Pulmonary modulation of benzo[a]pyrene-induced hemato- and hepatotoxicity in broilers[1]

I. K. Latif,* A. J. Karim,* A. B. Z. Zuki,* M. Zamri-Saad,* J. P. Niu,† and M. M. Noordin*[2]

*Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia; and †School of Public Health, Lanzhou University, 73000 Lanzhou, Gansu, China

ABSTRACT Aftermath in several air pollution episodes with high concentrations of polycyclic aromatic hydrocarbons did not significantly affect health and performance of broilers despite its renowned sensitivity to polycyclic aromatic hydrocarbons. The aim of the study was to elucidate the previous lack of response in birds exposed to such severe episodes of air pollution. Benzo[a]pyrene (BaP) was used to simulate the influence of air pollution on hematology, selected organ function, and oxidative stress in broilers. One-day-old chicks were assigned to 5 equal groups composed of a control group, tricaprylin group, and 3 groups treated with BaP (at 1.5 μg, 150 μg, or 15 mg/kg of BW). The BaP was intratracheally administered to 1-d-old chicks for 5 consecutive days. The hematology, liver and kidney function, P450 activity, and malondialdehyde level especially in the group receiving 15 mg of BaP/kg of BW demonstrated evidence of hemato- and hepatoxicity via BaP-induced oxidative stress. The deleterious effect of exposure to high concentration of BaP in broiler chickens was probably due to the anatomy of this species and the half-life of BaP. Although the effect of BaP may be transient or irreversible, pathogen challenges faced during the period of suppression may prove fatal.

Key words: benzo[a]pyrene, hematotoxicity, hepatoxicity, oxidative stress, broiler

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INTRODUCTION

The respiratory tract is constantly exposed to a variety of noxious materials and air pollutants including those by polycyclic aromatic hydrocarbons (**PAH**; Kendall et al., 2002). Industrialization has resulted in substantial annual global discharge of this compound (King et al., 2002) into the environment (Saegerman et al., 2006). Suppression of the antioxidant defense by PAH, especially benzo[a]pyrene (**BaP**) through aryl hydrocarbon receptor (**AhR**), leads to the generation of reactive oxygen species (Briedé et al., 2004). Much more worrying is that regardless of its route of entry, BaP produces a systemic effect (Sun et al., 1982). Metabolism or biotransformation through the phase I (P450 monooxygenase enzymes) and phase II (conjugating enzymes) pathway are requisites for detoxification and excretion of lipophilic chemicals such as BaP (Goksøyr and Förlin, 1992).

Advances in studies on AhR have begun to clearly define its exact role (Karchner et al., 2006; Okey, 2007). Nevertheless, differences in AhR properties contributed to the differential sensitivity with chicken (*Gallus gallus*) exhibiting dramatic sensitivity to PAH (Karchner et al., 2006; Okey, 2007). Even though it is known that some PAH are immunotoxic (Knuckles et al., 2001), little has been done to determine the effect of BaP as indicators of contamination by PAH in broiler chickens. Our previous work revealed that intratracheal (**i.t.**) administration of 15 mg/kg of BW BaP impairs the nonspecific respiratory defense mechanism (Latif et al., 2009), which may lead to colonization of bacteria (Lorenzoni and Wideman, 2008). The present study is the first to investigate the effects of BaP on hematological parameters, serum biochemistry, and oxidative stress in broiler chickens in an attempt to explain the susceptibility of broilers to BaP. Likewise, it is also aimed at establishing the minimum toxic dose of BaP in poultry via the i.t route. Such studies in birds are also of importance because the metabolism of BaP may be used as a biomarker of exposure to environmental pollutants.

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[2]Corresponding author: noordin@vet.upm.edu.my

MATERIALS AND METHODS

Chemicals and Experimental Birds

All chemicals used such as BaP (Sigma, St. Louis, MO), tricaprylin (ICN, Costa Mesta, CA), 1-chloro-2,4-dinitrobenzene (Fluka Chemika, Buchs, Switzerland), glutathione (Sigma), and other chemicals were of analytical grade.

One hundred twenty 1-d-old male broiler chicks (Cobb strain) were purchased from a local hatchery and kept at the farm of the Department of Animal Science, Universiti Putra Malaysia. Upon arrival, the chicks were weighed and divided randomly into 5 equal groups of 24 chicks each in 3-tiered battery cages. The chickens were raised according to routine management practice. All nutrients including water were supplied ad libitum to meet the requirements of the NRC (1994).

Experimental Design

Experimental procedures were approved by the Faculty Animal Care and Use Committee (approval no. 08R29/July08-Jun09). Tricaprylin (ICN) was used as the solvent for BaP (Sigma). In each treatment, the total volume of inoculum instilled to each chick was 100 μL. Chicks within different treatment groups were treated intratracheally by using a micropipette (Eppendorf, Hamburg, Germany) for 5 successive days as follows: control (untreated) group, tricaprylin group (100 μL), BaP (dissolved in 100 μL of tricaprylin) groups (1.5 μg, 150 μg, or 15 mg/kg of BW, respectively). Before being killed at d 7, 14, 21, and 35 postinstillation (**p.i.**), blood samples were collected via the heart into plain and heparinized vacutainer tubes and then 6 birds/group were killed.

Hematology and Serum Biochemistry

Part of the heparinized blood was subjected to hematological determinations in a blood counter (Cell-Dyn 3700, Abbott Diagnostics, Abbott Park, IL) with standard reagents (Abbott Diagnostics) suitable for the analyzer. These included erythrocytic (red blood cells, **RBC**) values, leukocytic (white blood cells, **WBC**) counts, hemoglobin (**Hb**) concentration, packed cell volume levels, mean corpuscular volume, mean corpuscular Hb, and mean corpuscular Hb concentration. The remaining blood was centrifuged at 2,325 × *g* for 10 min to obtain the plasma, which was stored at −20°C until further use for the determination of malondialdehyde (**MDA**). The serum was used for determination of the following serum biochemical parameters: activities of alanine aminotransferase (**ALT**), gamma-glutamyl transferase (**GGT**), lactate dehydrogenase (**LDH**), alkaline phosphatase (**ALP**), aspartate aminotransferase (**AST**), creatine kinase (**CK**), and the concentrations of total protein (**TP**) and albumin levels using a clinical chemistry automated analyzer (Hitachi 902, Tokyo, Japan) with recommended standard reagents (Roche, Basel, Switzerland) suitable for this analyzer. All samples were analyzed within 2 h of collection.

