Oxidative damage to cellular and isolated DNA by metabolites of a fungicide ortho-phenylphenol

Mariko Murata, Kosuke Moriya, Sumiko Inoue and Shosuke Kawanishi

Department of Hygiene, Mie University School of Medicine, Tsu, Mie 514-8507, Japan

Introduction

ortho-Phenylphenol (OPP) and its sodium salt, which are used as fungicides and antibacterial agents, have been found to cause carcinomas in the urinary tract of rats. To clarify the carcinogenic mechanism of OPP, we compared the DNA damage inducing ability of an OPP metabolite, phenyl-1,4-benzoquinone (PBQ) with that of another metabolite, phenylhydroquinone (PHQ). Pulsed field gel electrophoresis showed that PBQ and PHQ induced DNA strand breakage in cultured human cells, but PBQ did it more efficiently than PHQ. Significant increases in 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) were observed in cells treated with PBQ and PHQ, and the increase of 8-oxodG induced by PBQ was significantly higher than that induced by PHQ. Using 32P-5'-end-labeled DNA fragments obtained from human p53 tumor suppressor gene and c-Ha-ras-1 protooncogene, we showed that PBQ plus NADH, and also PHQ, induced DNA damage frequently at thymine residues, in the presence of Cu(II). The intensity of DNA damage by PBQ was stronger than that by PHQ, showing higher importance of PBQ than other OPP metabolites. Catalase and bathocuproine inhibited Cu(II)-mediated DNA damage by PBQ plus NADH and PHQ, suggesting that H2O2 reacts with Cu(I) to produce active species causing DNA damage. Electron spin resonance and UV-visible spectroscopic studies have demonstrated generation of semiquinone radical and superoxide from the reaction of PBQ with NADH or the Cu(II)-mediated autoxidation of PHQ. The present results suggest that these OPP metabolites cause oxidative DNA damage through H2O2 generation in cells, and the damage may lead to mutation and carcinogenesis. It is concluded that PBQ may play a more important role in the expression of OPP carcinogenicity than other OPP metabolites.

Abbreviations: 8-oxodG, 8-oxo-7,8-dihydro-2'-deoxyguanosine; DTPA, diethylenetriamine-N,N',N'',N'''-pentaacetic acid (DTPA) and bathocuproinedisulfonic acid were from Dojin Chemicals Co. (Kumamoto, Japan). Superoxide dismutase (SOD), 3000 U/ml from bovine erythrocytes, catalase (45 000 U/mg from bovine liver), methionyl, RNase A and bacterial alkaline phosphatase (BAP) were from Sigma. ABI lysis buffer was from Applied Biosystems. Nuclease P1 was from Yamasa Shoyu Co. (Chiba, Japan). Acrylamide, bisacrylamide and piperdine were from Wako Chemicals Co. (Osaka, Japan). Phenyl-1,4-hydroquinone (PHQ) was from Tokyo Kasei Co. (Tokyo, Japan). Ethanol solutions of PHQ and PBQ were freshly made up each time.

Detection of cellular DNA damage by pulsed field gel electrophoresis

HL60 cells were grown in RPMI 1640 supplemented with 6% fetal calf serum (FCS) at 37°C under 5% CO2 in a humidified atmosphere. HL60 cells were treated with either PBQ or PHQ at 37°C. The medium contained 0.05% ethanol as the solvent of PBQ and PHQ. Control condition also contained 0.05% ethanol. After the incubation, the medium was removed and the cells
were washed twice with phosphate-buffered saline (PBS) and resuspended in PBS. The cell suspension was solidified with agarose, followed by treatment with proteinase K according to the method described previously (19). Electrophoresis was performed in 0.5× TBE buffer (45 mM Tris, 45 mM boric acid, 1 mM EDTA, pH 8.0) by CHEF-Mapper pulsed field electrophoresis system (Bio-Rad) at 200 V and 14°C. Switch time was 60 s for 15 h followed by 90 s switch time for 9 h. The DNA in the gel was visualized in ethidium bromide.

