obsolete PCBs and pesticide storage. There is also a certain similarity in the relatively high levels of DDT and its metabolites in sample BAL-EGG-14-4 and Lhenice, although the total values of DDT in each pooled egg sample is different.

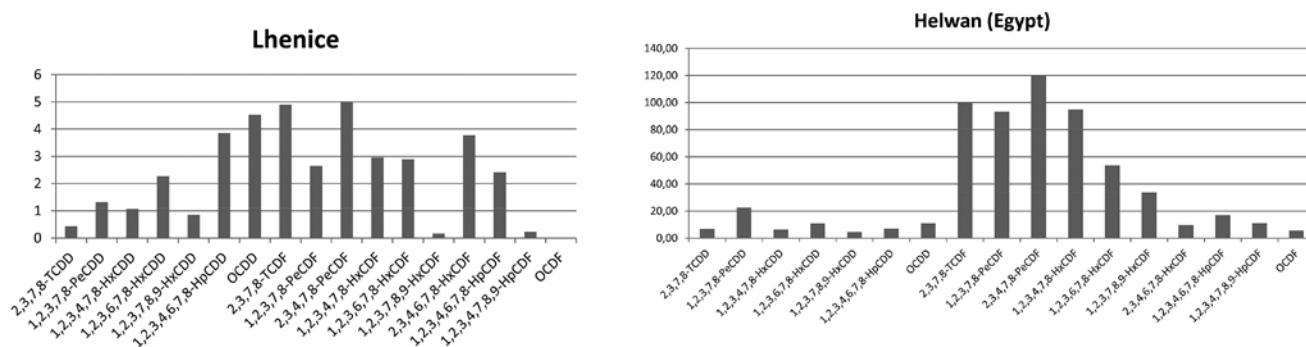
BAL-EGG-14-3 is close to the PCDD/Fs pattern in a sample from Helwan, Egypt (see graph in Figure 19) (IPEN Dioxin PCBs and Waste Working Group, Day Hospital Institute et al. 2005). Combustion sources, including the metallurgic industry, were marked as potential contamination sources in the case of the Helwan egg sample. We didn't find any potential single pattern

for the rest of the samples from the Balkhash city area, but the prevalence of PCDFs show on some combustion sources or obsolete POPs stockpiles as sources of dioxins which can vary from open burning of waste to metallurgy.

A high level of dioxins of 263.78 pg WHO-TEQ/g was found in dust from a copper smelter in Balkhash, according to data published in the Kazakhstani National Implementation Plan on the Stockholm Convention (Republic of Kazakhstan 2009); however, raw data about the analysis are not publicly available. A significant level was also measured in an air sample in the smelter.