Preparation of Homogenate

Lung and liver samples were carefully washed in ice-cold 0.9% NaCl and homogenized in ice-cold 1.15% potassium chloride in 50 m*M* potassium phosphate buffer solution (pH 7.4) to yield a 10% (wt/vol) homogenate using an Ultra Turrax T25 (IKA, Staufen, Germany). The homogenate was centrifuged at 15,000 × *g* for 30 min at 4°C and the supernatant was used for determination of MDA.

MDA Assay

Thiobarbituric acid reactive species were determined in duplicate. This method was described by Ohkawa et al. (1979).

Determination of Microsomal Cytochrome P450

The lung and liver microsomal fractions were prepared as described by Rivière et al. (1985). The pellet of the second centrifugation was resuspended in 0.25 *M* sucrose containing 10 m*M* phosphate buffer, pH 7.4, and was used for the determination of microsomal cytochrome P450.

The concentration of lung and liver cytochrome P450 homogenates was estimated from the dithionite-reduced difference spectrum of CO-bubbled samples as described by Omura and Sato (1964), using an absorption coefficient of 91 $mM^{-1}\cdot cm^{-1}$. Aliquots of tissue homogenate and microsomal pellets from each subfraction were analyzed for protein concentration using the BCA Protein Assay (Pierce Biochemical Co., Rockford, IL).

Glutathione S-Transferase Assay

The cytosolic glutathione S-transferase (**GST**) activity in lung and liver samples was determined in duplicate spectrophotometrically at 25°C according to the procedure of Habig et al. (1974). The specific activity of GST is expressed as micromoles of GSH-2,4-dinitrobenzene conjugate formed per minute per milligram of protein using an extinction coefficient of 9.6 $mM^{-1}\cdot cm^{-1}$.

Liver and Lung Weight, Hepatosomatic Index, and Pulmonosomatic Index

Because substantial BW change is anticipated in this study, hepatosomatic index (**HSI**) and pulmonosomatic index (**PI**) are of importance (Sellers et al., 2007). Liver and lungs procured at the stipulated p.i. interval were lightly blotted and individually weighed. Hence, the HSI and PI were calculated as follows:

$$\text{HSI} = \text{liver weight (g)/BW (g)} \times 100$$

$$\text{PI} = \text{lung weight (g)/BW (g)} \times 100.$$

Statistical Analysis

The data were subjected to a 1-way ANOVA using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL). Differences between means were determined using Tukey's test, in which the significance level was designated at $P < 0.05$. All data were checked for normality and homogeneity of variances.

RESULTS

The selected hematology of broilers during the experimental period is as shown in Table 1. A trend of an increase of selected erythrocytic values is seen in all groups during the course of the experiment. Commencing from d 7 through 21, broilers from the 15 mg of BaP group had the lowest ($P < 0.05$) total RBC and Hb values. Although the packed cell volume of the latter group was only significantly ($P < 0.05$) lower than that of the other groups at d 14, such differences were comparable to that of the 1.5 and 150 μg of BaP groups on d 7 and d 7 to 21, respectively. On the other hand, the mean corpuscular volume values remained comparable between all groups; those of the mean corpuscular Hb and mean corpuscular Hb concentration were the lowest in the 15 mg of BaP group at d 14. However, at d 35, no significant changes were seen in any group except the Hb value in the 15 mg of BaP group when compared with tricaprylin group. The morphology of RBC in chicks from the 15 mg of BaP group was that of normocytic and microcytic type.

Similarly, an increasing pattern of WBC was seen in all groups as time advanced (Table 2). However, such increment of WBC counts in the 15 mg of BaP group was the slowest and lowest ($P < 0.05$) commencing from d 7 until 21. The heterophil:lymphocyte ratio was higher ($P < 0.05$) in the 15 mg of BaP group at d 7 and 14 p.i. when compared with the other groups.

Table 1. Selected blood parameters of broilers during the trial (mean ± SD)

