Should apple snail Pomacea canaliculata *(Caenogastropoda, Ampullariidae) be used as bioindicator for BDE-209?*

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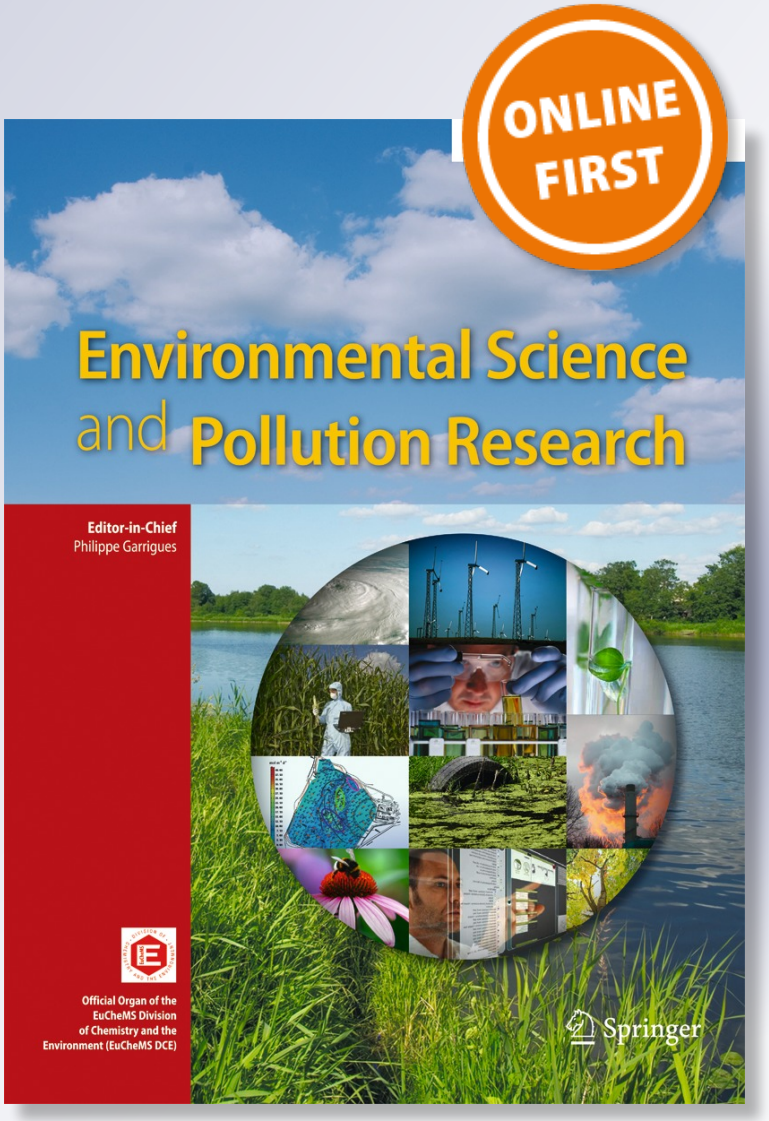

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SHORT RESEARCH AND DISCUSSION ARTICLE

Should apple snail *Pomacea canaliculata* (Caenogastropoda, Ampullariidae) be used as bioindicator for BDE-209?

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Abstract Apple snail *Pomacea canaliculata* has been reported to accumulate polybrominated diphenyl ethers (PBDEs) and was recently proposed as PBDE bioindicator. This work investigates the ability of *P. canaliculata* to accumulate BDE-209 by dietary exposure under controlled experimental conditions. A 30-day long enrichment feeding assay was carried out using 30 adult apple snails, placed in individual aquaria. Food was enriched at three BDE-209 concentrations (400, 4,700, and 8,300 μg g^{-1} lipid weight). Correlation between BDE-209 values in food and snail tissue were estimated according to Stockholm Convention suggested criteria for chemicals with K_{OW} >5. All animals survived with no evident physical alterations, and all of them accumulated BDE-209. BDE-209 levels in tissue samples increased exponentially with the exposure concentration. The bioaccumulation factor vs. food concentration plot showed a peculiar pattern, in which at intermediate concentrations the snails accumulated less BDE-209 than expected. Our results suggest that *P. canaliculata* would present a detoxification mechanism for BDE-209 different from the most commonly reported metabolic pathways.

