Effects of pentachlorophenol on the quail (*Coturnix japonica*) liver detoxification pathway

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HIGHLIGHTS

- The amount of PCP found decreased in the order quail kidney > liver > muscle.
- Quail exposed to PCP showed significant increase in CYP450 gene expression.
- PCP triggered the antioxidant system in the quail liver.
- Transcription factors had preventive effects on PCP caused oxidative damage.

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ABSTRACT

Pentachlorophenol (PCP), an extensively used pesticide and biocide, is of critical environmental concern due to its toxicity and recalcitrance to degradation. In this study, the effect of PCP on induction of transcription factors, cytochrome P450 (CYP450) genes, and the antioxidative enzyme system were investigated in the quail liver. A total of 60 (4- to 6-week-old) male quails (*Coturnix japonica*) were administered 0, 0.05, 0.5, and 5 mg/kg/d PCP orally for 42 d. Following exposure, both absolute and relative liver weights were significantly lower than those of the control. Using gas chromatography-mass spectrometry, PCP accumulation was, from highest to lowest, kidney > liver > muscle for all exposure groups. The expressions of CYP1A5, CYP1B1, CYP2C18, nuclear translocator 1 (ARNT1), and aryl hydrocarbon receptor 1 (AHR1) were induced after PCP treatment, and increases were found in the activities of hepatic superoxide dismutase (SOD) and catalase (CAT), and the content of hepatic malondialdehyde (MDA). In addition, exposure to PCP induced an increase in liver 8-hydroxydeoxyguanosine (8-OHdG) and significantly elevated ethoxyresorufin O-deethylase (EROD), methoxyresorufin O-demethylase (MROD), and 7-ethoxycoumarin-O-deethylase (ECOD) activity, but decreased that of glutathione peroxidase (GSH-Px), benzyloxyresorufin O-debenzylase (BROD), pentoxyresorufin O-depentyase (PROD), and erythromycin N-demethylase (END). No significant responses were observed for benzyloxy-trifluoromethyl-coumarin (BFC). The protein level of liver nuclear factor κB (NF-κB) was higher, whereas that of nuclear factor E2-related factor 2 (Nrf2) was lower for exposed quail. These results suggest that PCP affects quail oxidative stress by modulating CYP450 enzymes and nuclear transcription factors.

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1. Introduction

The broad-spectrum biocide pentachlorophenol (PCP) is widely used in wood preservatives, pesticides, and disinfectants (Thakur et al., 2001). Although PCP has been restricted or banned in various countries since the 1980s due to its toxic nature, its use as a

wood preservative remains permitted due to a lack of suitable alternatives (Muir and Eduljee, 1999). Consequently, environmental monitoring continues to demonstrate the existence of PCP in various environmental matrices, such as surface water, soil, and sediment, as well as wildlife and human samples (Gao et al., 2008; Montano et al., 2013). PCP, a typical uncoupler of oxidative phosphorylation, is a representative environmental pollutant that was once a crucial pesticide and fungicide (Xu et al., 2016). Zheng W et al. (Zheng et al., 2011) revealed that in water and freshwater

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sediments in western countries, PCP levels have declined over time, with half-lives ranging from 2.0 years to 11.1 years. The average level of PCP in river sediments was 7.93 ng/g based on dry weight, ranging from 1.44 to 34.4 ng/g in the Pearl River Delta (Hong et al., 2005). The concentrations of PCP in soils irrigated by wastewater were in the range of 1.38–179 ng/g (Liu et al., 2006). PCP is absorbed by oral, dermal, and inhalation routes (Karlsson et al., 2013). PCP was also detected in human breast milk with an average of 2.15 ng/g. The concentrations of PCP in the serum of weaned Northern elephant seals increased from 103 ± 7 pg/mL at early fast to 246 ± 41 pg/mL at late fast (Louis et al., 2015). Therefore, potential for exposing humans and wild animals to the PCP through these resources is very high and could have profound deleterious effects on humans, livestock, and wildlife health through a disturbance of detoxification system (Besser et al., 2016). The persistent use of PCP worldwide fosters its potential to contaminate the environment, thus posing likely health risks to humans and wildlife.