	Days p.i.[2]			
Parameter[1]	7	14	21	35
Total RBC ($\times 10^{12}$/L)				
Control	2.332 ± 0.057[a]	2.568 ± 0.054[a]	2.572 ± 0.076[a]	2.602 ± 0.104[a]
Tricaprylin	2.358 ± 0.054[a]	2.588 ± 0.055[a]	2.586 ± 0.039[a]	2.622 ± 0.077[a]
1.5 μg of BaP	2.342 ± 0.034[a]	2.508 ± 0.093[a]	2.514 ± 0.067[a]	2.558 ± 0.026[a]
150 μg of BaP	2.296 ± 0.049[a]	2.490 ± 0.092[a]	2.510 ± 0.066[a]	2.530 ± 0.060[a]
15 mg of BaP	2.110 ± 0.102[b,3]	2.242 ± 0.164[b,3]	2.338 ± 0.125[b,3]	2.470 ± 0.090[a,3]
Hb (g/L)				
Control	10.56 ± 0.677[a]	12.26 ± 0.272[a]	12.26 ± 0.32[a]	12.46 ± 0.392[ab]
Tricaprylin	10.48 ± 0.643[a]	12.20 ± 0.424[a]	12.40 ± 0.178[a]	12.60 ± 0.282[a]
1.5 μg of BaP	10.32 ± 0.713[ab]	11.88 ± 0.318[a]	12.34 ± 0.407[a]	12.30 ± 0.404[ab]
150 μg of BaP	10.18 ± 0.507[ab]	11.66 ± 0.870[a]	12.12 ± 0.667[a]	12.28 ± 0.386[ab]
15 mg of BaP	8.98 ± 0.727[b]	9.24 ± 0.516[b]	10.74 ± 0.920[b]	11.70 ± 0.435[b]
PCV (%)				
Control	29.2 ± 0.979[ab]	32.6 ± 0.489[a]	33.0 ± 1.095[a]	33.0 ± 0.894[a]
Tricaprylin	29.4 ± 1.019[a]	32.4 ± 0.800[a]	33.0 ± 0.894[a]	33.2 ± 0.979[a]
1.5 μg of BaP	28.6 ± 1.624[ab]	32.0 ± 1.264[a]	32.4 ± 1.019[a]	33.0 ± 0.894[a]
150 μg of BaP	29.2 ± 0.748[ab]	31.4 ± 1.356[a]	32.0 ± 1.414[ab]	32.4 ± 1.019[a]
15 mg of BaP	26.6 ± 1.624[b]	28.0 ± 0.894[b]	29.6 ± 1.200[b]	31.8 ± 1.166[a]
MCV (fL)				
Control	125.2 ± 3.427[a]	126.9 ± 1.389[a]	128.2 ± 0.835[a]	128.3 ± 3.759[a]
Tricaprylin	124.6 ± 1.841[a]	125.2 ± 4.566[a]	127.5 ± 2.069[a]	126.6 ± 3.365[a]
1.5 μg of BaP	122.0 ± 5.893[a]	127.6 ± 4.078[a]	128.8 ± 2.313[a]	129.0 ± 3.751[a]
150 μg of BaP	127.1 ± 1.789[a]	126.0 ± 2.099[a]	127.4 ± 2.432[a]	128.0 ± 2.197[a]
15 mg of BaP	125.9 ± 1.872[a]	125.2 ± 5.763[a]	126.5 ± 4.923[a]	128.7 ± 1.166[a]
MCH (pg)				
Control	45.33 ± 3.485[a]	47.75 ± 1.232[a]	47.64 ± 0.838[ab]	48.38 ± 0.791[a]
Tricaprylin	44.48 ± 3.179[a]	47.13 ± 0.869[a]	47.95 ± 0.391[ab]	48.06 ± 0.642[a]
1.5 μg of BaP	44.07 ± 3.182[a]	47.38 ± 0.724[a]	49.04 ± 0.760[a]	48.04 ± 1.168[a]
150 μg of BaP	44.33 ± 1.949[a]	46.77 ± 2.049[a]	48.24 ± 1.402[ab]	48.48 ± 1.087[a]
15 mg of BaP	42.50 ± 1.653[a]	41.26 ± 0.800[b]	45.87 ± 2.068[b]	47.40 ± 3.663[a]
MCHC (%)				
Control	36.16 ± 1.947[a]	37.61 ± 0.836[a]	38.00 ± 0.451[a]	37.70 ± 0.965[a]
Tricaprylin	35.72 ± 3.041[a]	37.67 ± 1.502[a]	38.03 ± 0.442[a]	37.97 ± 0.933[a]
1.5 μg of BaP	36.18 ± 3.013[a]	37.15 ± 0.914[a]	38.04 ± 0.920[a]	37.30 ± 1.700[a]
150 μg of BaP	34.87 ± 1.832[a]	37.09 ± 1.568[a]	37.80 ± 0.618[a]	37.86 ± 0.574[a]
15 mg of BaP	33.79 ± 0.792[a]	32.98 ± 0.987[b]	36.32 ± 2.574[a]	36.80 ± 1.093[a]

[a,b]Values bearing similar superscripts in the same column do not differ ($P < 0.05$).

[1]RBC = red blood cell; Hb = hemoglobin; PCV = packed cell volume; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; BaP = benzo[a]pyrene.

[2]p.i. = postinstillation.

[3]Red blood cell morphology was of the normochromic microcytic type.

Table 2. The white blood cell (WBC) counts and heterophil:lymphocyte (H:L) ratio of broilers during the trial (mean ± SD)

	Days p.i.[1]			
Parameter	7	14	21	35
Total WBC ($\times 10^9$/L)				
Control	19.02 ± 0.899[a]	21.56 ± 1.263[a]	21.48 ± 0.880[a]	21.79 ± 1.618[a]
Tricaprylin	19.40 ± 0.460[a]	20.76 ± 0.445[a]	20.32 ± 0.754[ab]	20.66 ± 0.605[a]
1.5 μg of BaP[2]	18.96 ± 0.300[a]	21.21 ± 0.792[a]	21.52 ± 1.244[a]	21.57 ± 1.323[a]
150 μg of BaP	19.02 ± 0.899[a]	20.66 ± 0.995[a]	20.34 ± 0.610[ab]	21.88 ± 0.851[a]
15 mg of BaP	16.26 ± 1.013[b]	16.40 ± 1.412[b]	18.86 ± 0.787[b]	20.83 ± 0.752[a]
H:L				
Control	0.457 ± 0.055[b]	0.388 ± 0.060[b]	0.408 ± 0.032[a]	0.383 ± 0.044[a]
Tricaprylin	0.467 ± 0.027[b]	0.437 ± 0.060[b]	0.449 ± 0.037[a]	0.438 ± 0.042[a]
1.5 μg of BaP	0.483 ± 0.037[b]	0.438 ± 0.060[b]	0.401 ± 0.041[a]	0.400 ± 0.018[a]
150 μg of BaP	0.462 ± 0.050[b]	0.445 ± 0.015[b]	0.417 ± 0.058[a]	0.409 ± 0.051[a]
15 mg of BaP	0.627 ± 0.119[a]	0.579 ± 0.059[a]	0.484 ± 0.043[a]	0.431 ± 0.040[a]

[a,b]Values bearing similar superscripts in the same column do not differ ($P < 0.05$).

[1]p.i. = postinstillation.

[2]BaP = benzo[a]pyrene.

Although there was an ascending increment of TP and albumin in all groups as time advanced (Table 3), those at d 7 until 21 were the lowest ($P < 0.05$) in the 15 mg of BaP group.

The hepatic enzyme activities manifested fluctuations without any clear pattern in all groups during the course of the experiment (Table 4). However, on d 7 to 14 p.i., the AST and GGT in the 15 mg of BaP group were significantly ($P < 0.05$) increased. Likewise, on d 7, increased levels of ALT, ALP, and LDH in the 15 mg of BaP group were the highest ($P < 0.05$). Nevertheless, no significant change was seen in the activity of CK at any instant.

MDA Levels

The levels of MDA in the plasma, lung, and liver are shown in Table 5. However, higher ($P < 0.05$) levels of plasma, lung, and liver MDA were only seen in the 15 mg of BaP group throughout the entire experimental period except at d 35 in plasma MDA. Nevertheless, the lung MDA level in the 15 mg of BaP group was comparable to that of the 1.5 μg of BaP group at d 7 and to that of the 150 μg of BaP groups at d 7, 14, and 35. It was only at d 7 that the liver MDA level in the 15 mg of BaP group was comparable to that of the 150 μg group. After this, the highest liver ($P < 0.05$) level of MDA was attained in the 15 mg of BaP group until the end of the experimental period.