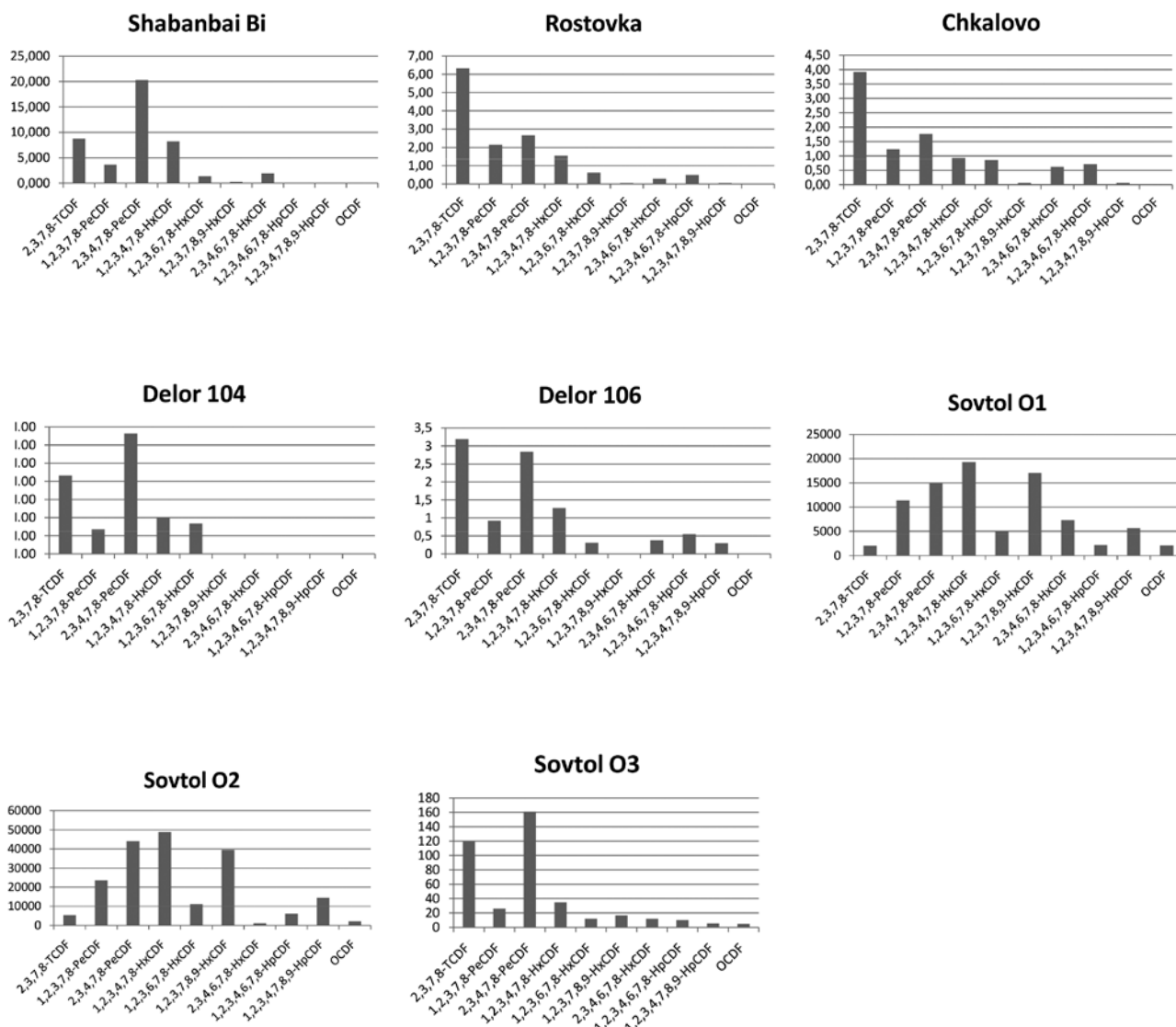
Figures 14 - 17: Dioxin congener patterns for free range chicken samples from the Balkhash city area.



Figures 18 - 19: Dioxin congener patterns in free range chicken eggs in a sample from Lhenice, Czech Republic (left graph) and in samples from Helwan, Egypt (right).

5.2.2 AREA OF RIVER NURA AND SHABANBAI BI

Polychlorinated dibenzofuran (PCDF) congener patterns in samples from Kazakhstan were compared with those for mixtures of PCBs: Sovtol described by Brodsky et al. (2005), Delor 103, 104, 105 and 106 as described by Taniyasu et al. (2003). A similar profile for PCDF congener fingerprints for a free range chicken egg sample from Shabanbai Bi is very close to the Sovtol O3 sample or Delor 104, while profiles for chicken egg samples from Rostovka and Chkalovo are closer to Delor 106 (see graphs in Figures 20 - 24). In all of these three samples, DL PCBs also prevailed in comparison with PCDD/Fs as major contributors to total WHO-TEQ (see graph in Figure 11).



Figures 20 - 27: PCDF congeners patterns for free range chicken egg samples from Shabanbai Bi, Rostovka and Chkalovo in comparison with Delor 104 and Delor 106, according to data available in Taniyasu et al. (2003), and Sovtol O1, O2 and O3 samples (Brotsky, Evdokimova et al. 2005)

It seems that potential obsolete PCBs used in transformer oils or their stockpiles can be potential source of free range chicken egg contamination in Shabanbai Bi and river Nura area, or they can be present in feed used for chicken. A team from EcoMuseum has visited Shabanbai Bi in order to find the potential source of high contamination of eggs in this village, but its search was not successful. It could also be from an already removed obsolete POP stockpile, but contamination of buildings or soil left in the village. There are obsolete PCBs stockpiles as well as old transformers used in Temirtau and the river Nura area.

The highest mean level of 6 indicator PCB congeners was measured in ambient air in Temirtau 884.58 pg m⁻³ in comparison with 5 other locations in Kazakhstan in 2008 (UNEP GMP 2013). A high level of dioxins 607.7 pg WHO-TEQ/g was found in dust from a sinter plant of Mittal Steel in Temirtau, according to data published in the Kazakhstani National Implementation Plan on the Stockholm Convention (Republic of Kazakhstan 2009). A significant level was also measured in an air sample in the plant. Detailed PCDD/F congener patterns for these measurements were not available to us.

