Int. J. Environ. Res., 7(3):807-816,Summer 2013
ISSN: 1735-6865

Integrated Biomarker Responses of *Carassius auratus* Exposed to BDE-47, BDE-99 and Their Mixtures

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Received 19 Aug. 2012; Revised 20 Dec. 2012; Accepted 2 Feb. 2013

ABSTRACT: Polybrominated diphenyl ethers (PBDEs) have been widely used in many products as flame retardants, which resulted in their release into the environment. Little is known about the impact of coexisting PBDEs on organisms. In this study, the in vivo effects of BDE-47 and BDE-99 on a suite of biomarkers, acetyl cholinesterase (AChE), ethoxyresorufin-O-deethylase (EROD), glutathione-S-transferase (GST), superoxide dismutase (SOD) and catalase (CAT), in goldfish (*Carassius auratus*) were investigated. The enzyme activities were significantly altered by the two PBDEs (alone and in combination) after 2, 4, and 7 days of exposure, and obvious dose-response and time-response relationships were observed at most cases. The results suggest that these biomarkers could be used to assess ecological risks of PBDEs on fish. An integrated biomarker response (IBR) was calculated by combining multiple biomarkers to single value and used to quantitatively evaluate the toxicological effects of different chemicals. In general, BDE-99 showed more adverse biological effects than BDE-47. The joint action of mixtures seemed to be synergism at low dosage and antagonism at high dosage with regard to IBR variation.

Key words: PBDEs, *Carassius auratus*, Biomarker, Co-exposure, Joint action

INTRODUCTION

Polybrominated diphenyl ethers (PBDEs), a group of brominated flame retardants formed by 209 congeners, are used as additives in plastics, textiles, foams and electronic appliances (Alonso *et al*., 2010). It is believed that PBDEs (mainly tetra- to octa-BDEs) are chemically and biologically persistent and lipophilic, and can be accumulated in organisms and humans (Chen *et al*., 2010; Feng *et al*., 2010). Some of these PBDE congeners are even toxic for organisms, and cause developmental neurotoxic effects (Cheng *et al*., 2009), impair learning/ memory abilities (Fischer *et al*., 2008), and induce oxidative stress (Bellés *et al*., 2010). Recently, tetra- to hepta-BDEs have been added to the POPs list of the Stockholm Convention**.** 2,2',4,4'-Tetrabromodiphenyl ether (BDE-47) and 2,2',4,4',5-pentabromodiphenyl ether (BDE-99) are two kinds of lower molecular PBDE congeners and are frequently detected in aquatic biota, sediments, and waters. The contents of BDE-47 ranged from 0.02 to 1.48 ng/g dry weight in sediment, and from 0.14 to 1.01 ng/g dry weight in mussel in Bo Sea, China, while BDE-99 from 0.01 to 1.62, and from 0.02 to 0.13 respectively (Wang *et al*., 2009b). Comparing with BDE 47, BDE 99 possesses higher molecular weight, lower solubility in water and similar octanol/water partition coefficient (Braekevelt *et al*., 2003). Due to the bioaccumulation, several studies have reported high concentrations of PBDEs in farm-raised and wild fish, and consistently indicate that BDE 47 is a predominant residue and can comprise up to 70% of the fish body burden of total PBDEs (Hale *et al*., 2001; Tittlemier *et al*., 2004; Stone, 2006). Previous studies showed that BDE-47 was more toxic than BDE-99. Tagliaferri *et al*. (2010) determined the effects of BDE-47 and BDE-99 on cellular viability of human neuroblastoma, and the observed median inhibitory concentration were 10.8 and 33.9 μmol/L, respectively.

Among the plethora of biological responses to pollution that have been observed in the last decades, those based on biomarkers seem to be the most sensitive, reliable and able to represent the earliest signals of environmental disturbance (Binelli *et al*., 2010). Biochemical biomarkers are increasingly used in early warning to identify the adverse effects caused by harmful organic pollutants. Some studies reported the biomarker responses of single PBDE exposure on rodent or fish (Cheng *et al*., 2009; Lema *et al*., 2007), but no information was available on the joint effects of coexisting PBDEs. The purpose of this study was to investigate the effects of BDE-47, BDE-99 and their

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mixtures on acetyl cholinesterase (AChE), 7-ethoxyresorufin-O-deethylase (EROD), glutathione-S-transferase (GST), superoxide dismutase (SOD) and catalase (CAT) activities in goldfish (*Carassius auratus*), and to study their concentration-response and time-response relationships during the exposure period. An integrated biomarker response (IBR) was computed with biomarker measurements to assess the comprehensive toxic effects of BDE-47 and BDE-99.

MATERIALS & METHODS

BDE-47 (98.9% purity) and BDE-99 (98.5% purity) were purchased from AccuStandard, Inc. (New Haven, CT, USA). Nicotinamide adenine dinucleotide phosphate, 5,5'-dithiobis (2-nitrobenzoic acid), 1-chloro-2,4-dinitrobenzene, resorufin, ethoxyresorufin, glutathione, and ammonium molybdate were purchased from Sigma Chemical Company (St. Louis, MO, USA) and the stated purities were >99.9%. Bovine serum albumin, acetylthiocholine iodide and pyrogallic acid were purchased from Shanghai Huixing Biochemistry Reagent Co., Ltd. (Shanghai, China) and the purity was >98%. Coomassie brilliant blue G-250 (Ultra Pure Grade) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All other chemicals were of analytical grade and were obtained from Nanjing Sunshine Biotechnology Co., Ltd. (Nanjing, China).
The goldfish was chosen due to its economical importance and wide distribution in freshwaters in China. Juvenile goldfish of both sexes weighing 21.26 ± 3.45 g were obtained from Nanjing Institute of Fishery Science, China. The fish were acclimatized for two weeks in dechlorinated municipal water prior to experimentation and their mortality was below 1% during the acclimation period. Fish were fed every day with commercial fish food. Feces and uneaten food were removed every day by suction. Fish were not fed for 24 hr prior to the experiments, and no food was provided during the test period.