Liver and Lung Weight and HSI and PI

The liver and lung weight and HSI and PI indices of broilers during the experimental period are shown in Table 6. The liver weight consistently increased as time advanced in all of the groups throughout the trial. In the 15 mg of BaP group, a significantly higher ($P < 0.05$) liver weight was observed when compared with the other groups except at d 35. A decreasing trend of HSI was seen in all groups over time, but the HSI of the 15 mg of BaP group was always the highest ($P < 0.05$) during the course of the experiment except at d 35 p.i.

Table 3. The total protein (TP) and albumin concentration of broilers during the trial (mean ± SD)

	Days p.i.[1]			
Parameter	7	14	21	35
TP (g/L)				
Control	31.9 ± 0.626[a]	32.1 ± 1.066[a]	32.7 ± 0.742[a]	33.9 ± 1.244[a]
Tricaprylin	32.0 ± 0.606[a]	31.8 ± 1.142[a]	32.5 ± 0.735[a]	32.4 ± 0.489[a]
1.5 μg of BaP[2]	31.6 ± 1.101[a]	31.7 ± 0.847[a]	32.9 ± 1.053[a]	33.1 ± 0.869[a]
150 μg of BaP	31.3 ± 0.836[a]	32.9 ± 0.629[a]	31.8 ± 1.510[a]	32.9 ± 1.053[a]
15 mg of BaP	27.8 ± 1.070[b]	28.2 ± 1.284[b]	29.8 ± 0.886[b]	32.7 ± 1.053[a]
Albumin (g/L)				
Control	14.64 ± 0.449[a]	14.8 ± 0.442[a]	14.90 ± 0.593[a]	15.46 ± 0.711[a]
Tricaprylin	14.74 ± 0.422[a]	14.46 ± 0.338[a]	14.80 ± 0.596[a]	14.32 ± 0.386[a]
1.5 μg of BaP	14.48 ± 0.793[a]	14.60 ± 0.583[a]	14.74 ± 0.677[a]	15.08 ± 0.519[a]
150 μg of BaP	14.16 ± 0.665[a]	14.74 ± 0.355[a]	14.46 ± 1.142[ab]	14.74 ± 0.677[a]
15 mg of BaP	11.20 ± 0.918[b]	11.80 ± 0.905[b]	13.04 ± 0.631[b]	14.88 ± 0.735[a]

[a,b]Values bearing similar superscripts in the same column do not differ ($P < 0.05$).

[1]p.i. = postinstillation.

[2]BaP = benzo[a]pyrene.

Table 4. The selected enzyme activities of broilers during the trial (mean ± SD)

	Days p.i.[2]			
Parameter[1]	7	14	21	35
AST (U/L)				
Control	201 ± 28.73[b]	190 ± 21.54[b]	195 ± 21.33[a]	187 ± 22.10[a]
Tricaprylin	216 ± 31.54[b]	203 ± 27.65[b]	186 ± 13.91[a]	207 ± 22.79[a]
1.5 μg of BaP	184 ± 28.69[b]	187 ± 19.01[b]	199 ± 20.17[a]	195 ± 20.26[a]
150 μg of BaP	213 ± 43.92[b]	197 ± 22.74[b]	188 ± 21.73[a]	197 ± 22.36[a]
15 mg of BaP	310 ± 52.29[a]	276 ± 38.95[a]	201 ± 29.27[a]	204 ± 29.15[a]
ALT (U/L)				
Control	8.4 ± 2.634[b]	8.7 ± 1.231[a]	9.4 ± 1.304[a]	9.6 ± 1.4333[a]
Tricaprylin	9.8 ± 1.911[b]	9.7 ± 1.758[a]	10.2 ± 1.92[a]	11.0 ± 1.631[a]
1.5 μg of BaP	7.2 ± 1.975[b]	8.4 ± 1.309[a]	8.9 ± 1.851[a]	10.4 ± 2.034[a]
150 μg of BaP	8.8 ± 2.234[b]	8.7 ± 1.431[a]	9.9 ± 1.820[a]	10.7 ± 1.860[a]
15 mg of BaP	20.2 ± 4.996[a]	9.3 ± 2.726[a]	10.2 ± 1.498[a]	11.1 ± 1.689[a]
ALP (U/L)				
Control	1,756 ± 252.0[b]	1,676 ± 310.8[a]	1,743 ± 236.4[a]	1,823 ± 232.5[a]
Tricaprylin	1,875 ± 193.1[b]	1,709 ± 171.1[a]	1,669 ± 201.0[a]	1,783 ± 199.1[a]
1.5 μg of BaP	1,812 ± 209.9[b]	1,731 ± 279.4[a]	1,785 ± 229.4[a]	1,875 ± 247.5[a]
150 μg of BaP	1,873 ± 387.7[b]	1,846 ± 264.8[a]	1,763 ± 211.8[a]	1,903 ± 290.4[a]
15 mg of BaP	3,992 ± 295.8[a]	1,874 ± 247.7[a]	1,863 ± 290.2[a]	1,983 ± 260.9[a]
GGT (U/L)				
Control	8.9 ± 0.815[b]	8.2 ± 1.594[b]	10.6 ± 2.093[a]	9.9 ± 1.547[a]
Tricaprylin	8.0 ± 1.585[b]	10.0 ± 1.793[b]	9.1 ± 1.844[a]	10.7 ± 1.511[a]
1.5 μg of BaP	9.5 ± 1.621[b]	9.1 ± 1.702[b]	10.7 ± 1.866[a]	9.4 ± 1.521[a]
150 μg of BaP	10.0 ± 1.861[b]	11.0 ± 2.591[b]	11.4 ± 2.100[a]	10.9 ± 1.980[a]
15 mg of BaP	24.5 ± 6.625[a]	19.5 ± 2.994[a]	11.6 ± 2.064[a]	11.0 ± 1.923[a]
LDH (U/L)				
Control	1,281 ± 256.8[b]	1,285 ± 256.2[a]	1,277 ± 220.9[a]	1,278 ± 189.8[a]
Tricaprylin	1,193 ± 178.7[b]	1,301 ± 262.8[a]	1,315 ± 212.7[a]	1,316 ± 200.1[a]
1.5 μg of BaP	1,271 ± 212.2[b]	1,335 ± 265.0[a]	1,358 ± 216.1[a]	1,298 ± 233.1[a]
150 μg of BaP	1,321 ± 274.0[b]	1,347 ± 229.9[a]	1,377 ± 259.2[a]	1,318 ± 255.4[a]
15 mg of BaP	2,142 ± 455.7[a]	1,405 ± 288.8[a]	1,416 ± 275.8[a]	1,332 ± 253.7[a]
CK (U/L)				
Control	1,019 ± 34.03[a]	969 ± 67.78[a]	992 ± 66.12[a]	961 ± 72.95[a]
Tricaprylin	931 ± 78.25[a]	944 ± 73.23[a]	1,017 ± 39.18[a]	985 ± 70.55[a]
1.5 μg of BaP	1,048 ± 141.0[a]	1,050 ± 149.0[a]	959 ± 52.88[a]	1,011 ± 105.1[a]
150 μg of BaP	993 ± 160.6[a]	1,016 ± 98.80[a]	911 ± 34.84[a]	995 ± 87.34[a]
15 mg of BaP	984 ± 137.0[a]	1,054 ± 146.2[a]	1,066 ± 122.5[a]	943 ± 83.07[a]