5.2.3 EKIBASTUZ

There are different PCDD/F congener patterns in pooled samples from Ekibastuz, which also shows in more different sources of contamination by dioxins at this locality; however, DL PCBs cause a bigger portion of total dioxin-like toxicity in egg samples.

It can be linked to historic contamination by PCBs at abandoned Ekibastuz electricity substation, although no specific PCB mixture pattern for PCDF congeners matched that found in chicken eggs in Ekibastuz.

5.3 OCPs AND THEIR PUTATIVE SOURCES

High levels of currently banned POPs pesticides, such as DDT or HCHs, in samples from Shetpe, Tauchik, Shabanbai Bi and Balkhash show a potential presence of ongoing use of these obsolete pesticides or their presence in unknown stockpiles, either at the sites or at the location of origin of feed used for chickens.

5.4 BFRs AND THEIR PUTATIVE SOURCES

Findings of high levels of HBCD and PBDEs in free range chicken samples from Balkhash, Shetpe, Tauchik and Baskuduk show that chicken most likely have access to sites contaminated with BFRs such as for example places with car wrecks, plastic waste (including polystyrene) and/or e-waste. Another potential source of contamination could be re-processing of these wastes in some facility in the city which can emit BFRs in dust as result of handling these wastes. BFRs are most likely not used intentionally in metallurgic industries located in Balkhash but can be in metal scrap processed in metallurgic plants. BFRs should be further monitored in Balkhash city environment compartments and more attention should be paid to waste management in the city and all facilities.

High level of HBCD in chicken eggs from controlled farm sold in supermarket in Karaganda is hard to explain without further investigation. It also requires attention of state food control, although there are no limits established for BFRs in food in Kazakhstan.

6. DIETARY INTAKE OF SELECTED POPs THROUGH CONSUMPTION OF FREE RANGE CHICKEN EGGS

The egg share in total food consumption in Kazakhstan in 2007 was close to 1% of total food basket per day according World Atlas - Food Security data ⁵ (Knoema 2012), and raised by approximately 1/3 of it total amount per day (18 g per person per day) every 5 years. It would mean that 2017 consumption can be about 28 g per person per day if the trend remained. If we count 50 g per one chicken egg as average weight it would mean consumption of ½ egg per person per day as general consumption pattern for Kazakhstani population these days.

We tried to calculate dietary intake for the following groups of contaminants per day: 1) PCDD/Fs plus DL PCBs; 2) HCHs, 3) PBDEs and 4) HBCD. Calculation was made by using measured levels of certain chemical(-s) per gram of fresh weight and calculation of its daily intake by consumption of half egg per day (25 grams of eggs weight). An average was calculated in case of more samples per municipality. Results are summarized in Table 8.

Results were then compared with available information about daily intake of evaluated chemicals: 1) **HCHs**: We used dietary intake data available for population of two Chinese cities: Beijing and Shenyang (Yu, Wang et al. 2013). 2) **PCDD/Fs + DL PCBs**: Calculations for PCDD/Fs and DL PCBs were compared with TWI suggested by WHO at level of 14 pg WHO-TEQ/kg of body weight per week = 2 pg WHO-TEQ/kg of b. w. per day (European Commission 2001, Gies, Neumeier et al. 2007). 3) **PBDEs and HBCD**: There is an estimate available of daily intake of these two groups of BFRs in Sweden at the beginning of this century (Lind, Aune et al. 2002). We compared our data with those available in Swedish study for eggs consumption. See the comparison in Table 8.

⁵ The food consumption refers to the amount of food available for human consumption as estimated by the FAO Food Balance Sheets. However the actual food consumption may be lower than the quantity shown as food availability depending on the magnitude of wastage and losses of food in the household. Food consumption per person is the amount of food, in terms of quantity, for each individual in the total population. Food from eggs relates to the quantity of eggs used also for preparation the food such as bakery products.