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Introduction

The apple snail *Pomacea canaliculata* (Lamarck 1822) is an internal fecundation gonochoric Ampullariidae that spawns above water level. It is a fresh water amphibian species with polyphagous habits that includes feeding on algae, macrophytes, vegetal, detrital, and animal matter (Estebenet and Martín 2002). *P. canaliculata* originates from the southernmost part of South America, where it inhabits lentic and lotic waters. This species was introduced in North America and Asia via aquarium trade and for human consumption. Soon, it became an agricultural pest of aquatic crops and a source of ecosystemic changes in wetlands. This led to its inclusion among the worst 100 world invasive species, being the sole freshwater snail on the list (Lowe et al. 2000). Due to the species ubiquity and abundance, added to its invasive condition, there is plenty of literature about *P. canaliculata*'s biology, ecology, and control, among other topics. Species of Ampullariidae family were proposed as bioindicators for several environmental contaminants. *Pomacea canaliculata* was tested for metals and organometallic compounds (Giraud-Billoud 2010; Piyatiratitivorakul et al. 2006; Province 2006; Takeda 2000; Vega et al. 2012) as well as for persistent organic pollutants (POPs) (Fu et al. 2011; Harmon 2009).

Polybrominated diphenyl ethers (PBDEs) are used in commercial flame retardants polymer additives. Penta- and Octa-BDE commercial mixtures are listed in the Stockholm Convention POPs list (http://chm.pops.int/) with the consideration that higher BDE congeners may be converted to lower, and possibly more toxic, congeners. PBDEs have become an issue of global concern due to their ubiquity and persistence in the environment, as well as to their adverse effects on wildlife and human health. Among the 209 PBDE congeners, the decabrominated BDE

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(BDE-209) is the principal constituent in the widely used Deca-BDE commercial mixture. While there are reports on the occurrence of BDE-209 in *P. canaliculata* (Fu et al. 2011; Liu et al. 2008; She et al. 2013; Wang et al. 2009), no information is currently available regarding the relationship between exposure to BDE-209 and organismal concentrations in snails. Similarly, no controlled exposure assays have been undertaken to estimate the bioaccumulation of PBDEs on *P. canaliculata* or other related species.

The aim of this work was to investigate the ability of *P. canaliculata* to accumulate BDE-209 by prolonged exposure administrated through diet under controlled experiments. The obtained results would indicate whether *P. canaliculata* could be considered as a bioindicator species for BDE-209 as it was proposed for lower brominated PBDEs congeners (Fu et al. 2011).

Materials and methods

The 30 adult snails (10 females and 20 males; average shell length, 41 mm; average wet mass, 18.23 g) used in the present study were obtained from a *P. canaliculata* cultured strain originated from Lake Rosedal (Palermo, Buenos Aires, Argentina) population. Voucher (ethanol preserved) specimens of the original population and cultured strain were deposited at the collection of Museo Argentino de Ciencias Naturales (Buenos Aires, lots MACN-In 35707 and MACN-In 36046, respectively). Commercial fish pellet from Shulet® (Peixe Car, SENASA no. 02-089/A) was used for the exposure assays. A stock solution 1,700 mg l^{-1} BDE-209 (DE-83R, Great Lakes Chemical Corporation, West Lafayette, IN, technical Deca-BDE 98.5 % BDE-209) in toluene was used to prepare the enriched food. Additional toluene was added in order to imbibe the whole pellet mass and to achieve the same carrier solvent volume in procedural blank (B), as well as enriched food groups. The food was smoothly mixed, left to stabilize for 2 h and further dried at 40 °C overnight. Prepared food was stored in amber containers at 4 °C in the dark. BDE-209 concentrations were corroborated in each batch of enriched food and mean values were considered for statistical analysis.

