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Polychlorinated Biphenyls Induce Oxidative DNA Adducts in Female *Sprague–Dawley* Rats

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Abstract

Polychlorinated biphenyls (PCBs) are organic chemicals that were traditionally produced and widely used in industry as mixtures and are presently formed as byproducts of pigment and dye manufacturing. They are known to persist and bioaccumulate in the environment. Some have been shown to induce liver cancer in rodents. Although the mechanism of the toxicity of PCBs is unknown, it has been shown that they increase oxidative stress, including lipid peroxidation. We hypothesized that oxidative stress-induced DNA damage could be a contributor for PCB carcinogenesis and analyzed several DNA adducts in female *Sprague–Dawley* rats exposed to 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126), 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB 153), and a binary mixture (PCB 126 + 153) for 14, 31, and 53 wks. Eight adducts were measured to profile oxidative DNA lesions, including 8-oxo-deoxyguanosine (8-oxo-dG), 1,N^6-ethenodeoxyadenosine (1,N^6-εdA), N^2,3-ethenoguanine (N^2,3-εG), 1,N^2-ethenodeoxyguanosine (1,N^2-εdG), as well as malondialdehyde (M_1dG), acrolein (AcrdG), crotonaldehyde (CrdG), and 4-hydroxynonenal-derived dG adducts (HNEdG) by LC–MS/MS analysis. Statistically significant increases were observed for 8-oxo-dG and 1,N^6-εdA concentrations in hepatic DNA of female rats exposed to the binary mixture (1000 ng/kg/day + 1000 μg/kg/day) but not in rats exposed to PCB 126 (1000 ng/kg/day) or PCB 153 (1000 μg/kg/day) for 14 and 31 wks. However, exposure to PCB 126 (1000 ng/kg/day) for 53 wks significantly increased 8-oxo-dG, 1,N^6-εdA, AcrdG, and M_1dG. Exposure to PCB 153 (1000 μg/kg/day) for 53 wks increased 8-oxo-dG, and 1,N^6-εdA. Exposure to the binary mixture for 53 wks increased 8-oxo-dG, 1,N^6-εdA, AcrdG, 1,N^2-εdG, and N^2,3-εG

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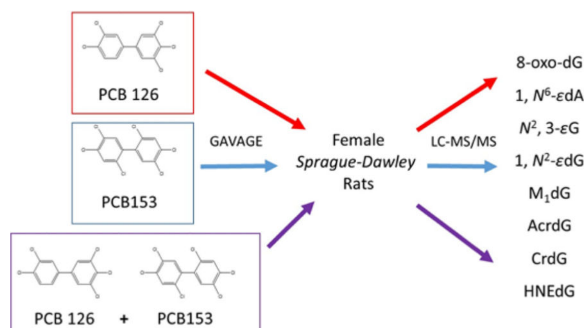
significantly above control groups. Increased hepatic oxidative DNA adducts following exposure to PCB 126, PCB 153, or the binary mixture shows that an increase in DNA damage may play an important role in hepatic toxicity and carcinogenesis in female *Sprague–Dawley* rats.

Graphical Abstract



INTRODUCTION

Polychlorinated biphenyls (PCBs) are industrial chemicals manufactured as mixtures that have been commercially available since 1929.[1] These compounds are relatively stable under a broad range of chemical, thermal, and electrical conditions. This stability has fostered their wide use in commercial applications, including dielectrics in transformers and capacitors, cooling fluids in hydraulic systems, and plasticizers in paints, copying paper, adhesives, sealants, plastics, and so forth.[1] Although production of PCBs was banned in 1979, some of these chemicals are still detectable in waters contaminated by paint manufacturing facilities and commercial paint pigments.[2] Because of their high lipophilicity and stability, these chemicals persist in the environment. Chronic exposure to PCBs has been shown to adversely affect the immune, reproductive, nervous, and endocrine systems in animals and, specifically, to induce cancer.[3,4] Additionally, the International Agency for Research on Cancer has recently classified PCBs, as a group, as a Group 1 Human Carcinogen due to their carcinogenic effects on animals and humans.[5]

