Bioavailability and toxicity variation of benzo[a]pyrene in three soil-wheat systems: Indicators of soil quality

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Running title: variation of benzo[a]pyrene in three soil-wheat systems

Bioavailability and toxicity variation of benzo[a]pyrene in three soil-wheat systems: Indicators for soil quality

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Abstract: Benzo[a]pyrene (B[a]P) as a representative polycyclic aromatic hydrocarbons is concerned by global scientists in various fields, but its biological and biochemical actions in soil-wheat systems are still rarely reported. The B[a]P as a ubiquitous soil pollutant possesses varied contents in real environment, and herein was studied in systems of soil and wheat to obtain relative results to reveal their variations in different systems. Its bioavailability (extractability and bioaccumulation) and basic biological toxicity were tested based on three typical soil types (red, black, and brown) in China and spiked amounts (0.1, 1, and 10 mg/kg) with several orders of magnitude. Results showed that B[a]P concentrations in soil-wheat systems extracted by HPCD were insignificantly ($p > 0.05$) higher than Tenax-TA, and varied with soil types and spiked concentrations. Besides, the root and shoot length were mostly inhibited, in a range of -21.85%-26.35% and -0.48%-54.85%, respectively, by B[a]P in different soil types and increased with its increasing concentration. Comparatively, higher bioconcentration factor and translocation factor values were observed under lower group in red soil-wheat systems, and higher spiked groups in black and brown soil-wheat systems. Moreover, inhibitive effects posed by B[a]P were mainly targeted at wheat shoots in these soils. The simultaneous studies provided a comparable knowledge of B[a]P in ecosystems of different soil types combined with different plant species due to lots of variations, further to serve for contaminated soil remediation and sustainable agricultural management.

Keywords: Benzo[a]pyrene; Soil bioavailability; Biological toxicity; Biochemical indicator; Soil-wheat system

1. INTRODUCTION

Benzo[a]pyrene (B[a]P) with five fused benzene rings, is a ubiquitous soil pollutant, which has been regarded as a representative indicator of polycyclic aromatic hydrocarbons, due to its persistence, biological toxicity, carcinogenicity, mutagenicity, and potential for bioaccumulation (IARC, 2012; Du et al., 2020; Lemieux et al., 2015). Reports showed that there exist no significant mineralization of

B[a]P in soils in the rates and extents even over a 90 d incubation period (Anyanwu et al., 2018). Although non-extractable residues (NER) could be formed after 33 d of aging, the remobilization risks still exist depending on the aging time, spiked concentrations, and soil properties (Luo et al., 2012; Umeh et al., 2018). Nowadays, several methods (physical, chemical and biological) have been developed to extract or degradate B[a]P. For instance, it is found that B[a]P absorption in roots, stems, leaves and shoots of ornamental plants (*T. patula* and *M. jalapa*) was positively related to its concentrations in soil, and plant roots could effectively promote phytoremediation of B[a]P contaminated soils (Sun & Zhou, 2016). Besides, the growth of ryegrass plants could be enhanced by B[a]P in acid sandy soil, which may help to dissipate B[a]P in soil at concentrations over 50 mg/kg soil (Xing et al., 2006).

Till now, the studies on bioavailability and biological toxicity of B[a]P in soil-wheat systems are still far from sufficiency, and it is hard to obtain a comparable knowledge due to several variations from applied methods, soil types, growing periods. Reports also showed that the increased pH and EC in sandy soil and ageing in clayey soil caused >55% and >85% of the changes in B[a]P extractability, respectively (Meng et al, 2019). It is found that the addition method (one-time or multiple-time addition) could affect the bioavailability of B[a]P in soils, its uptake by earthworms, as well as the enzyme activities in earthworm coelomocytes, due to the B[a]P age in soil (Ye et al., 2019). With the accumulation of B[a]P in receptors, varying degrees of damage were observed, such as the ultrastrutural damage in spring barley tissues depending on concentrations (Fedorenko et al., 2020). Nowadays, several methods have been developed to evaluate chemical bioavailability, including the extraction method and bioconcentration test (Li et al., 2016). And it has been demonstrated that the stimulative/inhibitory effects on wheat root and shoot

elongation as well as endogenous phytohormones, were sensitive bioindicators to biological toxicity of B[a]P (Li et al., 2007; Liu et al., 2012).

