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Organochlorine pesticides in placenta in Kyrgyzstan and the effect on pregnancy, childbirth, and newborn health

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Abstract

Organochlorine pesticides (OCPs) were determined by gas chromatography in 241 placentas from cotton-growing regions, 121 placentas from an urban area (city of Osh), and 146 placentas from unpolluted mountain regions of Kyrgyzstan. Manifestations of disease were recorded in the mothers during pregnancy and parturition and in their newborns during the first 6 days of life. OCPs were detected in 240 out of 508 placentas (47.2%), with increased incidence in the two polluted regions (65%), particularly in placentas from women living near former pesticide storehouses and agro air-strips (99%), but only in 2.7% of placentas from the unpolluted region. α-, β-, and γ-hexachlorocyclohexane (HCH); DDT; DDE; aldrin; and heptachlor were detected. The sum of concentrations of all OCPs (total OCPs) was calculated for each of the 240 placentas with detectable OCPs (median 9.5 μg/kg placenta, mean 88.3 μg/kg, range 0.1–3070 μg/kg). The incidence of health problems in four subgroups of this data set, with increasing levels of total OCPs, was compared with the incidence of health problems in the group of 268 placentas, where OCPs were undetectable. Relative risk of health problems in both, mothers and newborns, increased significantly, in a concentration-dependent manner, with increasing levels of total OCPs ($p < 0.0001$). Health complications with increased incidence in OCP-exposed newborns included, i.e., low birth weight, congenital malformations, infections, and stillbirths, in OCP-exposed mothers preterm delivery, (pre-)eclampsia/gestosis, and frequency of hospitalizations after delivery (infections). Women living near former pesticide storehouses and agro airstrips should be considered as being at risk. Reduction of exposure is urgently needed.

Keywords Organochlorine pesticides · Placenta · Pregnancy · Newborn · Adverse effects · Pesticide landfill · Pesticide storehouse · Agro airstrip · Kyrgyzstan

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Introduction

Due to the health impact of persistent organic pollutants (POPs) (Carpenter 2013), the Stockholm Convention has been established to globally protect human health from POPs and has meanwhile been ratified by 181 countries. The management of organohalogen pesticides (OCPs) and wastes/stockpiles is a particular challenge in the Stockholm Convention implementation (Dollimore and Schimpf 2013; Vijgen et al. 2013). The Eastern Europe and the Caucasus and Central Asian (EECCA) region including the Russian Federation countries has, in addition to POP contamination at former organochlorine production sites (Amirova and Weber 2015; Lysychenko et al. 2015; Revich and

Shelepchikov 2008), particular management and control challenges with the large stockpiles of unused POP pesticides from historic distribution amounting to approximately 240,000 t with associated risk for health and the environment (Pieterse et al. 2015; Toichuev et al. 2017; Vijgen et al. 2013).

During Soviet times, Kyrgyzstan was one of the main suppliers of cotton and tobacco for the Soviet Union. A large amount of pesticides was used in the 1970s and 1980s to maintain productivity and to control cotton and tobacco pests (Kyrgyz Republic 2006). OCPs were mainly used accounting for 66% of total pesticides used. These include a range of POP pesticides with major use of dichlorodiphenyltrichloroethane (DDT) and hexachlorocyclohexane (HCH) (Kyrgyz Republic 2006). The chemicals were mostly sprayed on tobacco and cotton fields, contaminating not only the cultivation areas but also the neighboring territories (Yablokov 1988). The long-term use of OCPs in agriculture results in the accumulation of pesticides in the environment and can lead to contamination of cattle and chicken/egg by bioaccumulation in biota, resulting in human exposure (Langenbach 2013; Toichuev et al. 2017; UNEP 2006; Kyrgyz Republic 2006).

The historic long-term use of POP pesticides is therefore of high concern for southern Kyrgyzstan, since, due to their persistence, OCPs are still present at sites of former pesticide application and in particular present in high levels at and around former pesticide storehouses, agro airstrips, and pesticide dumpsites (Toichuev et al. 2017). The health risks of the population at and around sites contaminated by POPs and other pollutants are of particular concern (Weber et al. 2008; WHO 2013) and require assessment of the impact on the population at those sites. Some of this potentially contaminated land in southern Kyrgyzstan has been transferred to the private sector as land parcels (Toichuev 2007). Initial securing measures have been implemented for a major OCP disposal site (Toichuev et al. 2017) and studies on the biodegradation OCPs in Kyryztan were conducted (Doolotkeldieva et al. 2017).