Analysis of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) formation in HL60 cells treated with OPP metabolites

HL60 cells (1×10⁶ cells/ml) were incubated with either PHQ or PBQ in 5.0 ml of RPMI 1640 supplemented with 6% FCS at 37°C. After the incubation, the medium was removed and the cells were washed twice with PBS. The cells were suspended in 0.05 mg/ml RNase A, 0.5 mg/ml Proteinase K and 500 µl of ABI lysis buffer and incubated for 60 min at 60°C. After ethanol precipitation, DNA was digested to nucleosides with nuclease P1 and bacterial alkaline phosphatase and analyzed by electrochemical detection coupled to high-performance liquid chromatography (HPLC–ECD), as described previously (19). The amount of 8-oxodG was measured by a modified method of Takeuchi et al. (20).

Preparation of 3'-P-5'-end-labeled DNA fragments

DNA fragments were obtained from the human p53 tumor suppressor gene (21). Two fragments from the p53 gene containing exons were amplified by the PCR method using an Omnipol Gene Temperature Cycling System. The PCR products were digested with Smal I and ligated into Smal-cleaved pUC 18 plasmid, and then transferred to Escherichia coli JM 109. The plasmid pUC 18 was digested with EcoRI and HindIII, and the resulting DNA fragments were fractionated by electrophoresis on 2% agarose gels. The 5'-end-labeled 650 bp fragment (HindIII*13972–EcoRI*14621) was obtained by dephosphorylation with calf intestine phosphatase and repolyphosphorylation with [γ-32P]ATP and T4 polynucleotide kinase (*, 32P-labeled). The 650 bp fragment was further digested with Apal to obtain singly labeled 443 bp fragment (Apal 14179–EcoRI*14621) and the 211 bp fragment (HindIII*13972–Apal 14182), as described previously (22). DNA fragment was also obtained from human c-Ha-ras-1 protooncogene (23). A DNA fragment was prepared from plasmid pbcNL, which carries a 6.6 kb BamHI chromosomal DNA segment containing c-Ha-ras-1 gene, and a singly labeled 337 bp fragment (PstI 2345–Avul*2681) were obtained according to the method described previously (24). Nucleotide numbering starts with the BamHI site (23).

Detection of DNA damage by OPP metabolites in the presence of NADH and Cu(I)

The standard reaction mixture (in a microtube; 1.5 ml; Eppendorf) contained PHQ or PBQ plus NADH and CuCl₂. 32P-5' end-labeled DNA fragments (sonicated calf thymus DNA (25 µM per base) in 200 µl of 10 mM sodium phosphate buffer (pH 7.8) containing 2.5 µM DTPA. DTPA was added to remove metal ions, which may be contained in sodium phosphate buffer. After incubation at 37°C for 1 h, the DNA fragments were heated at 90°C in 1 ml pipetidine for 20 min where indicated and treated as described previously (25). The preferred cleavage sites were determined by direct comparison of the positions of the oligonucleotides with those produced by the chemical reactions of the Maxam–Gilbert procedure (26) using a DNA-sequencing system (LKB 2010 Macrophor). The relative amounts of oligonucleotides from the treated DNA fragments were measured with a laser densitometer (LKB 2222 UltraScan XL).

Analysis of 8-oxodG formation in calf thymus DNA by OPP metabolites in the presence of NADH and Cu(I)

The amount of 8-oxodG was measured by a modified method of Kasai et al. (27). Native or denatured DNA fragments (25 µM per base) from calf thymus were incubated with PHQ or PBQ in the presence and absence of NADH and CuCl₂ for 1 h at 37°C. For the experiment with denatured DNA, calf thymus DNA fragments was treated for DNA extraction. DNA was digested to nucleosides enzymatically, and analyzed by HPLC–ECD, as described in the Materials and methods. Means (PBQ, square; PHQ, circle) and standard deviations (bar) are in values of two to four individual experiments. * (P < 0.05) and ** (P < 0.01) indicate significant differences compared with control, and # (P < 0.05) indicates significant difference compared with the same concentration between PBQ and PHQ by Student’s t-test.

Detection of semiquinone radical derived from OPP metabolites

The generation of semiquinone radical from PHQ or PBQ plus NADH in the presence and absence of CuCl₂ in 10 mM sodium phosphate buffer (pH 7.8) was detected by using an ESR (JEOL model JES-TE100) with 100 kHz field modulation at room temperature (25°C). Reaction mixtures were taken up in a flat cell and spectra were recorded immediately after mixture with a microwave power of 16 mW; a modulation amplitude of 0.1 mT and a receiver gain of 500. No spin trapping agent was used.