Table 8: Summarized results of calculation of dietary intake of selected POPs by eating half egg (25 g) from chicken raised at some Kazakhstani hot spots or eggs bought in supermarket in Karaganda from chicken raised at a large commercial farm. Half of chicken egg is approximate current average consumption per person per day in Kazakhstan based on calculation from available data (Knoema 2012). Total daily intakes of selected POPs from literature are given for comparison (see explanation in third paragraph of Chapter 6). Dietary intake data just for eggs were available only for BFRs in Sweden (Lind, Aune et al. 2002).

Dietary intake from 1/2 egg	Balkhash	Ekibastuz	Rostovka	Chkalovo	Shabanbai Bi	Baskuduk	Tauchik	Shetpe	Karaganda superm.	Total DI Beijing (China)	Total DI Shenyang (China)	EFSA TWI	Sweden DI from eggs
β-HCH (ng)	81	46	151	45	1767	197	3053	3376	0.25	NA	NA	NA	NA
sum of HCHs (ng)	114	62	171	53	2087	217	3087	3594	1.75	4620	6580	NA	NA
PCDD/Fs + DL PCBs (pg WHO-TEQ)	52	25	110	95	607	53	NA	25	3	NA	NA	140	NA
PBDEs (ng)	257	NA	NA	NA	NA	108	12	0	33	NA	NA	NA	1.88
HBCD (ng)	377	NA	NA	NA	NA	713	514	37 004	3625	NA	NA	NA	42

Dietary intake of HCHs is most critical in Shetpe followed by Tauchik and Shabanbai Bi. By consumption of single half egg from these locations people can reach one third respectively half of total daily intake of these chemicals in Chinese cities Shenyang respectively Beijing per person (calculated for 70 kg weight of adult). The same comparison with situation in western European countries would look very different as in Germany in 1995 median levels of three HCH isomers in total for 70 kg man could be approximately 882 ng (Wilhelm, Schrey et al. 2002).⁶ This level would be then several times exceeded by eating half free range chicken eggs from above mentioned localities in Kazakhstan.

Brominated flame retardants were measured at the end of our research and therefore we were able to receive analyses only for limited number of four samples from Balkhash, one sample from both Baskuduk and Tauchik, two samples from Shetpe, and Karaganda – supermarket sample. By eating half egg from all these sites one should exceed many times total daily intake by eating eggs in Sweden in 1998 – 99 as researched by Lind, Aune et al. (2002). Intake of HBCD from eggs obtained in Shetpe respectively bought in supermarket in Karaganda is 1734-fold respectively 86-fold in comparison with Swedish ones at the end of 20-th century (see Table 8).⁷

Dietary intake of dioxins (PCDD/Fs) and DL PCBs in half egg for different localities is shown in Table 8. Eating of half egg from Shabanbai Bi would exceed daily intake derived from the tolerable weekly intake (TWI) suggested by EFSA more than 4-times and half eggs from Rostovka and Chkalovo were close to that level as well. In Table 9 is calculated total number of eggs according concentrations of PCDD/Fs and DL PCBs needed to be consumed in order to reach tolerable daily dose derived from TWI suggested by EFSA at level of 14 pg WHO-TEQ kg⁻¹ b.w. (European Commission 2001, Gies, Neumeier et al. 2007). These figures are assumptions based on limited number of samples, although in some localities were taken several pooled samples of eggs as documented in chapter 2 of this study but they illustrate how serious is the pollution by dioxins and DL PCBs at evaluated hot spots. Most serious contamination was found in Shabanbai Bi, where mainly DL PCB congeners contribute to overall dioxin-like toxicity in both samples taken from the same farm in the period of two year. Also contamination of eggs from pooled samples in Rostovka and Chkalovo followed by Baskuduk and Balkhash is alarming.

⁶ In ng kg⁻¹ b.w. per day: 1.9 for α-HCH, 1.2 for β-HCH, 9.5 for γ-HCH. These levels were calculated for children at North Sea island Amrum.

⁷ The study by Törnkvist et al. (2011) shows that the levels, and intake, of different POPs (including HBCD) from food of animal origin, in the Swedish market basket of 2005 seem to have decreased since the market basket study in 1999. Törnkvist, A., A. Glynn, M. Aune, P. O. Darnerud and E. H. Ankarberg (2011). "PCDD/F, PCB, PBDE, HBCD and chlorinated pesticides in a Swedish market basket from 2005 – Levels and dietary intake estimations." *Chemosphere* **83**(2): 193-199..