PCBs are generally classified as either dioxin- or nondioxin-like depending on the location of the chlorination. Dioxin-like PCBs act through a similar mechanism of action as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) by binding to the aryl hydrocarbon receptor (AhR), which upon activation has been reported to cause severe weight loss, thymic atrophy, hepatotoxicity, immunotoxicity, and enzyme induction in rodents.[4,6] Available in vivo and in vitro data show that 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) has a toxic equivalency factor (TEF) value of 0.1 relative to that of the TCDD toxicity.[6] As the most potent PCB in the environment, PCB 126 accounts for 40–90% of the toxic potency of dioxin-like PCBs.[7] A nondioxin-like PCB, 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB 153), is shown to have the highest concentrations in human samples on a molar basis. Nondioxin-like PCBs are not included in the TEF methodology, and therefore, PCB 153 has no TEF value.[6]

The role of DNA adducts in the tumorigenesis of nongenotoxic chemicals such as PCBs has been studied, leading to one possible mechanism of PCB carcinogenesis occurring through

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the production of reactive oxygen species (ROS) or reactive oxygenated metabolites (ROM).[8,9] Generation of ROS or ROM is followed by oxidative attack on DNA or proteins.[4] ROS-induced damage during PCB exposure/metabolism has been reported in numerous in vitro[10–23] and in vivo studies.[3,24–45] Bioaccumulation of PCBs in target tissues may cause upregulation of CYP450 and subsequent DNA damage due to oxidative stress. Although other mechanisms of PCB carcinogenesis have been suggested, such as induction of metabolic enzymes, inhibition of gap junction intercellular communication, stimulation of mitosis or inhibition of apoptosis, and enhanced cell proliferation, the accumulation of promutagenic lesions in DNA may also be a key factor.[46–49]

In this study, we collaborated with the National Toxicology Program (NTP) to understand the oxidative DNA adduct profile in hepatic DNA of female *Sprague–Dawley* rats that were exposed to PCB 126, PCB 153 (Figure 1), and a binary mixture of PCB 126 and PCB 153 for 14, 31, and 53 wks. To date, several DNA adducts have been specified as potential key endogenous oxidative DNA adducts in cancer studies and include 8-oxo-deoxyguanosine (8-oxo-dG), 1,N^6-ethenodeoxyadenosine (1,N^6-εdA), N^2,3-ethenoguanine (N^2,3-εG), 1,N^2-ethenodeoxyguanosine (1,N^2-εdG), malondialdehyde (MDA)-derived dG adducts (M_1dG), acrolein-derived dG adducts (AcrdG), crotonaldehyde-derived dG adducts (CrdG), and 4-hydroxynonenal (HNE)-derived dG adducts (HNEdG).[39,41–44,50–61] These adducts are summarized in Figure 2. Site-directed mutagenicity studies found that these DNA adducts could induce specific transition and/or transversion point mutations in bacteria and/or mammalian cells.[50–57] Growing evidence supports that these DNA adducts are significantly induced in patients or animals with various chronic inflammatory diseases, including cancer.[39,52,58–61] Because these adducts have different formation and repair pathways in vivo and can cause different mutations in cells, their profile study will provide more comprehensive information on oxidative DNA lesions to estimate the toxicity of PCBs. The knowledge gained from this study will improve the scientific basis of human risk assessment of PCBs in the environment.

EXPERIMENTAL PROCEDURES

Chemicals

Nucleic acid purification grade lysis buffer, protein precipitation solution, and proteinase K were purchased from Gentra Systems (Minneapolis, MN). HPLC grade water and methanol were from Thermo Fisher Scientific Company (Raleigh, NC). $^{15}N_5$-8-oxo-dG, $^{15}N_5$-dG and $^{13}C_{10}$-dG were purchased from Cambridge Isotope Laboratories (Andover, MA, USA). Other chemical reagents were from Sigma-Aldrich Chemical Company (St. Louis, MO). $^{15}N_5$-1,N^6-εdA standard was synthesized as described by Ham et al.[62] 1,N^2-εdG and $^{13}C_{10}$-1,N^2-εdG were synthesized as reported by Kusmierek et al.[63] MDA-modified $^{15}N_5$ and $^{14}N_5$ DNA were made by the method in Jeong's study.[64] AcrdG, CrdG, and HNEdG standards and their $^{15}N_5$-labeled internal standards were synthesized according to previously published methods.[65–67]