Results

Detection of DNA damage in cultured cells treated with OPP metabolites

Figure 1 shows DNA strand breakage in cultured cells treated with OPP metabolites, detected by pulsed field gel electrophoresis. DNA strand breakage to produce 1000–2000 kb fragments was observed in cells treated with 10–20 µM PBQ and PHQ, but not with PHQ under the condition used. Formation of 50 kb fragments was increased at 10–20 µM PBQ and PHQ. These results reveal that PBQ caused cellular DNA damage more efficiently than PHQ.

Analysis of 8-oxodG formation in HL60 cells induced by OPP metabolites

Figure 2 shows the amount of 8-oxodG in cells induced by OPP metabolites. The formation of 8-oxodG was significantly
higher in cells treated with 20 µM PHQ (P < 0.05) and 20 µM PBQ (P < 0.01) than that of the control. The increase of 8-oxodG induced by PBQ was significantly (P < 0.05) higher than that induced by PHQ under 20 µM.

**Damage of ³²P-labeled DNA fragments by OPP metabolites in the presence of NADH and Cu(II)**

Figure 3 shows an autoradiogram of DNA fragments treated with PHQ or PBQ plus NADH in the presence of Cu(II). Oligonucleotides were detected on the autoradiogram as a result of DNA cleavage. In the absence of PHQ and PBQ, DNA damage was not observed in the presence of NADH and Cu(II) (lane 1) under the conditions used. When NADH or Cu(II) was omitted (lanes 2 and 3), no DNA damage was observed. Whereas PHQ-induced DNA damage required Cu(II) alone, PBQ-induced DNA damage required both NADH and Cu(II). The intensity of DNA damage increased with time (data not shown) and increasing concentration of PHQ (lanes 4–6) or PBQ (lanes 7–9). The intensity of Cu(II)-dependent DNA damage induced by PBQ was stronger than that of PHQ under the same concentration, although PBQ required 100–200 µM NADH.

**Effects of scavengers and bathocuproine on DNA damage by PBQ**

Figure 4 shows the effects of scavengers and bathocuproine, a Cu(I)-specific chelator, on DNA damage induced by PBQ plus NADH in the presence of Cu(II). Inhibition of DNA damage by catalase (lane 10) and bathocuproine (lane 11) indicates the involvement of H₂O₂ and Cu(I). Methional inhibited the DNA damage (lane 8), although other typical hydroxyl free radical (OH⁻) scavengers such as ethanol (lane 5), mannitol (lane 6) and sodium formate (lane 7), and superoxide dismutase (SOD) (lane 9) showed little or no inhibitory effect on DNA damage. When denatured DNA was used, formation of oligonucleotides increased (lane 12).

**Increase of oligonucleotides with piperidine treatment**

Lane 1, control; lane 2, Cu(II) + NADH; lane 3, 20 µM PBQ; lane 4, Cu(II) + NADH + 20 µM PBQ; lane 5, Cu(II) + NADH + PBQ + 5% (v/v) ethanol; lane 6, Cu(II) + NADH + PBQ + 0.1 M mannitol; lane 7, Cu(II) + NADH + PBQ + 0.1 M sodium formate; lane 8, Cu(II) + NADH + PBQ + 0.1 M methional; lane 9, Cu(II) + NADH + PBQ + 30 U SOD; lane 10, Cu(II) + NADH + PBQ + 30 U catalase; lane 11, Cu(II) + NADH + PBQ + 50 µM bathocuproine; lane 12, DNA was denatured by 90°C for 5 min and quickly chilled before incubation with Cu(II) + NADH + PBQ; lane 13, Cu(II) + NADH + PBQ without piperidine treatment.

