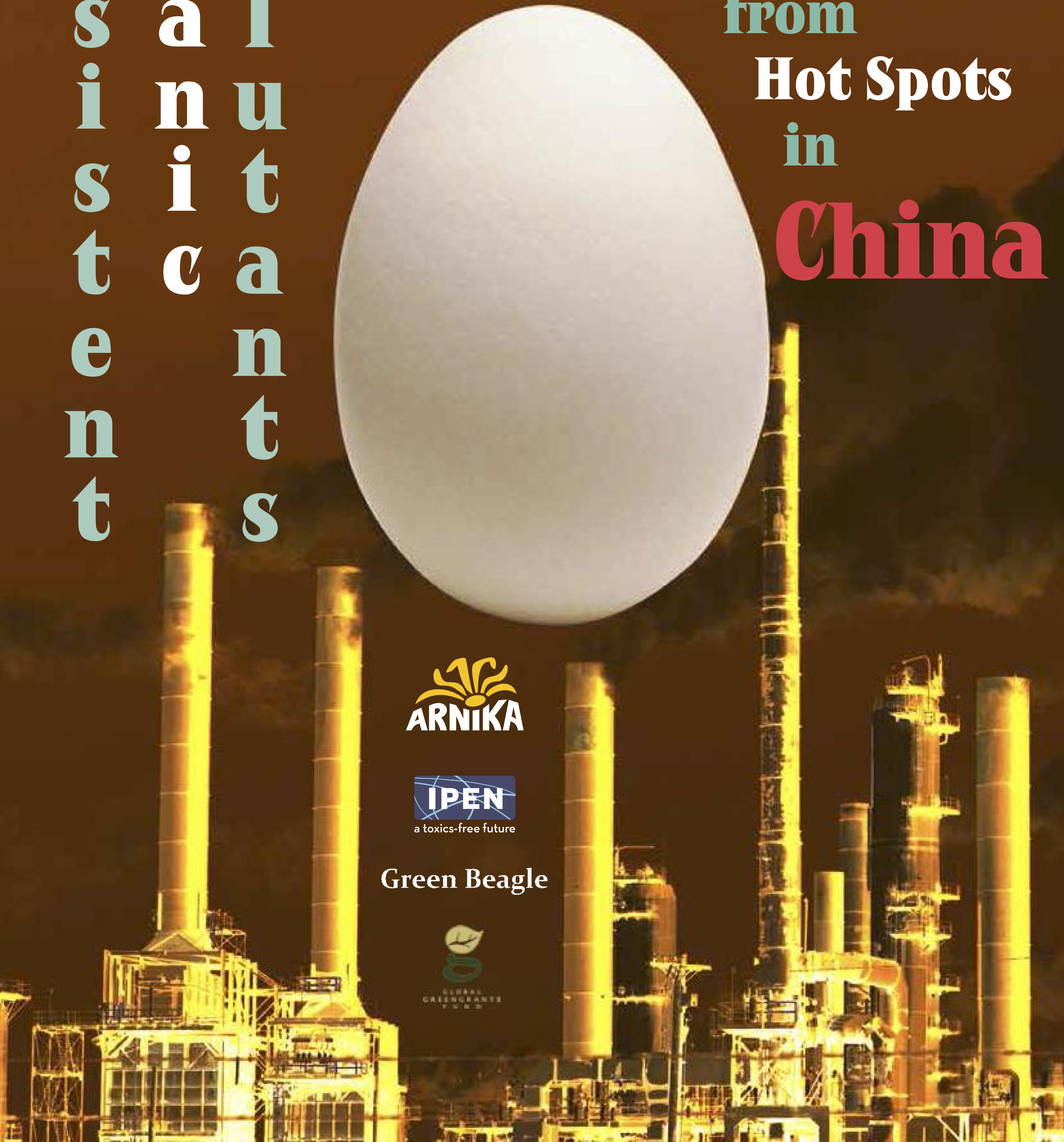
POPs in Chicken Eggs from Hot Spots in China

Persistent Organic Pollutants



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Persistent Organic Pollutants (POPs) in Chicken Eggs from Hot Spots in China

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Contents

1. Abbreviations

ADI – acceptable daily intake
AMA - advanced mercury analyser
BDS – BioDetection Systems (laboratory in Netherlands)
BEQ – bioanalytical equivalent
CALUX - chemically activated luciferase gene expression
CAS – chemical abstracts service registry number (a unique numerical identifier asigned to every chemical substance described in the open scientific literature)
BTBPE - 1,2-bis(2,4,6-tribromo-fenoxy)ethane
DDD – dichlorodiphenyldichloroethane (a metabolite of DDT)
DDE - dichlorodiphenyldichloroethylene (a chemical compound formed by the loss of hydrogen chloride from DDT)
DDT – dichlorodiphenyltricholoroethane (pesticide)
DL PCBs – dioxin-like PCBs
d.w. – dry weight
EU – European Union
f.w. – fresh weight
GC – gas chromatography
GEF – Global Environment Facility
GoK – Government of Kazakhstan
GPC - gel permeation chromatography
GPS - global positioning system
HBB - hexabromobenzene
HCB - hexachlorobenzene
HCHs – hexachlorocyclohexanes (pesticides and their metabolites)
HRGC-HRMS – high resolution gas chromatography – high resolution mass spectroscopy
IARC - International Agency for Research on Cancer
INC – Intergovernmental Negotiating Committee (normaly set up for negotiations of new international convention)
IPEN – International POPs Elimination Network
LOD – limit of detection
LOQ – limit of quantification
MAC – maximum acceptable (allowable) concentration
ML – maximum level
MRL – maximum residue level
NA – not analyzed
NGO – non-govermental organization (civil society organization)
NIP – National Implementation Plan
NOAEL - no observed adverse effect level
OBIND – octabromotrimethylfenylindane
OCPs – organochlorinated pesticides
PBDD/Fs – polyfrominated dibenzo-p-dioxins and furans
PBEB – pentabromoethylbenzene
PBT – pentabromotoluen
PCBs – polychlorinated biphenyls
PCDD/Fs – polychlorinated dibenzo-p-dioxins and furans

PCDDs – polychlorinated dibenzo-p-dioxins
PCDFs – polychlorinated furans
POPs – persistent organic pollutants
SC – Stockholm Convention on Persistent Organic Pollutants
SOP - standard operating procedures
TEF – toxic equivalency factor(-s)
TEQ – toxic equivalent
UNDP – United Nations Development Programme
UNEP – United Nations Environment Programme
US EPA – United States Environmental Protection Agency
WHO-TEQ – toxic equivalent defined by WHO experts panel in 2005
w.w. – wet weight

2. Introduction

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 with exceeding 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 project ''Strengthening the capacity of pollution victims and civil society organizations to increase chemical safety in China''.

The data and analyses of free range chicken eggs discussed in this report were obtained during a two and quarter year-long joint project of three NGOs: IPEN (Sweden), Green Beagle (China) and Arnika – Toxics and Waste Programme (Czech Republic). They were obtained from local people and/or sampled by Chinese NGOs Nature University and Green Beagle during field visits in 2013 and 2014 from seven different localities in China. Additional sampling was done in Likeng in November 2015.