Snails were randomly distributed in five experimental groups with six individuals each (two of which were females): control (C neither BDE-209 stock solution nor carrier solvent was added to the pellets), procedural blank (B, only carrier solvent added to pellets) and enriched foods (C_1, C_2, and C_3: ca. 400, 4,700 and 8,300 μg g^{-1} lipid weight (lw) BDE-209, respectively). The food was *ad libitum* delivered daily, resulting in ca. ten pellets per day per experimental group. Animals were individually kept in 800 ml glass aquariums containing ca. 200 ml tap water for 30 days controlling ambient conditions (14 light–10 dark hours, ambient temperature of 20.5±2 °C). Water was renewed daily at the same scheduled time. The discarded water was filtered (Whatman paper filter no. 40; pore, 8 μm) collecting the debris from nondigested food and feces. A 7-day acclimation period was carried out for all animals before starting the exposure assays feeding the animals with untreated pellets. In order to establish a prolonged exposure, a 30-day period was selected after longevity described for *P. canaliculata* under natural conditions in southern China ranges from 23 to 28 months (Liu et al. 2012). At the end of the exposure assays, animals were sacrificed and their soft parts were lyophilized *in toto*. The dried snails, as well as the collected filters, were used for further BDE-209 determination.

The analytical method used was previously described (Roosens et al. 2008; Van den Steen et al. 2007) and is briefly presented below. Snail tissues (~2 g), food (~2 g), and filters (~0.2 g) were homogenized individually with sodium sulfate and enriched with 50 ng ^{13}C-BDE-209 used as internal standard (IS). The homogenate was extracted by hot Soxhlet with 80 ml *n*-hexane/acetone (3:1) for 2.5 h. An extract aliquot was used for the determination of lipid content gravimetrically. The remaining volume was further cleaned up by loading it on ca. 8 g acidic silica column (H_2SO_4, 44 %, *w*/*w*). BDE-209 was eluted with 20 ml hexane and 15 ml dichloromethane. The extract was further rotary evaporated to ca. 2 ml, dried under a gentle N_2 stream, and reconstituted with 200 μl isooctane. For analysis, an aliquot of 1 μl was injected into a gas chromatograph coupled to a mass spectrometer operated in electron capture negative ionization mode. Instrumental conditions were the same as previously reported. Ions with *m*/*z* 79 and 81 were monitored during the entire run, while ions *m*/*z* 486.7 and 494.7 were monitored for BDE-209 and ^{13}C-BDE-209, respectively.

Diet was preferred as administration way (Arnot and Gobas 2006) due to the high K_{OW} (octanol-water partition coefficient) of BDE-209. To correlate BDE-209 levels between tissue and food, bioaccumulation factor (BAF) was used. The criteria to select this ratio are in accordance with Stockholm Convention Annex D for chemicals with K_{OW} >5. BAF was calculated as BAF = conc. BDE-209 tissue/conc. BDE-209 diet (both lipid normalized). Although bioaccumulation consider diet and ambient environmental sources (Arnot and Gobas 2006), the experimental design proposed makes diet the principal BDE-209 uptake.

For comparison among the different concentration groups and BAF values and to identify whether there are differences among them, Kruskal–Wallis (after Shapiro–Wilk normality test) and Dunn's Multiple comparisons posttests, respectively, were used (Prism 5, GraphPad Software Inc.). Outlier values were identified by Dixon's *Q* test. BDE-209 concentration values were expressed as lipid weight content.

Results

All 30 specimens survived with no deterioration sign on their general condition (no significant variations on weight,

behavioral alterations, visible malformations, nor spawning differences among treatments, all females spawned eventually), and in all cases, BDE-209 tissue concentration was quantifiable. Results are expressed independent to sex since no significant difference was observed between them (Kruskal–Wallis, $P<0.05$). Table 1 shows BDE-209 levels in analyzed snail and filter samples. Data from snail no. 18, B no. 1, and C no. 12 were excluded after running a Dixon's Q test. The BDE-209 levels for control and blank snail groups were under the quantification limit of analytical methodology (LOQ= 160 ng g^{-1} lw). BDE-209 levels in tissue samples increased as the exposure concentration did. Nevertheless, such increment was not strictly proportional. Linear ($y=0.2112x-214.56$), quadratic ($y=4E-05x^2-0.1144x+115.97$), and exponential trend lines ($y=59.811$ exp $0.0004x$) lead to R^2 of 0.648, 0.759, and 0.921, respectively, when plotting diet vs. tissue levels (Fig. 1). Moreover, comparing the square sum of each trend line residuals resulted in the lowest value for the exponential trend line (2880172), followed by the quadratic (3085623) and finally the linear one (4154983). Significant differences among the tissue, filter, and BAF data were confirmed ($P=0.0008$; Kruskal–Wallis). The only difference detected in tissue was between the C_1 and C_3 assays ($P<0.05$, Dunn's Multiple Comparison Test), with C_3 values being higher than C_1. Similar results were obtained for the filter concentrations. On the other hand, the BAF for C_2 differed with respect to C_1 and C_3 ($P<0.05$, Dunn's Multiple Comparison Test, Fig. 2) and was lower than expected, while the BAF values for C_1 and C_3 did not differ among each other.