**Site specificity of DNA cleavage by OPP metabolites**

To examine the DNA cleavage site, ³²P-5'-end-labeled 337 bp DNA fragments, 25 µM/base of sonicated calf thymus DNA, were incubated with PHQ or PBQ plus 200 µM NADH, 20 µM CuCl₂ in 200 µl of 10 mM sodium phosphate buffer (pH 7.8) containing 2.5 µM DTPA. Scavenger or bathocuproine was added where indicated. After incubation at 37°C for 1 h, followed by piperidine treatment, the DNA fragments were analyzed by the method described in the legend to Figure 3. Lane 1, control; lane 2, Cu(II) + NADH; lane 3, 20 µM PBQ; lane 4, Cu(II) + NADH + 20 µM PBQ; lane 5, Cu(II) + NADH + PBQ + 5% (v/v) ethanol; lane 6, Cu(II) + NADH + PBQ + 0.1 M mannitol; lane 7, Cu(II) + NADH + PBQ + 0.1 M sodium formate; lane 8, Cu(II) + NADH + PBQ + 0.1 M methional; lane 9, Cu(II) + NADH + PBQ + 30 U SOD; lane 10, Cu(II) + NADH + PBQ + 30 U catalase; lane 11, Cu(II) + NADH + PBQ + 50 µM bathocuproine; lane 12, DNA was denatured by 90°C for 5 min and quickly chilled before incubation with Cu(II) + NADH + PBQ; lane 13, Cu(II) + NADH + PBQ without piperidine treatment.

**Figure 3. Autoradiogram of ³²P-labeled DNA fragments incubated with PHQ and PBQ plus NADH in the presence of Cu(II).** The reaction mixture contained ³²P-5'-end-labeled 337 bp DNA fragments, 25 µM/base of sonicated calf thymus DNA, the indicated concentrations of PHQ or PBQ plus 200 µM NADH, 20 µM CuCl₂ in 200 µl of 10 mM sodium phosphate buffer (pH 7.8) containing 2.5 µM DTPA. The mixture was incubated at 37°C for 1 h, and then treated with 1 M piperidine at 90°C for 20 min. The DNA fragments were electrophoresed on an 8% polyacrylamide–8 M urea gel and the autoradiogram was obtained by exposing X-ray film to the gel. Lane 1, control; Cu(II) + NADH; lane 2, 10 µM PHQ; lane 3, NADH + 10 µM PBQ; lane 4, Cu(II) + 2 µM PHQ; lane 5, Cu(II) + 5 µM PHQ; lane 6, Cu(II) + 10 µM PBQ; lane 7, Cu(II) + NADH + 2 µM PBQ; lane 8, Cu(II) + NADH + 5 µM PBQ; lane 9, Cu(II) + NADH + 10 µM PBQ.

**Figure 4. Effects of scavengers or bathocuproine on DNA cleavage induced by PBQ in the presence of NADH and Cu(II).** The reaction mixture contained ³²P-5'-end-labeled 443 bp DNA fragments, 25 µM/base of sonicated calf thymus DNA, 20 µM PBQ, 200 µM NADH and 20 µM CuCl₂ in 200 µl of 10 mM sodium phosphate buffer (pH 7.8) containing 2.5 µM DTPA. Scavenger or bathocuproine was added where indicated. After incubation at 37°C for 1 h, followed by piperidine treatment, the DNA fragments were analyzed by the method described in the legend to Figure 3. Lane 1, control; lane 2, Cu(II) + NADH; lane 3, 20 µM PBQ; lane 4, Cu(II) + NADH + 20 µM PBQ; lane 5, Cu(II) + NADH + PBQ + 5% (v/v) ethanol; lane 6, Cu(II) + NADH + PBQ + 0.1 M mannitol; lane 7, Cu(II) + NADH + PBQ + 0.1 M sodium formate; lane 8, Cu(II) + NADH + PBQ + 0.1 M methional; lane 9, Cu(II) + NADH + PBQ + 30 U SOD; lane 10, Cu(II) + NADH + PBQ + 30 U catalase; lane 11, Cu(II) + NADH + PBQ + 50 µM bathocuproine; lane 12, DNA was denatured by 90°C for 5 min and quickly chilled before incubation with Cu(II) + NADH + PBQ; lane 13, Cu(II) + NADH + PBQ without piperidine treatment.
PHQ (Figure 7B) in the presence of Cu(II). The amount of 8-oxodG increased with PBQ and PHQ concentration. When denatured DNA was used, the formation of 8-oxodG was increased. The increase of 8-oxodG produced by PBQ was higher than that produced by PHQ at the same concentrations.

Detection of $O_2^-$ derived from PBQ plus NADH in the presence and absence of Cu(II)

Figure 8 shows $O_2^-$ produced by PBQ plus NADH in the presence and absence of Cu(II). These results indicate that $O_2^-$ was generated by PBQ reduction even in the absence of Cu(II). When Cu(II) was added, the content of $O_2^-$ detected by this system was decreased, suggesting rapid reaction between $O_2$ and Cu(II). Production of $O_2^-$ by the autoxidation of PHQ was also detected (date not shown).