Oxidative stress can generate reactive oxygen species (ROS) via various pathways after exposure to exogenous chemicals (Klaunig and Kamendulis, 2004). Oxidative DNA damage due to metabolically-generated ROS is considered a contributor to PCP-induced carcinogenesis. Within cells exposed to PCP and its metabolites, 8-hydroxydeoxyguanosine (8-OHdG), a biomarker of oxidative DNA damage, is reported to be significantly elevated (Dahlhaus et al., 1996). Previous study suggests long-term exposure to PCP can cause oxidative DNA damage accumulation in the liver in male B6C3F mice, which can, in turn, result in mutations that eventually lead to carcinogenesis (Sai-Kato et al., 1995). Here, we investigated whether PCP can lead to 8-OHdG formation in the quail liver and clarified the degree of involvement of oxidative DNA damage in PCP toxicity.

Downstream of signaling cascades, transcription factors allow cells to respond to biological and environmental stimuli by controlling the transcription process (Sahin et al., 2012). Nuclear factor- κ B (NF- κ B) is one such factor responsible for controlling DNA transcription. It is activated by different stresses to protect or damage the liver in context-specific manners (Lu et al., 2015). Nuclear factor erythroid 2-related factor 2 (Nrf2) is a principal cytoprotective transcription factor that induces the expression of numerous enzymes for detoxification and biotransformation, as well as transporter proteins for xenobiotic efflux (Yang et al., 2013a). Oxidative or electrophilic stress can activate Nrf2, and subsequently detoxify and eliminate exogenous chemicals and their metabolites (Kawai et al., 2011). Studies on the effects of Nrf2 against exogenous chemical exposure have demonstrated that Nrf2 plays an important role in oxidative stress induced by PCP (Umemura et al., 2006).

The major site for xenobiotic detoxification is the liver. Known to be hepatocarcinogenic in rodents, PCP metabolism involves the redox cycle and cytochrome P450 (CYP450) induction (Fang et al., 2015). Therefore, hepatotoxicity generated by oxidative damage is of importance in PCP-induced liver damage. Although studies have revealed that PCP can be metabolized by cytochrome P450 enzymes into tetrachlorohydroquinone (TCHQ), causing cell and tissue oxidative stress (Mehmood et al., 1996), identification of *in vivo* preventive mechanisms against PCP and discovery of key defense factors remain unknown; yet both are critical for accurate hazard risk estimation.

Although there has been considerable research (Umemura et al., 2006; Zhang et al., 2008) concerning the toxicity of humans and wildlife, hepatotoxicity effects are still being explored, especially with regards to non-mammalian species. Birds are one of the most important ecological pollution indicators due to their strong sensitivities to environmental pollutants (Du et al., 2017). Thus,

research about the effect of PCP on birds might be of significance to evaluations of the adverse effects of PCP in the environment. Although we have displayed the effect of pentachlorophenol on the detoxification system in white-rumped munia, how the oxidative stress is mediated by modulating CYP450 enzymes and nuclear transcription factors, as well as the underlying mechanisms of PCP hepatotoxicity in the liver remain unclear. Here, we chose quail as a model of wild birds to reveal preventive mechanisms against PCP and discover key defense factors *in vivo*. Quails are representative for terrestrial birds and an accepted model for assessing both the acute and chronic effects of pesticides and other chemicals (Ahmed et al., 2015). The objective of the present study was to investigate the detoxification system and further elucidate the underlying toxicological mechanism of PCP in birds. We examined the effect of PCP on quail P450 and transcription factor as well as on the modification of the antioxidative enzyme system to explore PCP hepatotoxicity in quails. This *in vivo* study also explored the effects of PCP on the mRNA expressions of CYP monooxygenases (CYP1A4, CYP1A5, CYP1B1, and CYP2C18) and transcription factors (aryl hydrocarbon receptor 1 (AHR1) and AHR1 nuclear translocator 1 (ARNT1)), and assessed the PCP-induced activity of antioxidative enzymes in quail.