Already in the 1970s, research studies showed that the contact of pregnant women with pesticides increases the frequency of complications during pregnancy and childbirth, causes anomalies in newborns, and leads to deterioration of integrated indicators of children's health (Dollimore and Schimpf 2013). OCPs have a wide range of adverse effects on human health including the development of tumors (Beyond Pesticides 2016; Mrema et al. 2013), impacting the gene structures (Toichuev et al. 2014); congenital malformations in children (Toichuev and Tostokov 2006); immunodeficiency (Corsini et al. 2013; Mrema et al. 2013); reproductive and developmental disorders (Hanke and Jurewicz 2004); endocrine effects (Croes et al. 2014); hepatitis (Nikolaeva et al. 2013; Toichuev et al. 2012, 2014); neurotoxicity (Mrema et al. 2013); and development of placental pathologies of pregnant women, fetus, and newborns, including preterm birth (Tyagi et al. 2015). Prenatal exposure to the major pesticides used in Kyrgyzstan—hexachlorocyclohexane (HCH) with its isomers α-HCH, β-HCH, and γ-HCH, and dichlorodiphenyltrichloroethane (DDT) and its metabolite dichlorodiphenyldichloroethylene (DDE)—may impair fetal development, reducing birth weight, body length, head circumference, chest circumference, and ponderal index (Dewan et al. 2013). Elevated levels of DDT and metabolites in placenta were correlated with impaired fetal growth also in another study (Al-Saleh et al. 2004). Prenatal exposure to α-HCH, β-HCH, DDD, DDE, DDT, and dieldrin, determined in maternal blood and placental tissue, was associated with premature birth and low birth weight in India (Tyagi et al. 2015).

Since the placenta absorbs xenobiotics including OCPs (Al-Saleh et al. 2013; Tyagi et al. 2015; Toichuev et al. 2007), it is a good biomarker to study OCP and other environmental pollution. Studies of the levels of OCPs in placenta and their influence on the course of pregnancy, childbirth, fetus, and newborns can reveal vulnerable populations at risk and contribute to the development of preventive measures.

In this study, we analyzed placenta samples from areas of historic OCP use in southern Kyrgyzstan and compared the levels with levels in placenta samples from areas without specific pesticide application. Pregnant mothers and their children were assessed for clinical and laboratory manifestations of disease, and levels of OCP in placenta in impacted groups were assessed for correlation to heath impacts. The study demonstrates that with the simple analytical equipment and tools available and with limited resources, studies of major pollutants and related effects on humans from contaminated sites are possible, but that analytical capacities and epidemiological tools should be extended for more detailed investigations.

Materials and methods

Collection of placenta samples,

In order to detect and identify effects of OCPs in placenta, clinical and toxicological examinations were carried out on 508 women in labor, their placentas, and their children, including stillborn cases. Medical cards were developed of placentas. Placenta samples were collected from different cohorts from areas with and without specific OCP pollution sources between 2011 and 2015 (Table 2):

- Two hundred forty-one placenta samples were collected from the maternity hospital located in Kara-Suu and Aravan districts. In this area, where pesticides including OCPs were formerly intensively used for pest control, 83 samples of placentas were obtained from women residing near former pesticide warehouses, dump sites, and agro airstrips. Several exposure pathways were revealed for this population group (Toichuev et al. 2017).

- One hundred twenty-one placenta samples were collected from the Osh maternity hospital. Women living in the city partially consumed vegetables, fruits, and melons bought from polluted cotton-growing areas and might have consumed meat from animals impacted from these areas.
- One hundred forty-six placenta samples were collected from hospitals located in the unpolluted areas of Alay and Kara-Kulzha districts, a mountain region. The local population cultivates leguminous plants and is involved in animal husbandry.

The study was approved by the Ethical Committee of the South Branch of the National Academy of Sciences.

Assessment of health outcomes

The health of mothers (from the beginning of pregnancy and during the postnatal period) was assessed by obstetricians/gynecologists, therapists, dentists, hematologists (since we have a high percentage of anemia in pregnant women), endocrinologists (Kyrgyzstan belongs to an iodine deficiency area, and diabetes increased during recent years), and gastroenterologists including hepatologists (since latent forms of hepatitis including toxic hepatitis are regularly reported in southern Kyrgyzstan in cotton- and tobacco-growing areas). The mothers were also examined by nephrologists (high number of gestational pyelonephritis), surgeons (pregnancy hemorrhoids, varix dilatation), cardiologists, neuropathologists, and other specialists, if necessary. Newborns were examined by neonatologists, neuropathologists, pediatric surgeons, hepatologists, and other specialists.