Environmental behaviors of B[a]P, and its mechanisms of bioaccumulation and phytodegradation were investigated in a number of studies. It is demonstrated that the migration of B[a]P in soils and its accumulation in plants mainly depended on soil and plants interactions (Sushkova et al., 2018). The differentiated physicochemical and geochemical properties especially soil organic carbon in different soil types, affected the adsorption of organic chemicals including B[a]P (Xing et al., 2006). Meanwhile, plants provided root surface for the microbial population growth, and the interactions between root exudates, microbes and contaminants stimulated the growth of soil microbes (microbial activities, enzyme-catalyzed processes, cometabolic processes etc.) which caused the degradation of B[a]P (Sun & Zhou, 2016). Its metabolites have been identified in some studies, corvering B[a]P-1,6- and 3,6-quinones, 3- and 9-hydroxyl B[a]P, trans-9,10-dihydroxy-9, 10-dihydro B[a]P, trans-7,8-dihydroxy-7, 8-dihydro B[a]P, and trans-4,5-dihydroxy-4,5-dihydro B[a]P, some of which may be more toxic than their parent B[a]P and may be the optimum co-metabolic substances (Zang et al., 2007). Besides, through plant cells or intercellular spaces, B[a]P could involve into plant metabolic processes, and induce adverse effects, covering the damage of plant membrane functions and structures, and further the disturbance of plant photosynthetic systems (Sushkova et al., 2018). In this study, we mainly concerned the content of parent B[a]P in different types of soil and wheat roots and shoots, and its overall effects on wheat.

Herein, we tested the bioavailability (extractability and bioaccumulation) and basic biological toxicity of B[a]P in different soil-wheat systems, based on three typical soil types (red, black and brown) in China, and spiked concentrations (0.1, 1,

and 10 mg/kg) with several orders of magnitude. We measured its extractability through two widely applied methods (HPCD and Tenax-TA extraction), its biological accumulation (BCF) and biochemical translocation (BTF), as well as responses of wheat (germination, root and shoot length) to B[a]P. We carried out this study aiming to provide a comparable knowledge of its bioavailability and basic biological toxicity in soil-wheat systems under the same experimental condition, and to provide some reference for contaminated soil remediation and sustainable agricultural management.

2. MATERIALS AND METHODS

2.1 Tested samples and materials

The brown, black and red soil samples were collected/purchased from Tianjin (forest park), Jilin (Changbai Mountain) and Hunan (azalea soil) Province, respectively. Their physical-chemical properties were measured, including pondus Hydrogenii (pH), soil organic matter (SOM), cation exchange capacity (CEC), soil salinity (SS), total nitrogen (TN), total phosphorus (TP), total potassium (TK) (Table 1). The B[a]P contents in different soil types were not determined as for the varied levels in real environment, and specifically concerned in soil-wheat systems to obtain relative results to reveal variation in different systems. The Tenax-TA beads and Hydroxypropyl-beta-cyclodextrin (HPCD) were purchased from ANPUL Laboratory Technology Inc. in Shanghai, China. The B[a]P, mixed standard solutions of 16 PAHs (500 μg/mL), and 5 deuterated PAHs (200 μg/mL) were bought from J&K China Chemical Ltd. in Beijing, China. And the tested wheat seeds *(Triticum aestivum* L.) were bought from a seed shop in Tianjin. The solvents (n-hexane, dichloromethane, acetone etc.) were chromatographic grade. High purity water was prepared from a Milli-Q Water System.

2.2 Experimental processes

Four treatments (CK, 0.1, 1 and 10 mg/kg) were designed for soil-wheat systems, and three parallels were prepared for each group. Soils were air-dried and passed through a 2-mm sieve to ensure soil homogeneity. Firstly, B[a]P was dissolved using acetone, added into soils, stirred evenly, and then placed in a fume hood until the acetone was fully volatilized. Secondly, 50-g soil was put into a petri dish (90 mm), 15 seeds were added after sterilization by 3% H_2O_2, and 10 mL water were added. Next, petri dishes were put in a constant temperature incubator at 25 ^oC in dark, and added same volume water daily to maintain soil water content. Soil and wheat samples were sampled after 10 days for the analyses.