2. Materials and methods

2.1. Reagents

The purity of the PCP was $\geq 99\%$ (Dr. Ehrenstorfer, Ausberg, Germany). We obtained corn oil from Sigma-Aldrich (USA) and ^{13}C -PCP ($\geq 99\%$) standard from AccuStandard (USA). HPLC grade chemicals were used for PCP chemical analysis.

2.2. Animals

Sixty male common quails (*Coturnix japonica*, 4–6 weeks, 130 g) were purchased from a commercial breeder (Deling Wu) in Beijing. The animals were kept in suspended, unattached cages in a mass air-displacement room controlled at $20\text{ }^{\circ}\text{C}$ – $26\text{ }^{\circ}\text{C}$. Dry seeds and fresh water were provided *ad libitum*. After 14 d of acclimation, the quails were randomly separated into four groups and orally administered corn oil with 0, 0.05, 0.5, or 5 mg/kg/d of commercial PCP for 42 d, respectively. The doses were selected based on PCP toxicity studies on wildlife and rat (Hudson et al., 1984; Blakley et al., 1998). All quails were euthanized at the end of the experiment, and serum and tissue (liver, kidney, and muscle) samples were collected and stored at $-80\text{ }^{\circ}\text{C}$ until further use.

2.3. PCP content in tissues

We conducted PCP quantification via extraction into an organic solvent and then gas chromatography with electron capture detection. Details on the method detection limit (MDL) and procedural blanks are as described in previous research (Jiang et al., 2016).

2.4. Effect of PCPs on transcriptional levels of genes in liver

We performed qPCR to analyze the responses of certain genes, including P450 isomers, AHR1, and ARNT, to environmental pollutants. Table A1 (Supplementary Data) shows the selected gene expression levels and their PCR primers. TRIzol reagent was used to isolate total RNA from quail liver, and β -actin was selected as the internal standard. The Stratagene Mx3000P q-PCR system (Stratagene, USA) and SYBR Green PCR Master Mix reagent kit (Tiangen, Beijing, China) were used for qPCR. The relative fold changes in the

expressions of target genes were calculated according to previous study (Livak and Schmittgen, 2001).

2.5. Hepatic 8-hydroxy-2'-deoxyguanosine

The content of 8-OHdG in the liver samples was detected using a commercial ELISA kit (Beijing Elisa Technology Ltd., China). Calibration, curve fitting, and data analysis were performed, and the detection limit of 8-OHdG was 28.75 pg/mL.

2.6. Enzyme activity analyses

The activities of EROD, MROD, BROD, PROD, END, ECOD, BFC, glutathione peroxidase (GSH-Px), and glutathione S-transferases (GST) were detected using appropriate kits (Genmed Scientifics Inc., USA). The activities of CAT and SOD and the content of MDA and GSH were measured using commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.7. Effect of PCP exposure on NF- κ B and Nrf-2 protein levels

Homogenization of liver tissue was carried out with RIPA buffer (Thermo Scientific, Waltham, MA, USA). The tissue homogenates were centrifuged at 12 000 g for 30 min at 4 °C. After detection of protein concentrations, proteins were separated via 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred onto polyvinylidene fluoride membranes (Millipore, Billerica, MA, USA), which were blocked and incubated with chicken antibodies against NF- κ B (ab16502) and Nrf2 (ab31163) (Abcam, Cambridge, UK) (1:1000). The protein loading process was controlled via a monoclonal mouse antibody against β -actin (CW0096A; Kangwei, China). An enhanced chemiluminescence Western blot detection kit (Tiangen, Beijing, China) was used to detect protein bands following probing with goat polyclonal to rabbit IgG for NF- κ B and Nrf2 (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Bands were visualized by exposure to X-ray film (Kodak, NY, USA), and band intensity was quantified by Quantity One software (Bio-Rad, Hercules, CA, USA).

2.8. Statistical analysis

Analyses of the data were performed with SPSS (version 16.0, SPSS Inc., Chicago, USA). One-way analysis of variance (ANOVA) followed by Duncan's multiple range tests were used to evaluate the differences between the control and treatment groups.

3. Results

No signs of toxicity or morbidity were observed among the quails during exposure to PCP. No significant changes in final body weight were observed, but absolute and relative liver weights of the PCP treated groups were significantly lower than those of the control (Table 1).

