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Chen, Y-P., Maltby, L. orcid.org/0000-0003-3817-4033, Liu, Q. et al. (5 more authors) (2016) Captive pandas are at risk from environmental toxins. Frontiers in Ecology and the Environment, 14 (7). pp. 363-367. ISSN 1540-9295

https://doi.org/10.1002/fee.1310

Captive pandas are at risk from environmental toxins

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Ex situ conservation efforts are the last resort for many critically endangered species, and captive breeding centers are thought to provide a safe environment for producing individuals for eventual re-introduction to the wild. The giant panda (*Ailuropoda melanoleuca*) is one of the world's most endangered animals and is a widely recognized symbol for conservation. Here, we report that captive pandas in China experience environmental and dietary exposures to high concentrations of persistent organic pollutants (polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls) and heavy metals (arsenic, cadmium, chromium, and lead). In the short term, those animals exhibiting elevated levels of such toxins should be relocated to breeding centers in less contaminated areas. Ensuring the long-term survival of both captive and wild pandas depends in part on reducing atmospheric emissions of toxic pollutants throughout China.

Front Ecol Environ 2016; 14(7): 363–367, doi:10.1002/fee.1310

The giant panda (*Ailuropoda melanoleuca*) is one of the world's most endangered animals and is well recognized as a symbol for conservation. The panda lineage is at least 11.6 million years old (Abella *et al.* 2012); fossil evidence and historical records have revealed that pandas were once distributed in at least 18 of China's 23 provinces (Zhu and Long 1983). Until the mid-19th century, giant pandas still inhabited most of eastern and southern China (Hunan, Hubei, Sichuan, Shaanxi, and Gansu provinces), but their range has declined in recent years as a result of hunting and habitat degradation/destruction, including natural resource exploitation (eg logging) and tourism-related activities (Zhang *et al.* 2013). Within China, giant pandas now survive only in small, fragmented conservation zones in the Qinling, Bashan, and Qionglai mountains (Zhang *et al.* 2013), and in ex situ breeding facilities, including the zoos of Beijing and the breeding centers of Wolong and Chengdu.

Conservation areas and captive breeding centers are widely assumed to protect giant pandas from the adverse impacts of human activities. However, their presumed safety may be compromised by the widespread dissemination of pollutants into conservation zones or by the proximity of heavily polluted urban areas to breeding centers. For example, perfluorinated compounds used in consumer and industrial products as surfactants, surface protectors, and fire-retardant foams have been found in serum samples taken from giant pandas in the Beijing zoo as well as from red pandas (*Ailurus fulgens*) in several other zoos and wild animal parks in China (Dai *et al.* 2006). Yet the extent to which wild and captive giant pandas are exposed to persistent organic pollutants (POPs) and heavy metals – which can accumulate in their body tissues, compromise their health, and may affect the success of ongoing conservation programs – remains unknown.

Here, we investigate whether giant pandas at selected captive sites in China have been exposed to greater concentrations of POPs and heavy metals as compared with their wild counterparts.

Materials and methods

Fecal droppings, which can be used as a non-invasive means to detect pollutant exposure (Christensen *et al.* 2013), were collected from wild pandas in the Wolong and Foping National Nature Reserves, and from captive pandas housed in the China Conservation and Research Center for the Giant Panda (CCRCGP) and the Shaanxi Wild Animal Research Center (SWARC) (Figure 1). The former site is the largest captive panda breeding center for the Sichuan subspecies of giant panda, while the latter site is the only breeding center for the Qinling subspecies. Samples of bamboo (*Fargesia qinlingensis* and *Bashania fargesii*), the primary food for giant pandas, were collected in the wild from Foping and from plants grown at SWARC. Mixed feedstuff, fed to pandas as a nutrient supplement, was also sampled from SWARC.