2.3 Bioavailable extraction in soils planted with wheats

The 1-g freeze dried soil was put into a tube and extracted with 20 mL of HPCD (50 mmol), and 1 mg $HgCl_2$ was added to prevent the microbial degradation. The tube was put in a constant temperature shaker for 24h at 150 rpm and 25 ^oC, and centrifugated for 20 min at 3000 rpm. Then, 10 mL HPCD solution was extracted with 10 mL hexane, mixed for 2 min, and the organic solvent was collected. The extraction was repeated three times, and the solvents were condensed to 1 mL for the the determination of B[a]P.

Another 1-g soil was put into a tube, with 0.2-g Tenax-TA, 20-mL high purity water, and 1-mg $HgCl_2$. The tube was put in a constant temperature shaker for 24h at 150 rpm and 25 ^oC, and centrifugated for 20 min at 3000 rpm. Then, Tenax-TA beads were collected, washed with water, and extracted with 15 mL of hexane/acetone (3:1, v/v) for 30 min. The extraction was repeated three times, and the solvents were concentrated to dryness, redissolved with hexane, and determined for B[a]P. Increased ratios were calculated based on the formula: Increased ratio = (T-C-O)/O, where T is the average concentration of B[a]P extracted from the spiked group, C is the average

value from CK, and O is the original spiked concentration.

2.4. Exhausted extraction in soils, wheat roots and shoots

The freeze-dried 5-g soil and wheat samples (<1 g) were weighted to determine the exhausted contents of B[a]P. Each sample was extracted with 20 ml dichloromethane by ultrasonication for 15 min. The suspension was centrifuged at 4000 rpm for 20 min, and the supernatant was collected. The extraction was repeated three times, and all of the extracts were concentrated to dryness with a rotary evaporator at 40 ^{o}C. After that, 2-mL n-hexane was added before column purification. The columns were filled with silicone (1 g), alumina (1 g), and anhydrous sodium sulfate (1 g) from bottom to up, and activated with 10 mL n-hexane. The samples were loaded, washed with 3 mL n-hexane, and eluated with 12 mL of n-hexane/dichloromethane (1:1, v/v). The extracts were dried and redissolved with hexane for the determination of B[a]P with a recovery of 80-110%. The bioconcentration factor (BCF) and biochemical translocation factor (BTF) were calculated based on the formulas:

$$BCF_{root/shoot} = C_{root/shoot}/C_{soil} \quad (1)$$

and

$$BTF = C_{shoot}/C_{root} \quad (2)$$

Where $C_{root/shoot}$, C_{soil}, C_{shoot} and C_{root} are the concentration in wheat root/shoot, soil, shoot and root, respectively.

2.5 Toxic indicators for wheats

The germinations were counted, and the wheat samples were washed with tap and distilled water, dried with filter paper, and then separated into roots and shoots. the root and shoot lengths were measured using rhizograph software (EPSON, STD4800). The germination rates and (root and shoot) inhibition rates were calculated based the formulas: Inhibition rate (%) = [(a-b)/a]×100%, Germination rate (%) = n/15×100%,

where a is the average value of root/shoot length in CK, b is the average value in the spiked group, n is the germination number in each group.

2.6 Determining methods and statistical analysis

The B[a]P concentration was determined through the gas chromatography mass spectrometry (GC-MS, Agilent 7890A-5975) with a TR5-MS capillary column. The helium was applied as the carrier gas at a constant flow of 1 mL/min. The column temperature was started at 70 °C and held for 1 min, and then was increased at 10 °C/min to 260 °C and held for 4 min, which was followed by 5 °C/min to 300 °C and held for 4 min. Data were statistically processed with the Microsoft Excel software, Origin 9.0 and IBM SPSS 22, and were expressed as mean ± standard deviation (SD). Difference between HPCD and Tenax-TA extraction was analyzed through the Wilcoxon Signed-Rank test (* $p < 0.05$).

3. RESULTS

3.1 Extractability of B[a]P in soils planted with wheat

The B[a]P in soil samples was extracted by HPCD and Tenax-TA, respectively. Its averaged concentrations and increased ratio were depicted in Figure 1, and provided in Table S1. Results showed that there was not significant difference ($p > 0.05$) between the concentrations extracted by HPCD and Tenax-TA in these soil-wheat systems, but extracted concentrations by HPCD were generally higher than that by Tenax-TA, except for the brown soil samples. Generally, results indicated that two methods were equivalently efficient on the evaluation of B[a]P bioavailability, but varied with soil types.