3. Sampling and analytical methods

Samples of mostly free range chicken eggs were collected at seven localities in China. One sample, from a supermarket in the city of Beijing, was from chickens that did not have free range and was considered as a background sample, as practiced in other studies (DiGangi and Petrlik 2005). Six localities were expected to be contaminated by POPs to a certain level. A basic description of these six localities can be found later in this report (see chapter 3).

Pooled samples of more individual egg samples were collected at each of the selected sampling sites in order to get more representative samples. Table 1 summarizes the basic data about the size of samples and the measured levels of fat content in each of the pool samples. Ten pool samples of free range chicken eggs were taken in total. Two samples were taken in 2013, eight in 2014, and one in 2015.

It was determined to analyze free range chicken eggs from the eight pool samples (the majority sampled before June 2014) and one pool sample of commercial eggs from Beijing for PCDD/Fs and dioxin-like PCBs using the DR CALUX method. These were sent to a Dutch ISO 17025 certified laboratory (BioDetection Systems B.V., Amsterdam). The procedure for the BDS DR CALUX® bioassay has previously been described in detail (Besselink H 2004). Briefly, rat liver 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 (37ºC, 5% CO_2, 100% humidity). Cells were exposed in triplicate on 96-well microtiter plates containing the standard 2,3,7,8-TCDD calibration range, a reference egg sample (analysed by GC-HRMS; for the bioassay apparent recovery), a procedure blank, a DMSO blank and the sample extracts in DMSO. Following a 24-hour incubation period, cells were lysed. A luciferin containing solution was added and the luminescence was measured using a luminometer (Berthold Centro XS3).

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

No	Locality (sample)	Month/year of sampling	Number of eggs in pooled samples	Number of chicken fanciers	Fat content in %
1	Beijing - supermarket	10/2014	3	Big farm	10.14
2	Beihai I	4/2014	2	2	18.50
3	Beihai II	4/2014	2	1	18.20
4	Beihai III	4/2014	2	2	21.90
5	Likeng	9/2013	8	1	22.80
6	Likeng - II	11/2015	7	1	16.00
7	Wuhan 1	3/2014	6	6	15,50
8	Wuhan 2	9/2014	3	2	12,46
9	Quihua 1	8/2013	8	2	15.80
10	Ziyang	9/2014	3	3	8.05
11	Shenzhen	5/2014	4	NA	8.40

The DR CALUX bioassay method is proven for screening analyses that can give a good picture about the level of pollution[1]; 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 sampling period (September 2014) and additional eggs sample from Likeng (November 2015) 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. Some of the samples were from at least the same area as those from the first field sampling.

[1] "Bioanalytical methods" means methods based on the use of biological principles like cell-based assays, receptorassays 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 (2012). Commission Regulation (EU) No 252/2012 of 21 March 2012 laying down methods of sampling and analysis for the official control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs and repealing Regulation (EC) No 1883/2006 Text with EEA relevance European Commission. Official Journal of the European Communities: L 84, 23.83.2012, p. 2011–2022.

The later egg samples were also analyzed for content of non-dioxin-like PCBs and OCPs 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.

Two free range chicken eggs samples from Wuhan and the control group sampled in the Beijing supermarket were also analysed for polybrominated dibenzo-p-dioxins and dibenzofurans (PBDD/Fs) in MAS laboratory, Muenster, Germany. Accredited method MAS_PA002, ISO/IEC 17025:2005 was used to determine PBDD/Fs. The basic steps of the analyses can be summarized as follows:

- Addition of $^{13}C_{12}$-labelled PBDD/F internal standards to the sample extract
- Multi-step chromatographic clean-up of the extract
- Addition of $^{13}C_{12}$-labelled PBDD/F - recovery standards
- HRGC/HRMS analysis
- Quantification via the internal labelled PBDD/F-standards (istope dilution technique and internal standard 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. Description of hot spots

Localities chosen for sampling are located in different parts of China. Their selection was based on environmental advocacy cases of Chinese NGOs Green Beagle and Nature University. Most of them were also chosen for case studies within the project ''Strengthening the capacity of pollution victims and civil society organizations to increase chemical safety in China''. The location of the sites in China can be found on maps at Figures 1 and 2. All hot spots were chosen as potential sources of POPs releases.

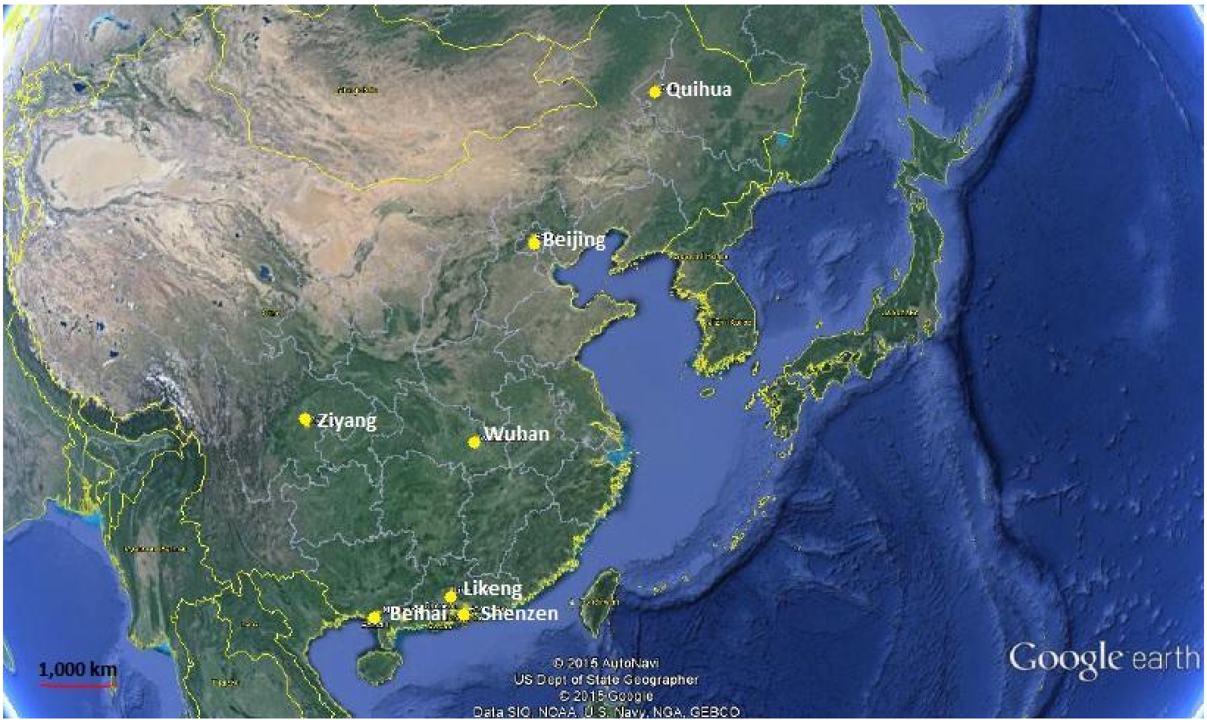


Figure 1: Location of chosen hot spots on a Google Earth map of China.

Figure 2: Google Earth Map of the southern part of China with three hot spots: Beihai (metallurgical complex in Xinggang), Likeng (waste incinerator) and Shenzen (waste incinerator).