[a,b]Values bearing similar superscripts in the same column do not differ ($P < 0.05$).

[1]AST = aspartate aminotransferase; ALT = alanine aminotransferase; ALP = alkaline phosphatase; GGT = gamma-glutamyl transferase; LDH = lactate dehydrogenase; CK = creatine kinase; BaP = benzo[a]pyrene.

[2]p.i. = postinstillation.

Table 5. The malondialdehyde levels of broilers during the trial (mean ± SD)

	Days p.i.[1]			
Parameter	7	14	21	35
Plasma (nmol/mL)				
Control	2.08 ± 0.223[b]	2.05 ± 0.175[b]	2.25 ± 0.089[b]	2.29 ± 0.107[a]
Tricaprylin	2.01 ± 0.120[b]	2.11 ± 0.184[b]	2.25 ± 0.079[b]	2.36 ± 0.111[a]
1.5 μg of BaP[2]	2.18 ± 0.115[b]	2.12 ± 0.195[b]	2.30 ± 0.095[b]	2.37 ± 0.184[a]
150 μg of BaP	2.20 ± 0.154[b]	2.20 ± 0.214[b]	2.30 ± 0.095[b]	2.41 ± 0.115[a]
15 mg of BaP	2.81 ± 0.292[a]	2.90 ± 0.262[a]	2.57 ± 0.089[a]	2.42 ± 0.108[a]
Lung (nmol/mg of protein)				
Control	2.43 ± 0.502[b]	2.50 ± 0.726[b]	2.41 ± 0.337[b]	2.39 ± 0.351[b]
Tricaprylin	2.72 ± 0.366[b]	2.63 ± 0.520[b]	2.18 ± 0.257[b]	2.55 ± 0.320[b]
1.5 μg of BaP	2.89 ± 0.821[ab]	3.08 ± 0.895[b]	2.64 ± 0.390[b]	2.42 ± 0.510[b]
150 μg of BaP	3.02 ± 0.958[ab]	3.37 ± 0.584[ab]	2.61 ± 0.548[b]	2.45 ± 0.166[ab]
15 mg of BaP	4.13 ± 0.539[a]	4.65 ± 0.806[a]	3.58 ± 0.234[a]	3.25 ± 0.445[a]
Liver (nmol/mg of protein)				
Control	4.12 ± 0.446[b]	3.97 ± 0.467[b]	4.18 ± 0.546[b]	3.59 ± 0.636[b]
Tricaprylin	4.09 ± 0.417[b]	3.96 ± 0.613[b]	3.92 ± 0.635[b]	3.64 ± 0.646[b]
1.5 μg of BaP	4.45 ± 0.840[b]	4.10 ± 0.592[b]	4.38 ± 0.653[b]	3.66 ± 0.318[b]
150 μg of BaP	4.99 ± 0.934[ab]	4.71 ± 0.238[b]	4.54 ± 0.707[b]	3.77 ± 0.456[b]
15 mg of BaP	6.21 ± 0.609[a]	6.42 ± 0.626[a]	5.87 ± 0.477[a]	4.74 ± 0.323[a]

[a,b]Values bearing similar superscripts in the same column do not differ ($P < 0.05$).

[1]p.i. = postinstillation.

[2]BaP = benzo[a]pyrene.

Nonetheless, an increasing pattern of lung weight was seen in all groups as time advanced. A decrement of PI was seen in all groups over time, whereas at all instances, the PI of the 15 mg of BaP group was the highest ($P < 0.05$). Significantly ($P < 0.05$) higher PI in the 150 μg of BaP group than the remaining lower level group was recorded at d 7 and 14 only.

Liver Microsomal Protein, Cytochrome P450, and GST Levels

The liver microsomal protein, cytochrome P450, and GST levels are shown in Table 7. The high ($P < 0.05$) level of microsomal protein in the 15 mg of BaP group was only significantly different from the control and tricaprylin groups from d 7 until 21. Such differences were comparable to those of the 1.5 and 150 μg of BaP groups on d 7 to 14 and d 7 to 21, respectively.

Although the control group showed an increment of liver P450 levels as time advanced, fluctuations were seen in the tricaprylin, 1.5 μg of BaP, and 150 μg of BaP groups. At d 7, the 15 mg of BaP group had the highest ($P < 0.05$) concentration of P450 than any other group. This change continued until d 21 except that the P450 concentration was comparable to that of the 150 μg of BaP group at d 14 and 21. Again at d 7 p.i., changes in P450 concentration in the 150 μg of BaP group were always higher ($P < 0.05$) than those of the control and tricaprylin groups but insignificant to that of the 1.5 μg of BaP group.

The liver GST levels in the 15 mg of BaP group were significantly higher ($P < 0.05$) than the control group only at d 7 and to all other groups commencing from d 14 until the end of the experiment.