Table 1
Effect of PCP on body weight, liver weight, and relative liver weight in quail.

Dose (mg/kg/d)	Final BW (g)	Liver weight (g)	Relative liver weight (%)
0	132.72 $\pm$ 10.48	2.32 $\pm$ 0.42	17.52 $\pm$ 2.86
0.05	129.54 $\pm$ 9.32	2.02 $\pm$ 0.34*	15.61 $\pm$ 2.21*
0.5	127.28 $\pm$ 6.32	1.97 $\pm$ 0.23**	15.49 $\pm$ 2.08*
5	127.43 $\pm$ 7.34	2.03 $\pm$ 0.24*	15.47 $\pm$ 0.95*

Data are means $\pm$ SEM (n = 12). Relative liver weight (%) = liver weight (g)/final body weight (g) $\times$ 100%. BW, body weight. Statistical significance is indicated by * P < 0.05 and ** P < 0.01 compared with the control.

3.1. Tissue distribution of PCP

The PCP concentrations in quail tissues were quantified using GC-MS. We detected PCP content in the serum and tissues (liver, kidney, muscle) of all groups, indicating that PCP was assimilated into different tissue. PCP concentrations increased significantly in the liver, kidney, and muscle in a dose-dependent manner, with the maximum accumulation of PCP found in the kidneys of the 5 mg/kg/d group (5.19 ± 0.28 μ g/g w.w.) at nearly 12-fold that detected in muscle (Fig. 1a). PCP concentration in serum also increased in a dose-dependent manner, with the highest PCP content (2.65 ± 0.21 μ g/mL) detected in the 5 mg/kg/d group (Fig. 1b).

3.2. Effect of PCP on CYP1A4, CYP1A5, CYP1B1, CYP2C18, AHR1, and ARNT mRNA expressions in quail liver

The expressions of CYP450 (CYP1A4, CYP1A5, CYP1B1, and CYP2C18), AHR1, and ARNT were detected in quails after exposure to PCP (Fig. 2). A significant upregulation in the CYP1A5 mRNA expression was observed in the quail liver, and was approximately 2-, 5-, and 4-fold higher in the PCP treatment groups, respectively. The mRNA levels of CYP2C18 in the 0.5 and 5 mg/kg/d PCP-treated groups were increased compared with those in the control group. CYP1B1 mRNA expression in the 0.05 mg/kg/d PCP-treated group was significantly upregulated. However, there were no significant differences in CYP1A4 mRNA expression among the PCP treatment and control groups. AHR1 and ARNT1 transcripts were also detected in quail exposed to PCP for 42 d. Compared with the control, the mRNA expressions of AHR1 and ARNT were significantly elevated in all PCP treatment groups. The highest expression levels of AHR1 and ARNT were found in the 0.5 mg/kg/d PCP-treated group.

3.3. Effect of PCP on CYP450 enzyme activity in quail liver

Alkoxyresorufin O-dealkylation (AROD), EROD, MROD, BROD, PROD, BFC, END, and ECOD activities were examined in quail exposed to PCP for 42 d (Fig. 3). A significant induction of quail hepatic microsomal EROD activity was observed, and an approximate 2-fold increase in all PCP treatment groups was found. In the 5 mg/kg/d PCP exposure group, MROD activity was 1.4-fold higher than that in the control (P < 0.05), whereas BROD activity significantly decreased by 27.5%, 40.0%, and 35.0% in all treatment groups, respectively. Compared with the control group, a significant 6-fold higher induction of hepatic ECOD activity was observed after exposure to 0.5 and 5 mg/kg/d of PCP for 42 d. In addition, in the low dose group (0.05 mg/kg/d), PROD and END activities showed significant decreases of 25.0% and 30.0% (P < 0.05), respectively, compared with the control. As for mean hepatic BFC activity, there were no significant differences between the control and PCP treatment groups.

