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1 **PBDEs (polybrominated diphenyl ethers) pose a risk to captive giant pandas**

2 **Yi-ping Chen [a, b]*, Ying-juan Zheng [a], Qiang Liu [a], Aaron M. Ellison[c], Yan Zhao[a], Qing-yi Ma[d]**

3 [a] State Key Laboratory of Loess and Quaternary Geology, Institute of Earth Environment, CAS, Xi'an, 710075,

4 China

5 [b] College of Life Science, Northwest Normal University, Lanzhou 730070, China

6 [c] Harvard University, Harvard Forest, Petersham, Massachusetts, 01368, USA

7 [d] Shaanxi Wild Animal Research Center, Zhouzhi, Xi'an, 710402, China

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9 *Corresponding author: Dr YP Chen, Institute of Earth Environment, Chinese Academy of Science, No. 97,

10 Yan-Xiang Road, Xi'an, 710061, China; Tel：+86-29-62336269; Fax：+86-29 -62336269; E-mail:

11 chenyp@ieecas.cn

12 **Abstract**

13 The Qinling subspecies of giant panda (*Ailuropoda melanoleuca qinlingensis*) is highly

14 endangered; fewer than 350 individuals still inhabit the Qinling Mountains. Previous research

15 revealed that captive pandas were exposed to bromine, so we hypothesized that captive pandas

16 also were exposed to, and affected by, polybrominated diphenyl ethers (PBDEs). To test this

17 hypothesis, we sampled blood and feces of captive and wild pandas, their drinking water, food

18 (bamboo leaves) from the Shaanxi Wild Animal Research Center (SWARC) and the Foping

19 National Nature Reserve (FNNR), and supplemental feedstuff fed to captive pandas at SWARC.

20 We found 13 congeners of PBDEs in fecal samples, of which BDE47, BDE66, BDE71, BDE99,

21 and BDE154 predominated; total PBDE concentration in feces of captive pandas was 255%

22   higher than in wild pandas. We found nine PBDEs congeners in blood samples: BDE153 and

23   BDE183 predominated, and concentrations of PBDEs in blood from captive pandas also were

24   significantly higher than in wild pandas. The primary source of PBDEs appears to be the bamboo

25   fed to the pandas: total concentration of PBDEs were 5473 and 4835 $pg \cdot g^{-1}$ in the bamboo

26   *Fargesia qinlingensis*; 2192 and 1414 $pg \cdot g^{-1}$ in the bamboo *Bashannia fargesii*; 0.066, 0.038

27    $pg \cdot mL^{-1}$ in drinking water; and 28.8 $pg \cdot g^{-1}$ in supplemental feedstuff for captive and wild pandas,

28   respectively. BDE99 and BDE47 could threaten the health of captive pandas, whereas other

29   PBDE congeners may pose additional health risks to the captive pandas. In the short term, this

30   risk may be ameliorated by strict control of food quality. In the long term, however, reducing air,

31   water and soil contamination to improve environmental quality will best reduce these risks.

32

33   Keywords: PBDEs; Captive Panda; Feces and Blood; Food; Health risk

34

35   **Capsule:** Captive pandas were exposed to higher concentrations of toxic PBDEs than wild

36   pandas. PBDEs are most prevalent in the bamboo fed to the pandas, highlighting the need for

37   quality control on the food supply of captive pandas.

38

39   **Introduction**

40   The giant panda (*Ailuropoda melanoleuca*) is one of the rarest animals in the world.

41   Approximately 1800 individuals remain in anthropogenically fragmented habitats (SFA, 2015), of

42   which < 350 individuals are of the Qinling subspecies (*A. melanoleuca qinlingensis*) living in the

43   Qinling Mountains of China (SFA, 2015). In the last several decades, two strategies have been

44   used to protect this species. One strategy is *ex-situ* breeding in, for example, the Beijing Zoo, the

45   Wolong Breeding Center, and the Shaanxi Wild Animal Research Center (SWARC). The other

46   strategy is the establishment of natural conservation zones to preserve panda habitat. In the last

47   several decades, 67 conservation zones, with a total area $> 43,600$ km^2, have been established

48   (SFA, 2015).

49   It is generally assumed that captive breeding centers can effectively protect giant pandas

50   from the adverse impacts of human activities. However, canine distemper virus has killed at least

51   four pandas at SWARC (Mara, 2015) suggesting that new measures are needed to protect captive

52   individuals of this iconic endangered species. Environmental pollution further stresses captive

53   animals. For example, we have shown that captive pandas are exposed to heavy metals including

54   cadmium, zinc, chromium, arsenic and lead (Chen et al., 2016). We also found that chlorine and

55   bromine were 690% and 330% higher in feces of captive pandas than in those of wild pandas

56   (Chen and Ma, 2017), and we therefore hypothesized that captive pandas may be exposed to and

57   affected by polybrominated diphenyl ethers (PBDEs).