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Animal Exposure and DNA Isolation

Rat liver tissues were provided by Battelle Laboratories (Columbus, OH) and State University of New York at Buffalo from a series of studies conducted for the National Toxicology Program's Dioxin Toxic Equivalency Factor (TEF) initiative according to the NIEHS contract N01-ES-75411.[7,68,69] Female Harlan *Sprague–Dawley* rats were treated by gavage (2.5 mL/kg) with PCB 126 alone, PCB 153 alone, or the binary mixture of PCB 126 and PCB 153 five days per week for 14, 31, and 53 weeks. The *Sprague–Dawley* female rat was used for these studies based upon the prior observation of high hepatocarcinogenic potency of TCDD within this strain and the extensive literature on the effects of TCDD and related compounds in this model.[7,68–70] Dose formulations were prepared fresh on a monthly basis by formulating the test articles in a corn-oil vehicle containing 1% USP-grade acetone. Control groups, dosed with a corn-oil:acetone (99:1) vehicle were included in each of the studies. Doses used were based on values of the TEF selected by the World Health Organization.[6,71] The design and dosages for these studies are as outlined in the original NTP reports and were selected to evaluate the dose response of a constant ratio of each of the PCB congeners and evaluate the effect of increasing concentrations of PCB 153 on the PCB 126-induced responses. The dosages of PCB 126 were designed to match the dose range used for the studies of carcinogenicity of TCDD with an adjustment for the TEF of 0.1. The doses of PCB 153 are similar to those previously used in tumor promotion studies.[7,68,69] The doses were as follows: PCB 126 (0 and 1000 ng/kg/day for 14 and 31 wks; 0, 550, and 1000 ng/kg/day for 53 wks), PCB 153 (0 and 1000 μg/kg/day for 14 and 31 wks; 0, 300, and 1000 μg/kg/day for 53 wks), and the binary mixture of PCB 126 and PCB 153 (0 and 1000 ng/kg/day + 1000 μg/kg/day for 14 and 31 wks; 0, 300 ng/kg/day + 300 μg/kg/day, and 1000 ng/kg/day + 1000 μg/kg/day for 53 wks). Liver tissues were collected from 5–8 female rats per group/day following the final exposure and stored frozen at −80 °C. DNA was isolated as described previously.[72]

8-oxo-dG and 1,N^6-εdA Assay

The assay was performed as previously described by Pang et al. with minor modifications.[58] First, 100 μg of DNA in NaOAc buffer I (30 mM sodium acetate, 0.2 mM $ZnCl_2$, pH 5.6) was incubated with nuclease P1 (5 μg) at 37 °C for 1 h. Immediately after incubation, DNA solutions were spiked with 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO, 5 μL, 1.5 M), $^{15}N_5$-8-oxo-dG (500 fmol), and $^{15}N_5$-1,N^6-εdA (20 fmol) followed by the addition of NaOAc buffer II (30 mM sodium acetate, pH 8.1), alkaline phosphatase (20 units), and phosphodiesterase (0.012 units) and then incubated at 37 °C for an additional hour. Enzymes and undigested DNA were removed by Microcon-10 filtration (11500 rpm, 4 °C, 50 min), and the filtrate was concentrated by SpeedVac.

Samples were enriched for 8-oxo-dG and 1,N^6-εdA using an Agilent 1200 HPLC system equipped with an Atlantis T3 column (5 μm, 4.6 mm × 150 mm). The nucleosides were monitored at 264 nm. The column was eluted at a flow rate of 1 mL/min at 30 °C with a 5–80% methanol gradient in 10 mM ammonium acetate buffer as follows: hold at 5% methanol for 5 min, 5–10% methanol over 5 min, 10–20% methanol over 10 min, and 20–80% over 10 min and re-equilibrate at 5% for 5 min. 8-oxo-dG and 1,N^6-εdA fractions were collected at 24–26 min and 33–34 min, respectively.