3.4. Effect of PCP on antioxidation enzyme activities and contents of MDA, 8-OHdG, and GSH

In liver tissue, activity of CAT increased in all PCP exposure groups, activity of SOD significantly increased in the lowest PCP-treated group (0.05 mg/kg/d) (Fig. 4a), and GSH-Px activity decreased in all treatment groups compared with the control (Fig. 4a). In addition, decreases of 53.85% and 54.54% in GSH-Px activity were observed in the 0.5 and 5 mg/kg/d PCP groups, respectively. Enzymatic activity of GST did not show significant differences between the control and treatment groups (Fig. 4a). However, PCP significantly increased MDA levels in the livers of the 0.05 and 0.5 mg/kg/d PCP-treated groups (Fig. 4c), and decreased

Fig. 1. Levels of PCP (µg/g w.w. for liver, kidney, and muscle; µg/mL for serum) measured in the liver, kidney, muscle, and serum of quails exposed to PCP at doses of 0, 0.05, 0.5, and 5 mg/kg/d. (a) Content of PCP in liver, kidney, and liver; (b) Content of PCP in serum.

Fig. 2. Effects of PCP on CYP1A4, CYP1A5, CYP1B1, CYP2C18, AHR1, and ARNT mRNA levels in the liver of quails ($n = 6$). Gene expression levels represent relative mRNA expressions compared with those in the control. Statistical significance is indicated by * $p < 0.05$ and ** $P < 0.01$ compared with the control.

Fig. 3. Enzyme activities of EROD, MROD, BROD, PROD, BFC, END, and ECOD ($n = 6$). EROD, MROD, PROD, and BROD are expressed as pmol/mg/min, BFC, END, and ECOD activities are expressed as nmol/mg/min. Statistical significance is indicated by * $p < 0.05$ and ** $P < 0.01$ compared with the control.

GSH concentrations in all exposure groups (Fig. 4d). Furthermore, compared with the control, 8-OHdG levels in the liver after PCP administration showed significant 1.6-, 1.7-, and 1.9-fold increases in the 0.05, 0.5, and 5 mg/kg/d PCP groups, respectively (Fig. 4b).

3.5. Effect of PCP on nuclear transcription factors of NF-κB and Nrf2

The NF-κB and Nrf2 protein levels in the liver were lower and higher, respectively, in quails exposed to PCP compared with those in the control (Fig. 5). The NF-κB protein levels were inhibited by 32.1% and 37.7% in the 0.5 and 5 mg/kg/d PCP-treated groups, respectively, whereas Nrf2 levels increased with PCP dosage.

4. Discussion

In this study, PCP exposure resulted in lower absolute and relative liver weights in quails. This is in accordance with previous research, which showed that the body weights of *Anas platyrhynchos* and liver weights of broiler chickens significantly decreased following PCP exposure (Nebeker et al., 1994). We demonstrated that the primary site of PCP accumulation was the quail kidney, with the high levels of PCP in the kidney and liver possibly indicating key elimination and metabolism routes. These results are consistent with PCP accumulation studies on white-rumped munia (*Lonchura striata*), in which the kidney and liver exhibited the highest and second highest PCP content, respectively (Jiang et al., 2016).

We focused on PCP hepatotoxicity because the liver is the major site for xenobiotic detoxification. Cytochrome P450 (P450) is a heme-containing enzyme superfamily that participates in exogenous and endogenous substance metabolism and elimination (Ibrahim, 2015). Several drugs and other exogenous compounds can interact with CYPs, which might increase or decrease their expression and/or activity (Pelkonen et al., 2008). Among the various CYP enzymes, the CYP1A subfamily is the main agent of organic contaminant biotransformation (He et al., 2011). Birds possess two CYP1A genes (Goldstone and Stegeman, 2006), CYP1A4 and CYP1A5, which are orthologous to mammalian genes CYP1A1 and CYP1A2. The present study suggests that CYP1A expression, especially that of CYP1A5 mRNA, might be a better biomarker of quail PCP exposure. CYP1A genes can be induced via the aryl hydrocarbon receptor (AHR) signaling pathway (Marshall and Kerkvliet, 2010). Toxicity of several polycyclic/polychlorinated hydrocarbons can result from alterations in AHR gene transcription and the formation of heterodimers with ARNT (Walker et al., 1997). When activated, the AHR/ARNT heterodimers bind the promoter regions of a series of genes, causing downstream gene modulation, which includes both phase I and II enzymes (e.g. CYP1A1 and UGT1A1, respectively) (Sorg, 2014). Elevated levels of AHR and ARNT were observed in all PCP exposure groups, and were accompanied by significantly higher expression levels of the Ahr-controlled gene