After being dissolved in corn oil, BDE-47 and BDE-99 were administered via intraperitoneal injection at dosages from 0.04 to 10 mg/kg. In order to facilitate comparison with the results from individual exposures, BDE-47/BDE-99 mixtures were tested according to a quality ratio of 1:1 (*m*/*m*), and the total exposure doses were also 0.04, 0.2, 1, 5 and 10 mg/kg. A blank control and a solvent control (SC, corn oil) were included in the experimental design. Fish were weighed before injection to determine the volume of dosage per kilogram body mass of each fish. Masses and dosages were recorded. Fish were kept in groups of twelve in 30-L glass tanks containing dechlorinated municipal water under constant aeration. A 50% water change was performed every day. Water temperatures ranged from 20 to 22°C, with pH at 7.0±0.2, dissolved oxygen of 5.5±0.1 mg/L, and natural photoperiod during 7 days of exposure.

Three fish were collected for each treatment and control after 1, 2, 4, and 7 days of exposure. Fish were killed by cervical transaction and brain and liver tissues were collected. Tissues were washed in 0.15 mol/L of KCl, weighed, immediately frozen in liquid nitrogen, and stored at –80°C. Brain samples were homogenized in 5 volumes of cold sodium phosphate buffer (0.1 mol/L, pH 7.2, 0.1% Triton X-100) on ice and centrifuged for 20 min (10,000×g) at 4°C. The supernatants were used as the enzyme extract for AChE activity determination. AChE enzymatic activity was determined at 405 nm using the method described by Guilhermino *et al.* (1996).

Liver samples were homogenized in nine volumes of cold buffer (0.15 mol/L KCl, 0.1 mol/L Tris–HCl, pH 7.4) and centrifuged for 25 min (9,000×g) at 4°C. The supernatants were used as the extract for enzymatic activity determination. EROD activity was quantified at 572 nm using a microplate reader (Molecular Device VersaMax, USA) by the method of Lu *et al.* (2009). GST activity was determined at 340 nm by adapting to a microplate reader as described by Frasco and Guilhermino (2002), where 1-chloro-2,4-dinitrobenzene was used as a substrate. SOD activity was determined by measuring the inhibition of the auto-oxidant of pyrogallol using a modification of the method suggested by Marklund and Marklund (1974). CAT activity was determined using ammonium molybdate (Góth, 1991). Protein concentrations were determined at 595 nm using a method developed by Bradford (1976), with bovine serum albumin as the standard.

A method for integrating all the measured biomarker responses into one general "stress index", termed "Integrated Biomarker Response" (IBR) (Beliaeff and Burgeot, 2002), was used to evaluate the integrated impact of toxicants. The procedure for determining an IBR involved first the calculation of the mean and standard deviation for a biomarker response from each treatment. These data were then standardized for each treatment according to the equation $F_i' = (F_i - \text{mean } F)/S$, where F_i' is the standardized value of the biomarker, F_i is the mean value of a biomarker from each treatment, mean F is the mean of the biomarker calculated for all the treatments, and S is the standard deviation calculated for the treatment-specific values of each biomarker. Using standardized data, Z was computed as $+F_i'$ in the case of activation and $-F_i'$ in the case of an inhibition, and then the minimum value for all treatment for each biomarker was obtained and added to Z. Finally the score B was computed as $B=Z+|\min|$, where B e≥0 and $|\min|$ were the absolute values of the minimum. The corresponding IBR value is: $\{[(B_1 \times B_2)/$

$2]+[(B_2\times B_3)/2]+\cdots[(B_{n-1}\times B_n)/2]+[(B_n\times B_1)/2]\}$ (Damiens *et al*., 2007).

For each biomarker, results were expressed as mean ± S.D. All data from different treatments were compared by a one-way analysis of variance (ANOVA) and statistically different treatments were identified by Dunnett's test. All differences were considered significant at $P < 0.05$. Statistical analyses were performed using SPSS 11.5.

RESULTS & DISCUSSION

No mortality occurred during the experiments. In goldfish exposed to corn oil in the solvent controls, brain AChE and liver EROD, GST, SOD and CAT activities did not differ significantly from those in the water controls. Therefore, the enzyme activities of chemical-exposed fish were compared with those of solvent controls. Brain AChE responses in goldfish exposed to BDE-47 and BDE-99 during all exposure period (1, 2, 4, and 7 days) are shown in Fig. 1. Lower dosages of BDE-47 and BDE-99 (0.04 and 0.2 mg/kg) did not induce obvious effects on brain AChE activity. However, AChE activity was significantly inhibited by exposure to higher dosages tested ($P < 0.05$), and the inhibition rate of AChE activity matches the dosage increased with an exception of a slight decrease of AChE inhibition rate for the highest BDE-99 dosage (10 mg/kg) as compared with that of the exposure of 5 mg/kg at day 7. Regarding time response, brain AChE activity decreased continuously with exposure time