Observations were documented on medical cards. This is a case report form describing the results of examination during pregnancy, childbirth and postnatal period, and children's details, including mother's name, age, residence area (e.g., areas located near the former pesticide store houses, agro airstrips; availability of gardens with information on applied fertilizers), profession (husband's profession, possible contacts with pesticides), place of birth, ethnicity, weight, height, number of pregnancies, births, stillbirths, miscarriages, abortions, food diet (e.g., markets where urban women purchase foodstuff, or gardens/fields where rural women collect fruits or vegetables), and consumption of sour milk and meat products. For example, Uzbek women mostly consume vegetable products, and the Kyrgyz women—meat and sour milk products.

Case report forms also contain information on macroscopic, microscopic, and bacteriological examinations of placenta samples. Placenta samples were screened for the presence of granulomas, calcifications, abscesses, and surface conditions (maternal and fetal surfaces, umbilical cord, and its attachment site) (OCPs are mainly accumulated there and at the edges of the placenta), and the presence of any changes on the section, weight, volume, thickness, etc. The same data are also entered in case report forms for newborn children.

Measurements of OCPs

The analyses were conducted according to *Methodological recommendations on determination of pesticides trace amounts in foodstuffs, animal feed and environment*, Part 17, Moscow 1988. The laboratory has been accredited by proficiency test KG417/KAC TL 105 (KAC-Kyrgyz Accreditation Center, TL-Testing laboratory) Registration No. 0055 KG-Kyrgyz Republic.

Briefly, HCH and the isomers α-HCH, γ-HCH, β-HCH, and δ-HCH; DDT, DDE and DDD; and aldrin, dieldrin, and heptachlor were analyzed in placenta samples by a thin-layer gas-liquid chromatography in the Laboratory of Toxicology, Radiology, Morphology and Ecology of the Institute of Medical Problems, South Branch of the Kyrgyz National Academy of (Osh) and Osh Province SES (Osh), following the "Guidelines on the detection of trace amounts of pesticides in food stuffs, biological media, feed and environment" (Aleksandrova et al. 1992).

A total of 50–60 g of placental tissue (approx. 10 g from five parts of the placenta) was sampled. The samples were placed in a glass dish with a glass lid, filled with hexane, and transported to the laboratory for analysis.

Reagents are as follows: n-hexane, acetone, petroleum ether, sodium sulfate (anhydrous), silica gel (activated), reagent grade benzene, sulfuric acid (reagent grade, special purity grade), special purity grade nitrogen, and organochlorine pesticides, standard solutions.

The placenta samples were minced, and portions of placenta (10–15 g) were added to 50 ml of concentrated sulfuric acid and shaken up during 5 min. Then, 50 ml of n-hexane were added and stirred for 30 min. The hexane layer was separated, new portion of n-hexane was added to the sample, and the extraction was repeated. The extracts were transferred to a device for distilling solvent, and the solvent was reduced to 15–20 ml. The solution was quantitatively transferred to a separating funnel, and 30–45 ml of hexane-saturated dimethylformamide or acetonitrile was added. The ratio of hexane solution to dimethylformamide was not less than 1:2. The funnel was shaken for a minute, and the layers were allowed to separate and transferred dimethylformamide or acetonitrile layer to another separating funnel containing 350–400 ml of 2% sodium sulfate solution and 50 ml of hexane (for dimethylformamide) or 350–400 ml of saturated sodium chloride solution and 50 ml of hexane (for acetonitrile).The funnel was shaken three to four times, allowing the layers to separate. The hexane layer was separated; the aqueous layer was extracted once again with 50 ml of hexane. Hexane fractions were combined, washed with 100 ml of the distilled water, and evaporated to a small volume (2–3 ml). The combined hexane extracts were added to a column with alumina or activated silica gel. Finally, the samples were purified with concentrated sulfuric acid. The solvent was reduced in a rotary evaporator at 30 °C and then subjected to instrumental analysis for quantification.