58   PBDEs are brominated flame retardants that are used in electronic equipment, textiles,

59   cabinets for television and computers, and in many plastic products (WHO, 1994; Darnerud et al.,

60   2001; Kim et al., 2012). PBDEs are lipophilic, are released slowly into the environment, can

61   bioaccumulate in tissues of humans and other mammals, and are toxic to them (Hooper and

62   McDonald, 2000; De Wit, 2002; Hu et al., 2008). Exposure of laboratory animals to high

63   concentrations of PBDEs can suppress production of antibodies and proliferation of lymphocytes

64 (Darnerud and Thuvander, 1998), decrease thymic weights (Fowles et al., 1994), cause

65 immunomodulatory turbulence, and lead to hormonal deficits (Eriksson et al., 2001; Branchi et

66 al., 2003). Modulating effects of PBDE exposure on endocrine systems of wild animal also have

67 been documented (Legler and Brouwer, 2003; Darnerud, 2003). Recent research showed that

68 giant pandas were exposed to PCDDs, PCDFs, PCBs, and heavy metals from the bamboo they

69 eat (*Fargesia qinlingensis* and *Bashania fargesii*) in both captive breeding centers and *in situ* in

70 conservation areas (Chen et al., 2016). However, there has been no research on exposure of

71 captive or wild pandas to PBDEs, or their concentrations in panda feces and blood.

72   The objective of this study was to (1) test whether captive or wild pandas are exposed to

73 PBDEs. (2) document and compare the concentrations of PBDEs in wild and captive pandas; and

74 (3) identify possible sources of PBDEs contamination. Feces, drinking water, and food (bamboo)

75 were collected from SWARC and the Foping National Nature Reserve (FNNR), and blood

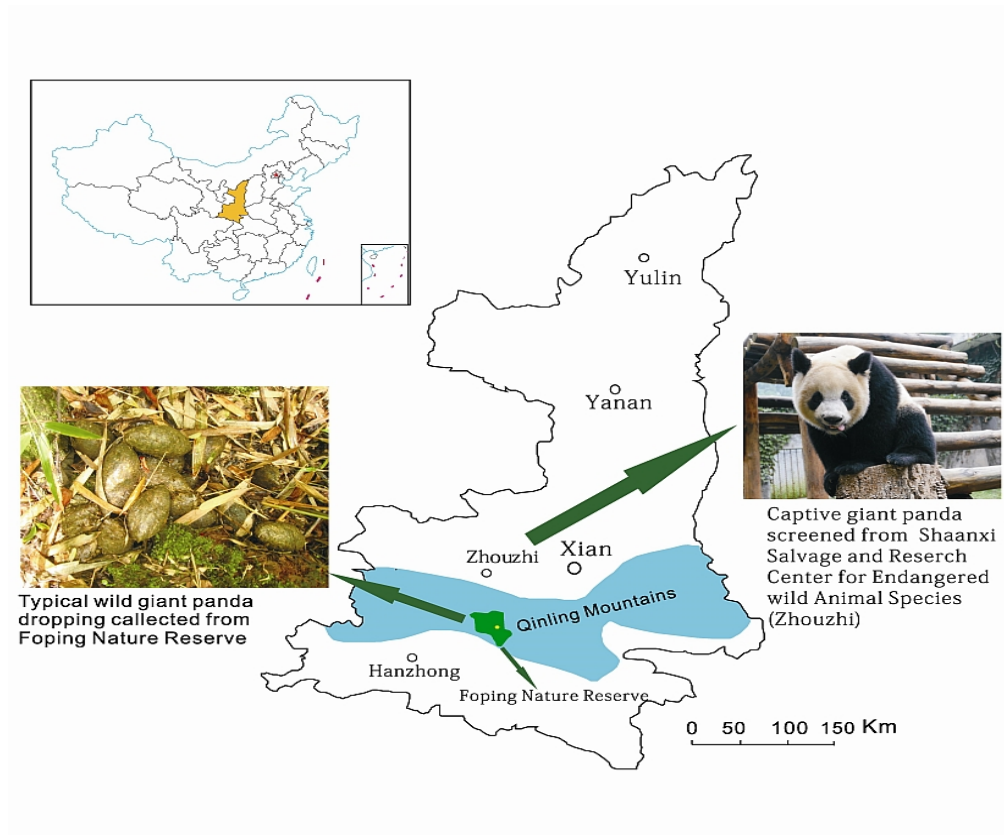76 samples and supplemental feedstuff were sampled at SWARC (Fig.1).

77

Figure 1. Sample Collection Sites. The Shannxi Wild Animal Research Center (SWARC) is located at 34° 04′ N, 108°19′ E in Zhouzhi County, Shaanxi province. The Foping National Nature Reserve (green shaded area) is located in the area bounded by 33° 33′ – 33 44′ N, 107° 40′ – 107° 55′ E within the Qinling Mountains (blue shaded area).

78

Materials and methods

Sample collection

Giant pandas are protected in China, capturing them is illegal, and so samples from wild animals must be collected non-invasively. Fecal samples (droppings: Fig. 1) were collected from 16 different locations within FNNR. Sampling locations were spaced 10-km apart and every four independent samples were pooled into a mixed sample. Droppings of 16 captive pandas were

85   collected from SWARC, which was established in 1987 to conserve the Qinling panda. Each set

86   of 16 fecal samples were pooled for analysis into four samples each consisting of four

87   independent samples.