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AcrdG, 1,N^2-εdG, M_1dG, CrdG, and HNEdG Assay

A similar assay to that of 8-oxo-dG and 1,N^6-εdA was applied to AcrdG, 1,N^2-εdG, M_1dG, CrdG, and HNEdG with minor modifications. First, 100 μg of DNA in NaOAc buffer I was incubated with nuclease P1 (5 μg) at 37 °C for 1 h. Immediately before incubation, DNA solutions were spiked with TEMPO (5 μL, 1.5 M), $^{15}N_5$–AcrdG (50 fmol), $^{13}C_{10}$-1,N^2-εdG (100 fmol), MDA-modified internal standard corresponding to 400 fmol $^{15}N_5$-M_1dG, $^{15}N_5$–CrdG (50 fmol), and $^{15}N_5$–HNEdG (50 fmol) followed by the addition of NaOAc buffer II, alkaline phosphate (20 units), and phosphodiesterase (0.012 units) and then incubated at 37 °C for an additional hour. Enzymes and undigested DNA were removed by Microcon-10 filtration (11500 rpm, 4 °C, 50 min), and the filtrate was concentrated by SpeedVac.

Samples were enriched for AcrdG, 1,N^2-εdG, and M_1dG by the same HPLC method as described for 8-oxo-dG and 1,N^6-εdA with a 100% methanol and 5 mM ammonium formate–0.1% formic acid mobile phase. CrdG and HNEdG were eluted at a flow rate of 0.5 mL/min with a 35–70% methanol mobile phase in 10 mM ammonium acetate buffer over 25 min. Fraction collection times for AcrdG, 1,N^2-εdG, CrdG, and HNEdG were 28–30, 32–34, 15–18, and 34–36 min, respectively.

LC–MS/MS Analysis

8-oxo-dG was analyzed by a Waters Acquity UPLC coupled to a Thermofinnigan TSQ Quantum Ultra triple-quadrupole mass spectrometer in a positive selected reaction mode (SRM) monitoring the signals m/z 284.1 → 168.0 for 8-oxo-dG and m/z 289.1 → 173.0 for $^{15}N_5$-8-oxo-dG. Separation was performed on a T3 HSS column (1.7 μm, 2.1 mm × 100 mm) with a flow rate of 200 μL/min using mobile phase (A) 0.1% acetic acid in water and (B) 0.1% acetic acid in methanol. MS settings were as follows: electrospray voltage (3000 V), ion transfer capillary temperature (285 °C), vaporizer temperature (250 °C), sheath and auxiliary gas pressures (35 and 30 arbitrary units), and collision energy (12 eV). 1,N^6-εdA, AcrdG, 1,N^2-εdG, M_1dG, CrdG, and HNEdG were analyzed by nanoAcquity UPLC coupled to a Thermofinnigan TSQ Quantum Ultra triple-quadrupole mass spectrometer in positive SRM monitoring the signals m/z 276.0 → 160.0 for 1,N^6-εdA; m/z 281.0 → 165.0 for $^{15}N_5$-1,N^6-εdA; m/z 304.0 → 188.0 for M_1dG; m/z 308.0 → 193.0 for $^{15}N_5$-M_1dG; m/z 292.0 → 176.0 for 1,N^2-εdG; m/z 302.0 → 181.0 for $^{13}C_{10}$-1,N^2-εdG; m/z 424.0 → 308.0 for HNEdG; m/z 429.0 → 313.0 for $^{15}N_5$- HNEdG; m/z 338.0 → 222.0 for CrdG; m/z 343.0 → 227.0 for $^{15}N_5$- CrdG; m/z 324.0 → 208.0 for AcrdG; and m/z 329.0 → 213.0 for $^{15}N_5$- AcrdG. Separation was performed on a UPLC BEH C18 column (1.7 μm, 100 μm × 100 mm) with a flow rate of 1 μL/min using mobile phase (A) 5 mM ammonium formate in water and (B) 1% formic acid in acetonitrile for 1,N^6-εdA and (A) 0.1% formic acid in water and (B) acetonitrile for AcrdG, 1,N^2-εdG, M_1dG, CrdG, and HNEdG. MS settings were as follows: emitter tip voltage (1500 V), ion transfer capillary temperature (285 °C), and collision energy (12 eV). N^2,3-εG was analyzed as previously described.[58]