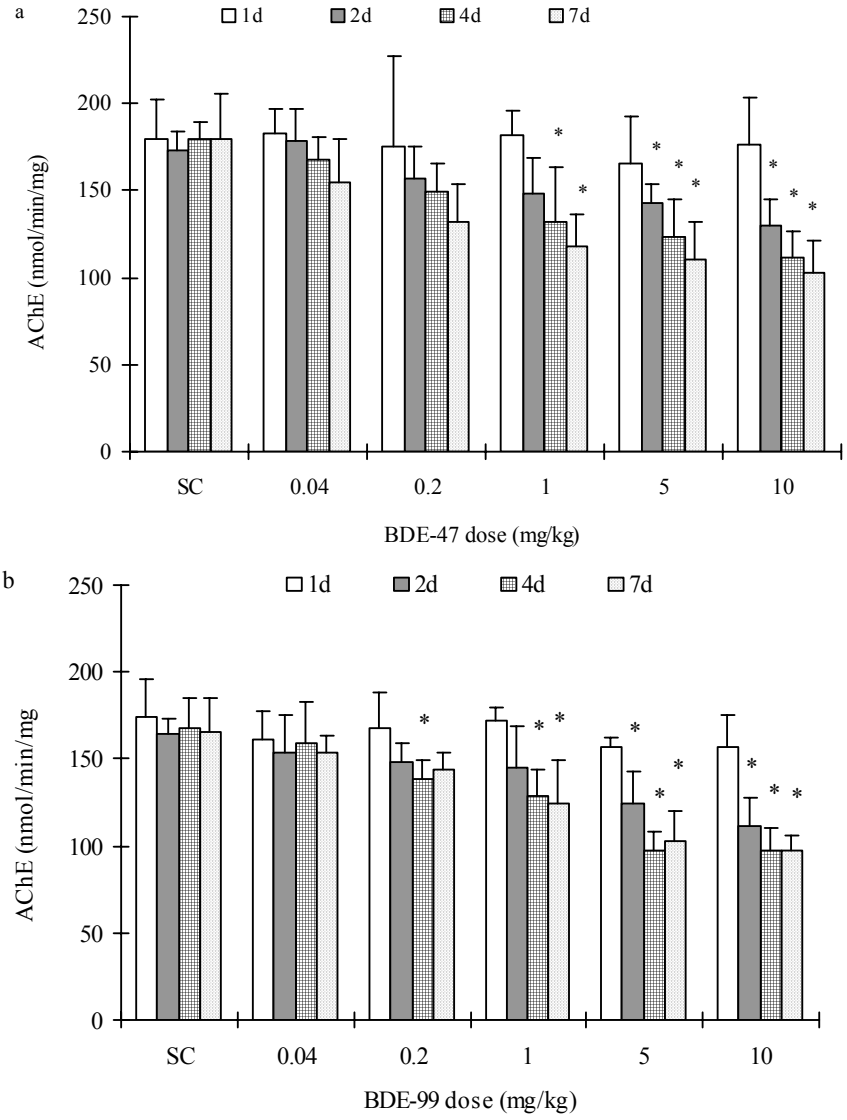

Fig. 1. Brain AChE responses in goldfish exposed to BDE-47 and BDE-99. Asterisks indicate values that are significantly higher than control values ($P < 0.05$)

for all doses of BDE-47, and exhibited an obvious time–response relationship. However, BDE-99 induced a similar AChE inhibition at day 4 and 7, thus time dependence was not as apparent as BDE-47.

The in vivo effects of BDE-47 and BDE-99 on liver EROD are shown in Fig. 2. Liver EROD activities did not change significantly at day 1 for all concentrations of BDE-47 and BDE-99. However, EROD activity increased by exposure to most dosages at day 2, 4 and 7 exposure period. The induction level of EROD elevated with increased dosages, whereas an obvious reduction was observed after 7 days of exposure in response to 5 and 10 mg/kg for both BDE-47 and BDE-99. BDE-47 and BDE-99 exhibited similar time dependence and dosage dependence on liver EROD activity.

The responses of liver GST activity exposed to BDE-47 and BDE-99 are shown in Fig. 3. The response pattern of GST was similar to that of EROD. GST activity was significantly induced by all tested dosages, with the exception of 0.04 mg/kg. GST activity was continuously increased for all concentrations after 1, 2 and 4 days of exposure, with the exception of a decreasing for the highest dosage (10 mg/kg) at day 4. It was found that GST activity was markedly induced at concentration of 0.2 mg/kg, and then the level of

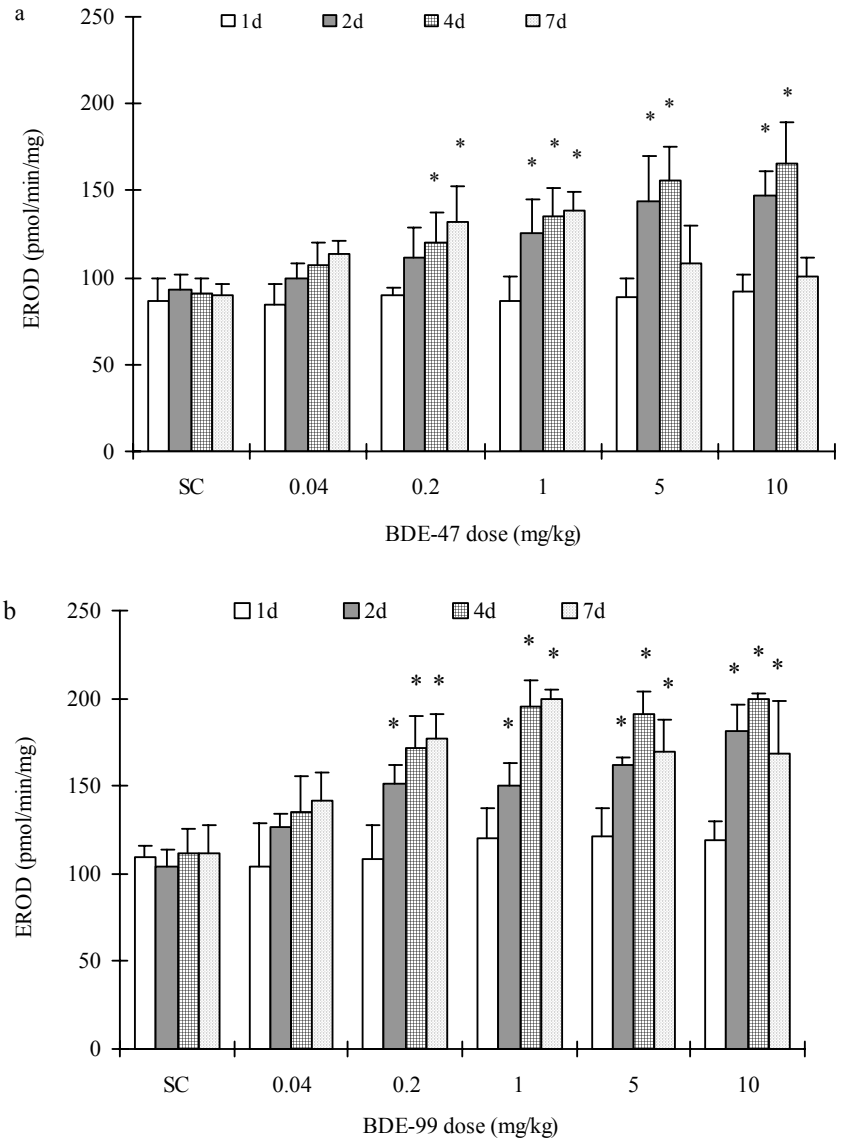

Fig. 2. Liver EROD responses in goldfish exposed to BDE-47 and BDE-99

induction decreased continuously with the dosages at day 7. Furthermore, the most significant induction of GST activity was found at 5 mg/kg after 4 days of exposure to BDE-47 and BDE-99.