Deducting the B[a]P concentration extracted from the un-spiked planted soil samples, the extracted concentrations by HPCD and Tenax-TA in the lower spiked groups were generally higher than the original spiked amounts (0.1 and 1 mg/kg),

while lower in the higher spiked group (10 mg/kg). The increased amounts were one order of magnitude larger than 0.1 mg/kg in the red and black soils, and ≤2.92-time as much as 1 mg/kg. Moreover, the increased ratios in red and black soils (black > red in 0.1 mg/kg group, red > black in 1 mg/kg group) were generally higher than that in brown soil. In brown soil samples, the extracted concentrations of B[a]P by HPCD were consistent with the aforementioned trend. Results indicated that there may stimulating effects on wheats in the lower spiked groups, and it seemed that both the effects were stronger in the black and red soils.

3.2 Accumulation of B[a]P in wheat roots and shoots

The B[a]P contents in wheat roots and shoots were depicted in Figure 2 and provided in Table S2, and its BCF and BTF were calculated and provided in Table 2. In the spiked groups, the contents in wheat roots were generally lower than those in the un-spiked planted soil group (CK), except for the 10 mg/kg group in black soil. While the contents in wheat shoots were generally higher than CK, except for the groups in brown soil. According to the BCF values, it showed that BCF_{root} and BCF_{shoot} were generally lower than those in CK, except for BCF_{root} in brown soil (1 mg/kg group), and BCF_{shoot} in red soil (0.1 and 1 mg/kg group). The BTF values in the spiked groups were generally more than one and higher than those in CK. In the spiked groups, the BCF and BTF values in red soil-wheat systems were generally higher than those under the lower spiked groups, while were generally higher under the higher spiked groups in black and brown soil-wheat systems. Results indicated that B[a]P was more likely accumulated and translocated into shoots in red soils below 1 mg/kg in this study, while higher accumulation and translocation in wheat in the black and brown soils under higher spiked groups.

3.3 Response of wheat germination, root and shoot length

The germination, root and shoot inhibition rates were provided in Figure 3 and Table S3. In the spiked groups, the rates generally increased in the red soils and decreased in the brown soil with an increasing concentration, except for the 1 mg/kg group. Nevertheless, the rates in the black soil increased under the group of 0.1 mg/kg, and decreased under the higher spiked groups. The root lengths were stimulated in the red and brown soils under the lower spiked groups (0.1 and 1 mg/kg), while inhibited under the group of 10 mg/kg. And both the stimulating and inhibitive effects were stronger in the red soil. In the black soil, the root lengths were inhibited under all of the spiked groups, which increased with an increasing concentration. However, the shoot lengths in red and brown soils were inhibited under all of the spiked groups. The inhibitive effect generally increased with an increasing concentration, and was stronger in the brown soil under the lower spiked groups. The shoot lengths in black soil were stimulated under the group of 0.1 mg/kg, while inhibited under the higher spiked groups and the inhibitive effect increased with an increasing concentration. Results indicated that inhibitive effects within the tested concentrations mainly targeted at the shoots in the red soil, the germination and shoots in the brown soil, the germination, root, and shoot in the black soil.

4. DISCUSSION

4.1 Indicator selection and criteria derivation for B[a]P

For the protection of soil environment, various types of soil-environmental standards were issued in most of the countries and regions around the world, such as quality standards, screening levels, cleanup levels, investigation values, in the consideration of soil type, land use, soil depth or extraction methods (Teng & Zhou, 2018; Morales & Zuleta, 2019). For instance, the risk screening value of B[a]P in China for the agricultural land is 0.55 mg/kg, and its risk screening and intervention values for the

first type of development land (residential, greening areas etc.) are 0.55 and 5.5 mg/kg, respectively (GB 15618/36600-2018). While the background level of B[a]P usually fluctuates within 0.1-5 μg/kg in majority of mineral soils, and 15-20 μg/kg in some chernozems and peaty soils due to the increase in the concentration of organic chemicals and specific structure of soil microbial community (Sushkova et al., 2018).