Discussion

The available information of PBDEs on Ampullariidae (Liu et al. 2008) and specifically *P. canaliculata* (Fu et al. 2011; She et al. 2013; Wang et al. 2011) focused on data from e-waste areas of southern China. Liu et al. (2008) determined 19 PBDE congeners, including BDE-209, in tissues from *Pomacea gigas* (as *Ampullaria gigas* in Liu et al 2008); however, specific data for BDE-209 was not presented. Wang et al. (2011) reported seven PBDE congeners, together with BDE-209, in water and sediment samples, as well as aquatic organisms, including *P.*

Table 1 Recorded data for enriched feeding assay for *Pomacea canaliculata*

Treatment		Snail lipid percentage (on dry weight)	Snail dry weight (g)	Snail concentration (μg g^{-1} lw)	Filter weight (g)	Filter concentration (μg g^{-1} dw)	BAF
C_1	Snail 13	3.96	1.79	100.40	0.20	22.58	0.25
	Snail 14	4.62	1.53	89.27	0.13	26.87	0.25
	Snail 15	3.80	1.59	81.12	0.15	33.79	0.20
	Snail 16	3.47	1.84	33.22	0.21	49.00	0.08
	Snail 17	4.51	1.63	74.04	0.24	32.50	0.18
	Average	*4.07*	*1.67*	*75.61*	*0.18*	*32.95*	*0.19*
	Standard dev.	*0.49*	*0.13*	*25.65*	*0.05*	*10.04*	*0.06*
C_2	Snail 19	3.30	1.51	705.96	0.21	305.23	0.15
	Snail 20	4.81	1.29	261.73	0.34	254.85	0.06
	Snail 21	3.47	1.51	245.00	0.16	272.68	0.05
	Snail 22	3.52	1.79	380.67	0.19	322.51	0.08
	Snail 23	3.75	1.88	399.97	0.29	230.17	0.08
	Snail 24	4.26	1.86	473.99	0.19	302.49	0.10
	Average	*3.85*	*1.64*	*411.22*	*0.23*	*281.32*	*0.09*
	St. dev.	*0.57*	*0.24*	*168.43*	*0.07*	*34.95*	*0.04*
C_3	Snail 25	3.57	1.19	1,759.85	0.22	1,071.49	0.21
	Snail 26	4.51	1.06	3,092.53	0.15	1,267.90	0.37
	Snail 27	3.09	1.74	1,004.14	0.17	1,082.10	0.12
	Snail 28	4.33	1.67	1,218.95	0.21	962.04	0.15
	Snail 29	3.43	1.80	1,887.51	0.27	851.98	0.23
	Snail 30	3.23	2.21	1,544.38	0.12	1,494.59	0.19
	Average	*3.69*	*1.61*	*1,751.23*	*0.19*	*1,121.68*	*0.21*
	Standard dev.	*0.59*	*0.42*	*735.12*	*0.06*	*229.24*	*0.09*

BAF= [BDE-209 tissue]/[BDE-209 diet]. Enriched food concentration: C_1, 406 μg g^{-1} lw; C_2, 4741 μg g^{-1} lw; and C_3, 8335 μg g^{-1} lw BDE-209, respectively
Numbers were presented in italics to highlight averages and SD

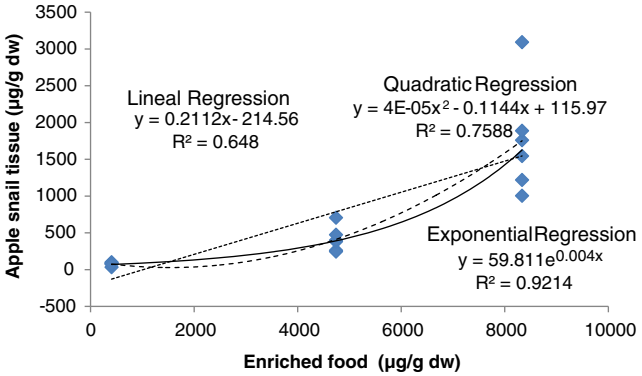

Fig. 1 Linear (*small broken line*), quadratic (*large broken line*), and exponential (*straight line*) regressions between apple snail tissue and enriched food BDE-209 levels