Analyses by DR CALUX method were not included in these calculations in Table 9 as they might reflect dioxin-like effects of some other chemicals as well. The same calculation for DR CALUX analyses results are summarized in Table 10. Samples from Balkhash seem to be more contaminated in this table because they include also sample with very high concentration of PCDD/Fs and DL PCBs 101 pg BEQ g⁻¹. Also dietary intake of dioxin-like compounds from eggs in samples from Temirtau – Samarkand seem to be very high. Eggs from Ekibastuz seemed to be less risky in comparison with measured levels by GCMS-HRMS. We need to take into account that we were not able to take samples at exactly same places as during first round of sampling so the information obtained by this additional evaluation draws Ekibastuz as less contaminated locality with regards to dioxin-like compounds.

The eggs from Shabanbai Bi do not seem to be so polluted according DR CALUX analysis. It could be explained by fact that only first pooled sample was analyzed by this method while the second one was not (see explanation in chapter 8) so Table 10 can show results calculated only for first sample (ARAI-EGG). First pooled sample from Shabanbai Bi had much lower concentration of DL PCBs (28.6 pg WHO-TEQ g⁻¹) in comparison with second one (150.4 pg WHO-TEQ g⁻¹), however still DR CALUX method has shown lower BEQ toxicity than WHO-TEQ toxicity measured by GCMS-HRMS. This result just underlines need to do more research in this locality at which unexpectedly high levels of PCBs and obsolete OCPs were discovered.

Table 9: Total number of eggs according concentrations of PCDD/Fs and DL PCBs needed to be consumed in order to reach tolerable daily dose derived from TWI suggested by EFSA at level of 14 pg WHO-TEQ kg⁻¹ b.w.

Localities	Balkhash	Ekibastuz	Rostovka	Chkalovo	Shabanbai Bi	Baskuduk	Shetpe	Karaganda su-perm.
PCDD/Fs + DL PCBs (pg WHO-TEQ in half egg)	52	25	110	95	607	53	25	3
Number og eggs to reach 140 pg WHO-TEQ per day	1.4	2.8	0.6	0.7	0.1	1.3	2.8	22.3

Table 10: Total number of eggs according concentrations of PCDD/Fs and DL PCBs as measured by DR CALUX (see Table 3) needed to be consumed in order to reach tolerable daily dose derived from TWI suggested by EFSA at level of 14 pg WHO-TEQ kg⁻¹ b.w.

Localities	Balkhash	Ekibastuz	Rostovka	Chkalovo	Shabanbai Bi	Temirtau - Samar-kand	Karaganda su-perm.
PCDD/Fs + DL PCBs (pg WHO-TEQ in half egg)	132	16	38	56	41	105	0
Number og eggs to reach 140 pg WHO-TEQ per day	0.5	4.3	1.8	1.2	1.7	0.7	NA

7. CONCLUSIONS AND RECOMMENDATIONS

POPs contamination in some free range chicken eggs from Kazakhstani hot spots shows potentially undiscovered obsolete POPs stockpiles, or so far publicly unknown sources of contamination. These results suggest the same conclusion as Muntean made for Uzbekistan in 2003: *“Second, although this study and others provide some provisional data, not enough is known about the environmental fate of historical pesticide use and its current impact on human health. Research should therefore be conducted to document the environmental transformation and fate of certain pesticides and to assess their health impact. Environmental analysis should evaluate the degradation and environmental behavior of parent pesticides and their degradation or transformation products.”* (Muntean, Jermini et al. 2003). We can only add that for Kazakhstan such research should also be done more properly for obsolete PCB oils and other potential use of PCBs (paints, plaster etc.) although we are aware that a basic PCBs inventory is underway. Sites contaminated by POPs should be properly remediated afterwards in order to get rid of POP contamination sources throughout the country.

High levels of PCDD/Fs and DL PCBs were found in free range chicken egg samples. Four-fifths of the samples exceeded EU MAC for PCDD/Fs and DL PCBs in chicken eggs. More regular monitoring of dioxins and DL PCBs in food samples should be undertaken by national authorities. Taking into account its limitations, the DR CALUX method has shown to be effective for estimation of overall contamination of food stuff (eggs) by PCDD/Fs and DL PCBs collected from Kazakhstani hot spots.

Both Russian and EU MAC for PCDD/Fs and DL PCBs for chicken eggs used in this study were exceeded more often than those for 6 PCB congeners and OCPs. One-third of the samples exceeded EU MAC for β -HCH.