4.1 Beihai – metallurgical plant

Before a metallurgical complex was built, Xinggang Town was a quiet fishing village. The quietness of this village disappeared rapidly after Chengde Ferronickel Stainless Steel Co. began operations in 2011. The company is one of four subsidiaries of the Chengde group, which also includes Beihai Chengde Metal Stamping Co. (hot rolling mill), Beihai Chengde Zongwei Co. (processing of steel slag into cement), and Guangxi Chengde International Trade Co. (raw material purchasing and product distribution). According to the company, Chengde produces 1.2 million tonnes of nickel-chromium alloy slab per year. It is a large operation that includes sintering, rotary kiln- submerged arc furnace, initial refining, AOD refining and rolling systems, plus auxiliary utilities for the main production facilities, such as oxygen stations, water treatment, power supply and distribution, etc. Chengde severely polluted the local environment during production due to phased construction and incomplete environmental measures. More information about this hot spot can be found in the case study by IPEN and Green Beagle (2015).

The Stockholm Convention has identified sinter plants in the iron and steel industry as a sector *"for comparatively high formation and release"* of persistent organic pollutants such as dioxins, furans, PCBs, hexachlorobenzene, and pentachlorobenzene.[2]

Figure 3: Location of free range chicken egg samples taken in the vicinity of the Chengde group metallurgical complex in Xinggang. Google Earth Map was used as a basis for this map.

4.2 Likeng – waste incinerator

The large French transnational corporation Veolia built the Likeng waste-to-energy incinerator and started operations in 2006. The Likeng incinerator burns about 1000 tonnes of garbage per day, generating 320 – 350 MW power – 80% of which is exported. Like other incinerators in China, Likeng is trying to burn wet waste which is about 60– 70% organic matter (EnviroCentre 2008).

A newspaper reported that a poll taken in the village by the Guangdong Situation Study and Research Center found that, *"...92 percent felt the plant posed a serious danger to their health and the local natural environment, while 97 percent said they opposed its construction. Almost all respondents said they opposed the incinerator projects over fears about dioxins"* (Qian and Jing 2009).

The negative community response to the incinerator reflected the dramatic change in health statistics after the incinerator began operation. In the six years between 1989 and 2005, only nine people died of cancer in a nearby village of 8,000 people. In contrast, from 2005 – 2009,

[2] Stockholm Convention Annex C, Part II

after the incinerator began operation, 42 people died of cancer. Common ailments included nasopharyngeal cancers and asthma. An analysis of health records from three villages near the incinerator found zero cases of respiratory cancer between 1993 and 2005. However, three years after the incinerator began operating, 13 respiratory cancer cases were found.

Local government officials and Veolia insisted that the incinerator operated according to EU standards and that the high temperature used in the facility would destroy all pollutants, including dioxins. However, a 2009 news investigation of the ashes surprisingly found intact rope, cloth, red plastic bags, and shoes, indicating incomplete combustion (News.sina.com 2009).

The Stockholm Convention has identified waste incineration as a sector *"for comparatively high formation and release"* of persistent organic pollutants such as dioxins, furans, PCBs, hexachlorobenzene, and pentachlorobenzene.[3] More information about this hot spot can be found in the case study by IPEN and Green Beagle (2015a).

4.3 Quihua – PVC plant

In Ang'angxi Industrial Park of the Harbin-Daqing-Qiqihar Industrial Corridor, production of PVC was run by Heilongjiang Haohua Chemical Co., Ltd., affiliated to the China Haohua Chemical (Group) Corporation. Haohua describes itself as promoting *"harmonious and healthy development"* in its production of caustic soda (350,000 tonnes annual capacity), PVC (380,000 tonnes annual capacity), liquid chlorine, hydrochloric acid, and sodium hypochlorite.

The Qiqihar Chemical Group (QCG) and Haohua Chemical have utilized the calcium carbide method for PVC synthesis. Most of the world replaced this process in the 1960s due to its high energy use and excessive waste production. However, in China this process is favored for economic reasons due to its use of coal as a starting material. In the process, coal-derived coke is heated with caustic at 2000C to create calcium carbide. Calcium carbide is then hydrolyzed with a lot of energy to generate acetylene and enormous amounts of calcium hydroxide wastes. Acethylene is then reacted with hydrogen chloride and a mercury catalyst to make vinyl chloride, which is polymerized to create PVC.

Mercury plays a significant role in vinyl chloride monomer plants in China such as QCG/Haohua. In 2005 the Chinese industry consumed 700-800 metric tons of mercury and the rate of growth of mercury use in this industry has been estimated at 25-30 percent per annum, although this may be affected by economic growth rates over time. Statistics by the China Chlor Alkali Industry Association show that by the end of 2010, China had 94 PVC manufacturing enterprises with a total capacity of over 20 million tonnes. Calcium carbide process plants represent approximately 80% of capacity (IPEN Heavy Metals Working Group 2013).

Villager Wang Fucheng's family is the only one that still lives south of Dapaozi pond, near waste dump. He has planted about one hectare of corn, but has abandoned about 5.5 other hectares of land because of pollution. In 2001, QCG and Yushutun Village signed a 28.57-hectare land lease agreement for storing calcium carbide-derived slurry from the PVC production process.

[3] Stockholm Convention Annex C, Part II

More information about this hot spot can be found in the case study by IPEN and Green Beagle (2015b). The production of PVC by QCG/Haohua Chemical also raises concerns due to the toxic lifecycle of the product, including its use of additives and its link to the formation and release of dioxins, listed under the Stockholm Convention.[4]

4.4 Shenzhen – waste incinerator

A selected hot spot of the Longgang municipal solid waste incinerator (MSWI), which is located at a hillside called Honghualing and has a processing load of 300 tons of municipal solid waste (MSW) per dayin central Shenzhen and has a processing load of 300 tons of municipal solid waste (MSW) per day , is the second oldest incinerator in Shenzhen City. Shenzhen is a fast developing and urbanizing city in the estuary of the Pearl River Delta, China. In 1988 a MSWI plant was built, imported from Japan to Shenzhen. From 1988 - 2005, the annual amount of local MSW increased dramatically to 3.329 million tons (228 times that of year 1985) (Deng 2006), with more than 90% disposed of via incineration in six MSWIs. (Wang, Zhao et al. 2011).

Solid residues from Shenzhen waste incinerators were studied and used for several studies about stabilization of waste incineration residues; however, those studies were focused mainly on heavy metals (Jianguo, Jun et al. 2004, Li, Xiang et al. 2004, Jianguo, Maozhe et al. 2009).

Until 2006, there were eight MSW incineration power plants in operation in Shenzhen, treating 6,000 tons of MSW daily. Longgang incinerator was a subject of criticism by local residents in Shenzhen in 2009 (Bradsher 2009), because the thermal process in MSW incineration has the potential to emit diverse types of organic pollutants to the environment, such as PAHs, PCBs, and PCDD/Fs, which may endanger the ambient environment and people's health (Liu, Tong et al. 2010).