Fig. 5. Comparison of site specificity of DNA cleavage induced by PBQ in the presence of 200 µM NADH and 20 µM CuCl$_2$. The $^{32}$P-5' end-labeled 337 bp fragment (PstI 2345–AvaI 2681) of c-Ha-ras-1 gene, 25 µM/base of sonicated calf thymus DNA, 5 µM PBQ plus 200 µM NADH and 20 µM CuCl$_2$ in 200 µl of 10 mM sodium phosphate buffer (pH 7.8) containing 2.5 µM DTPA. Reaction mixtures were incubated at 37°C for 1 h. Native DNA (A) and denatured DNA (B) were used. The DNA fragments were treated with 1 M piperidine for 20 min at 90°C, and then electrophoresed on an 8% polyacrylamide–8 M urea gel. The autoradiogram was obtained by exposing X-ray film to the gel. The relative amounts of DNA fragments were measured by scanning the autoradiogram with a laser densitometer (LKB 2222 UltroScan XL). The horizontal axis shows the nucleotide number of human c-Ha-ras-1 protooncogene starting with the BamHI site (23).

Fig. 6. Comparison of site specificity of DNA cleavage induced by PBQ plus 200 µM NADH and PHQ in the presence of 20 µM CuCl$_2$. The $^{32}$P-5' end-labeled 211 bp fragment (HinflI*13972–ApaI 14182) from human p53 tumor suppressor gene, 25 µM/base of sonicated calf thymus DNA and 5 µM PBQ plus NADH and Cu(II) (A), or 10 µM PHQ plus Cu(II) (B), in 200 µl of 10 mM sodium phosphate buffer (pH 7.8) containing 2.5 µM DTPA. Reaction mixtures were incubated at 37°C for 1 h. After the piperidine treatment, the DNA fragments were analyzed as described in the legend to Figure 4. The relative amounts of oligonucleotide were measured by scanning the autoradiogram with a laser densitometer (LKB 2222 UltroScan XL). The horizontal axis shows the nucleotide number of human c-Ha-ras-1 protooncogene starting with the BamHI site (23).

Fig. 7. Formation of 8-oxodG in calf thymus DNA by PBQ plus NADH and PHQ in the presence of Cu(II). DNA fragments were incubated with PBQ plus 200 µM NADH or PHQ, and 20 µM CuCl$_2$ for 1 h at 37°C. After ethanol precipitation, DNA was digested to the nucleosides enzymatically and analyzed by HPLC–ECD as described in the Materials and methods. PBQ and PHQ concentrations are as follows: 0, 5, 10, 15 and 20 µM.
Production of free radicals from OPP metabolites

Figure 9 shows ESR spectra of radicals generated by OPP metabolites. PBQ plus NADH showed the formation of a 1:3:3:1 spectrum radical with hyperfine splitting constant of $\alpha^H = 0.23$ mT, and PHQ also showed the similar signal. The ESR four-line signal was assigned to the PHQ semiquinone radical, as described in our previous study (11). The addition of Cu(II) to the reaction systems resulted in a marked increase in the signal intensity of the semiquinone radical.

Discussion

Pulsed field gel electrophoresis study showed that PBQ and PHQ caused cellular DNA damage, but PBQ did so more efficiently than PHQ. Significant increase of 8-oxodG was observed in cells treated with PBQ and PHQ. The formation of 8-oxodG by PBQ was significantly higher than that by PHQ, at a concentration of 20 µM. These results suggest that PBQ may play a more important role in damage to cellular DNA than does PHQ, although both of these OPP metabolites induced oxidative damage.

To clarify the mechanism of cellular DNA damage induced by OPP metabolites, we investigated damage to $^{32}$P-labeled DNA fragments obtained from the human p53 tumor suppressor gene and c-Ha-ras-1 protooncogene. PBQ required both NADH and Cu(II) for DNA damage, whereas PHQ required only Cu(II). Inhibitory effects of catalase and bathocuproine on the DNA damage indicate the involvement of reactive oxygen species generated from H$_2$O$_2$ and Cu(I). Inhibitory effects of catalase and bathocuproine on the DNA damage, because the conversion of Cu(II) into Cu(I) was required for the DNA damage, whereas the semiquinone radical was formed by PBQ plus NADH even in the absence of Cu(II).