Statistical Analysis

Statistical analyses were performed using R (2.11). Considering the limited sample size in certain groups, the nonparametric test was used to assess the differences between control and PCB-treated rats or various control groups for the number of adducts by Wilcox Rank Sum

test.[72] Two-sided and one-sided p values were considered significant if they were less than 0.05.

RESULTS AND DISCUSSION

In this study, we examined the relationship between PCB exposure and oxidative DNA adduct formation. Female *Sprague–Dawley* rats were exposed to PCB 126, PCB 153, or the binary mixture of PCB 126 and PCB 153 by gavage five days per week for 14, 31, and 53 wks.

8-oxo-dG and 1,N^6-εdA formation in the liver of female rats exposed to PCB 126, PCB 153, and binary mixture are shown in Table 1. After exposure to only PCB 126 or PCB 153 for 14 or 31 wks, neither 8-oxo-dG nor 1,N^6-εdA was significantly increased. For the longer exposure period of 53 wks, PCB 126 or PCB 153 alone showed significant increases for both 8-oxo-dG and 1,N^6-εdA (Table 1). However, exposure to the binary mixture showed a statistically significant increase in each exposure group for both adducts. Specifically, exposure to the binary mixture (1000 ng/kg + 1000 μg/kg) produced roughly a 1.5–2.5-fold increase in 8-oxo-dG in hepatic DNA after 14 wks ($p = 0.005$), 31 wks ($p = 0.009$), and 53 wks ($p = 0.029$) compared to their respective control groups. More impressively, a 6–20-fold increase of 1,N^6-εdA was observed in the same exposure groups.

The induction of the DNA adduct N^2,3-εG in hepatic DNA of female rats exposed to PCB 126, PCB 153, or the binary mixture for 53 wks are shown in Table 2. Although there was no increase observed for the lower doses of PCB 126 (550 ng/kg/day), PCB 153 (300 μg/kg/day), or the binary mixture (PCB 126 + PCB 153, 300 ng/kg/day + 300 μg/kg/day), approximately a 1.5–2-fold significant increase was observed for higher doses of PCB 126 (1000 ng/kg/day, $p < 0.05$), PCB 153 (1000 μg/kg/day, $p < 0.01$), and the binary mixture (PCB 126 + PCB 153, 1000 ng/kg/day + 1000 μg/kg/day, $p < 0.01$) compared to their respective control groups.

In this study, we also analyzed several other lipid peroxidation-induced DNA adducts such as AcrdG, 1,N^2-εdG, M_1dG, CrdG, and HNEdG in the hepatic DNA of female rats exposed to PCB 126 (1000 ng/kg/day) and the binary mixture (PCB 126 + PCB 153, 1000 ng/kg/day + 1000 μg/kg/day) for 53 wks (Table 3). Concentrations of AcrdG ($p = 0.029$) and 1,N^2-εdG ($p = 0.029$) were significantly higher in animals exposed to the binary mixture (PCB 126 + PCB 153, 1000 ng/kg/day + 1000 μg/kg/day) compared to their respective control groups. M_1dG ($p = 0.026$) and AcrdG ($p = 0.009$) were observed to increase in animals that were exposed to PCB 126 (1000 ng/kg/day) alone for 53 wks.