High levels of dioxins and dioxin-like PCBs in free range chicken eggs from Balkhash confirm serious contamination of the city by these pollutants. Our finding is in agreement with Kazakhstani NIP, which suggests the results of epidemiological studies among Balkhash population provide proof of the cancerogenicity and malignancy of POPs. The highest oncological disease rate in the period of 1999 – 2003 was observed in Balkhash (in comparison with several other locations and the Kazakhstani average) (Republic of Kazakhstan 2009). Measures to reduce dioxin and dioxin-like PCB releases from the metallurgic industry in Balkhash city, as well as in Temirtau, are crucial to overall reduction of releases of U-POPs in the studied region of Kazakhstan.

This study revealed serious contamination of free range chicken eggs from Balkhash city with brominated flame retardants (BFRs), PBDEs and α -HBCD in particular. Also commercially distributed eggs sample from supermarket in Karaganda contained surprisingly high level of α -HBCD. The level of 18 ppm (in fat) of α -HBCD, to our knowledge ever measured highest concentration of HBCD in chicken eggs was found in free range chicken eggs from Shetpe. These chemicals are not regularly monitored in Kazakhstani environment. They should be monitored although they are not probably widely used in industrial facilities but imported products such as cars, electronics, products from recycled plastics and/or insulation materials can contain these chemicals and they can appear in waste afterwards and contaminate food chain when they are not controlled and regulated.

Chicken eggs are important part of diet. The eggs from localities polluted by POPs can significantly contribute to body burden by these chemicals as demonstrated in chapter 6 of this report, although the eggs are less common in food of Kazakhstani population in comparison with some other countries. Solution is not to discourage public from eating chicken eggs, close the chicken farms down or start punishing the farmers, but to prevent further contamination of food chain at certain hot spots as well as in food from large commercial farms. We used chicken eggs as they were proven as good indicator of potential contamination of food chain. We didn't sample meat but results of other study focused on camel milk (Petrlik, Kalmykov et al. 2016) have shown that problem is broader and products from fatty tissues of animals raised at certain hot spots can be contaminated as well. Some other studies demonstrated simultaneous contamination of chicken eggs and meat from contaminated sites (Chang, Hayward et al. 1989, Lovett, Foxall et al. 1998).

8. LIMITATIONS OF THE STUDY

The major limitations of the study were the limited financial, temporal and personal resources. Therefore, only limited number of chicken egg samples could be taken and limited scale of analyses could be done. We couldn't repeat whole range of analyses for each sample what created some loopholes in data available for our final evaluation.

We also lack data about level of total dietary intake of different contaminants in Kazakhstan as well as information about average diet of Kazakhstani population. We worked with limited information available instead. Still, an impression of the situation including the identification of major issues in relation to potential pathways of contaminants into food chain (represented by free range chicken eggs in this study) in Central and Western Kazakhstan was obtained. However, future

investigations in this field are still necessary. The results presented here cannot be considered exhaustive, rather expressing the need for an extended research in future.

The comparison of pollutant concentration levels found in the samples with legal standards has also its limitations. Each of the legal standards is defined in a different way and for a different purpose. In addition, there are no existing legal standards for some of the pollutants and some legal limits or TDI levels might be outdated. The estimation of a potential risk to humans and the environment cannot be conducted by consulting legal standards only, an extensive risk analysis based on a sufficient number of samples and detailed description of the state of the area and the potential risk receivers is crucial. We tried to draw basic evaluation of health risk expressed as daily intake of some crucial pollutants through consumption of eggs from free range chicken raised at selected hot spots in order to give at least basic idea about level of human exposure to different pollutants.

We believe that most important is to start to address overall pollution by such contaminants as PCBs, PCDD/Fs, OCPs as well as by some BFRs in Kazakhstan.

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PHOTOGRAPHIC SUPPLEMENT



An obsolete leaking transformer at the entrance of the landfill in Baskuduk, north from the sampling site.

Collection of chicken eggs samples.





Collection of chicken eggs samples.

Old Aktau city waste landfill is not being reclaimed and neither is it fenced off.





Koshkar Ata, uranium processing tailing pond at Aktau is freely accessible to domestic animals. They come here because of lack of water in surrounding semi-desert area.

Below: CaspiCement plant is the only cement production site in Mangystau region and with production capacity of 800 thousand tons of cement per year. The plant is situated 7 kilometers from centre of Shetpe.



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