88   Fresh leaves of living plants (500 g) of the two bamboo species (*Fargesia qinlingensis,*

89   *Bashania fargesii*) that are the primary food of the panda were collected in proximity to where

90   the droppings were collected at FNNR and around SWARC. Water samples (500 mL) were

91   collected into Pyrex borosilicate amber glass bottles from streams at FNNR near where we

92   collected the droppings, and from the SWARC water supply. At both FNNR and SWARC, 12

93   samples of each bamboo species and of freshwater were collected. They subsequently were

94   pooled to produce four mixed samples each consisting of three independent samples from each

95   site. In addition, four samples of mixed feedstuff, provided as a nutrient supplement for captive

96   pandas, also were collected from SWARC.

97   Finally, blood samples were obtained from three similarly-aged pandas rescued from the

98   Qinling Mountains and three captive pandas bred at SWARC. These blood samples were

99   residuals from regular, routine physical examinations of the individual pandas. Prior to

100   examination, the pandas were anesthetized with 25% ketamine (dosage: 8 mg·kg^{-1}). After

101   collection, the blood was placed in EDTA tubes and frozen at –80 °C for analysis of PBDEs.

102

103   **Sample preparation and extraction**

104   PBDE congeners were analyzed using US Environmental Protection Agency (EPA)

105   method 1614 with minor modifications (Li et al., 2008). Bamboo, feces, and feedstuff samples

106   were freeze dried and then homogenized by passing them through a stainless steel sieve (0.5-mm

107   mesh). Each 3-g homogenized sample was spiked with a ^{13}C-labeled surrogate standard (EPA

108   methods 1613B and 1668A) and extracted using accelerated solvent extraction (ASE) for 24 h

109   with dichloromethane (150 mL) and hexane (150 mL) at 55 °C. After ASE, acidic silica (15 g,

110   30% w/w) was added to the sample to remove lipids. Then, 5 g of anhydrous sodium sulfate was

111   added to the extract. The extract sample was rotary evaporated to 2 ml and then passed through a

112   multi-layered silica-gel column that had been pre-cleaned by hexane (100 mL). After the sample

113   was loaded, the PBDE congeners were eluted with 70 ml hexane followed by 70 mL

114   dichloromethane. The eluant was then concentrated to 2 ml on the rotary evaporator. Its volume

115   was further reduced with a gentle nitrogen flow and the solvent was changed to 20 µL nonane in

116   a minivial.

117   PBDEs in water samples were extracted using US EPA method 1614. Prior to extraction,

118   1 L of the liquid samples were filtered using 0.45-µm microporous membranes to remove the

119   particle phase and then spiked with a ^{13}C-labeled BDE-LCS standard. Organic halogen pollutants

120   were adsorbed by siphon, 50 mL of acetone was used to flush the pillars and a 100-mL mixture

121   of acetone and water was collected. Resins were extracted using ASE for 24 h with

122   dichloromethane (300 mL) and hexane (300 mL) at 55 °C. After ASE, a 15-mL acetone-cleaned

123   soxhlet extractor and zeolite was used to obtain a 430-mL solution, which was transferred to a

124   1000-mL separatory funnel, washed with 100 mL of ultrapure water for 3 times, and extracted

125   with 30-mL n-hexane for 3 times to obtain a clear and transparent organic phase solution. This

126   organic phase solution was evaporated to 2–3 mL and purified on the silica gel column. After

127   adding 50 ml n-hexane and evaporateding to 2–3 mL, we added 15 mL isooctane, evaporated to

128   2–3 mL and used 6-mL isooctane to clean. The solution was purged by nitrogen stream and

129   diluted to 1 mL in a brown bottle at –20 °C for further analysis.

130

131   **Instrumental analysis**

132   BDEs 17, 28, 47, 66, 71, 85, 99, 100, 138, 153, 154, 183, and 190 were analyzed using

133   gas chromatography (Agilent 6890, USA) coupled with a high-resolution mass spectrometer

134   (HRMS). The HRMS (Waters Micromass, Manchester, UK) operated in selected ion

135   monitoring (SIM) mode with resolution >10,000. The m/z ratio of all the PBDE congeners was

136   79 and 81 except for BDE47 (m/z 325 and 327), BDE99 (m/z 404 and 406) and BDE183 (m/z

137   562 and 564). Exactly 1 µL of the sample was injected with a CTC PAL autosampler in splitless

138   mode into an HB-5 (30×250-µm i.d. $\times 0.1$-µm film thickness) capillary column for separation.

139   The flow rate of the carrier gas was 1.2 mL/min and the carrier gas was Helium. The program

140   was as follows: the injector was temperature programmed to ramp from 60 °C to 320 °C at

141   150 °C/min. The oven started at 80 °C held for 1 min, increased to 200 °C at 10 /min, held at

142   200 °C for 1 min, increased to 300 °C at 20 °C min, and then held at 300 °C for 5 min. The

143   temperature of the ion source was 150 °C.