Lung Microsomal Protein, Cytochrome P450, and GST Levels

The lung microsomal protein, cytochrome P450, and GST levels are shown in Table 8. The highest ($P < 0.05$) level of microsomal protein was only seen in the 15 mg of BaP group throughout the entire experimental period. However, at d 7, lung microsomal protein in the 150 μg of BaP group was significantly higher ($P < 0.05$) than that of the tricaprylin group only.

The lung P450 levels in the control, tricaprylin, and 1.5 μg of BaP groups remained unchanged throughout the experimental period and that of the 15 mg of BaP group remained significantly ($P < 0.05$) higher from all other groups except at d 21 and 35. At these 2 instants, the lung cytochrome P450 levels of the 15 mg of BaP group were similar to that of the 1.5 μg of BaP group at d 21 and to that of the 150 μg of BaP group at d 21 to 35.

The lung GST levels in the 15 mg of BaP group were significantly ($P < 0.05$) different from all other groups at d 7 and d 35. At d 14, the level in the 15 mg of BaP group was only comparable to that of the 150 μg of BaP group.

Table 6. The lung, liver weight, and indices of broilers at postmortem (mean ± SD)

	Days p.i.[2]			
Parameter[1]	7	14	21	35
Liver weight (g)				
Control	4.923 ± 0.209[b]	13.758 ± 1.151[b]	23.931 ± 1.879[b]	40.25 ± 1.709[a]
Tricaprylin	4.883 ± 0.235[b]	13.492 ± 0.783[b]	24.006 ± 1.264[b]	40.75 ± 1.890[a]
1.5 μg of BaP	4.911 ± 0.286[b]	13.810 ± 0.692[b]	25.513 ± 1.359[b]	41.20 ± 1.858[a]
150 μg of BaP	5.147 ± 0.585[b]	13.809 ± 0.671[b]	26.010 ± 1.518[b]	41.71 ± 1.396[a]
15 mg of BaP	6.279 ± 0.446[a]	16.093 ± 1.917[a]	30.555 ± 2.073[a]	42.08 ± 2.073[a]
HSI (mean $\times 10^2$)				
Control	2.793 ± 0.151[b]	2.599 ± 0.162[b]	2.423 ± 0.130[b]	2.009 ± 0.112[a]
Tricaprylin	2.723 ± 0.173[b]	2.572 ± 0.147[b]	2.437 ± 0.124[b]	2.041 ± 0.125[a]
1.5 μg of BaP	2.828 ± 0.267[b]	2.647 ± 0.850[b]	2.576 ± 0.158[b]	2.074 ± 0.096[a]
150 μg of BaP	3.011 ± 0.445[b]	2.788 ± 0.261[b]	2.673 ± 0.132[b]	2.106 ± 0.075[a]
15 mg of BaP	4.077 ± 0.463[a]	3.667 ± 0.540[a]	3.321 ± 0.274[a]	2.211 ± 0.144[a]
Lung weight (g)				
Control	1.602 ± 0.072[b]	4.344 ± 0.255[b]	7.826 ± 0.139[b]	12.803 ± 0.538[a]
Tricaprylin	1.586 ± 0.079[b]	4.419 ± 0.251[b]	7.747 ± 0.186[b]	12.839 ± 0.295[a]
1.5 μg of BaP	1.607 ± 0.062[b]	4.444 ± 0.243[b]	7.701 ± 0.386[b]	12.596 ± 0.529[a]
150 μg of BaP	1.714 ± 0.075[b]	4.628 ± 0.155[b]	8.005 ± 0.408[b]	12.774 ± 0.307[a]
15 mg of BaP	2.092 ± 0.154[a]	5.405 ± 0.425[a]	9.245 ± 0.441[a]	13.699 ± 1.230[a]
PI (mean $\times 10^2$)				
Control	0.910 ± 0.022[c]	0.820 ± 0.016[c]	0.793 ± 0.014[b]	0.638 ± 0.018[b]
Tricaprylin	0.883 ± 0.013[c]	0.841 ± 0.024[c]	0.786 ± 0.016[b]	0.642 ± 0.018[b]
1.5 μg of BaP	0.923 ± 0.032[c]	0.851 ± 0.016[c]	0.777 ± 0.029[b]	0.634 ± 0.022[b]
150 μg of BaP	0.998 ± 0.025[b]	0.932 ± 0.047[b]	0.822 ± 0.033[b]	0.645 ± 0.015[b]
15 mg of BaP	1.351 ± 0.036[a]	1.226 ± 0.038[a]	1.004 ± 0.054[a]	0.721 ± 0.017[a]

[a–c]Values bearing similar superscripts between columns do not differ ($P < 0.05$).

[1]BaP = benzo[a]pyrene; HSI = hepatosomatic index: liver weight (g)/total weight (g) × 100; PI = pulmonosomatic index: lung weight (g)/total weight (g) × 100.

[2]p.i. = postinstillation.

Table 7. The liver microsomal protein, cytochrome P450, and glutathione S-transferase (GST) levels of broilers at postmortem (mean ± SD)