Preparation of the chromatography column

Application of the liquid phase 2 g of silicone oil was dissolved in a small amount of organic solvent, usually chloroform, and poured into a porcelain cup with 18 g of the carrier gas which was completely filled out with the same solvent. The entire mass was thoroughly mixed and the solution was evaporated in a water bath. The residue was evaporated during 3–5 h in a drying box at a temperature of 80 °C. The prepared medium was stored in a bottle with a glass stopper.

Chromatography of OCPs was performed by gas chromatography (GC) with an electron capture detector (ECD) (Perkin Elmer) on a column 51.SE-30 on Chromatone N-AW-DCM (0.12–0.16 mm). The temperature of the column was 200 °C, and the temperature of the injector was 220 °C. Nitrogen flow rate was 120 ml/min. The voltage and sensitivity were set in a way that the current strength in the detector cell was 1.6-10-10-a.

For external calibration amounts of 0.001 to 0.1 μg of the individual OCPs (HCH isomers, DDT, DDD, DDE, aldrin, and dieldrin) dissolved in hexane were injected. The peak area was used for quantification. The retention time for the individual pesticides was measured from the time at which the sample is injected to the point at which the display shows a maximum peak height for the respective compound (Table 1).

Quality assurance and quality control

For quality assurance/quality control (QA/QC), the retention times were checked daily before analyzing the samples. For setting up the retention time, the standard solution was repeatedly injected and the date of final sample extract (2.3 ml) was set up. Selected samples were measured in duplicate. The deviation of repeated analysis was in the range of ± 30%. Laboratory blanks for all OCPs were below the detection limits.

Data analysis

In order to assess possible relations between OCP levels and adverse effects, the data from all regions were combined. Placenta samples were divided into two main groups. Group A comprises all placenta samples with detectable amounts of OCPs collected in the three regions (Osh, Kara-Suu/Aravan, and Alay/Kara-Kulzha; Table 2). Total OCP concentration (the sum of the concentrations of all OCPs detected in a given placenta sample) was calculated (Table 3). Only values above LOD were included in the calculation. Group A was further subdivided according to total OCP concentrations: group A1 > 100 μg/kg of placenta, A2 10–99 μg/kg, A3 1–9.9 μg/kg, and A4 0.1–0.99 μg/kg. The upper limit of group A4 (0.99 μg/kg) roughly corresponds to the 9.8 percentile (1.00 μg/kg) of group A, the upper limit of group A3 (9.9 μg/kg) to the 50.3 percentile (9.86 μg/kg), and the upper limit of group A2 (99 μg/kg) to the 78.8 percentile (99.4 μg/kg).

Table 1 Retention time, sensitivity, and number of chromatographic plates in the analysis

Pesticide type	Sensitivity (μg/ml)	Retention time (RT)	*N*
Aldrin	0.5	5.2	780
HCHs	0.5	2.3–2.7	250
Heptachlor	0.5	4.0	780
4′4′DDD	0.1	13.0	–
4′4′DDT	0.5	15.6	304
4′4′DDE	0.5	9.6	260
Methoxychlor	2.0	20.0	195

Group B consists of the OCP-negative placenta samples from all regions studied (Table 2, total number of placentas collected minus placentas with OCPs): 47% (126/268) of OCP-negative placentas originated from the polluted regions (Osh (43), Kara-Suu/Aravan (83)), and 53% (142/268) from the unpolluted region (Alay/Kara-Kulzha).

Mean, standard error of the mean (S.E.M.), median, and percentiles were calculated for total OCP of group A and its subdivisions, as well as for individual OCPs, using GraphPad Prism6 software. Frequency distributions of total OCP levels were positively (right-) skewed (not shown). In order to investigate possible relations (1) between domicile of the mother and complications in mothers and their newborn and (2) between placental OCP levels (total OCPs) and number of mothers with complications during pregnancy and delivery, as well as number of newborns with complications, relative risk and odds ratio were calculated (1) for the city of Osh and the cotton-producing regions (locations 1 and 2, Table 2) as compared to the unpolluted regions (location 3) and (2) for group A and for its subdivisions A1, A2, A3, and A4, as compared to group B (MedCalc software). The comparison included all complications reported in mothers and in their newborns, without subclassification according to specific symptoms.