Fig. 4. Enzyme activities of GST, GSH-Px, SOD, and CAT (a); Content of 8-OHdG (b); MDA (c); GSH (d) ($n = 6$). Concentrations of MDA and GSH are expressed as nmol/mg protein and μmol/mg protein, respectively. Activities of GST, GSH-Px, CAT, and SOD are expressed as U/mg protein. Statistically significant differences between the control and treatments are indicated by * $P < 0.05$ and ** $P < 0.01$.

Fig. 5. Protein levels of NF-κB and Nrf2 in quail livers after PCP exposure for 42 d. (upper panel) Western blot analyses of NF-κB and Nrf2. (lower panel) Densitometry analysis results of Western blots shown in (a) ($n = 6$). Statistical significance is indicated by * $p < 0.05$ and ** $P < 0.01$ compared with the control.

CYP1A. These results indicate that PCP induced CYP1A, possibly via an AhR-mediated pathway. Previous experiments have suggested that PCP can form dimers, leading to production of tetrachlorodibenzo-p-dioxin, an efficient AhR agonist and CYP1A

inducer (Jung et al., 2001). However, it is unknown whether this occurs within the quail, which deserves further research (Jiang et al., 2016).

CYP1B1 expression is also found in other bird species, and embryonic chicken (*Gallus gallus*) and quail CYP1B1 mRNA constitutive localization have been previously determined (Chambers et al., 2007). The current study is the first to report on alternation of CYP1B1 gene expression caused by xenobiotic administration. Following the administration of xenobiotics, such as phenobarbital, rifampicin, and hyperforin, CYP2C enzymes can be induced by activation of pregnane X receptor or constitutive androstane receptor (Kamata et al., 2010). In the present study, CYP2C18 induction by PCP might emerge via specific intracellular receptor responses. Due to the roles of CYP450 in xenobiotic detoxification and activation, changes in CYP450 expression can significantly affect xenobiotic potential risk and is of importance to toxicological studies (Mortensen and Arukwe, 2007).

PCP can induce P450 activities in insect and animal tissues, including that of humans, rats, and fish (Dubois et al., 1997; Livingstone, 1998; Suwanchaichinda and Brattsten, 2001), and is reportedly transformed into tetrachlorohydroquinone by a human P450 (CYP 3A4) (Suwanchaichinda and Brattsten, 2001). EROD is a commonly used biomarker for mammalian, bird, and fish CYP1A catalytic activity (Whyte et al., 2000), but it appears possible for other forms of CYP450 1 to deethylate ethoxyresorufin (Scornaienzi et al., 2010). A recent study indicated that CYP1A5 in chickens prefers shorter alkoxyresorufins, like CYP1A2 (the

mammalian orthologue), and that CYP1A4 and CYP1A5 both have similar catalytic activity toward MROD (Yang et al., 2013b). In the current study, PCP exposure was responsible for the significant increase in EROD and MROD activity in both the 0.05 and 5 mg/kg/d groups. Studies focusing on CYP1A isoform enzymatic activity have shown differences in their substrate preferences within bird species (Antos et al., 2015). Taken together with the present results, quail CYP1A5 might exhibit higher EROD and MROD activities, and induction of EROD could be a more specific and sensitive biomarker for quails treated with PCP.