The ecological criteria in different countries and regions are usually derived based on hazardous concentrations (HC_5/HC_{25}/HC_{50}) that were derived from various indictors applying the species sensitivity distribution (SSD) method, such as no observed effect concentration (NOEC), the lowest observed effect concentration (LOEC), and effect concentrations ($EC_{10/20/25/50}$). Collected from the studies on biological or ecological toxicity of B[a]P in different soil types and receptors, the indicators of NOEC, LOEC, EC_{10}, and EC_{50} were in the range of 1-293, 1-977, 1.3-106, and 1.24-1.83 mg/kg, respectively (Table S4). The available indicators were quite limited, and fluctuated by various orders of magnitude due to different endpoints, receptors, and soil types. In this study, our results exhibited variations on extractability, bioaccumulation and biological toxicity of B[a]P in different soil types-wheat systems under the exposure of concentrations with several orders of magnitude (0, 0.1, 1, and 10 mg/kg). Various simultaneous studies were required to provide more further comparable results, to promote the revision and development of soil standards to serve for clean soil protection and contaminated soil remediation.

4.2 Efficiency of extraction methods to test bioavailability of B[a]P

Bioavailability is critical to ecological risk assessment, criteria development and other aspects, which determines the exposure of organisms to chemicals associated with soils (Semple et al., 2004). It played an important role in its toxic effects both on plants, invertebrates and microorganisms. Results found that there was no significant

296mineralization of B[a]P in soils in the rates and extents even over a 90 d incubation
297period (Anyanwu et al., 2018), and existed the remobilization risk of nonextractable
298residues (NERs depending on the aging, spiked concentration, and soil properties
299(Luo et al., 2012; Umeh et al., 2018). It is reported that significantly greater (p <0.05)
300DNA percentage (%) in the tails and olive tail moments of earthworms were observed
301under the exposure to readily available B[a]P in soils, than B[a]P NERs (Umeh et al.,
3022019). In this study, consistent conclusions were obtained based on the results through
303HPCD and Tenax-TA extraction, while the extractability of HPCD seemed higher
304than Tenax-TA.

305**4.3 Possible mechanisms of B[a]P effects in soil-wheat systems**

306As shown in many studies, plant species (root exudates and morphology) and soil
307types (properties and soilborne microorganisms) were the major influencing factors
308on the structure and function of microbial communities especially in the rhizosphere
309(Berg and Smalla, 2009; Lauber et al., 2008). The migration of B[a]P in soil and its
310accumulation in plants were mainly affected by adsorptive properties of a soil matrix,
311and its own properties (Sushkova et al., 2018). Different soil types (red, black and
312brown soils in this study) possessed discriminable physicochemical properties and
313effects especially from SOM (Xing et al., 2006). Even in a similar soil type (pH-6.51,
314SOM-1.67%, and CEC-16.29 cmol/kg) with this work, only inhibitive effects on the
315wheat root and shoot length could be observed in the range of 0-2 mg/kg (Liu et al.,
3162012).

317 Different plant species had different exposure tolerance and degradative ability to
318B[a]P. Similarly, the content of B[a]P in different tissues (roots, stems, leavers and
319shoots) of ornamental plants (*Tagetes patula* and *Mirabilis jalapa*) was positively (p
320<0.01) related with that in soils (meadow burozem). Meanwhile, their dry biomass

increased at low B[a]P contaminated soil and then inhibited with increasing B[a]P concentrations, but they had low BF and BTF values. 2.7-26.8%, and 0.4%-33.9% of B[a]P removal rates in rhizosphere soils were observed at their different growing stages, mainly through stimulating soil microbe growth, which was caused by the interactions between root exudates, microbes and contaminants (Sun and Zhou, 2016). So the effects exhibited variation due to the interactions between B[a]P and specific soil-plant systems.

5. CONCLUSIONS

In this work, we simultaneously investigated the variations on bioavailability and biological toxicity of B[a]P in different soil-wheat systems, by considering different soil types, wheat planting and exposed concentrations with different orders of magnitude. Results showed that there exist no significant difference (p >0.05) between the HPCD and Tenax-TA extraction methods, but the extracted contents were generally higher through HPCD. Besides, the root (-21.85%-26.35%) and shoot length (-0.48%-54.85%) were mostly inhibited by B[a]P in different soil types and increased with an increasing concentration. There are higher wheat BCF and BTF values of B[a]P in red soil under the lower spiked groups, and in black and brown soils under the higher spiked groups. Meanwhile, within the tested concentrations of B[a]P, its inhibitive effects mainly targeted at the shoots in the red soil, shoots and germination in the brown soil, shoot, root and germination in the black soil. Generally, this study simultaneously provided a knowledge about the bioavailability and basic biological toxicity of B[a]P in different soil-wheat systems, and new insight of soil biology and biochemistry for clean soil protection and contaminated soil remediation.