PCDD/Fs and DL PCBs were measured in ambient air in Shenzhen in 2009. For all samples, the levels of PCDD/Fs were in the range of 11.45~370.12 fg I-TEQ m^{-3}, with a median of 151.24 fg I-TEQ m^{-3}. The concentrations of the 12 DL-PCBs were in the range of 1.81~19.55 fg WHO-TEQ m^{-3}, with a median of 12.82 fg WHO-TEQ m^{-3}. The level of atmospheric PCDD/Fs in Shenzhen was close to the average level of Sao Paulo of Brazil, higher than Taiwan, Hong Kong and the cities in Japan and Europe, but lower than Korea's cities and Beijing, Guangzhou and Shanghai of China. The level of DL-PCBs was close to that of Manchester, but lower than Gyeonggi-do of Korea and Taizhou of China (Zhang J 2011).

Sun et al. (2011) suggested that waste incineration is one of the sources of Cl-/BrPAHs in surface water sediments in the city.

4.5 Wuhan – waste incinerator

Wuhan is the capital of Hubei Province, and is the most populous city in Central China. The city of Wuhan has a population of 10,220,000 people (as of 2013). It lies in the eastern

[4] Stockholm Convention Annex C, Part II

Jianghan Plain at the intersection of the middle reaches of the Yangtze and Han rivers. It has arisen out of the conglomeration of three cities; Wuchang, Hankou, and Hanyang.[5]

There are two waste incinerators in Hanyang, Wuhan. One is municipal waste incinerator and the other is a medical waste incinerator. The former one burns 1500 tons of waste per day with circulating fluidized bed technology. The latter one burns 50 tons of medical waste each day. The MWI started operation in December 2012. The medical waste incinerator started in 2013. The medical waste incinerator is in the north of the MWI and next to it.

The municipal waste incinerator uses the bottom ash to make bricks and dumps the fly ash in the Chenjiachong landfill of Wuhan, which lacks the appropriate environmental protection measures. The residues from the medical waste incinerator are managed as follows: the bottom ash is disposed of at Chenjiachong landfill and the fly ash is sent to Xianning landfill (also in Wuhan).

The incineration plants have long been subjects of complaints from local residents. There were more than 25 cancer patients reported in the community in the past two years, although it is unclear whether the diseases are related to the plants (Kun and Wei 2015).

One plant, run by Borui Energy Environmental Protection in Wuhan, was ordered to halt operations in December 2013, after local authorities failed to demolish all the residential buildings in the required buffer areas, according to documents online at the Wuhan environmental authority. It has since resumed operation. The plant also received a warning from the Ministry of Environmental Protection in October 2013 for transferring ash to unauthorized units for handling (Kun and Wei 2015).

4.6 Ziyang – PCBs stockpile

The State-owned Ziyang Locomotive Plant in Sichuan Province produced locomotives in the 1960s and used at least 500 power capacitors containing PCBs that often leaked, resulting in human exposure. An abnormally high percentage of cancers and other serious illnesses among workers at the locomotive plant was observed by a local resident and worker in the plant, Guo Ruzhong. Some of them even died at young ages, in their thirties or forties. Guo suspected links to occupational exposures and electrical capacitors containing PCBs became the prime suspect. This led to an investigation into the use and disposal of these capacitors as well as his colleagues' exposure to PCBs.

In 2003, the factory admitted that more than 200 PCB-containing capacitors were still in use at the factory. In 2009, the factory delivered them to Tianjin Hejia Veolia Environmental Services, for disposal. However, Guo reminded the company and public that due to their persistence, the leaked PCBs would continue to pose a threat. Study by researchers at the State Key Laboratory of Marine Environmental Science in Xiamen University confirmed results of serious PCBs contamination at the factory. Results showed high levels of PCBs in soil from the front gate of the capacitor storage tunnel (~227 ppm) and in dust from the window sill of the iron foundry (>10 ppm) (Wu, Yin et al. 2011).

In March 2014, Guo Ruzhong discovered 47 insulating paper coils that he recognized formed part of the electrical capacitors containing PCBs sitting in a waste pile at the factory. Other industrial waste was also found at the site. Green Beagle personnel requested photos and

[5] https://en.wikipedia.org/wiki/Wuhan

samples for testing and tests were commissioned. The results of analyses ordered by Green Beagle at the SGS-CTSC laboratory in Shanghai in 2014 showed PCB levels as high as 8.11 mg l^{-1}, 4,000 times higher than permitted under Chinese law.

More information about this hot spot can be found in the case study by IPEN and Green Beagle (2015c).

5. Results and discussion

Eight samples of chicken eggs collected at Chinese hot spots and a control sample from a supermarket in Beijing were screened for dioxins and dioxin-like PCBs using the DR CALUX method in BDS laboratory, Amsterdam. The results of the analyses by using DR CALUX are summarized in Table 3 and the graph in Figure 4. The results of the analyses for other POPs and congener analyses by using HRGC-HRMS are summarized in Table 4. Free range chicken eggs from Kazakhstan and Belarus were also analyzed using the same methods, so we can compare data from Chinese hot spots with similar locations in other countries as well. The results for samples from Kazakhstan and Belarus are presented in Table 3 for DR CALUX analyses and in Table 5 for other analyses.

GC-HRMS analyses were chosen for dioxins and dioxin-like PCBs analyses in the sample of commercial eggs from a supermarket in Beijing and three pool samples of free range chicken eggs from two localities in China: the PCBs-contaminated site in Ziyang and the MSWI and medical waste incineration site in Hanyang, Wuhan. The same samples were also analyzed for other POPs, a group of OCPs (hexachlorobenzene (HCB), hexychlorocyclohexanes (HCHs) and DDT) and its metabolites. HCB is also considered to be an 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.

Eggs from the control group (supermarket, Beijing) and from sample Wuhan 1 were also analysed for PBDD/Fs and brominated flame retardants (PBDEs, PBT, PBEB, HBB, BTBPE and OBIND). Results are summarized in Table 6.

5.1 The EU and other limits for POPs in eggs

Chicken eggs are a quite common part of the diet in China; however, we didn't find any limits for PCDD/Fs and PCBs in eggs used in China, although there are limits for OCPs in food established (The People´s Republic of China 2007). Therefore we have chosen legislative limits established in the European Union in order to be able to compare measured levels of POPs in chicken eggs with them, although they don't exist for all measured chemicals in this study. There is no limit established for BFRs or PBDD/Fs in chicken eggs yet. Limit values for eggs are summarized in Table 2.

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

	Hen eggs	
	EU ML[1]	EU MRL[2]
Unit	pg g^{-1} fat	ng g^{-1} fat
WHO-PCDD/Fs TEQ	2.5	
WHO-PCDD/Fs-dl-PCB TEQ	5.0	
PCBs[5]	40	
DDT total[3]		50
γ-HCH (lindane)		10
α-, **β**-HCH		20, 10*
HCB		20

[1]EU Regulation (EC) N°1259/2011. Maximum level (ML) – food with PCDD/Fs and DL PCBs concentrations above this level is considered to be contaminated and is not suggested for consumption.
[2]Regulation (EC) N°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.
[3] There is a limit for DDT residues in eggs in Chinese legislation set at the level of 0.1 mg kg^{-1} fat, according to *Limits of Pollutants in Foods* (GB2763-2005) (The People´s Republic of China 2007).
*for each congener MRL is set separately

5.2 Dioxins (PCDD/Fs) and dioxin-like PCBs measured by DR CALUX

Table 3: Results of DR CALUX bioassay analyses for both PCDD/Fs and DL PCBs for samples from China, Kazakhstan and Belarus. Data are in pg BEQ g^{-1} fat. Each sample above EU maximum levels (ML) is in bold.