CYP2A-, CYP2B-, and CYP3A-associated activities, as measured by fluorometric assays for ECOD, PROD, and END, respectively, have been reported in previous research (Murtomaa-Hautala et al., 2012; Liang et al., 2013). Our results showed that PCP exposure significantly induced ECOD expression at dosages of 0.5 and 5 mg/kg/d, whereas PROD and END activities were markedly inhibited in the 0.5 mg/kg/d PCP-treated group. The increase in ECOD activity observed in the present study confirms the findings of (Dubois et al., 1996), who reported PCP treatment induced ECOD activity in fetal quail hepatocytes, suggesting that PCP enhances the levels of CYP2A apoprotein and related activities. The significant decreases in the levels of PROD and END enzymes following PCP exposure indicates that CYP2B and CYP3A expressions were downregulated by PCP. Furthermore, BROD activities were significantly decreased after PCP exposure. *In vivo* stimulation with PCP did not alter CYP3A4-mediated metabolism of 7-benzyloxy-4-trifluoromethyl-coumarin (BFC) in quail. These findings are consistent with past data demonstrating that PCP can induce cytochrome 3A4 in rat hepatocytes, but not quail hepatocytes, suggesting possible species specificity (Thummel and Wilkinson, 1998). These results indicate that PCP can alter the activity of quail liver enzymes, which function in the xenobiotic activation and/or detoxification pathway.

PCP is associated with increased free radical production and alterations in antioxidant system scavenging capacity (Kan et al., 2015). For aerobic metabolism, ROS are unavoidable (Lushchak, 2011), and when ROS exceed the amount that can be removed by an organism's antioxidant defense mechanisms, oxidative stress occurs. The widely-used endpoint of ROS-induced lipid peroxidation is MDA, with 8-OHdG considered a biomarker for free radical-induced oxidative lesions. Both molecules, as biomarkers for oxidative stress, can reflect the degree of DNA oxidative damage, and are positively correlated with the degree of oxidative stress *in vivo* (He et al., 2016). Here, administration of PCP caused considerable MDA and 8-OHdG accumulation in the quail liver, as also reported in earlier research (Umemura et al., 1996; Dong et al., 2009). Antioxidant enzymes, such as CAT, GST, SOD, and GSH-Px, allow an organism to cope with ROS. We found that GSH levels decreased in all PCP exposure groups, whereas SOD activity in the low PCP exposure group and CAT activity in all PCP exposure groups increased. GSH depletion causes ROS increase, which can subsequently elevate the intensity of lipid peroxidation (Chiou et al., 2003). Furthermore, increased SOD and CAT activities can indicate enhanced superoxide and hydrogen peroxide influx, which can then trigger SOD activation and CAT enzyme elevation. A decrease in GSH-Px activities was observed in the PCP-treated quail liver. Using GSH as a substrate, GSH-Px can catalyze the conversion of hydrogen peroxide to hydrogen oxide (Kaptaner, 2015). A decrease in GSH-Px activity could be a protective response to hydrogen peroxide overproduction. Here, GST activity did not show significant change following PCP exposure for 42 d. Similarly, PCP exposure has shown no significant effect on GST enzyme activity in *Chironomus riparius* (Morales et al., 2014).

NF- κ B and Nrf2 play key roles in the detoxification/antioxidant defense mechanism of phase II induction by enhancing the

expression of certain enzymes, such as heme oxygenase-1, to fight oxidative stress (Sahin et al., 2012). Recently, Nrf2, a regulator of phase II and antioxidant enzyme induction, has received considerable attention in regards to its potential role in cellular defense against oxidative stress. It can help prevent PCP-induced oxidative damage and hepatocyte proliferation induction (Umemura et al., 2006) and lipopolysaccharide-induced NF- κ B activation can be significantly diminished by PCP treatment (Yoshitake et al., 2008). The increased NF- κ B and decreased Nrf2 expression following PCP exposure observed in this study could be related to minimizing PCP-induced oxidative stress through a series of activation and translocation pathways.

In conclusion, high concentrations of PCP were found in the kidney and liver of quail, indicating that these tissues exhibit high bioaccumulation capability. The induction of CYP1A4, CYP1A5, CYP1B1, CYP2C18, AHR1, and ARNT1 mRNA levels, as well as CYP450 enzyme activities, following PCP exposure suggests that the CYP-dependent drug-metabolizing system might be fully activated. Furthermore, changes in NF- κ B and Nrf2 suggest that transcription factors are involved in the hepatotoxicity of PCP. Given the induction of antioxidant enzymes, lipid peroxidation, and DNA oxidation, the potential toxicity induced by PCP metabolic processes and whether PCP results in chronic effects in other organs will be subject to further investigation.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.chemosphere.2017.02.154>.

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