Results and discussion

Regional differences and levels of OCPs in placentas

In a total of 508 surveyed placenta samples, OCPs were found in 240 placentas (47.2%) with the available detection limits (Table 3). DDT, DDE, HCH (α-HCH, β-HCH, γ-HCH),

Table 2 Origin of placentas, number of placentas containing measurable amounts of OCPs (DDT, DDE, α-HCH, β-HCH, and γ-HCH, aldrin, heptachlor), and number of complications in mothers and newborns

Location/sample collection site[1]	Placentas		Mothers			Newborns		
	Total number	Placentas with OCPs detected, N (%)[2]	Total number	Mothers with complications, N (%)[2]	Relative risk versus location 3, p value)	Total number	Newborns with complications, N (%)[2]	Relative risk versus location 3, p value
1 City of Osh	121	78, (64.5)	121	14 (11.6)	1.877, $p = 0.124$	121	21 (17.35)	1.949, $p = 0.0436$
2 Kara-Suu and Aravan districts: production of cotton	241	158, (65.6)	241	111 (46.06)	7.472, $p < 0.0001$	241	144 (59.751)	6.711, $p < 0.0001$
Location 2, women living in cotton-growing areas	158	76, (48.1)	158	44 (27.8)	4.518, $p < 0.0001$	158	62 (39.2)	4.407, $p < 0.0001$
Location 2, women living near former pesticide storehouses and agro airstrips	83	82, (98.8)	83	67 (80.72)	13.095, $p < 0.0001$	83	82 (98.8)	11.096, $p < 0.0001$
3 Alay and Kara-Kulzha districts: unpolluted areas	146	4, (2.7)	146	9 (6.164)		146	13 (8.9)	
Total	508	240, (47.2)	240	134 (26.4)		508	178 (35.0)	

Odds ratios (mothers/newborns): city of Osh: 1.991 (n.s.)/2.149 ($p = 0.043$); location 2, Kara-Suu and Aravan districts:12.99 ($p < 0.0001$)/15.19 ($p < 0.0001$); location 2, women living in cotton-growing areas: 5.875 ($p < 0.0001$)/6.607 ($p < 0.0001$); location 2, women living near former pesticide storehouses and agro airstrips: 63.74 ($p < 0.0001$)/838.9 ($p < 0.0001$)

[1] Placentas were collected between 2011 and 2015

[2] Number and percentage of total number of placentas from the corresponding region

aldrin, and heptachlor were detected. DDD, δ-HCH, and dieldrin were not detected in any of the placentas.

The percentage of placentas positive for OCPs strongly depended on the residential area of the women surveyed (Table 2). A high percentage of placentas with OCPs was found in pregnant women living in cotton-growing regions (65.6%), with the highest percentage of OCP-positive placentas, 98.8%, in pregnant women living near pesticide dump sites, former pesticide storehouses, and agro airstrips that were in use until the 1990s. A description of environmental OCP contamination levels at these sites is given elsewhere (Toichuev et al. 2017). In contrast, OCPs were detected in only 2.7% of the placentas from pregnant women living in unpolluted areas.

In order to get an overview of the exposure to OCPs, the sum of the concentrations of all OCPs detected in a given placenta sample was defined as "total OCP concentration." Total OCP concentrations ranged from 0.1 to 3070 μg/kg placenta (Table 3). Data for individual OCPs are shown in Table 4. HCH isomers (α-, β-, γ-HCH) were found in the highest number of cases, in 116 placentas or 48.3% of placentas where OCPs were detected, in a concentration range of 0.1 to 880 μg/kg. γ-HCH (lindane) showed the highest detection frequency with 78 placentas, followed by α-HCH (38 placentas) and β-HCH (17 placentas). Highest levels were reached by β-HCH which is more persistent than the other two congeners in the environment and in biota (Table 4). This corresponds to placenta data from an Indian study (Tyagi et al. 2015). It is interesting that γ-HCH was found in a higher number of placentas than β-HCH, albeit at much lower levels. This might indicate that mainly lindane has been applied in Kyrgyzstan. In India, the technical HCH mixture has mainly been used (Jit et al. 2010; Vijgen et al. 2011).

p,p′-DDE was detected in 65 cases or 27.1% of OCP-positive placentas at levels between 1.1 and 2560 μg/kg, with a mean concentration of 263.3 μg/kg, and o.p′-DDT in 18 cases (7.5%) at levels between 3.1 and 120 μg/kg with a mean level of 51.8 μg/kg (Table 4). The DDE levels in the placenta were considerably higher than DDE levels observed in Spain (2.37 ± 2.80 μg/kg) (Lopez-Espinosa et al. 2007) and in a study in India with sum of DDTs 14.8 μg/kg (Tyagi et al. 2015). This shows that a subpopulation in the exposed area of the Kyrgyz Republic has been and still is highly exposed to DDT and metabolites.