The feces, plant tissue, and feedstock samples were all dried to constant mass, digested, and analyzed using standard methods. Concentrations of POPs in the samples were determined by high-resolution mass spectrometry

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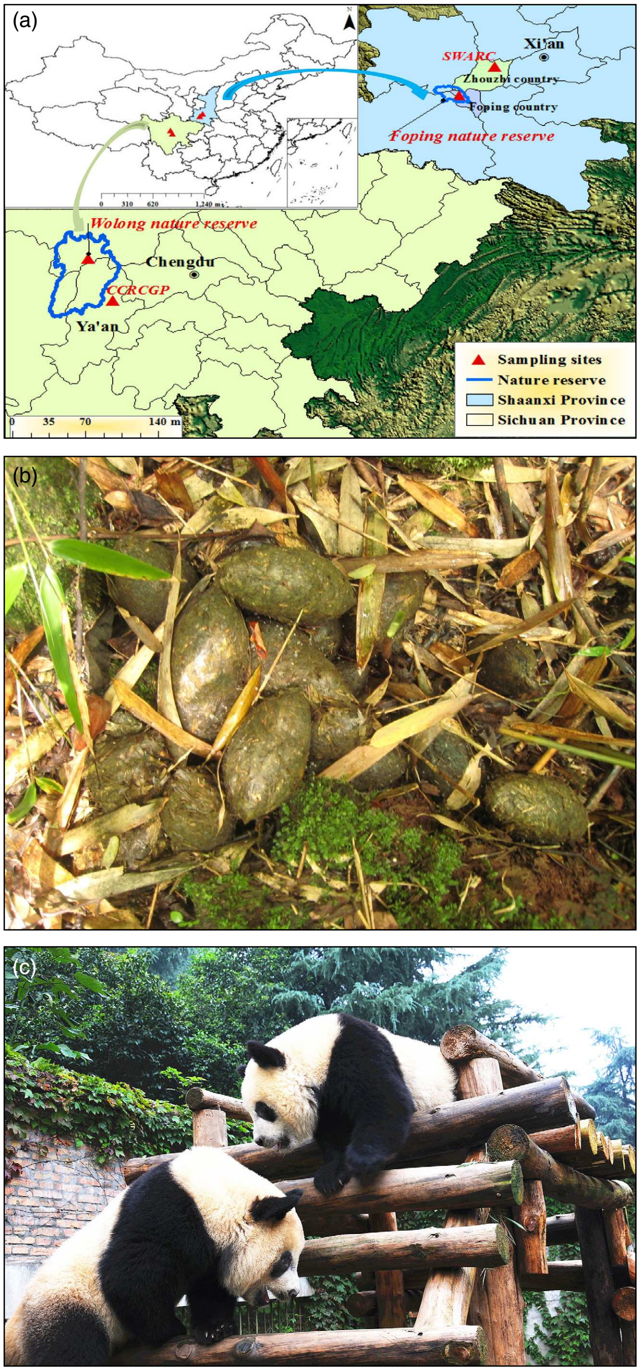

Figure 1. *(a) Sites of sample collection. (b) Typical droppings of wild giant pandas. (c) Captive giant pandas at the Shaanxi Wild Animal Research Center (SWARC).*

(Liu *et al.* 2006; Li *et al.* 2008) at the Research Center for Eco-Environmental Sciences of the Chinese Academy of Sciences. Heavy metal concentrations were ascertained by atomic absorption or fluorescence spectrometry at the Institute of Earth Environment–Chinese Academy of Sciences (IEE CAS). Additional details on sample collection and analytical methods, including quality assurance/quality control protocols, are provided in WebPanel 1.

Data were analyzed using the SPSS software version 19.0 (Armonk, NY: IBM Corp). Contaminant concentrations in droppings from wild and captive giant pandas within and between the two subspecies were compared using *t* tests.

Results and discussion

Pandas in captive breeding centers are generally thought to be better protected from human activities than are wild pandas in nature conservation zones, primarily because these zones have become more fragmented and less suitable for supporting this species over time (Liu *et al.* 2001). However, captive breeding centers are often located near or within urban areas, and there is an increasing concern that ex situ conservation efforts are being compromised due to environmental pollution associated with urbanization. With China's rapid industrialization and urbanization, environmental pollution is increasing in scale and magnitude, following a similar trajectory to that previously seen in developed countries (Seinfeld 2004). This pollution is having major impacts on public health, as evidenced by, for example, the presence of more than 200 "cancer villages" in China (Yang 2013).