The responses of liver SOD activity exposed to BDE-47 and BDE-99 during the four exposure period (1, 2, 4 and 7 days) are shown in Fig. 4. No significant change in liver SOD activity occurred at day 1 for all exposure dosages, whereas SOD activity continuously increased with the dosages at day 2. The response pattern of SOD activity was similar to that of GST activity after 4 and 7 days of exposure to BDE-99. However, SOD activity showed the trend of "lower concentrations being induced, higher concentrations recovered" after 4 and 7 days of exposure to BDE-47. In addition, the most significant SOD induction was observed at day 4 at 1 mg/kg to BDE-47, and at 5 mg/kg to BDE-99.

The effects of BDE-47 and BDE-99 on liver CAT activity during all periods of exposure are shown in Fig. 5. Both chemicals at low dosage (0.04 mg/kg) and/or short exposure duration (1 day) did not significantly alter CAT activity. Percentage inhibition of CAT activity increased continuously with the dosages and exhibited good dosage dependence for both compounds. The responses of CAT activity after 4 day exposure were almost the same as those of 7 day exposure to both BDE-47 and BDE-99 for all dosages, and time dependence was not apparent, especially for BDE-47 exposure.

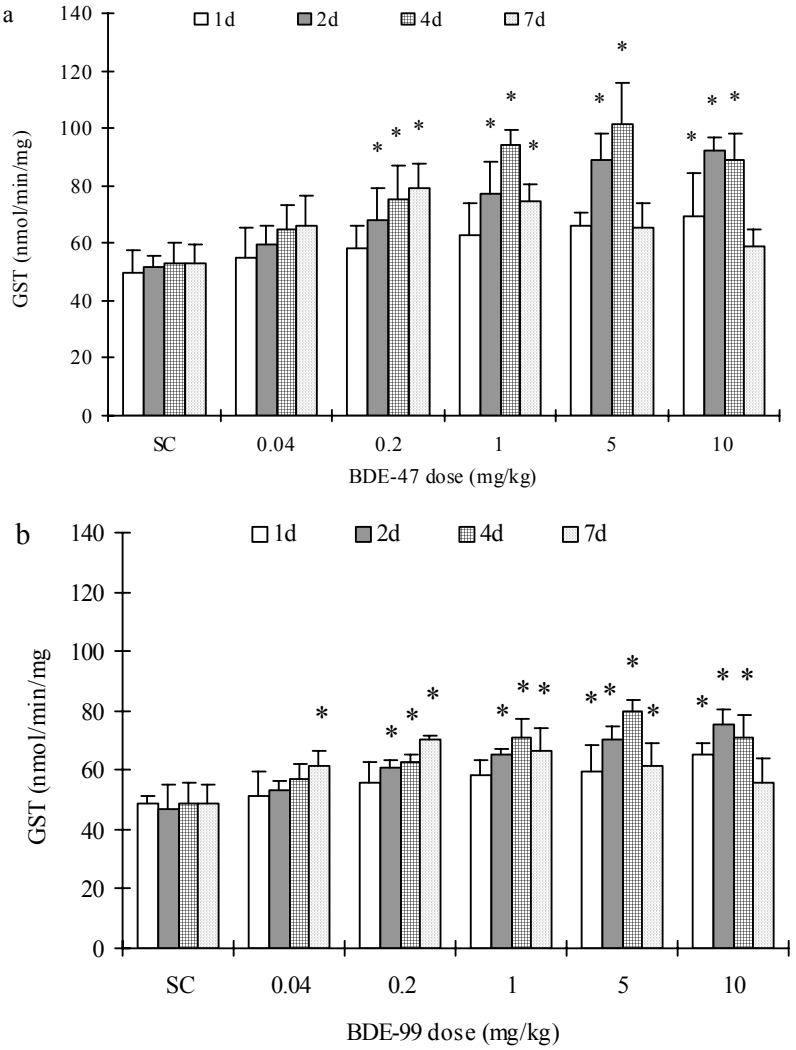

Fig. 3. Liver GST responses in goldfish exposed to BDE-47 and BDE-99

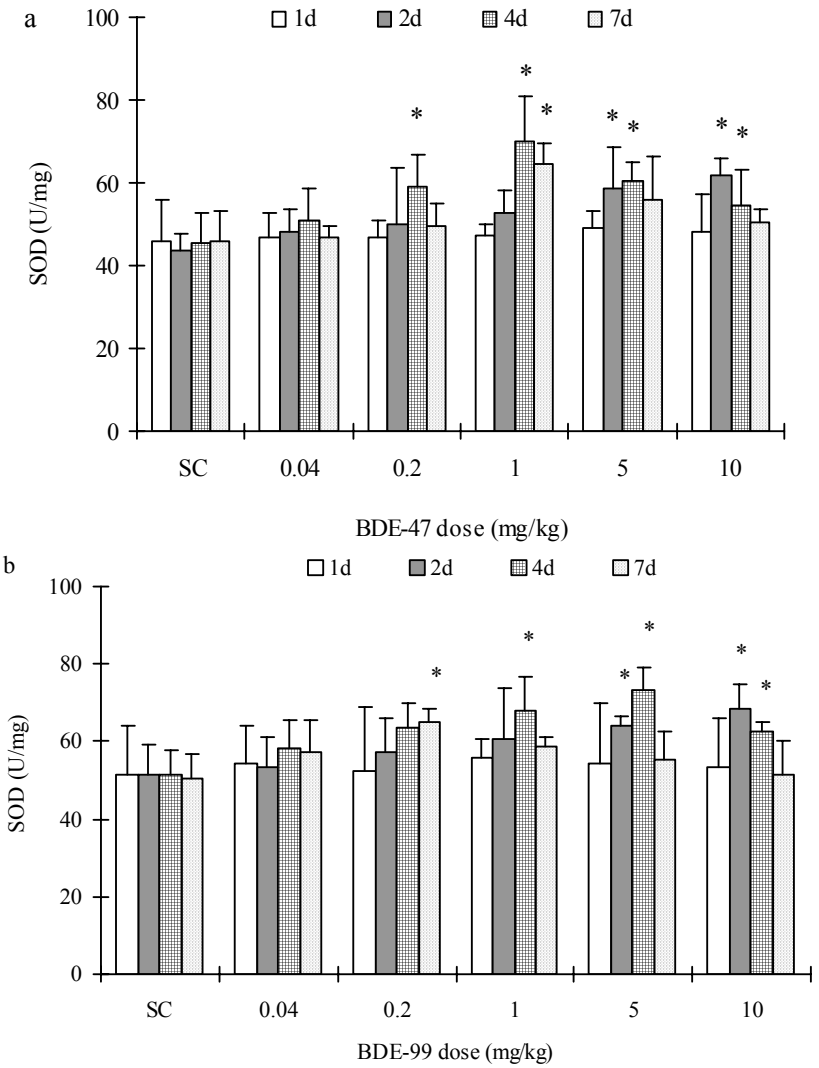

Fig. 4. Liver SOD responses in goldfish exposed to BDE-47 and BDE-99

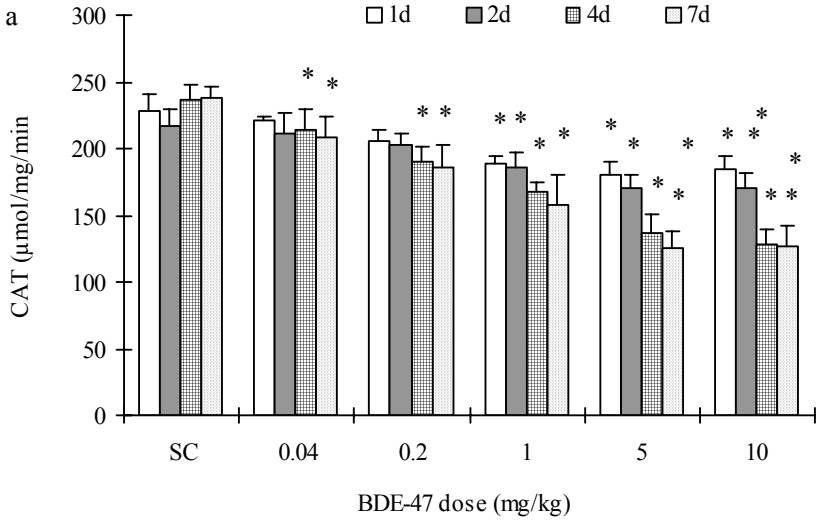

Fig. 5. Liver CAT responses in goldfish exposed to BDE-47 and BDE-99

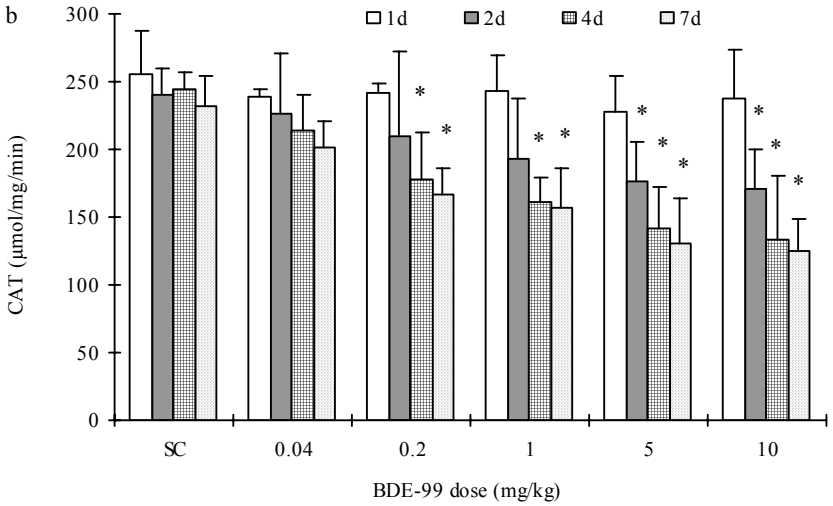

Fig. 5. Liver CAT responses in goldfish exposed to BDE-47 and BDE-99