144

145   **Quality assurance and quality control**

146   All solvents were pesticide-residue grade and were purchased from Fisher (Hampton, NH,

147   USA). Silica gel was obtained from Merck (silica gel 60, Darmstadt, Germany). [13]C labeled

148   surrogate and labeled injection standards were purchased from Wellington Laboratories (Guelph,

149   Canada).

150   All analytical procedures were checked by the strict quality assurance and control

151   measures to avoid sample- and cross-contamination. Reference material and 3 blank control

152   samples (ultrapure water) were analyzed using the same methods as described above. Triplicate

153   samples were analyzed to determine repeatability and reproducibility. To monitor analytic losses,

154   all samples were spiked with internal standards of ^{13}C-labeled BDE47, 99, and 153. The mean

155   recoveries of ^{13}C-labeled surrogate PBDE congeners 47, 99 and 153 were in the range of 54.2 $\pm$

156   12.1%, 66.0 $\pm$ 10.1%, 102.2 $\pm$ 20.1%, respectively, which were well in the limits according to

157   US EPA Method 1614; all the content of PBDEs in the control (blank) samples were below the

158   limit of detection (LOD), which for BDEs 17, 28, 47, 66, 71, 85, 99, 100, 138, 153, 154, 183,

159   and 190 are 5, 4, 3, 15, 6, 7, 7, 7, 4, 1, 12, and 4 pg·g^{-1}, respectively). If concentrations of actual

160   samples fell below LOD, 1/2 of the LOD values were used in subsequent statistical analysis.

161

162   **Data analysis**

163   Correlation analysis (CA) and principal components analysis (PCA) were used to analyze

164   the association between 13 PBDE congers in different samples. Paired samples were analyzed

165   using *t*-tests. All statistical analyses were done using the IBM statistical package SPSS 20.0

166   (IBM Corp., USA).

167

168   **Evaluation methods**

169   The giant panda's health risk evaluation was calculated using following equations

170   detailed in the Exposure Factors Handbook (US EPA 1997). Assuming that giant pandas only

171   feed on bamboo leaves, average daily dose (ADD) was calculated as:

$$ADD = \frac{C \times IR_S \times EF \times ED}{BW \times AT}$$

172

173   where C is concentration of PBDEs (mg/kg), IR_S is the ingestion rate of bamboo, EF is the

174   exposure frequency (350 day/year), ED is the exposure duration (10.36 years), BW is the average

175   body weight (105 kg, from mid-range of 80-130 kg [Zhang and Wei 2006]), and AT is the

176   averaging time (3781.4 days).

177   Noncancer toxic risk was determined by the model hypothesis of HQ (Hazard Quotient):

$$HQ = \frac{ADD}{RfD_o}$$

178

179   where RfD_o is the reference dose of PBDEs (US EPA, 1997). Risk increases with HQ (Hang et al.

180   2009). If $HQ \leq 1$, risk exposure is relatively safe. If $1 < HQ \leq 10$, considerable threat is

181   suggested, Finally, if $HQ \geq 10$, high chronic risk is suggested.

182

183   **Results**

184   **Concentrations of PBDEs**

185   Total PBDEs concentrations were consistently and significantly greater in captive pandas

186   and their food supply than in wild pandas and their food and water supply (Fig. 2). In the fecal

187   samples, Σ13PBDE of captive pandas was 2.55 times greater than in wild pandas (Fig. 2A).

188   Σ13PBDE of *Fargesia qinlingensis* was 1.13 times higher, and of *Bashania fargesii* 1.55 times

189   higher, in leaves eaten by captive pandas (Figs. 2B, 2C). Water samples had low concentrations

190   of PBDEs (Fig. 2D).

191   Thirteen congeners of PBDEs were found in fecal samples; BDE47, BDE66, BDE71,

192   BDE99 and BDE154 predominated in captive pandas (Fig. 3A). Of the dozen congeners found in

193   the two bamboo species eaten by captive pandas, BDE47 and BDE99 predominated in *Fargesia*

194   *qinlingensis* and *Bashania fargesii*, respectively (Figs. 3B, 3C). Although captive pandas were

195   exposed to somewhat higher concentrations of PBDEs in their water supply (Fig. 2D), the

196   concentrations of each congener were quite low and none predominated (Fig. 3D). Ten PBDE

197   congeners were found in the supplemental feedstuff provided for captive pandas, with BDE28

198   and BDE183 predominating (Fig. 3E). Finally, nine PBDE congeners were found in the blood

199   samples collected from SWARC. BDE153 and BDE183 were the predominant congeners in

200   captive panda blood samples, and occurred in significantly higher concentrations than in blood

201   sampled from wild pandas (Fig. 3F).