Among the many pollutants, POPs and heavy metals are of major concern because they can be transported over long distances in air and water (Lohmann *et al.* 2007), their persistence in the environment, and their tendency to accumulate in fatty tissues, as well as their high toxicity to humans and other mammals (eg Qiu 2013; Sfriso *et al.* 2014; Eqani *et al.* 2015; Fernandez-Rodriguez *et al.* 2015). Three classes of POPs – PCDDs (polychlorinated dibenzo-*p*-dioxins), PCDFs (polychlorinated dibenzofurans), and PCBs (polychlorinated biphenyls) – were found in much higher concentrations in fecal droppings of captive giant pandas than in those of wild pandas (Figure 2; WebTables 1 and 2). Elevated levels of POPs were also detected in the bamboo that was fed to captive pandas and in their nutrient-supplement feedstock (WebFigures 1 and 2). A variety of forms ("congeners") of PCDDs and PCDFs are generated as by-products from various chemical processes, such as combustion, whereas PCBs were widely used as dielectric fluids in transformers and capacitors, as heat exchange fluids, and as additives in pesticides, adhesives, plastics, and paints because of their insulating and nonflammable properties (Fiedler 2007). Although their production ceased in 1974, PCBs are still released from old electrical equipment and can still be found in the environment (eg in soil, sediments, and water) and in human tissues (Mai *et al.* 2005; Imamura *et al.* 2007).

Because PCDDs, PCDFs, and PCBs occur as congeners that differ in toxicity and toxic equivalency factors, the World Health Organization has defined a single toxic equivalent (WHO-TEQ) that can be calculated to determine total POP exposure (Van den Berg *et al.* 2006). POP concentrations and corresponding WHO-TEQ values were higher in captive panda droppings as compared with wild panda droppings (Figure 3). Similarly, these toxins were more concentrated in bamboo and feedstocks offered as food items to captive pandas than they were in bamboo consumed by wild pandas (WebFigure 2).

Four heavy metals with known toxicity – arsenic (As), cadmium (Cd), chromium (Cr), and lead (Pb) (Brahmia *et al.* 2013; Neal and Guilarte 2013; Uddh-Soderberg *et al.* 2015) – were also found at elevated levels in droppings of captive pandas relative to those of wild individuals (Figure 4), as well as in their food and their nutrient-supplement feedstock (WebFigure 3). Unlike POPs, these heavy metals occur naturally but are readily mobilized by human activities such as mining, automobile use, and overuse of chemical fertilizer.

Our data provide direct evidence that giant pandas are exposed to PCDDs, PCDFs, PCBs, and heavy metals in both captive breeding centers and in situ conservation areas, but concentrations of these toxins are far greater for pandas in captivity. Previous studies have shown that PCDDs and PCDFs are associated with developmental toxicity, immunotoxicity, and reproductive toxicity in humans and other animals. Similarly, PCBs and their breakdown products are known endocrine disrupters, cause the loss of renal cell viability, and are associated with increased risk of chloracne, goiter, anemia, and cancer (Lohmann *et al.* 2007; Qiu 2013; Sfriso *et al.* 2014; Eqani *et al.* 2015; Fernandez-Rodriguez *et al.* 2015; Gustavson *et al.* 2015). Heavy metal exposure has been linked with increased incidence of cancer (Cr and As), nephrotoxicity and bone damage (Cd), and reduced reproductive function (Pb) (Neal