Considering that the most significant biomarker responses of single exposure occurred mainly at day 4, we investigated the changes of enzyme activities after 4 days of exposure to BDE-47/BDE-99 mixtures, and the results are shown in Fig. 6. The mixtures significantly inhibited brain AChE and liver CAT activities at all test dosages except at 0.04 mg/kg ($P < 0.05$). The dose-response curves of co-exposure of the mixtures on AChE and CAT activities were similar to those of single exposures. EROD activity was significantly induced by all the mixture dosages, with the exception of 0.04 mg/kg. Induction of EROD activity was elevated with increased mixture dosages, with the exception of slight reduction at 10 mg/kg. GST activity was induced markedly by BDE-47/BDE99 at the three lowest concentrations. However, the fold induction declined significantly at the two highest exposure dosages. The response of SOD exposed to the mixtures was similar to that of exposed to BDE-99, and the fold induction for mixtures was higher than that for both individuals. Dose dependence was apparent for each biomarker studied although the response patterns were not consistent.

AChE activity is frequently used as a biomarker of insecticide and pesticide toxicity. The activity of this enzyme is extremely important for many physiological functions, such as prey location, predator evasion, and orientation toward food (Miron *et al*., 2005). When AChE activity decreases, it may result in the incapable stop of receptor function of acetylcholine and nerve membranous posterior, making organisms at excitement status for a long-term and physiological process maladjustment as well as death finally (Han *et al*., 2010). In the present study, BDE-47