Aldrin was detected in 11 samples (4.6%) at concentrations up to 220 μg/kg and a mean value of 165 μg/kg (Table 4). Heptachlor was found in only one placenta at a concentration of 12.3 μg/kg.

Comparison of health impacts with maternal domicile and OCP levels

The relative risk of complications in both, pregnant mothers and newborns, was markedly increased in the cotton-growing Kara-Suu and Aravan districts, as compared to the unpolluted

Table 3 Concentration range of total OCPs in placenta samples

Group	Subgroup	Range of total OCP concentrations[1] (μg/kg) of placenta	OCPs (μg/kg) of placenta						Number of placentas	Percent of group A	Percent of all placentas
			Mean ± S.E.M.	Minimum	25 percentile	Median	75 percentile	Maximum			
Group A	A1	> 100	360.1 ± 65.3	100	150	230	380	3070 [2]	51	21.25	10.04
	A2	10–99	32.8 ± 3.1	10	14	24.5	39	95	68	28.3	13.4
	A3	1–9.9	5.10 ± 0.28	1.1	2.0	6.0	7.4	9.5	97	40.4	19.1
	A4	0.1–0.99	0.399 ± 0.056	0.1	0.1	0.5	0.6	0.9	24	10.0	4.7
	Total of group A, containing OCPs		88.3 ± 16.6	0.1	4.1		73	3070 [2)]	240	100.0	47.2
Group B, without OCPs (control group)			n.d.						268	100.0	52.75
Total of all samples									508	100.0	100.0

[1] Sum of concentrations of all OCPs detected (DDT, DDE, HCH (α-HCH, β-HCH, γ-HCH), aldrin, and heptachlor). n.d.: no OCPs detected. Detection limits (LOD): DDT and metabolites 0.1 μg/kg; HCHs 0.5 μg/kg, aldrin 3 μg/kg; heptachlor 5 μg/kg. DDD, δ-HCH and dieldrin were not detected in any of the placentas

[2] Woman living in the vicinity of a pesticide storehouse

mountain areas of the Alay and Kara-Kulzha districts (Table 2). A particularly high increase of relative risk was observed in areas close to former pesticide storehouses and agro airstrips (mother relative risk = 13, newborn relative risk = 11, $p < 0.0001$). A modest increase in relative risk was also noted in newborns of the urban area of the city of Osh (relative risk 1.95, Table 2), while no significant change in relative risk could be documented for pregnant mothers living in that area. These data identify the cotton-growing regions as areas of high concern for health of pregnant mothers and their newborns.

The complications registered in pregnant mothers and their newborns comprise a large array of adverse effects, to which a variety of factors (environment, life style, occupation, nutrition) may have contributed. One possible reason is exposure to pesticides. For comparison of OCP levels and observations of health implication in mothers and their newborns, the cases were grouped according to total OCP levels in the placenta (Table 5). Group A comprised all 240 placentas where OCPs were detected. It was further subdivided into four subgroups with different ranges of total OCP concentration: subgroup A1 exhibiting levels of total OCPs > 100 μg/kg, subgroup A2 10–99 μg/kg, subgroup A3 1–9.9 μg/kg, and subgroup A4 0.1–0.99 μg/kg (Table 3). Group B consisted of 268 women where no OCPs, or only traces around the detection limit (0.1 μg/kg), was found in the placenta with the available analytical method. As 47% of the (OCP-negative) placentas of group B originated from polluted and 53% from unpolluted regions, this group was well suited as a reference group for comparison with group A, whose placentas were also collected in all regions.