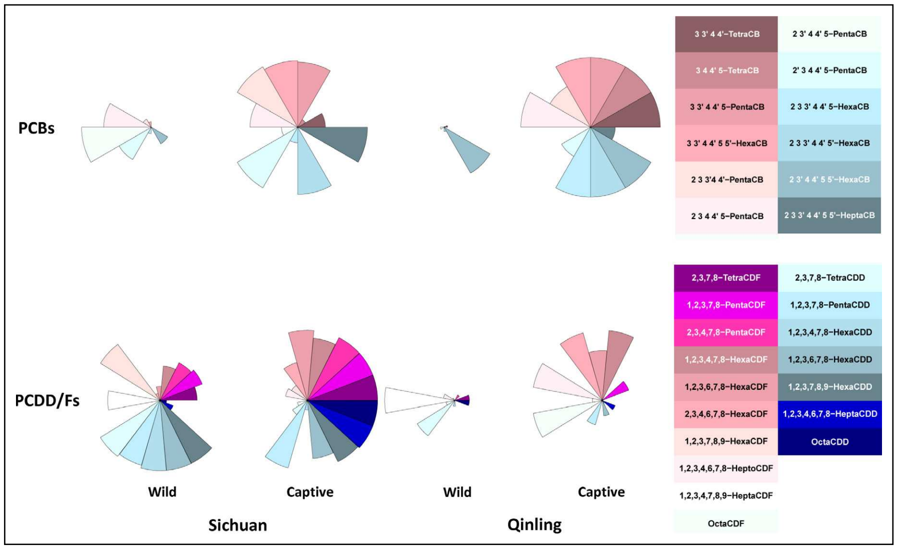

Figure 2. *Concentrations of 12 polychlorinated biphenyl (PCB) congeners (top) and 17 polychlorinated dibenzo-p-dioxin and furan (PCDD/F) congeners (bottom) in the droppings of wild and captive giant pandas of the Sichuan and Qinling subspecies. In each star plot, the radius is equal to the maximum observed concentration, and concentrations of each individual congener are scaled to the maximum. These plots reveal that fecal samples from captive pandas have both more congeners and higher concentrations of congeners as compared with fecal samples from wild pandas. Tabular data (actual mean concentrations and the standard errors of the means) are provided in WebTables 1 and 2.*

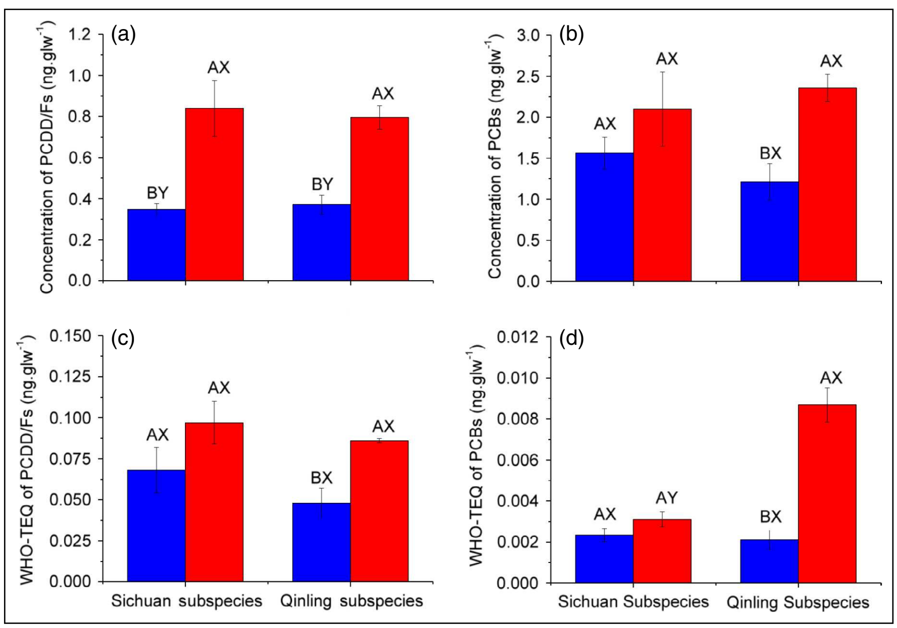

Figure 3. *Concentrations of POPs in fecal samples collected from two subspecies of wild (blue) and captive (red) giant pandas: (a) all (summed) PCDDs and PCDFs, (b) all (summed) PCBs, (c) WHO-TEQs of PCDDs and PCDFs, and (d) WHO-TEQs of PCBs. Bars (means ± 1 SE of the mean from n = 4 independent replicates comprising three or four pooled samples) with different letters between the wild and captive pandas for the same subspecies (A or B), or between Sichuan and Qinling subspecies (X or Y), are significantly different ($P < 0.05$, t test). ng.glw^{-1} = nanograms per gram lipid weights.*