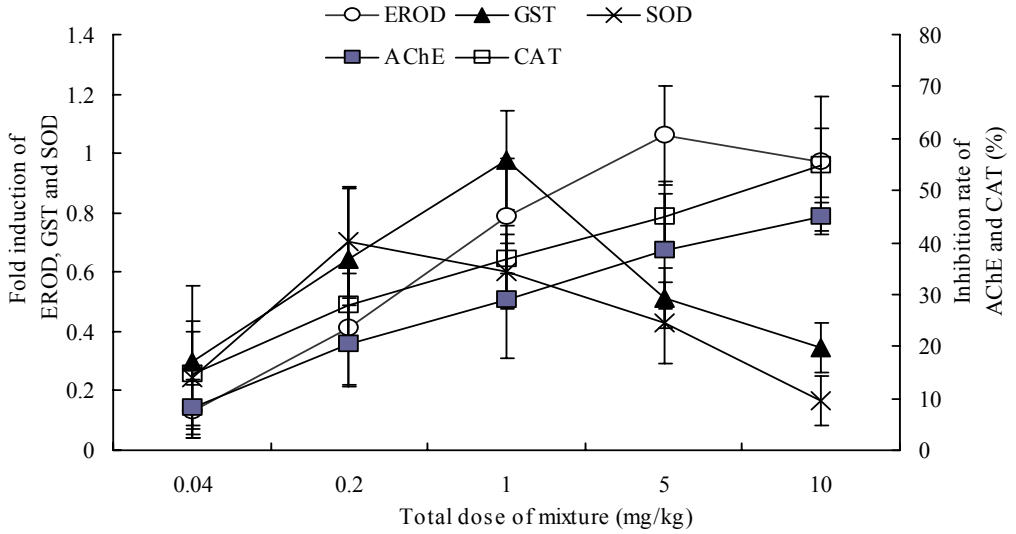

Fig. 6. Biomarker responses in goldfish exposed to BDE-47/BDE-99 mixtures

and BDE-99 (alone and in binary mixture) significantly inhibited AChE activity in brain ($P < 0.05$). The inhibitory effects of binary mixtures on AChE activity were a little higher than those of the corresponding individual exposures in same cases, suggesting that BDE-47 combined with BDE-99 may induce more than additive neurotoxicity in fish. Wang *et al*. (2009a) measured muscle and brain AChE inhibition in goldfish exposed to sub-lethal concentrations of two insecticides (propoxur and isoprocarb) and additive effects were observed. The cytochrome P4501A (CYP1A) is extremely important in the metabolism of many xenobiotics. EROD activity has been widely used as a biomarker in fish exposed to substances that bind to the aryl hydrocarbon receptor (Teles *et al*., 2005). In the present investigation, EROD activity was significantly induced 0.8-fold by both individual compounds and 1.1-fold by the mixture, suggesting two PBDEs (BDE-49 and BDE-99) produce a synergistic effect. It is reported that a technical PBDE mixture containing BDE-47 and BDE-99 induced the EROD activity in the mouse liver (Lundgren *et al*., 2007). Nevertheless, dietary exposure of a commercial PBDEs mixture, penta-BDE (mostly PBDE-47 and 99), with doses of 10 and 50 mg/kg body weight showed no significant EROD induction in Atlantic salmon (Boon *et al*., 2002).

GSTs are involved in the biotransformation of several pollutants, and may play an important role in detoxifying strong electrophiles with toxic, mutagenic and carcinogenic properties. It can catalyze the conjugation of the tripeptide glutathione with the xenobiotic in phase II of the biotransformation process and promote its elimination from the organism (Richardson *et al*., 2008). Therefore induction of GST activity has been widely used as an environmental biomarker (Rao, 2006; Peebua *et al*., 2007). Comparing with the individual exposures, the mixtures exhibited a stronger GST induction and a synergistic effect seemed to exist. In addition, EROD and GST induced by BDE-49 and BDE-99 at day 4 exhibited bell shaped dose-response curves. Bell-shaped curves have been reported on EROD and GST induction for in vivo or in vitro systems after exposure to PAHs (Bosveld *et al*., 2002; Lu *et al*., 2009). Although the mechanism that decreased EROD or GST induction has not been completely defined, it is likely that high concentrations of the inducer inhibit or inactivate the induced enzyme (Voorman and Aust, 1987).

SOD catalyzes the transformation of superoxide radicals to H_2O_2 and O_2, and is the first enzyme to deal with oxyradicals. CAT is a major primary antioxidant defense component responsible for catalyzing the decomposition of H_2O_2 to H_2O. SOD and CAT activities may be altered after exposure to environmental contaminants. Hence, they can serve as early monitor of exposure to contaminants eliciting oxidative stress (Palace *et al*., 1996). The joint action of these enzymes decreases the toxicity produced by the superoxide anion and radicals generated by action of different toxicants during their metabolism. SOD activity increased in goldfish treated with BDE-47 and BDE-99 (alone and in combination), while CAT activity showed dose-dependent decreases after treatment with both compounds in the present study. This result was consistent with the result of a study conducted by Albina *et al*. (2010); the authors observed a significant increase in SOD activity and decrease in CAT activity in adult rats exposed to BDE-99. However, Bellés *et al*. (2010) reported that SOD and CAT activities in cerebellum of rats were significantly decreased when exposed to BDE-99. The changes of SOD and CAT activities in liver revealed that severe oxidative damage might occur in goldfish after exposure to BDE-47, BDE-99 and their mixtures. Raldúa *et al*. (2008) determined PBDE concentrations in field exposed fish and measured several biochemical and histological markers, and found that these concentrations were not only associated with high activities of phase I and II metabolic enzymes, oxidative stress in liver, but also with neurotoxicity in brain.

No single biomarker can unequivocally measure environmental degradation. A pool of available biomarkers, by allowing information to be summarized in the form of a multivariate data set, can provide a more useful basis for interpretation of ecotoxicological surveys (Beliaeff and Burgeot, 2002). In the present work, the neurotoxical parameter AChE, biotransformation enzymes EROD and GST and antioxidant defense enzymes SOD and CAT were used to calculate IBR. In general, AChE and SOD scores were higher and contributed more to IBR values. However, the IBR values seemed not to depend on any of the individual biomarkers. In comparison, AChE for two individual chemicals, EROD for BDE-99 and mixtures, GST for BDE-47, SOD for BDE-99, and CAT for mixtures, showed higher scores. The IBR results are shown in Table 1. With regard to IBR variation of individual PBDE exposures, dose dependence and time dependence were apparent. The maximum IBR value was always observed at day 4 of exposure in all cases.

Given that the IBR is an indicator of environmental stress, BDE-99 appeared to be more stressful than BDE-47 toward the goldfish. However, the IBR results displayed different manners of joint action dependent to mixture dosages, and it seemed to be synergistic effect at 1 mg/kg and antagonistic effect at 5 mg/kg and 20 mg/kg. The results suggested that the IBR might be a useful tool for quantification of integrated responses induced by toxic chemicals toward fish. IBR method has been previously used as a useful tool for environmental risk assessment (Damiens *et al*., 2007; Wang *et al*., 2011). Recently, IBR was also used to identify the toxicological effects of organic pollutants toward fish including per fluorinated organic

Table 1. Integrated biomarker responses of two PBDEs (alone and in combination)

IBR	Compound	1 mg/kg	5 mg/kg	10 mg/kg
2 d	BDE-47	3.38	7.96	9.94
	BDE-99	2.78	7.02	11.35
4 d	BDE-47	10.83	15.87	15.61
	BDE-99	10.72	19.45	17.29
	BDE-47+BDE-99	13.67	10.51	9.36
7 d	BDE-47	7.28	6.39	4.73
	BDE-99	9.91	11.59	11.21

compounds (Kim *et al*., 2010), fungicide (Li *et al*., 2011a) and verapamil (Li *et al*., 2011b).