202
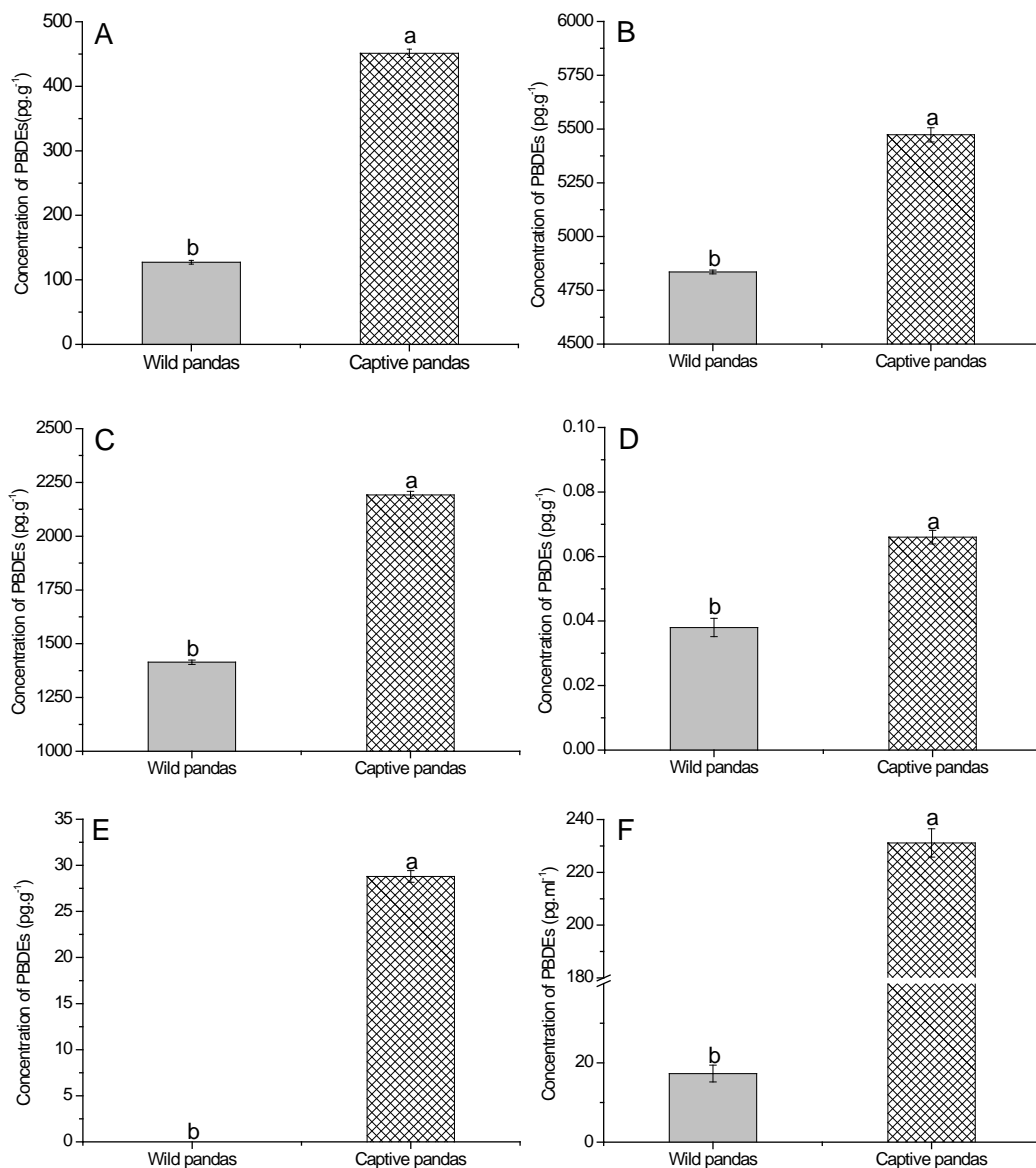
Fig. 2. Total concentrations of PBDEs (Σ13PBDEs) in (A) fecal samples; (B) leaves of *Fargesia qinlingensis*; (C) leaves of *Bashania fargesii;* (D) drinking water; (E) supplemental feedstuff; and (F) blood sample of wild (gray bars) and captive (cross-hatched bars) giant pandas. The concentrations of PBDEs in A, B, C and E are based on dry weight. In (F), the wild pandas were three 17-year old individuals rescued from Qingling and the captive pandas were 8–9-years old. Bars (means ± 1 SE of the mean from n = 4 independent replicates comprising three or four pooled samples). Different letters indicate significant differences between the wild and captive pandas identified using Tukey HSD test (all *P* < 0.01). pg · g $^{-1}$ lw^{-1} = nanograms per gram lipid weights.
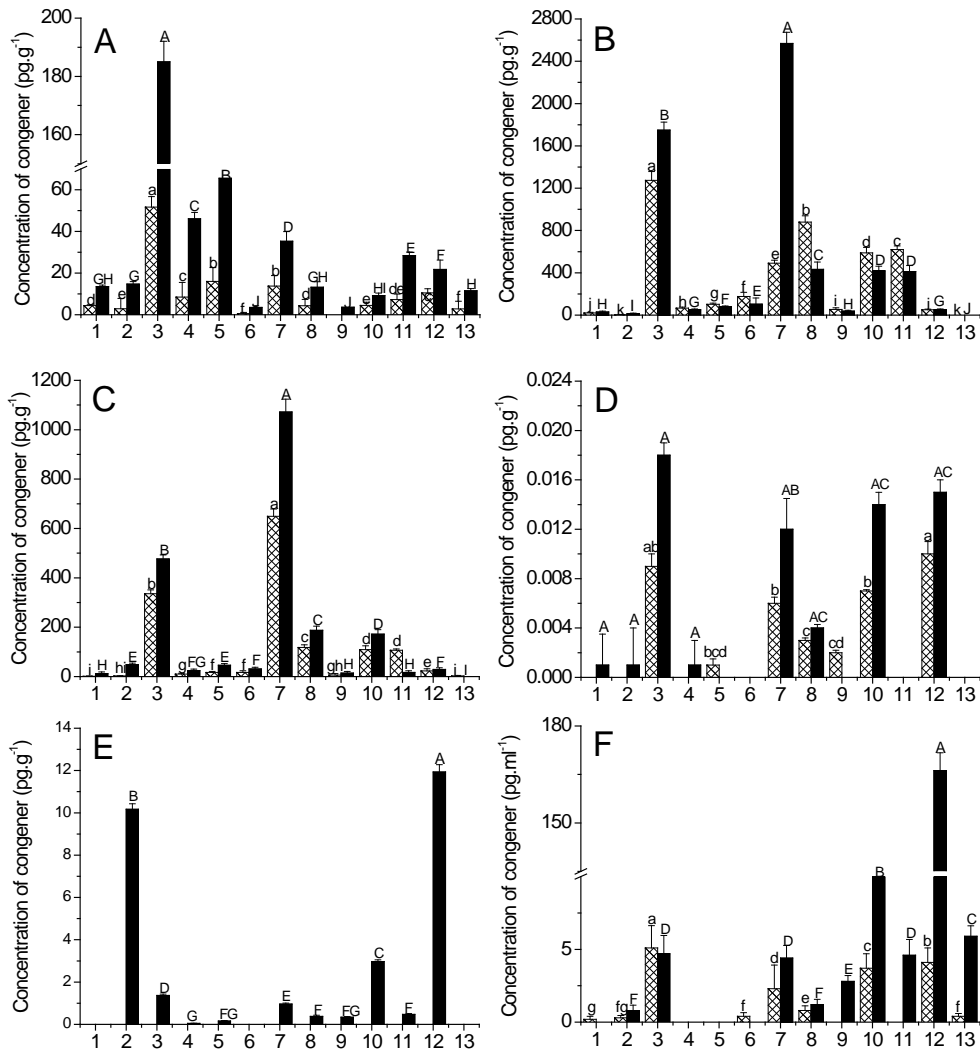
Fig. 3. Concentrations of individual PBDE congeners in (A) feces; (B) leaves of *Fargesia qinlingensis*; leaves of *Bashania fargesii*; (D) drinking water; (E) supplemental feedstuff; and (F) blood from wild (cross-hatched bars) and captive (black bars) giant pandas. Numbers 1 – 13 on the *x*-axis denote different congeners, respectively: BDE17; BDE28; BDE47; BDE66; BDE71; BDE85; BDE99; BDE100; BDE138; BDE153; BDE154; BDE183; BDE190. Different letters indicate significant differences between captive and wild pandas ($P < 0.05$).