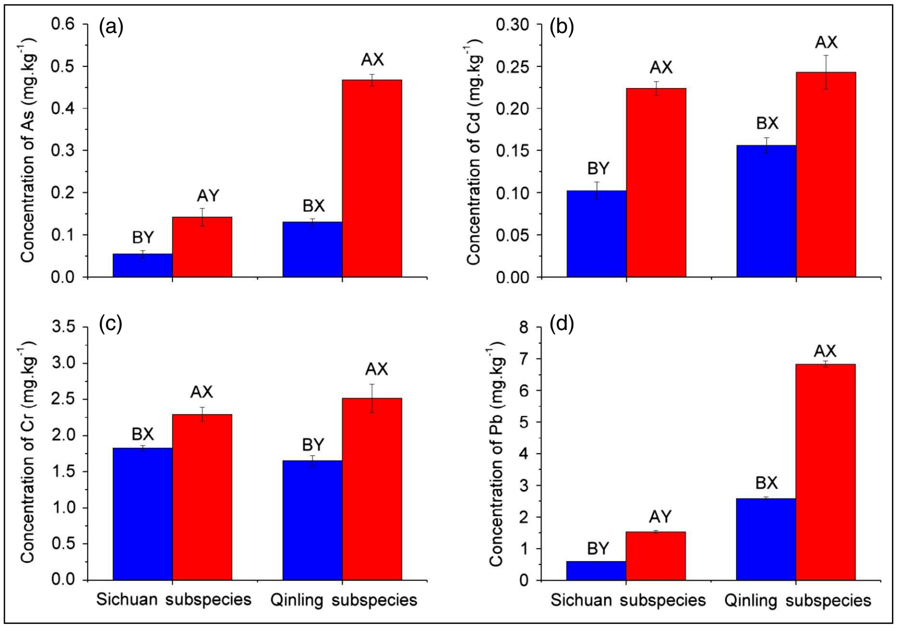

Figure 4. *Concentrations of heavy metals in fecal samples collected from two subspecies of wild (blue) and captive (red) giant pandas: (a) arsenic (As), (b) cadmium (Cd), (c) chromium (Cr), and (d) lead (Pb). Bars (means ± 1 SE of the mean from* n *= 4 independent replicates comprising three or four pooled samples) with different letters between the wild and captive pandas for the same subspecies (A or B), or between Sichuan and Qinling subspecies (X or Y), are significantly different (*$P < 0.05$*,* t *test).* $mg.kg^{-1}$ *= milligrams per kilogram.*

and Guilarte 2013; Brahmia *et al.* 2013; Uddh-Soderberg *et al.* 2015). Our results thus challenge the notion that captive breeding centers and zoos provide a safe haven for pandas, protecting them from human impacts.

Our findings also indicate that dietary exposure is the dominant, proximal pathway through which giant pandas are exposed to POPs and heavy metals (WebFigures 1–3). Although the food of both captive and wild pandas was enriched in POPs (WebFigures 1 and 2) and heavy metals (WebFigure 3), the concentrations of both POPs and heavy metals, and WHO-TEQs of POPs, were significantly greater in bamboo eaten by captive pandas (WebFigures 1–3). We note that the nutrient-supplemented feedstock (baked into steamed bread for the pandas) was enriched only in Cd, Cr, and Pb, but not in As, relative to fresh bamboo.

In sum, we provide clear evidence that giant pandas both in the wild and in captivity are exposed to PCDDs, PCDFs, PCBs, and heavy metals through their diet, and that exposure to these environmental toxins is greater in captive breeding centers than in nature reserves. Because exposure to these environmental toxins is likely to negatively affect the health of these animals, we suggest that urgent action is needed to safeguard these conservation icons. In the short term, captive breeding centers should be relocated to less contaminated areas, and the food provided to captive pandas should be strictly monitored to ensure that it lacks POPs and heavy metals, and is of consistent high quality. In the long term, however, a more sustainable solution will rely on improving air quality through the reduction of toxic pollutant emissions.

Acknowledgements

We thank the IEE CAS for financial support, the CCRCGP for research assistance, and Professor ZS An for valuable advice during the course of this study.

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Supporting Information

Additional, web-only material may be found in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/fee.1310/suppinfo