Locality	Country	PCDD/Fs and DL PCB (DR CALUX)	PCDD/Fs (DR CALUX)
Beijing – supermarket	China	1.2	NA
Shenzhen	China	7.7	5.7
Wuhan 2	China	8.8	5.8
Beihai II	China	8.9	7.4
Quihua	China	9.2	NA
Likeng	China	17	NA
Beihai III	China	24	20
Wuhan 1	China	35	31
Beihai I	China	37	30
Ekibastuz – Soyuz	Kazakhstan	3.8	1.3
Eikbastuz - dachas (south)	Kazakhstan	4.8	NA
Eikbastuz – substation	Kazakhstan	6.4	NA
Samarkand village; Nura	Kazakhstan	9.2	NA
Rostovka; Nura	Kazakhstan	9.4	NA
Balkhash 2	Kazakhstan	12	NA
Balkhash – southwest B3	Kazakhstan	15	NA
Shabanbai Bi	Kazakhstan	16	7.6
Chkalovo; Nura	Kazakhstan	18	NA
Balkhash – Rembaza B1	Kazakhstan	24	NA
Temirtau; Samarkand dam	Kazakhstan	28	NA
Balkhash – southwest B5	Kazakhstan	33	NA
Balkhash – north B4	Kazakhstan	101	NA
Gatovo	Belarus	8.1	5.2
EU standards (ML)		5.0	2.5

When PCDD/Fs 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/252/2012 (European Commission 2012). Screening methodologies are usually used to exempt 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/Fs TEQ level. When bioassays are used as screening tools, the interpretation of the obtained results should consider that other dioxin-like POPs (such as mixed-halogenated dioxins/ PCBs, N-dioxins) are also covered by such effect-based bioanalysis tools (van Overmeire, van Loco et al. 2004, Gasparini M 2011).

Nine pool egg samples from China were analyzed using the DR CALUX method for determination of dioxin activity in total. Among those, only the control group sample from the supermarket was below the 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 shown by the graph in Figure 4. The highest level of 37 was measured in chicken eggs marked as Beihai I, western from the metallurgical plant. The sample was taken in the first half of 2014 at a site where we were not able to repeat sampling later in the same year. Sample Beihai III, taken at a location closer to the metallurgical complex, was also high, at a level of 24 pg BEQ g^{-1} fat. These levels are comparable to those obtained for free range chicken eggs from Balkhash city, a similar hot spot with a metallurgical plant in Kazakhstan.

PCDD/Fs and DL PCBs content in samples from the vicinity of Wuhan waste incinerators was also high. A level of 35 pg BEQ g^{-1} fat was in the sample from an area in a distance of 300 meters from the MWI in Wuhan, while a more distant sample had a lower level of 8.8 pg BEQ g^{-1} fat. Thus it is in the same range as samples from Beihai (8.9 – 37.0 pg BEQ g^{-1} fat).

The eggs from Shenzhen have shown higher toxicity values than in a study of PCDD/Fs in the diet of local residents by Zhang, Jiang et al. (2008).

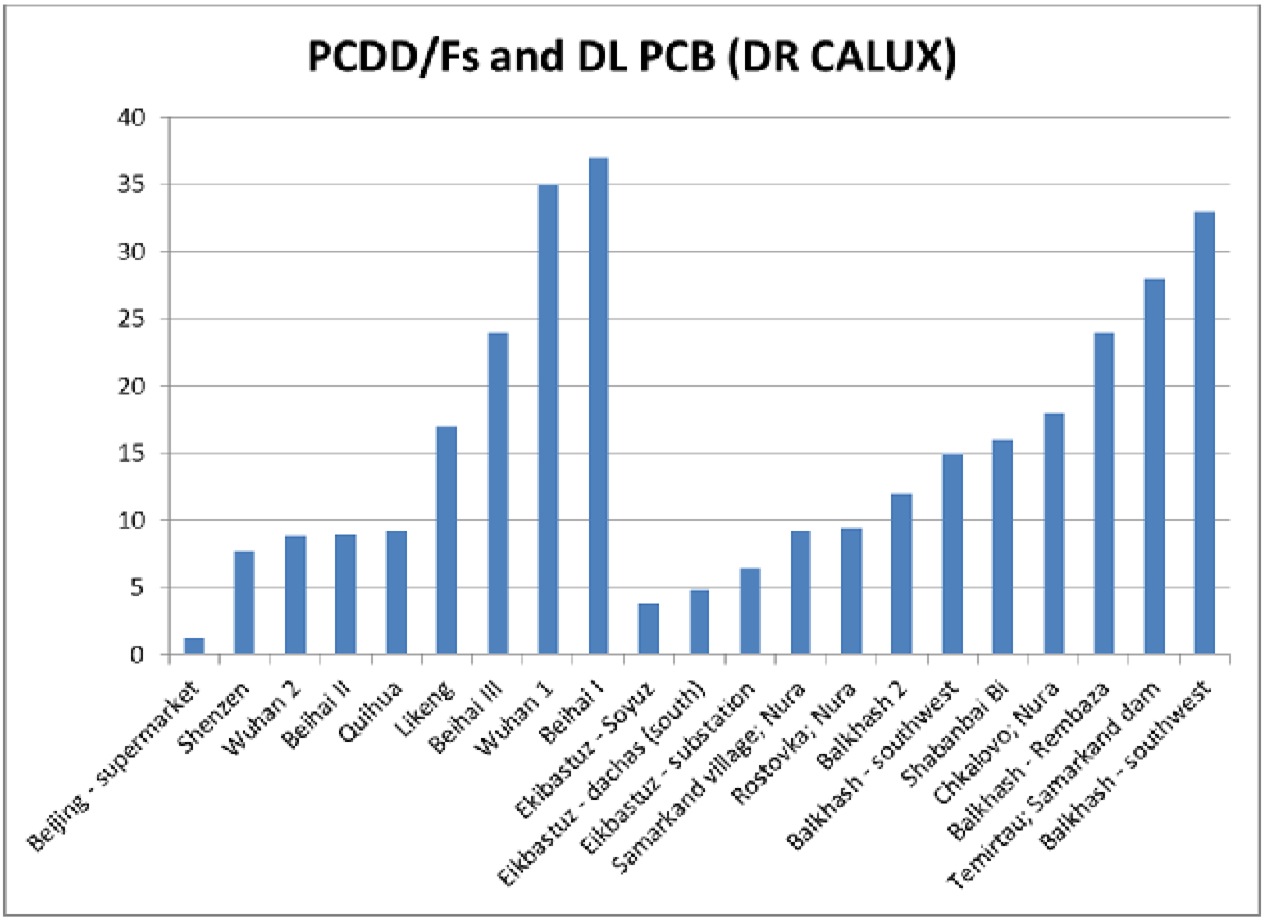


Figure 4: Graph showing comparison of total PCDD/Fs and DL PCBs in pg BEQ g^{-1} fat for different pooled chicken eggs samples from Kazakhstan, China and Belarus (full set of results is in Table 3).