Statistical results

The highest positive correlations in Σ13PBDEs were detected for feces and blood *vs.* bamboos ($r > 0.88$), and blood *vs.* feedstuff ($r = 0.88$). Concentration of PBDEs in water samples were not significantly correlated with any of the other samples (Table 1).

13

222   Three principal axes accounted for most of the variance in the groupings of PBDE

223   congeners (Fig. 4). In leaves of *Fargesia qinlingensis*, the first three principal axes accounted for

224   87.8% of the variance (Fig. 4A), whereas they accounted for 62.5% for *Bashania fargesii* (Fig.

225   4B), and 77.4% in feedstuff (Fig. 4C). Only one grouping was found for PBDEs in drinking

226   water (19.8% of the variance; Fig. 4D). The PCA identified clusters of the congeners BDE47,

227   BDE66, BDE71, BDE99, and BDE154 in leaves of *Bashania fargesii* that matched the

228   predominant congeners found in fecal samples (compare Fig. 4B with Fig. 3A).

229

230   **Table 1** Spearman correlation matrix for PBDEs measured in captive samples

	Fargesia qinglingensis	*Bashania fargesii*	Blood	Water	Feces
Bashania fargesii	0.89**				
Blood	0.92**	0.92**			
Water	-0.11	-0.10	0.15		
Feces	0.90**	0.88**	0.93**	-0.09	
Feedstuff	0.73**	0.75**	0.88**	0.309	0.79**