In the OCP-exposed group A, 98 out of 240 women (40.8%) had moderate or severe complications, whereas only 36 out of 268 women (13.4%) exhibited this kind of complications in group B (Table 5). In the exposed group (group A), the incidence of complications appeared to be related to the total OCP concentration in the placenta (Tables 3 and 5). While 96.1% of the women in subgroup A1, with a mean total

Table 4 Concentration range of individual OCPs in placenta samples

Chemical	OCP concentration in placenta (μg/kg)						Number of placentas	Percent of group A[1]
	Mean ± S.E.M.	Minimum	25 percentile	Median	75 percentile	Maximum		
o,p′-DDT	51.8 ± 10.7	3.1	3.68	46.0	94.0	120	18	7.5
p,p′-DDE	263.3 ± 50.7	1.1	7.0	72.0	385.0	2560	65	27.1
Total HCH	129.9 ± 24.3	0.1	1.1	2.0	17.0	880	116	48.3
α-HCH	128.6 ± 43.6	0.1	0.675	4.6	79.6	880	38	15.8
β-HCH	189.4 ± 72.0	0.1	15.5	77.0	170.5	880	17	7.1
γ-HCH	130.6 ± 29.5	0.1	1.25	2.0	16.3	880	78	32.5
Aldrin	165.0 ± 11.6	110	130	165	200	220	11	4.6

Heptachlor: 12.3 μg/kg, $n = 1$

[1] Percentage of placentas with OCPs detected

Table 5 Moderate and severe complications diagnosed in women and their newborn children in relation to the concentration of OCPs in placenta

Group	Subgroup	Total OCP concentration (μg/kg) of placenta	Number of placentas with OCPs	Mothers with complications during pregnancy and delivery			Newborns with complications			
				Number of cases with complications	Percent of complications	Relative risk (p value)	Number of newborns surveyed	Number of cases with complications	Percent of complications	Relative risk (p value)
Group A with OCPs	A1	> 100	51	49	96.1	7.153 $p < 0.0001$	51	50	98.0	6.737 $p < 0.0001$
	A2	10–99	68	35	51.5	3.832 $p < 0.0001$	68	57	83.8	5.760 $p < 0.0001$
	A3	1–9.9	97	12	12.4	0.921 $p = 0.792$	97	28	28.9	1.984 $p = 0.0016$
	A4	0.1–0.99	24	2	8.3	0.620 $p = 0.492$	24	4	16.7	1.145 $p = 0.777$
	Total of group A		240	98	40.8	3.040 $p < 0.0001$	240	139	57.9	3.980 $p < 0.0001$
Group B OCPs undetectable			268	36	13.4		268	39	14.5	
Total			508	134	26.4		508	178	35.0	

Odds ratio for total of group A: mothers OR = 4.448 ($p < 0.0001$). Newborns OR = 8.081 ($p < 0.0001$)

Relative risk, 95% confidence intervals: Mothers: group A4 0.159 to 2.420; group A3 0.500 to 1.696; group A2 2.616 to 5.612; group A1 5.252 to 9.742; total group A 2.164 to 4.271. Newborns: group A4 0.447 to 2.933; group A3 1.295 to 3.038; group A2 4.232 to 7.841; group A1 5.028 to 9.028; total group A 2.921 to 5.424

Table 6 Pathologies diagnosed in newborns of groups A and B

Newborns with complications	Group A ($n = 240$)		Group B ($n = 268$)	
	Number	Percent of total	Number	Percent of total
Newborns with low birth weight	44	18.3	14	5.2
Newborns with congenital malformations	12	5.0	2	0.75
Stillbirths	5	2.1	2	0.75
Total number of newborns with complications	139	57.9	39	14.5

OCP concentration of 360 μg/kg placenta (Table 3), exhibited moderate or severe complications, this percentage was reduced to 51.5% in subgroup A2 with a mean OCP concentration of 32.8 μg/kg, 12.4% in subgroup A3 with mean OCP level of 5.10 μg/kg, and 8.3% in subgroup A4 with mean OCP level of 0.399 μg/kg.

Relative risk of complications was significantly increased in mothers for the upper two OCP concentration groups A1 and A2 and in newborns for the concentration levels A1, A2, and A3, in comparison with reference group B comprising placentas with OCP levels below detection limit. Odds ratios were also significantly increased (Table 5). The increase in relative risk was concentration-dependent, and the data suggests that newborns, with increased relative risk in the concentration range 1–9.9 μg/kg placenta, may have been more sensitive than their mothers who exhibited an increased risk of complications at OCP levels of 10–99 μg/kg and above. These data point to a relevant association between exposures to OCPs on the observed pathologies. Complications were diagnosed in all women and newborns whose placentas contained aldrin or heptachlor.