CONCLUSION

The present study investigated the biological effects of two PBDEs on goldfish and a set of biochemical responses were determined in different tissues. Decreased AChE and CAT activities and increased EROD, GST and SOD activities were observed in the goldfish exposed to BDE-47 and BDE-99 (alone and in combination), and concentration dependence and time dependence were apparent. The results suggest that these biomarkers should be addressed in ecological risk assessments of PBDEs in fish. Finally, IBR method was used to identify the toxicological effects of different PBDEs and BDE-99 showed more adverse biological effects than BDE-47. Co-exposure of BDE-47 and BDE-99 produces a synergistic effect at low dosage and an antagonistic effect at high dosage with regard to IBR variation. It is shown that IBR might be a useful tool for quantification of integrated responses induced by toxic chemicals toward fish.

ACKNOWLEDGEMENT

This study was supported by the National Natural Science Foundation of China (51079049) and Qing Lan Project of Jiangsu Province.

REFERENCES

Albina, M. L., Alonso, V., Linares, V., Bellés, M., Sirvent, J. J., Domingo, J. L. and Sánchez, D. J. (2010). Effects of exposure to BDE-99 on oxidative status of liver and kidney in adult rats. Toxicology, **271**, 51-56.

Alonso, V., Linares, V., Bellés, M., Albina, M. L., Pujol, A., Domingo, J. L. and Sánchez, D. J. (2010). Effects of BDE-99 on hormone homeostasis and biochemical parameters in adult male rats. Food Chem. Toxicol., **48**, 2206-2211.

Beliaeff, B. and Burgeot, T. (2002). Integrated biomarker response: a useful tool for ecological risk assessment. Environ. Toxicol. Chem., **21**, 1316-1322.

Bellés, M., Alonso, V., Linares. V., Albina, M. L., Sirvent, J. J., Domingo, J. L. and Sánchez, D. J. (2010). Behavioral effects and oxidative status in brain regions of adult rats exposed to BDE-99. Toxicol. Lett., **194**, 1-7.

Binelli, A., Cogni, D., Parolini, M. and Provini, A. (2010). Multi-biomarker approach to investigate the state of contamination of the R. Lambro/R.Po confluence (Italy) by zebra mussel (*Dreissena polymorpha*). Chemosphere, **79**, 518-528.

Boon, J. P., van Zanden, J. J., Lewis, W. E., Zegers, B. N., Goksøyr, A. and Arukwe, A. (2002). The expression of CYP1A, vitellogenin and zona radiata proteins in Atlantic salmon (*Salmo salar*) after oral dosing with two commercial PBDE flame retardant mixtures: absence of short-term responses. Mar. Environ. Res., **54**, 719-724.

Bosveld, A. T. C., de Bie, P. A. F., van den Brink, N. W., Jongepier, H. and Klomp, A. V. (2002). In vitro EROD induction equivalency factors for the 10 PAHs generally monitored in risk assessment studies in The Netherlands. Chemosphere, **49**, 75-83.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., **72**, 248-254.

Braekevelt, E., Tittlemier, S. A. and Tomy, G. T. (2003). Direct measurement of octanol-water partition coefficients of some environmentally relevant brominated diphenyl ether congeners. Chemosphere, **51**, 563-567.

Chen, C. E., Chen, J. W., Zhao, H. X., Xie, Q., Yin, Z. Q. and Ge, L. K. (2010). Levels and patterns of polybrominated diphenyl ethers in children's plasma from Dalian, China. Environ. Int., **36**, 163-167.

Cheng, J. P., Gu, J. M., Ma, J., Chen, X., Zhang, M. C. and Wang, W. H. (2009). Neurobehavioural effects, redox responses and tissue distribution in rat offspring developmental exposure to BDE-99. Chemosphere, **75**, 963-968.

Damiens, G., Gnassia-Barelli, M., Loquès, F., Roméo, M. and Salbert, V. (2007). Integrated biomarker response index as a useful tool for environmental assessment evaluated using transplanted mussels. Chemosphere, **66**, 574-583.

Feng, C. L., Xu, Y. P., Zha, J. M., Luo, Q., Shan, X. Q. and Wang, Z. J. (2010). Toxicokinetics and the related metabolites in rainbow trout (*Oncorhynchus mykiss*) after exposure to decabromodiphenyl ether. Sci. China Chem., **53**, 2379-2386.

Fischer, C., Fredriksson, A. and Eriksson, P. (2008). Coexposure of neonatal mice to a flame retardant PBDE 99 (2,2',4,4',5-pentabromodiphenyl ether) and methyl mercury enhances developmental neurotoxic defects. Toxicol. Sci., **101**, 275-285.

Frasco, M. F. and Guilhermino, L. (2002). Effects of dimethoate and beta-naphthoflavone on selected biomarkers of *Poecilia reticulata*. Fish Physiol. Biochem., **26**, 149-156.

Góth, L., (1991). A simple method for determination of serum catalase activity and revision of reference range. Clin. Chim. Acta, **196**, 143-151.

Guilhermino, L., Celeste Lopes, M., Carvalho, A. P. and Soares, A. M. (1996). Inhibition of acetylcholinesterase activity as effect criterion in acute tests with juvenile *Daphnia Magna*. Chemosphere, **32**, 727-738.

Hale, R. C., La Guardia, M. J., Harvey, E. P., Matteson Mainor, T., Duff, W. H. and Gaylor, M. O. (2001). Polybrominated diphenyl ether flame retardants in virginia freshwater fishes (USA). Environ. Sci. Technol., **35**, 4585-4591.

Han, Z. X., Lv, C. X. and Zheng, Z. R., (2010). Toxicological effects of PBDEs on *Carassius aurats*. Chinese J. Geochem., **29**, 217-222.