A possible mechanism of DNA damage induced by OPP metabolites in the presence of NADH and Cu(II) is proposed as shown in Figure 10. PHQ is autooxidized to PBQ through the intermediate semiquinone. PBQ is reduced by an endogenous reductant, NADH, to form semiquinone radical. The generation of O$_2^-$ takes place during the Cu(II)-mediated autoxidation of PHQ or by the reaction of semiquinone radical with O$_2$. The generation of H$_2$O$_2$ by O$_2^-$ dismutation and the reduction of Cu(II) to Cu(I) occur concomitantly. H$_2$O$_2$ reacts with Cu(I) to form a metal–oxygen complex, such as Cu(I)–OOH causing DNA damage. Thus, the NADH-dependent redox cycle of PBQ generates reactive oxygen species, and mediates DNA damage. The production of semiquinone radical and O$_2^-$ from PHQ or PBQ plus NADH was confirmed by the data of ESR and UV-visible spectroscopies, respectively. However, the semiquinone radical is not an active species causing DNA damage, because the conversion of Cu(II) into Cu(I) was required for the DNA damage, whereas the semiquinone radical was formed by PBQ plus NADH even in the absence of Cu(II).

The biological importance of NADH as a nuclear reductant has been described (37). The possibility that some chemicals are non-enzymatically reduced by NADH in vivo has been shown (38–40). NADH can be a source of endogenous reductant, resulting in oxidative DNA damage. The present study showed that PBQ induced cellular DNA strand break at lower concentration than PHQ, indicating that PBQ has a higher potentiality to cause DNA damage in HL60 cells. This is supported by the report regarding DNA damage of bladder epithelium of rats treated with PBQ, but not with PHQ (18). It is considered that the NADH-dependent redox cycle is important to explain the higher potentiality of PBQ. The
concentration of NAD(P)H in certain tissues has been estimated to be as high as 100–200 μM (41), and NAD(P)H possibly plays important roles as a reductant.

Copper occurs in the mammalian cell nucleus, and may contribute to high order chromatin structures (42). Cu(II)/ascorbate/H2O2-mediated DNA damage in aerobic aqueous solutions is believed to be induced in vitro and in vivo (43) through formation of a DNA–Cu(I)–H2O2 complex (32). Copper caused much stronger ascorbate-mediated DNA damage than iron (44). Copper ions exhibit a very high affinity for DNA, and DNA-bound Cu(II) can undergo Cu(II)/Cu(I) redox cycling in a reducing environment, and also O2 reduced to O2−, generating H2O2. Also, the DNA–Cu(I) complex reacts with H2O2, inducing DNA damage through a Fenton-type reaction (45–47). Therefore, the copper-dependent DNA damage by OPP metabolites is of interest in connection with these observations.

Many studies have shown cytotoxicity and genotoxicity of OPP (1–8). Generation of reactive oxygen species from the redox cycle by OPP metabolites has been discussed in relation to OPP carcinogenicity. Although DNA–OPP metabolite adduct formation has been considered as one of the possible mechanisms of OPP carcinogenesis (9,10), a recent study (48) shows the lack of OPP–DNA adduct formation in bladder epithelium of rats exposed to OPP. This report may support the contribution of oxidative DNA damage to the expression of OPP carcinogenicity instead of DNA adduct formation. The present study suggests that OPP metabolites generate reactive oxygen species to induce cellular DNA damage, including 8-oxodG. It has been reported that 8-oxodG formation can cause DNA misreplication resulting in mutation (49,50), leading to carcinogenesis.

OPP is known to cause carcinomas in the urinary bladder and kidney of rats (1–7). Nakao et al. (51) estimated that non-conjugated forms of OPP and PHQ at a dose of 250–300 μM were excreted in the urine of rats receiving 2% OPP in their diet, with which the incidence of tumors was 90%. Interestingly, it is reported that high levels of prostaglandin H synthase are also localized in human bladder and kidney and have been proposed to play a role in the oxidation of PHQ to PBQ exhibiting toxicity to these organs (52). It is noteworthy to find that these OPP metabolites at a low concentration (20 μM) cause oxidative DNA damage in cells, which might lead to mutation and carcinogenesis. In addition, we showed that PBQ caused stronger damage to both cellular and isolated DNA than PHQ. Although there is some difficulty in extrapolating these findings to the whole animal, we concluded that PBQ might play a more important role in OPP carcinogenesis than other OPP metabolites.

References