Acknowledgments

The authors are grateful for the financial support by the National Natural Science Foundation of China as a general project (grant No. 21677080), and the Ministry of Education, People's Republic of China as the 111 program (T2017002).

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Table Captions

Table 1. The physicochemical properties of the tested soil samples. The abbreviations indicated pondus Hydrogenii (pH), soil organic matter (SOM), cation exchange capacity (CEC), soil salinity (SS), total nitrogen (TN), total phosphorus (TP), and total potassium (TK)

Table 2. The calculated BCF and BTF in the soil-wheat systems. The red, black and brown indicated the soil types. The CK, 0.1, 1, and 1 mg/kg indicated the spiked B[a]P concentrations; The 'a' and 'b' indicated the BCF_{root} and BCF_{shoot}, respectively.

Table S1. The exhausted and bioextractable contents (mg/kg) of B[a]P in soil samples planted with wheat.

Table S2. The B[a]P content data (mg/kg) in wheat roots and shoots.

Table S3. The inhibition rates (%) of the wheat root and shoot length and wheat germination rate (%) under the exposure of B[a]P.

Table S4. Indicators from biological or ecological toxicity studies on B[a]P in different receptors and soil types.

Figure Captions

Fig. 1. The averaged concentrations (mg/kg) and increased ratios of B[a]P in soil-wheat systems extracted by HPCD and Tenax-TA. (a-c) in red, black, and brown soil. The treatments include the CK, 0.1, 1, and 10 mg/kg groups.

Fig. 2. The averaged concentrations (mg/kg) of B[a]P in wheat roots and shoots in different soil types. (a) in wheat roots among red, black, and brown soils; (b) in wheat shoots among red, black, and brown soils; (c) in wheat roots and shoots of red soil; (d) in wheat roots and shoots of black soil; (e) in wheat roots and shoots of brown soil. The treatments include the CK, 0.1, 1, and 10 mg/kg groups.

Fig. 3. The inhibition rates (root and shoot length) (%) and germination rates (%) in different soil types and treatments. The soil types include the red, black, and brown soils. And the treatments include the CK, 0.1, 1, and 10 mg/kg groups.

Table 1. The physicochemical properties of the tested soil samples. The abbreviations indicated pondus Hydrogenii (pH), soil organic matter (SOM), cation exchange capacity (CEC), soil salinity (SS), total nitrogen (TN), total phosphorus (TP), and total potassium (TK)

Item	Red soil	Black soil	Brown soil
pH	6.15	6.67	8.50
SOM (%)	0.9	8.9	1.7
CEC (cmol/kg)	3.3	12.2	19.9
SS (g/kg)	0.3	0.7	1.7
TN (g/kg)	0.4	2.2	0.6
TP (g/kg)	13.1	3.6	4.2
TK (g/kg)	10.8	8.9	16.6

Table 2. The calculated BCF and BTF values in the soil-wheat systems. The red, black and brown indicated the soil types. The CK, 0.1, 1, and 1 mg/kg indicated the spiked B[a]P concentrations; The 'a' and 'b' indicated the BCF_{root} and BCF_{shoot}, respectively.

Treatment	BCF			BTF		
	Red	Black	Brown	Red	Black	Brown
CK	$381^{a}/95^{b}$	$234^{a}/680^{b}$	$149^{a}/278^{b}$	0.25	2.91	1.86
0.1 mg/kg	$52^{a}/110^{b}$	$103^{a}/95^{b}$	$46^{a}/124^{b}$	2.12	0.92	2.68
1 mg/kg	$61^{a}/153^{b}$	$33^{a}/122^{b}$	$265^{a}/249^{b}$	2.45	3.68	0.94
10 mg/kg	$42^{a}/35^{b}$	$114^{a}/125^{b}$	$84^{a}/205^{b}$	0.82	1.09	2.45