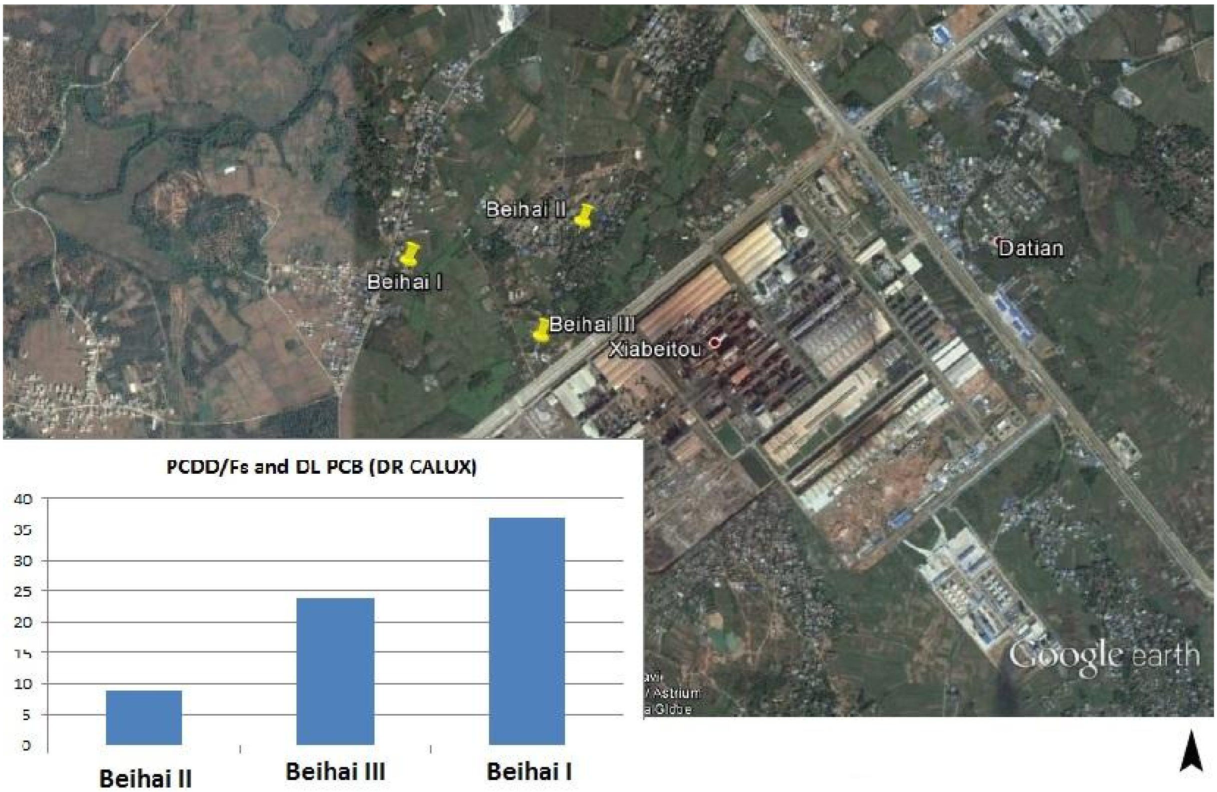


Figure 5: Map of samples location in Beihai and comparison of levels of PCDD/Fs and DL PCBs measured by DR CALUX in graph at bottom of the map.

5.3 Dioxins (PCDD/Fs), PCBs, HCB and OCPs in samples from China and Kazakhstan

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 that 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 PCBs' (NDL PCBs) (European Commission 2011). Levels of PCDD/Fs and DL PCBs are expressed in total WHO-TEQ calculated according to toxic equivalency factors (TEFs) set by a WHO experts panel in 2005 (Van den Berg, Birnbaum et al. 2006). These new TEFs were used to evaluate dioxin-like toxicity in pooled samples of chicken eggs from China (see Table 4) and Kazakhstan (see Table 5).

Table 4: Summarized results of analyses for U-POPs in chicken egg samples from China compared with EU standards. All data are per grams of fat.

Sample	Beijing (superm.)	Ziyang	Wuhan 2	Wuhan 1	Likeng II	EU standard
Fat content (%)	10.14	8.05	12.46	15.5	16.0	
PCDD/Fs (pg WHO-TEQ g^{-1})	0.20	3.77	8.59	12.17	3.22	2.50
DL PCBs (pg WHO-TEQ g^{-1})	0.28	1.04	4.70	3.79	0.95	
Total PCDD/F + DL PCBs (pg WHO-TEQ g^{-1})	0.48	4.82	13.29	15.96	4.17	5.00
PBDD/Fs (pg WHO-TEQ g^{-1})	<1.8		<3.6	27.3 – 29.2	NA	-
PCDD/Fs and DL PCB (DR CALUX) (pg BEQ g^{-1})	1.2	NA	8.8	35	NA	5.00
PCDD/Fs (DR CALUX) (pg BEQ g^{-1})	NA	NA	5.8	31	NA	2.50
HCB (ng g^{-1})	3.52	< 0.05	28.90	480.65	NA	- [1)]
6 PCB (EU) (pg g^{-1})	2.10	1.08	5.29	1.03	0.79	40.00

[1)] Standard is set per g of fresh weight at level of 20 ng g^{-1} f.w.

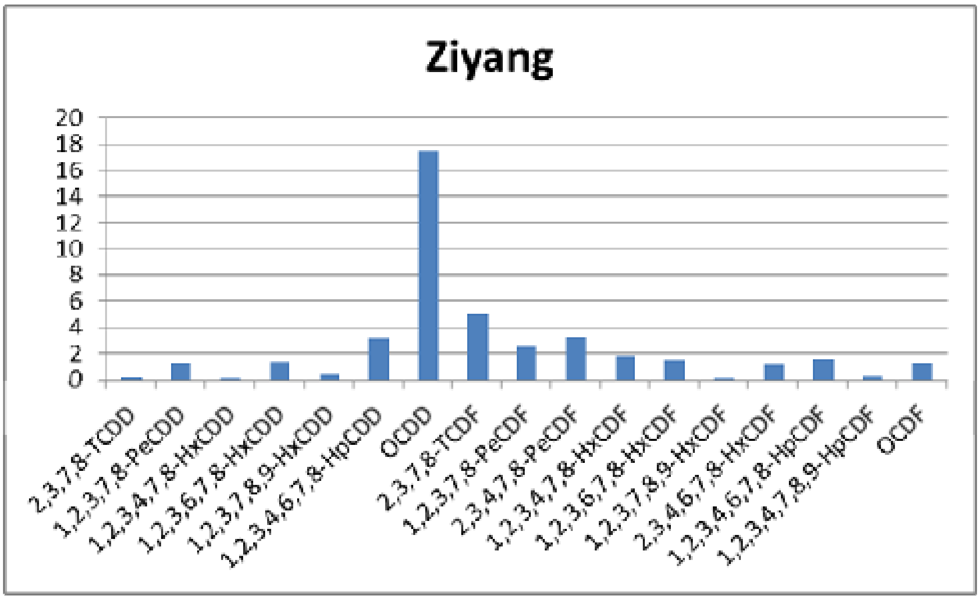
Table 5: Summarized results of analyses for POPs in chicken eggs from Kazakhstan for comparison.