Kim, W. K., Lee, S. K. and Jung, J., (2010). Integrated assessment of biomarker responses in common carp (*Cyprinus carpio*) exposed to perfluorinated organic compounds. J. Hazard. Mater., **180**, 395-400.

Lema, S. C., Schultz, I. R., Scholz, N. L., Incardona, J. P. and Swanson, P., (2007). Neural defects and cardiac arrhythmia in fish larvae following embryonic exposure to 2,2',4,4'-tetrabromodiphenyl ether (PBDE 47). Aquat. Toxicol., **82**, 296-307.

Li, Z. H., Velisek, J., Grabic, R., Li, P., Kolarova, J. and Randak, T. (2011a). Use of hematological and plasma biochemical parameters to assess the chronic effects of a fungicide propiconazole on a freshwater teleost. Chemosphere, **83**, 572-578.

Li, Z. H., Velisek, J., Zlabek, V., Grabic, R., Machova, J., Kolarova, J., Li, P. and Randak, T. (2011b). Chronic toxicity of verapamil on juvenile rainbow trout (*Oncorhynchus mykiss*): effects on morphological indices, hematological parameters and antioxidant responses. J. Hazard. Mater., **185**, 870-880.

Lu, G. H., Wang, C. and Zhu, Z. (2009). The dose–response relationships for EROD and GST induced by polyaromatic hydrocarbons in *Carassius auratus*. Bull. Environ. Contam. Toxicol., **82**, 194-199.

Lundgren, M., Darnerud, P. O., Molin, Y., Lilienthal, H., Blomberg, J. and Ilbäck, N. G. (2007). Viral infection and PBDE exposure interact on CYP gene expression and enzyme activities in the mouse liver. Toxicology, **242**, 100-108.

Marklund, S. and Marklund, G. (1974). Involvement of superoxide anion radical in the auto oxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem., **47**, 469-474.

Miron, D. D., Crestani, M., Shettinger, M. R., Morsch, M. V., Baldisserotto, B., Tierno, A. M., Moraes, G. and Vieira, V. L. (2005). Effects of the herbicides clomazone, quinclorac, and metsulfuron methyl on acetylcholinesterase activity in the silver catfish (*Rhamdia quelen*) (Heptapteridae). Ecotox. Environ. Safe., **61**, 398-403.

Palace, V. P., Dick, T. A., Brown, S. B., Baron, C. L. and Klaverkamp, J. F. (1996). Oxidative stress in Lake Sturgeon (*Acipenser fulvescens*) orally exposed to 2,3,7,8-tetrachlorodibenzofuran. Aquat. Toxicol., **35**, 79-92.

Peebua, P., Kosiyachinda, P., Pokethitiyook, P. and Kruatrachue, M. (2007). Evaluation of alachlor herbicide impacts on nile tilapia (*Oreochromis niloticus*) using biochemical biomarkers. Bull. Environ. Contam. Toxicol., **78**, 128-131.

Raldúa, D., Padrós, F., Solé, M., Eljarrat, E., Barceló, D., Riva, M. C. and Barata, C. (2008). First evidence of polybrominated diphenyl ether (flame retardants) effects in feral barbel from the Ebro River basin (NE, Spain). Chemosphere, **73**, 56-64.

Rao, J. V. (2006). Toxic effects of novel organophosphorus insecticide (RPR-V) on certain biochemical parameters of euryhaline fish, *Oreochromis mossambicus*. Pestic. Biochem. Phys., **86**, 78-84.

Richardson, B. J., Mak, E., De Luca-Abbott, S. B., Martin, M., McClellan, K. and Lam, P. K. (2008). Antioxidant responses to polycyclic aromatic hydrocarbons and organochlorine pesticides in green-lipped mussels (*Perna viridis*): do mussels "integrate" biomarker responses? Mar. Pollut. Bull., **57**, 503-514.

Stone, D. (2006). Polybrominated diphenyl ethers and polychlorinated biphenyls in different tissue types from chinook salmon (*Oncorhynchus tshawytscha*). Bull. Environ. Contam. Toxicol., **76**, 148-154.

Tagliaferri, S., Caglieri, A., Goldoni, M., Pinelli, S., Alinovi, R., Poli, D., Pellacani, C., Giordano, G., Mutti, A. and Costa, L. G. (2010). Low concentrations of the brominated flame retardants BDE-47 and BDE-99 induce synergistic oxidative stress-mediated neurotoxicity in human neuroblastoma cells. Toxicol. in Vitro, **24**, 116-122.

Teles, M., Pacheco, M. and Santos, M. A. (2005). *Sparus aurata* L. liver EROD and GST activities, plasma cortisol, lactate, glucose and erythrocytic nuclear anomalies following short-term exposure either to 17β-estradiol (E_2) or E_2 combined with 4-nonylphenol. Sci. Total Environ., **336**, 57-69.

Tittlemier, S. A., Forsyth, D., Breakell, K., Verigin, V., Ryan, J. J. and Hayward, S. (2004). Polybrominated diphenyl ethers in retail fish and shellfish samples purchased from Canadian markets. J. Agr. Food Chem., **52**, 7740-7745.

Voorman, R. and Aust, S. D. (1987). Specific binding of polyhalogenated aromatic hydrocarbon inducers of cytochrome P-450d to the cytochrome and inhibition of its estradiol 2-hydroxylase activity. Toxicol. Appl. Pharm., **90**, 69-78.

Wang, C., Lu, G. H., Cui, J. and Wang, P. F. (2009a). Sublethal effects of pesticide mixtures on selected biomarkers of *Carassius auratus*. Environ. Toxicol. Pharm., **28**, 414-419.

Wang, C., Lu, G. H., Wang, P. F., Wu, H., Qi, P. D. and Liang, Y. (2011). Assessment of environmental pollution of Taihu Lake by combining active biomonitoring and integrated biomarker response. Environ. Sci. Technol., **45**, 3746-3752.

Wang, Z., Ma, X. D., Lin, Z. S., Na, G. S., Yao, Z. W. (2009b). Congener specific distributions of polybrominated diphenyl ethers (PBDEs) in sediment and mussel (*Mytilus edulis*) of the Bo Sea, China. Chemosphere, **74**, 896-901.