	Days p.i.[1]			
Parameter	7	14	21	35
Liver microsomal protein (mg)				
Control	8.780 ± 1.169[b]	8.740 ± 1.184[b]	8.640 ± 0.864[b]	8.880 ± 0.909[a]
Tricaprylin	8.680 ± 1.540[b]	8.720 ± 1.056[b]	8.520 ± 1.091[b]	8.640 ± 1.352[a]
1.5 μg of BaP[2]	9.280 ± 1.544[ab]	9.160 ± 1.303[ab]	8.740 ± 1.064[b]	8.840 ± 1.158[a]
150 μg of BaP	9.640 ± 2.236[ab]	9.380 ± 1.430[ab]	9.740 ± 0.887[ab]	8.860 ± 1.613[a]
15 mg of BaP	12.48 ± 2.552[a]	11.48 ± 1.883[a]	10.76 ± 1.244[a]	9.240 ± 1.492[a]
Liver P450 (nmol/mg of protein)				
Control	0.238 ± 0.023[c]	0.242 ± 0.046[b]	0.244 ± 0.043[b]	0.258 ± 0.040[a]
Tricaprylin	0.246 ± 0.038[c]	0.255 ± 0.036[b]	0.246 ± 0.041[b]	0.257 ± 0.023[a]
1.5 μg of BaP	0.259 ± 0.027[bc]	0.284 ± 0.037[b]	0.252 ± 0.030[b]	0.253 ± 0.029[a]
150 μg of BaP	0.319 ± 0.051[b]	0.309 ± 0.049[ab]	0.259 ± 0.033[ab]	0.253 ± 0.035[a]
15 mg of BaP	0.403 ± 0.045[a]	0.369 ± 0.035[a]	0.334 ± 0.053[a]	0.297 ± 0.059[a]
Liver GST (μmol/min per mg of protein)				
Control	1.502 ± 0.285[b]	1.477 ± 0.085[b]	1.576 ± 0.073[b]	1.583 ± 0.083[a]
Tricaprylin	1.512 ± 0.100[ab]	1.514 ± 0.032[b]	1.559 ± 0.074[b]	1.563 ± 0.069[a]
1.5 μg of BaP	1.639 ± 0.054[ab]	1.521 ± 0.072[b]	1.569 ± 0.047[b]	1.575 ± 0.068[a]
150 μg of BaP	1.655 ± 0.062[ab]	1.559 ± 0.121[b]	1.593 ± 0.046[b]	1.602 ± 0.088[a]
15 mg of BaP	1.818 ± 0.346[a]	1.990 ± 0.110[a]	1.906 ± 0.093[a]	1.425 ± 0.043[b]

[a–c]Values bearing similar superscripts in the same column do not differ ($P < 0.05$).

[1]p.i. = postinstillation.

[2]BaP = benzo[a]pyrene.

DISCUSSION

This study represents an assessment of the widely used avian toxicology and biomonitoring blood profiles (Grasman, 2002; Garg et al., 2004) of broiler chickens exposed to BaP. It is strongly believed that the deleterious effects of BaP seen in this study were attributed to 2 main factors, namely the species used and metabolism of BaP.

The expressions of AhR under normal (Singh et al., 2009) and after exposure to xenobiotics (Hirabayashi and Inoue, 2009) on hemopoiesis have been comprehensively reviewed. In our study, AhR activation (de Oliveira et al., 2007) by the i.t. instilled BaP leading to P450 induction (Andrieux et al., 2004) generated systemic oxidative stress (Dalton et al., 2002) that later impaired hemopoiesis. This response reflected the widespread effect of BaP to be independent of its route of administration (Sun et al., 1982) because the lungs, liver, and blood were not spared. The reduction in RBC parameters (Knuckles et al., 2001) along with normochromic microcytic morphology in the 15 mg of BaP

Table 8. The lung microsomal protein, cytochrome P450, and glutathione S-transferase (GST) levels of broilers at postmortem (mean ± SD)

	Days p.i.[1]			
Parameter	7	14	21	35
Lung microsomal protein (mg)				
Control	1.486 ± 0.047[bc]	1.511 ± 0.053[b]	1.554 ± 0.086[b]	1.507 ± 0.045[b]
Tricaprylin	1.482 ± 0.041[c]	1.466 ± 0.060[b]	1.511 ± 0.053[b]	1.518 ± 0.046[b]
1.5 μg of BaP[2]	1.521 ± 0.058[bc]	1.513 ± 0.052[b]	1.562 ± 0.099[b]	1.534 ± 0.085[b]
150 μg of BaP	1.776 ± 0.103[b]	1.678 ± 0.175[b]	1.575 ± 0.117[b]	1.574 ± 0.075[b]
15 mg of BaP	2.119 ± 0.317[a]	1.959 ± 0.250[a]	1.891 ± 0.252[a]	1.959 ± 0.296[a]
Lung P450 (nmol/mg of protein)				
Control	0.021 ± 0.005[c]	0.026 ± 0.006[c]	0.027 ± 0.008[b]	0.029 ± 0.005[b]
Tricaprylin	0.026 ± 0.005[c]	0.027 ± 0.006[c]	0.027 ± 0.004[b]	0.028 ± 0.003[b]
1.5 μg of BaP	0.029 ± 0.004[bc]	0.030 ± 0.007[bc]	0.028 ± 0.004[ab]	0.030 ± 0.004[b]
150 μg of BaP	0.039 ± 0.007[b]	0.041 ± 0.009[b]	0.032 ± 0.006[ab]	0.031 ± 0.005[ab]
15 mg of BaP	0.063 ± 0.010[a]	0.059 ± 0.010[a]	0.042 ± 0.011[a]	0.040 ± 0.006[a]
Lung GST (μmol/min per mg of protein)				
Control	0.202 ± 0.013[b]	0.215 ± 0.019[b]	0.216 ± 0.017[a]	0.219 ± 0.013[a]
Tricaprylin	0.211 ± 0.018[b]	0.212 ± 0.020[b]	0.215 ± 0.015[a]	0.215 ± 0.014[a]
1.5 μg of BaP	0.204 ± 0.009[b]	0.207 ± 0.022[b]	0.213 ± 0.012[a]	0.217 ± 0.011[a]
150 μg of BaP	0.216 ± 0.028[b]	0.240 ± 0.010[ab]	0.210 ± 0.019[a]	0.206 ± 0.017[a]
15 mg of BaP	0.270 ± 0.025[a]	0.270 ± 0.018[a]	0.211 ± 0.017[a]	0.191 ± 0.014[b]

[a–c]Values bearing similar superscripts in the same column do not differ ($P < 0.05$).

[1]p.i. = postinstillation.

[2]BaP = benzo[a]pyrene.

group is indicative of PAH-induced anemia (Briggs et al., 1997). This might have emanated via cyclin-dependent pathway (Pang et al., 2008) and inhibitory primitive hemopoiesis (Wang et al., 2003; Pang et al., 2008). Alternatively, the changes seen implied that the broilers used were not AhR-deficient, conforming to our exhaustive search on the absence of documentation on AhR-deficient chickens (Yasui et al., 2007).

Leukocytes are the effectors of immune responses and have been assessed in many immunotoxicological studies of avian wildlife (Grasman, 2002). The deviation of WBC count and heterophil:lymphocyte ratio from normal as observed in this study was mainly due to a decreased lymphocyte count (De Jong et al., 1999). The decrement of WBC count suggests a direct effect of BaP on the hemopoietic tissues or an increase in the rate of destruction of the circulating leucocytes, or both (Mitchell and Johns, 2008). It also may be due to the mobilization of lymphocytes from the blood stream to the submucosa of the intrapulmonary conducting airways (Lorenzoni et al., 2009).