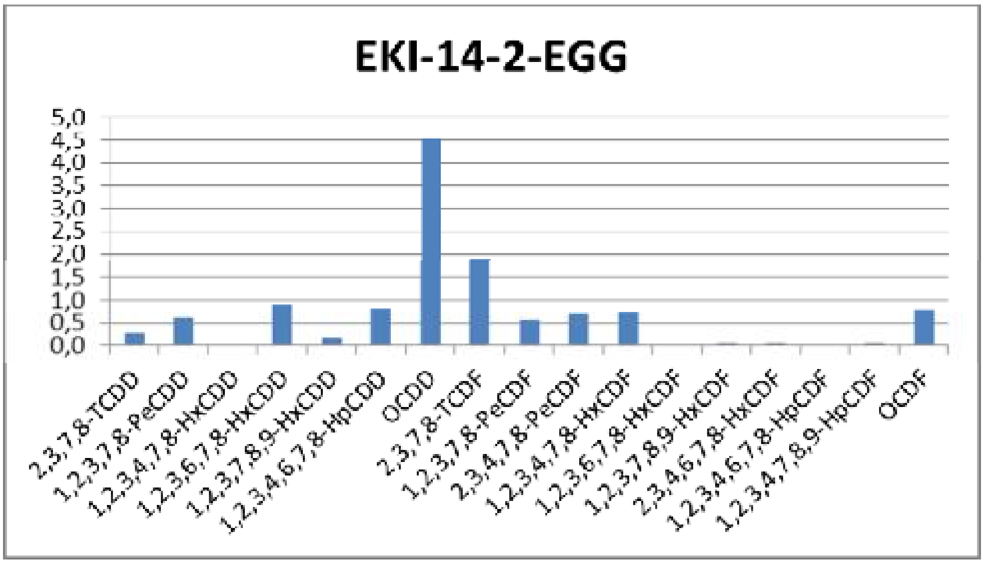
Locality	Balkhash	Ekibastuz	Nura river***	Shabanbai Bi
PCDD/Fs (pg WHO-TEQ g^{-1} fat)	4.3 – 9.8	1.6 - 5.7*	1.8 - 2.8	9.26
DL PCBs (pg WHO-TEQ g^{-1} fat)	2.9 – 22.3	2.9 - 6.5*	25.9 -26.5	28.62
Total PCDD/F + DL PCBs (pg WHO-TEQ g^{-1} fat)	12.7 – 30.1	4.5 - 12.2*	27.8 -29.3	37.88
HCB (ng g^{-1} fat)	1.7 – 4.4	1.3 - 5.4**	2.3 – 5.0	6.25
6 PCB (ng g^{-1} fat)	10.1 – 58.8	10.5 - 23.0**	275.5 – 360.4	1975.97

* two pool samples
** three pool samples

*** two pool samples from villages Rostovka and Chkalovo

The situation in Ziyang is comparable to that in Ekibastuz, Kazakhstan, where contamination is also caused by a site contaminated by PCBs (abandoned power electric substation). Also, PCDD/Fs congeners pattern in one sample of eggs from Ekibastuz is very close to one observed in free range chicken eggs collected in Ziyang (see graphs at Figures 6 and 7), although total TEQ value for PCDD/Fs was higher in the sample from Ziyang in comparison with sample EKI-14-2-EGG from Ekibastuz, Kazakhstan (1.57 pg WHO-TEQ g^{-1}). The level of PCDD/Fs in the eggs collected in Ziyang (3.77 pg WHO-TEQ g^{-1}) exceeded the EU limit value for dioxins in chicken eggs. Contamination of those eggs is most likely caused by PCDD/Fs, which are present as unintentional by-products in PCB mixtures.





Figures 6 and 7: PCDD/Fs congeners patterns in free range chicken egg samples from Ziyang and Ekibastuz (EKI-14-2-EGG).

Seven out of ten samples from Kazakhstan exceeded EU maximum levels of PCDD/Fs congeners in chicken eggs, and eight out of ten total samples exceeded the EU limit value for both PCDD/Fs and DL PCBs in chicken. There are higher levels of PCBs in free range chicken eggs sampled from Kazakhstan, while in general the eggs from China seem to be more contaminated by dioxins (also see the results for DR CALUX in Table 3).

The highest level of dioxins (9.81 pg WHO-TEQ g^{-1} fat) in eggs from Kazakhstan was measured in the sample from Balkhash – southwest (BAL-EGG-14-2), and almost the same level was measured in the sample from Shabanbai Bi, which had the highest level of total WHO-TEQ (37.88 pg WHO-TEQ g^{-1} fat), and toxicity of DL PCBs has prevailed in that sample. The highest level of dioxins in eggs from China was in sample Wuhan 1 (12.17 pg WHO-TEQ g^{-1} fat). There was level of 3.22 pg WHO-TEQ g^{-1} fat in chicken eggs pooled sample from Likeng. Also this sample exceeded EU standard level.

Levels of 6 PCB congeners were rather low in all egg samples from China, while contamination by non-dioxin like PCBs is more serious in Kazakhstan. The same applies to DDT (expressed as sum of 4 DDT metabolites) and HCHs. Both were one order lower than EU standards. The sum of 4 DDT metabolites was 3.3 – 5.2 ng g^{-1} f.w. in eggs from Wuhan and 0.01 ng g^{-1} f.w. in eggs from Ziyang.

5.4 The egg samples from Wuhan

There were two pooled samples taken in the vicinity of waste incinerators in Hanyang city, Wuhan (see their location on the map at Figure 8). The waste incinerators are described in chapter 3.5 of this report.

Figure 8: Map showing location of MSWI in Hanyang, Wuhan (medical waste incinerator is on north side of MSWI) and sites where pooled egg samples (Wuhan 1 and Wuhan 2) were taken in the vicinity of waste incinerators in Wuhan.

Both samples from the vicinity of Wuhan waste incinerators exceeded EU standards for dioxin content in chicken eggs (European Commission 2011) by almost three and five times, respectively (see Table 4). Also, total levels of PCDD/Fs and DL PCBs in these samples were three times higher than the EU standard. The level of HCB in sample Wuhan 1 was 74.5 ng g^{-1} fresh weight (in Table 4 the value is on lipid base only), which is almost four times higher than the standard set for HCB content in chicken eggs in the EU. The value of HCB in pooled eggs sample Wuhan 1 is almost double in comparison with the highest HCB level among the collection of samples from IPEN´s The Egg Report from 2005, which was 250 ng g^{-1} fat in eggs from Liberec, Czech Republic (DiGangi and Petrlik 2005).

The eggs in sample Wuhan 1 (which is the site closer to the waste incinerators in Hanyang city) showed extremely high levels of PBDD/Fs. This additional PBDD/F contamination is likely to be responsible for part of BEQ in the free-range eggs from Wuhan 1 and explains the large gap between BEQ and instrumental PCDD/F and dl-PCB TEQ in this case. PBDD/Fs contamination of eggs is discussed further in chapter 4.4 of this study.

Waste incinerators and management of wastes in that facility are putative sources of contamination of the eggs, although it is not possible to exclude other potential sources (despite the fact that they are so far unrecognized). High contamination by PBDD/Fs and PBDEs in the Wuhan 1 sample should show on some e-waste scrap, for example, but the team of Green Beagle didn't find any such site in the surroundings of the waste incinerator.