Maternal pathologies include the following complications: incidence of preterm delivery (babies born alive before 37 weeks of pregnancy are completed), 17.1% in group A versus 1.5% in group B; preeclampsia (a condition in pregnancy characterized by high blood pressure, sometimes with fluid retention and proteinuria); and eclampsia (a condition in which one or more convulsions occur in a pregnant woman suffering from high blood pressure, often followed by coma and posing a threat to the health of mother and baby), 7.5% in group A versus 0.75% in group B, gestosis 21.25 versus 11.9%, and bleeding during pregnancy and childbirth 8.3 versus 0.75%. Hospitalization of women with a variety of complications (mostly bacterial infections) in the postnatal period was reported in 5.4% in group A as compared to 0.77% in group B. The total number of cases with moderate and severe complications observed in group A was 98 (40.8%, Table 5). The maternal mortality rate was 0.8% in group A.

A relationship of pathologies to OCP exposure was also observed in the newborns during the first 6 days of life (Tables 5 and 6). The number of cases with complications diagnosed in the 240 children born to mothers from the OCP-exposed group A was 139 (57.9%), as compared to 39 (14.5%) of 268 newborns in group B with OCPs below detection limit in placenta. The percentage of children with low birth weight was 3.5 times higher in group A than in group B (Table 6). Congenital malformations were 6.7 times more frequent in group A, and stillborn children 2.8 times more frequent. Complications in newborns included low body weight, malnutrition, congenital malformations, stillbirths and perinatal mortality, cerebrovascular accident, encephalopathy, intrauterine hypoxia, respiratory disorders, hepatitis and bacterial infections, immune deficiencies, and intestinal dysbiosis. Complications were diagnosed by clinical symptoms or diagnostic devices, such as ultrasonography, encephalography, tomography, and X-ray analysis, and laboratory data.

In line with the change in relative risk, the observations in newborns of group A also showed a relation of complications to OCP concentrations. The percentage of newborns with low birth weight was 47.0% (24/51 observed newborns) in subgroup A1, 16.2% (11/68) in subgroup A2, and 7.2% (7/97) in subgroup A3. Congenital malformations occurred in 15.7% (8/51) of subgroup A1, 4.4% (3/68) of subgroup A2, and 1.03% (1/97) of subgroup A3. The percentage of perinatal mortality was 21.6% (11/51) in subgroup A1 and 2.9% (2/68) in subgroup A2. Congenital hepatitis and stillbirth were seen only in subgroup A1, both at 9.8% (5/51). In subgroup A4, pathologies were found in 16.7% (4/24) of the newborns, which is comparable to the percentage of newborns exhibiting complications in the unexposed group B (14.5%, Table 6).

Conclusions

The analysis of OCPs in placentas revealed that women living in cotton-growing areas of southern Kyrgyzstan and their children are highly exposed to OCPs, in particular when they live in the vicinity of former pesticide landfills, storehouses, or agro airstrips. Pregnant women and their newborns exposed to OCPs exhibited a higher percentage of complications in pregnancy and during the first postnatal week than women and newborns from areas with low pesticide pollution and exposure. The complications observed cover a wide range of pathologies.

The design of the present study did not allow to investigate whether additional differences between group A and group B, e.g., differences in nutrition, lifestyle, housing, occupation, or ethnicity, may have had any impact. Also, it was not possible to clarify which types of pathologies were directly correlated with increased OCP exposure. The analysis was based on the total of complications observed. However, the marked increase in relative risk of complications and in the odds ratio in mothers and their newborns, and in particular, the concentration dependency of relative risk increase (increased incidence of pathologies) points to a significant relationship between exposure to OCPs and at least some of the complications observed in mothers and their newborns.

The investigation identified the cotton-growing regions as areas of high concern for the health of pregnant mothers and their newborns. In particular, women living near the former pesticide storehouses, agro airstrips, and pesticide dump sites should be considered as being at high risk. The high levels of OCPs in placentas in these areas show that reduction of exposure is urgently needed in order to prevent further pathologies of pregnant women and newborns. Major sources of OCPs need to be secured and the exposure pathways reduced and eliminated. While the large pesticide dumpsites have been secured, most former pesticide storage sites have not been assessed for pollution level and exposure risk (Toichuev et al. 2017). For known contaminated sites like pesticide airstrip areas, the population should be advised not to cultivate vegetables, melons, legumes, and fodder crops, but rather, cultivate technical cultures or plant trees. Furthermore, cattle raising at potentially contaminated areas should be restricted. For future studies, analytical capacities need to be improved to reach lower detection limits in human studies and include additional pesticides and other pollutants.

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