The ironic response of lack of susceptibility of broilers to the lower BaP dose used might be related to its distinctive anatomy and the respirable size of BaP (<2 μm in diameter). Compared with other species, the existence of specialized lung and air sacs (Corbanie et al., 2006) has rendered avian to be less affected by BaP at low levels. Our earlier experience revealed that severe biomass burning-based air pollution episodes did not adversely affect poultry production compared with other species (Noordin et al., 1998). During that episode, the equivalent concentration of BaP was within that of 1.5 μg/kg of BW. It is possible that during that period although metabolism of BaP via AhR and P450 occurred, it was insignificant to elicit any discernible effect. Likewise, the amount inhaled could have been promptly eliminated by the body due to its low burden. Findings from our study also showed that the minimum dose of BaP to elicit toxic effect in poultry is 15 mg/kg (i.t) as opposed to mammals, which is 10 mg/kg (oral) (DeJong et al., 1999). Although this may indicate that poultry were less sensitive to BaP toxicity, effects arising from different routes of administration should not be dismissed. However, the chance of avian being exposed to the respiratory route is much more likely than the oral route. Similarly, the dose of 15 mg of BaP could be sustained if the birds were close to the source of the pollutant (Lim and Ooi, 1998).

In avian lungs, respirable particles such as BaP used in this study are deposited for long periods within the parabronchi and air sacs by sedimentation and Brownian movement (Brown et al., 1997). Furthermore, particle clearance in this region is largely accomplished by phagocytic cells and is significantly slower than in the lung (Kiama et al., 2008). Our documentation of the much longer elevated level of P450 in the lung compared with the liver supported the earlier mentioned findings (Brown et al., 1997; Kiama et al., 2008). The lung anatomy would have either retained or channeled inhaled BaP to other nonvital parts of the body. We believed that much of the BaP at low doses was localized in the poorly vascularized air sacs and may be entrapped by heterophils and macrophages (Crespo et al., 1998). Such stimuli and surveillance might have led to the administered BaP especially at the low dose being made unavailable and hence failure to elicit toxic response. The aftermath of such processes yielded inadequate biologically available concentrations of BaP. Such parts may be affected to a certain degree but the dose of BaP attained was insufficient to elicit overt signs, lesions, or system impairment. At higher doses, such scavenging systems might have been saturated, leading to an active biologically available BaP to elicit toxicity.

The possibility for the lack of susceptibility of broilers to a high dose of BaP to be due to the difference in the length and diameter of the avian trachea is eliminated (Frappell et al., 2001). In the design of this study, i.t. administration of BaP bypassed such barrier and no amelioration increases in resistance to flow is expected to exist as a variable.

The waning toxic effect of the BaP during the experimental period is closely related to the half-life of BaP, which is about 18 d (Sun et al., 1982). After cessation of administration, decaying levels or absence of cumulative effect have led to almost negligible effect on tissues. This explains the return to comparable levels with controls and other lower dose groups.

Undoubtedly, the elevation of ALT (Golet et al., 2002), AST, GGT (Harr, 2002), LDH (Lumeij, 1997), and ALP (Sallie et al., 1991) in chicks from the 15 mg of BaP group indicated hepatic damage. Here, the parallel elevation of AST and ALT coupled with the low activity of CK (Seiser et al., 2000) reinforces the diagnosis of hepatic damage and rules out muscular injury (Dabbert and Powell, 1993). Indirectly, the hepatic compromise explained the reduction in TP as a result of hepatic dysfunction leading to hypoalbuminemia. This further suggested the possibility that the observed increase in AST levels (Golet et al., 2002) would have been as a result of increased activity of the PAH-sensitive hepatic P450 (Kennedy et al., 1996; Trust et al., 2000). This in turn probably incited increased oxidative stress, as substantiated by increased MDA levels (Alonso-Alvarez et al., 2007) seen in the liver, lung, and plasma.

The key finding from these data is that only high levels of BaP may reduce efficiency of poultry production and increase susceptibility to infections due to their adverse effect on hematology and induction of oxidative stress. This has indirectly explained the failure to observe adverse changes in broiler production during the air pollution episodes (Noordin et al., 1998). During that episode, the equivalent concentration was within that of the 1.5 μg of BaP group. It is possible that during that period although metabolism of BaP via AhR and P450 occurred, it was insignificant to elicit any discernible effect.

The observation of higher HSI and PI in the 15 mg of BaP group signifies toxicity (Knuckles et al., 2001) and

hepatic response to injury (Sellers et al., 2007). In our case, such response is likely due to either proliferation of smooth endoplasmic reticulum (Szczesna-Skorupa et al., 2004), mononuclear infiltration, congestion, or sinusoidal dilatation due to oxidative stress generated by BaP (Garçon et al., 2001; Kepley et al., 2003). The GST is a critical detoxification enzyme that primarily functions in conjugating functionalized P450 metabolites with endogenous ligands (reduced glutathione) favoring their elimination from the body of the organisms (Hartman and Shankel, 1990). The cytotoxic effect of 15 mg of BaP involved alterations of GST enzyme and the activity of these enzymes was markedly increased in chickens exposed to 15 mg of BaP. This response is indicative of cellular oxidative stress because of the fact that the presence of lipid peroxidation products (Tables 7 and 8) apparently causes the induction of the activity of this enzyme that takes part in toxic compound removal. It has been observed that the presence of active oxygen species, as a primary response, induces GST gene expression (Zimniak et al., 1997) as similarly observed with other xenobiotics (Stewart et al., 1996). There is persuasive evidence to support the induction GST and protection against a wide spectrum of cytotoxic, mutagenic, and carcinogenic chemicals (Reed, 1990).

In conclusion, the avian lung anatomy and the half-life of BaP may yield differing results compared with those in mammals. However, i.t. administration of BaP would eventually lead to a systemic rather than a localized (pulmonary) effect despite being given through the respiratory route. Thus, an assessment of toxic effects of inhaled particulates in birds may warrant a systematic investigation of all possible tissues. Likewise, it is believed that during such exposure, birds are still at risk to infection with a detrimental outcome despite a rather transient or irreversible effect of inhaled BaP.

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