231   **. $P < 0.01$ level (2-tailed test).
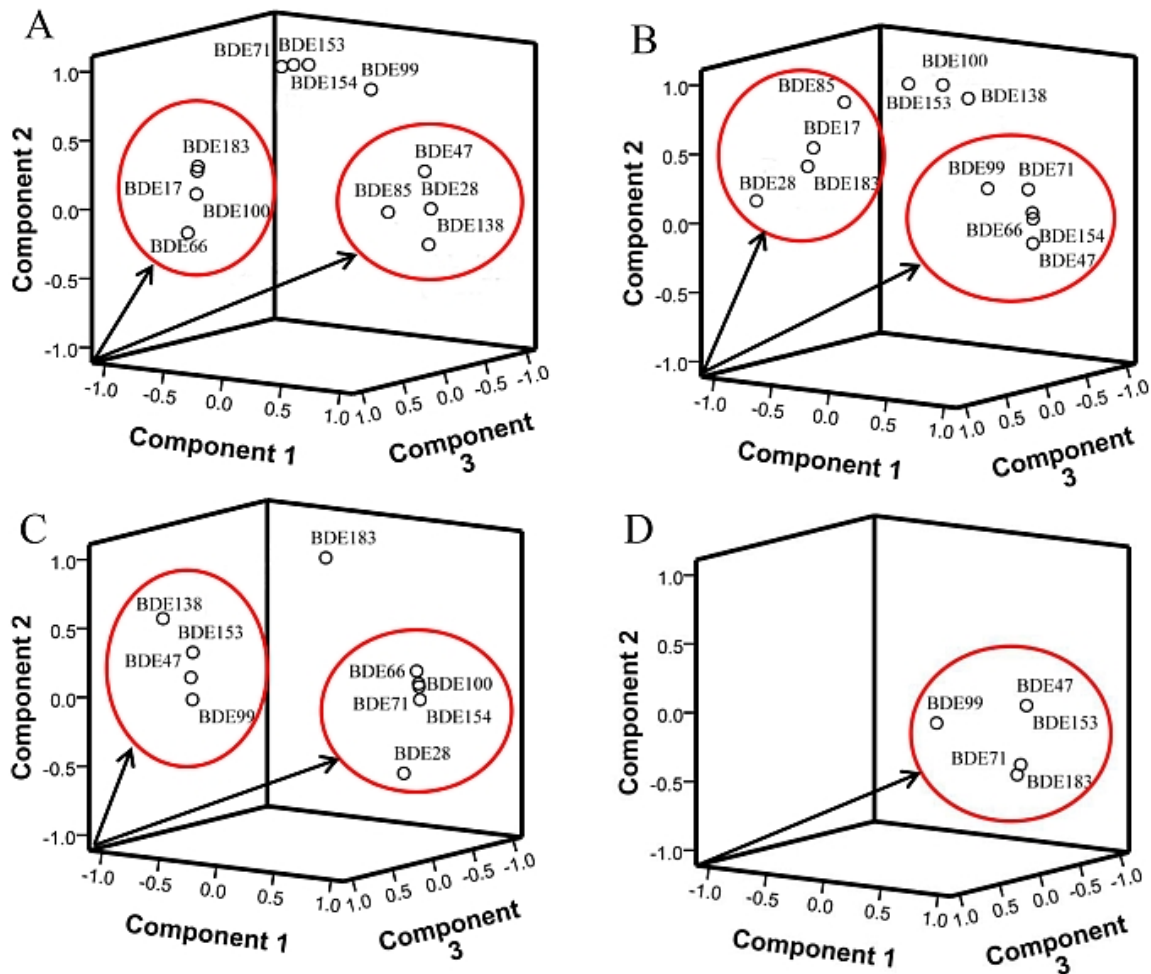
Fig. 4. Principal component triplot (principal axes 1−3) for 13 PBDE congeners in (A) leaves of *Fargesia qinglingensis*; (B) leaves of *Bashania fargesii*; (C) supplemental feedstuff; and (D) drinking water of samples taken at SWARC.

Health risk assessment

The ordering of hazard quotients (*HQ*) of the top five PBDE congeners in captive pandas was BDE99 (1.60) > BDE47 (1.10) > BDE100 (1.07) > BDE153 (0.97) > BDE154 (0.55), and was BDE47 (1.20) > BDE100 (0.99) > BDE154 (0.75) > BDE153 (0.57) > BDE99 (0.55) in wild pandas. with *HQ* values >1, BDE99 and BDE47 appear to pose a significant health risk to captive giant pandas, whereas BDE47 is congener putting wild pandas at risk.

15

Discussion

The first objective of this study was to test this hypothesis that giant pandas were exposed to PBDEs. Our previous research had found that Br in fecal samples of captive pandas were 3.3 times higher than in wild pandas (Chen et al., 2017), and data reported here supported the resulting hypothesis that captive pandas were exposed to PBDEs (Figs. 2A, 3A). In captive pandas, BDE47, BDE66, BDE71, BDE99, BDE154 were the major PBDE congeners in fecal samples, which matched the profile of PBDEs in leaves of the bamboo *Bashania fargesii* (Fig. 4B). This result suggests that the bamboo that is the primary food supply for pandas is the main source of PBDE exposure. Plants can accumulate high concentrations of toxicants in tissues that subsequently can accumulate and in the animals (Burreau et al. 2006; Voorspoels et al. 2007; McKinney et al. 2011; Krieger et al. 2016). Once in their body, PBDEs can lead to deficiencies in neural responses, thyroid hormone disorders, and carcinogenicity (McDonald 2002; Staskal et al. 2005; Lee et al. 2014).

Many studies have shown that PBDEs bioaccumulate in aquatic (Law et al., 2003) and terrestrial (Huwe et al., 2002; Jaspers et al., 2005; Pirard et al., 2007; Voorspels et al., 2006, 2007; Da Chen et al., 2012, 2013; Crosse et al., 2012; Andersen et al., 2015) species. However, we are aware of fes studies that sampled PBDEs in fecal samples (Zheng et al., 2015), which are an appropriately non-invasive method to sample PBDEs in a rare species like the giant panda.