5.5 Background levels of POPs in eggs

In October 2014 we sampled chicken eggs from a supermarket in Beijing from chickens raised on a large farm without access to open air space in order to obtain information about background levels of POPs in chicken eggs from China. The results of the analyses for this sample are in Tables 3, 4 and 6. The levels of POPs in this sample were similar for PCDD/Fs, PCBs (DiGangi and Petrlik 2005) or slightly higher, e.g. for HCB (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. The results for dioxins analysis of eggs published in National Implementation Plan for the Stockholm Convention on Persistent Organic Pollutants (The People´s Republic of China 2007) in eggs sampled in Hong Kong showed lower levels (0.137 pg TEQ g^{-1}) in comparison to our background sample from the supermarket in Beijing.

5.6 PBDD/Fs and BFRs in eggs

With broad use of brominated flame retardants, the question of the presence of polybrominated dibenzo-p-dioxins and dibenzofurans has arisen, as they were found in different environmental compartments (Kannan, Liao et al. 2012). It was also found that they exhibit similar toxicity to PCDD/Fs. The WHO expert panel has recently concluded that polybrominated dibenzo-p-dioxins (PBDDs), dibenzofurans (PBDFs), and some dioxin-like polybrominated biphenyls (dl-PBBs) may contribute significantly to daily human background exposure to the total dioxin toxic equivalencies (TEQs) (van den Berg, Denison et al. 2013).

PBDD/Fs are compounds that are not measured very often in the environment yet, although there are several studies focused on their presence in the Chinese environment. Studies have reported PBDD/F contamination in air samples from China. A report on PBDD/Fs in ambient air in the city of Shanghai intensively suggested that more detailed and deeper studies about PBDD/Fs should be carried out due to their relatively high atmospheric concentrations in Shanghai (Li, Feng et al. 2008). This obviously applies to more Chinese cities, including Wuhan. The concentrations of PBDD/Fs in ambient air samples collected from Shanghai, China, in 2006 ranged from 0.03 to 4.2 (mean: 1.0) pg m^{-3} (Li, Yu et al. 2008).

A study performed at an electronic waste (e-waste) dismantling area in 2005 showed the highest levels of PBDD/Fs in ambient air reported to date, at concentrations of 0.91 – 118 (mean: 25) pg m^{-3}. Release of PBDD/Fs from crude e-waste recycling operations was suggested (Li, Yu et al. 2007). Thus, the e–waste dismantling process can be a significant source of PBDD/Fs releases into the atmospheric environment. This highlights the importance of focusing future chicken egg sampling on e-waste dismantling sites in China. The high level of PBDD/Fs (27.3 pg WHO-TEQ g^{-1} fat) in free range chicken eggs from Wuhan shows that this could be the case in other Chinese cities surrounding waste ininerators, too.

Table 6: Summarized results of analyses for different BFRs in free range chicken eggs sample Wuhan 1 (from vicinity of MSWI in Hanyang city, Wuhan) and control group of eggs bought in a supermarket in Beijing.

BFRs ($ng\ g^{-1}$ fat)	Wuhan 1	Beijing (supermarket)
S PBDE	1053.61	0.2
PBT	< 0.1*	< 0.1*
PBEB	< 0.1*	< 0.1*
HBB	< 0.1*	3.72
BTBPE	51.3	< 0.5*
OBIND	< 10*	< 10*

*below LOQ

We have found only one study following PBDD/Fs in chicken eggs in other countries. A report from Ireland showed levels of 0.244 – 0.415 pg TEQ g^{-1} fat (Fernandes, Tlustos et al. 2009). It is two orders of magnitude lower than the level measured in free range chicken eggs sample Wuhan 1 presented in this study.

In the eggs sample from Wuhan 1, a high level of PBDEs was also measured (1053.6 ng g^{-1} fat), and is comparable to e-waste dismantling sites studied by Labunska et al. (2013). Results for all measured BFRs in samples from Wuhan 1 and Beijing are summarized in Table 6. A significantly increased level of 51.3 ng g^{-1} fat was also measured in sample Wuhan 1 for BTBPE[6]. It exceeded the background sample by two orders magnitude at least (see Table 6).

6. Conclusions and recommendations

High levels of PCDD/Fs and DL PCBs were found in free range chicken egg samples from hot spots in China. All of the free range chicken eggs samples exceeded the EU ML either for PCDD/Fs or for sum of both PCDD/Fs and DL PCBs. More regular monitoring of dioxins and DL PCBs in food samples should be undertaken by national authorities, as there is not much data about levels of POPs in this common part of the diet in China. The cell based screening test DR CALUX(R) method has shown to be effective to find new polluted areas as well as for an estimation of overall contamination of food stuff (eggs) by PCDD/Fs and DL PCBs collected from Chinese hot spots, although it can also show response to a broader range of contaminants such as brominated dioxins, for example.

An extremely high level of PBDD/Fs was revealed in free range chicken egg samples taken close to waste incinerators in Hanyang city, Wuhan, accompanied with high levels of BFRs and HCB in the same pool sample. This finding provokes questions about the level of our knowledge about contamination of food in China and other countries with high disposal volumes of e-waste containing PBDD/Fs and BFRs, as it is not the first indication of very high concentrations of PBDD/Fs in the environment. It also raises questions about the potential listing of PBDD/Fs under the Stockholm Convention, as this group of chemicals shows similar properties to other U-POPs already listed in its Annex C (Kannan, Liao et al. 2012, van den Berg, Denison et al. 2013).

[6] BTBPE stands for 1,2-bis(2,4,6-tribromo-fenoxy)ethane. It is one from the family of new brominated flame retardants used e.g. in electronics where replaced PBDEs. Its accumulation in the eggs highlights the need of more detailed screening of new retardants used as alternatives replacing PBDEs for their potential properties similar to POPs, otherwise we will continue to repeat the same mistake and will use new POPs to replace other POPs which is not intention of the Stockholm Convention.

High levels of PCDD/Fs and DL PCBs measured by bioassay analyses in samples from the vicinity of the metallurgical complex in Beihai, as well as in samples from the vicinity of three Chinese waste incinerators (Likeng, Shenzhe and Wuhan), highlight the importance of monitoring homegrown food sources in neighbourhoods of U-POPs source categories listed in Annex C to the Stockholm Convention; in particular in developing countries with a fast growing industry like China. To take appropriate measures to decrease releases of U-POPs from these sources is an even more important step.

High levels of POPs in free range chicken eggs from the vicinity of several waste incinerators underline the needs to 1) increase non-toxic waste recycling in China instead of massive building of new large waste incinerators, and 2) tighten the control of management of waste incineration residues and other pathways of U-POPs releases from waste incineration. The BAT/BEP Guidelines of the Stockholm Convention (Stockholm Convention on POPs 2008) contain a good list of potential measures for waste management which can improve the situation in that field.

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About the China Chemical Safety Project

The China Chemical Safety Project is an EU-funded project of IPEN with partners Green Beagle (China) and Arnika – Toxics and Waste Programme (Czech Republic) that aims to strengthen the capacity of civil society organizations and communities impacted by pollution and to increase chemical safety in China. The Project was implemented in China over two years with total EU funding of €344,580 and EU contribution of 77.84% of the total cost.

The Project included:

- Improving capacities of impacted communities and civil society organizations for involvement in policy making
- Training on public participation in environmental impact assessment
- Generating new publicly available data about pollution and impacted communities that contribute to increased implementation of local and national chemical safety policies
- Raising awareness on emissions-related pollution

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