canaliculata. Fu et al. (2011) reported 14 PBDE congeners and, although BDE-209 was determined, it was excluded from the correlation analysis between PBDE concentrations in sediments and *P. canaliculata* tissues. Although these studies focused on lower PBDEs, Fu et al. (2011) proposed apple snail as an appropriate bioindicator species for reporting the levels and profiles of PBDEs. This was based on a linear correlation between the PBDE concentrations in snail tissue and soil/sediment samples that would suggest bioaccumulation combined with insignificant metabolism.

In order to fulfill the aim of this work, enriched feeding assays (range, 8.5–190.3 µg g^{-1} dry weight (dw)) were carried out. The assay concentration range included the highest values reported by Fu et al. (2011) in *P. canaliculata* tissue (45.0 µg g^{-1} dw) and Wang et al. (2011) in soil/sediments samples (58.4 µg g^{-1} dw).

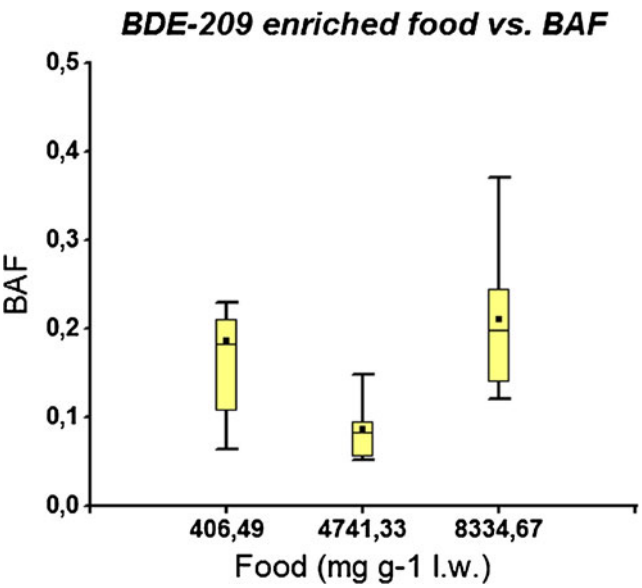

Fig. 2 BAF vs. enriched food BDE-209 levels

Besides the apparent innocuous effects, a lack of linear correlation between the levels of BDE-209 in the snail tissue and food was observed along with an unexpected accumulation pattern (Figs. 1 and 2, respectively). In particular, we have observed low BAF values for the C_2 exposure group. This would compromise the proposal of *P. canaliculata* as a good bioindicator since both characteristics mentioned for the other PBDE congeners, e.g., linear correlation and the apparent resistance to metabolism were not observed for BDE-209 in this study.

Possible explanations for the pattern shown in Fig. 2 are methodological or biological reasons. Regarding the methodological possibility, food pellets were delivered from a single lot, in which the concentration of BDE-209 was determined at the end of the enriched feeding study (Table 1). Thus, the underestimation of administrated doses is discarded. On the other hand, it should be mentioned that the concentration pattern observed in snail tissues was also found in the filter papers, which are representative for the remaining food and feces in the respective aquarium. Additionally, the reason for lower values from the C_2 assays could not be attributed to the selected analytical methodology since the same protocol was applied to all samples in a short period of time (days) when all samples (snails and filters) were processed and analyzed. Based on the foregoing, we discarded a possible loss of BDE-209 due to methodological issues.

Regarding the biological hypothesis, metabolism is an important detoxification process. The most reported metabolization processes for BDE-209 are debromination to lower brominated BDE congeners, methylation, hydroxylation, and/or their combinations. If any of these detoxification mechanisms would occur in *P. canaliculata*, it would lead to various brominated metabolites that should have been present in the resulting chromatograms. However, no peaks corresponding to other brominated compounds than BDE-209 were observed in the chromatograms. Also, a total debromination of BDE-209 is improbable. Our results suggest that *P. canaliculata* would present a detoxification mechanism for BDE-209 different from the described metabolic pathways.

This species could be an interesting biological model rather than a good bioindicator for BDE-209 studies.

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