The second objective of our research was to compare exposure of wild and captive pandas to PBDEs. Not surprisingly, given the anthropogenic origin of PBDEs, the Σ13PBDE was significantly higher in samples taken from captive pandas. Plants often reflect the content of

264   organic pollutants in the environment (Collins and Finnegan, 2010) because the soil-air-plant

265   pathway (pollutants volatilized from soils into the atmosphere are deposited onto plants)

266   describes uptake of organic pollutants from contaminated soils (e.g., Paterson et al., 1991; Trapp

267   and Matthies., 1997; Harrad et al., 2006; Collins and Finnegan., 2010; Ding et al., 2014). We

268   found that the Σ13PBDE of in *Fargesia qinlingensis* and *Bashania fargesii* growing around the

269   captive breeding center was significantly higher than that growing in the nature reserve (Fig. 2B

270   and 2C). The panda captive breeding center at SWARC is located close to the large city of Xi'an

271   and also to several potential sources of PBDE contamination: waste incinerators,

272   electronic-waste processing facilities, and industrial discharges (He et al., 2014; Kosior et al.,

273   2015; Wang et al., 2015).

274   The final objective of our research was to identify possible sources of exposure for PBDE

275   pollution by pandas. This study is the first to measure PBDE concentrations in bamboo, so we

276   can compare our results only with those from unrelated plant species. The Σ13PBDEs of

277   *Fargesia qinlingensis* and *Bashania fargesii* growing at FNNR and SWARC in our study were

278   higher than that of the moss *Pleurozium schreberi* in uncontaminated (755.6 pg/g dry mass) or

279   urban (3062.9 pg/g dry mass) areas. Pandas eat little besides bamboo. They can consume on

280   average 30 kg/day (Tuanmu et al., 2013) and bamboo accounts for > 99% of their diet (Hu, 1991,

281   2000), yet only 25% of the nutrients in bamboo can be assimilated (Zhou et al., 2008). This diet,

282   together with the similarities in congener profiles of panda fecal samples (Fig. 3A) and the

283   clustering in the PCA, (Fig. 4B) suggest that bamboo is the primary source of PDBE exposure

284   for pandas.

285   The very low concentrations of PBDEs in water (Figs. 2D, 3D, 4D) suggest that it is an

286   unlikely source of PBDEs for pandas. Likewise, the feedstuff appears an unlikely route of

287   exposure. In captivity, pandas are fed a steamed bread supplement ("feedstuff") that includes

288   additional ingredients, including milk powder, apple, carrot, steamed bran, rice flour, maize flour,

289   bean flour, fishmeal, bone meal, and mineral additives that provide supplemental nutrients

290   essential for successful breeding programs (Chen and Ma, 2016). The Σ13PBDE in feedstuff

291   (28.8 pg·g^{-1}; Fig. 2E) exceeded PBDEs concentrations in some farmland grains (13.7 pg·g^{-1}: Luo

292   et al., 2009) but not others (30–440 pg·g^{-1}: Zheng et al., 2015). This might be because the

293   feedstuff used in our research was purchased from local markets instead of having been made on

294   site from locally-grown ingredients.

295   To our knowledge, this is the first investigation of exposure of pandas to PBDEs, and one

296   of only a very few studies of PBDE exposure and bioaccumulation in a terrestrial species (Hoshi

297   et al., 1998; Christensen et al., 2005). There are few comparables, but the *HQ* model for

298   exposure risk suggests that BDE99, BDE47, and BDE153, each of which has an *HQ* >1, could

299   threaten the health of captive giant pandas.

300   Pandas in captive breeding centers generally are thought to be better protected from human

301   activities than are wild pandas in nature conservation zones. However, our previous research has

302   shown that captive pandas are exposed to a variety of environmental pollutants (Chen et al.

303   2016). The data presented here provide further evidence that the habitat and captive breeding

304   centers of giant pandas are polluted by PBDEs, and that exposure to PBDEs is significantly

305   greater in captive conditions.

306   PBDEs in pandas most likely come from the bamboo they eat. Every giant panda consumes

307   30 kg of shoots and leaves of bamboo, on average, every day (Tuanmu et al. 2013). Therefore,

308   even relatively low concentration of PBDEs in bamboo can still lead to a high dietary exposure

309   that threatens the health of the giant pandas. For mammals, PBDEs can be transferred to nursing

310   offspring via mother's milk (Travis and Hattermer-Frey, 1991; Darnerud, 2003; Beineke et al.,

311   2005; Beineke et al., 2007). PBDEs also have immunotoxicity and can be immunosuppressants

312   (Arkoosh et al., 2010; Frouin et al., 2010; Lv et al., 2015) that make pandas more vulnerable to

313   bacterial and viral infections.

314   **Conclusions and Recommendations**

315   Our data suggest that pandas are exposed to high levels of PDBEs in captive breeding

316   centers, and may represent a significant health risk for pandas in captivity. We recommend that

317   managers of these centers and captive breeding programs, including the Chinese State Forestry

318   Administration (SFA), seek strategies to minimize PDBE exposure by pandas lest decades of

319   successful *ex situ* conservation efforts become compromised by the increasing pollution

320   associated with Chinese economic development. A short-term solution to addressing this issue is

321   to reduce the supply of contaminated bamboo and to grow uncontaminated bamboo strictly for

322   captive pandas. In the long term, however, sustaining a successful captive breeding program for

323   pandas will require reduction of air, water, and soil pollution that will lead to improvements in

324   the environmental quality of the giant panda's natural habitat.

325

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