Similar findings that implicate the role of PCBs in creating oxidative stress are consistent with our data.[29,30,38,73,74] Fadhel et al.[38] and Lamartinier et al.[73] observed a significant increase in glutathione-*S*-transferase (GST) in *Sprague–Dawley* rats that were exposed to 150 μmol/kg PCB 153 for six days and an increase in hepatic lipid peroxidation with a single dose of PCB 153. Twaroski et al.[30] also reported similar findings from rats that received intraperitoneal injections of PCB 153 (100 mmol/kg for 3 wks). Previous reports also showed *Sprague–Dawley* rats to have significant increases in oxidized vitamin E (α-

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tocopheryl quionone) after intraperitoneal PCB 153 injections (100 mmol/kg/injection)[75] as well as a significant induction of hepatic vitamin A following subchronic exposure to 50 ppm of PCB 153.[74] These studies, and others that showed the enhancement of ROS by several pathways,[7] indicate the induction of oxidative stress by an AhR-independent pathway due to PCB 153 exposure.

Similarly, the significant induction of 8-oxo-dG, M_1dG, 1,N^6-εdA, and AcrdG that we observed in samples exposed to PCB 126 for 53 wks (1000 ng/kg/day) could be indicative of PCB 126-related oxidative stress. Although Hassaoun et al.[25,26] observed increased oxidative stress in hepatic tissues of female rats exposed to PCB 126 for 13 and 31 wks, we found no significant increase in 8-oxo-dG or 1,N^6-εdA in samples that were exposed to PCB 126 or PCB 153 for 14 and 31 wks. More severe accumulation of PCB 126 and PCB 153 were observed in the liver tissues of 53 wk exposure animals than in the 13 or 31 wk exposure ones.[5,6] Because the increase in formation of these DNA adducts was only observed in the 53 wk exposure groups, we assume that long-term chronic exposure is necessary for induction of these oxidative lesions. Aqil et al. also reported a significant increase of 8-oxo-dG in the liver and lung tissues of female *Sprague–Dawley* rats after exposure to PCB 126 via polymeric implants up to 45 days.[76]

In this study, we also illustrated that the exposure to PCB 126 (1000 ng/kg/day) for 53 wks induced slightly more 8-oxo-dG and 1,N^6-εdA adducts than those of PCB 153 (1000 μg/kg/day) for 53 wks. These findings are also in agreement with the induction of the milder hepatic lesions that are induced by exposure to PCB 153 compared to that of PCB 126 in these animals.[7,68] The relative toxicity of PCB 126 and PCB 153 that was evaluated by tumor incidence and cell proliferation in those studies is also in accordance with our oxidative DNA adduct profile. The NTP reported that although a significant induction of cell proliferation and dose-dependent increases of tumor incidences were observed in PCB 126 exposed animals with the highest labeling index detected in the longest exposure group (Figure 3), there was only one report of hepatocellular adenoma (3000 μg/kg/day group), and no cholangiocarcinomas were observed in PCB 153-exposed animals.[7,68]

Furthermore, we found that the binary mixture (PCB 126 + PCB 153, 1000 ng/kg/day + 1000 μg/kg/day) generally induced more DNA adducts in comparison to that of PCB 126 or PCB 153 alone, especially for the 53 wk exposure group. Similar results were also reported for M_1dG formation by Jeong et al.[39] Likewise, the histopathology results for the 2-year cancer bioassay from NTP (Figure 3) reported a higher hepatic tumor incidence in the animals that were exposed to the binary mixture in comparison to PCB 126 or PCB 153 alone. It is not known whether this reflects a pharmacokinetic or pharmacodynamic interaction. At 53 wks, the PCB 153 levels in the binary mixture (PCB 126 + PCB 153, 1000 ng/kg/day + 1000 μg/kg/day) exposed animals were over 3.5× higher than those in the comparable 1000 μg/kg/day PCB 153 group (6728 ng of PCB 153/g). In contrast, average liver PCB 126 levels showed less change in the rats exposed to the 1000 ng/kg/day + 1000 μg/kg/day binary mixture (780 ± 218 ng/g) than those exposed to 1000 ng/kg/day of PCB 126 alone (605 ± 19 ng/g). However, the EROD activities were higher with 3219 ± 271 and 2122 ± 132 pmol/min/mg in the livers of rats exposed to the binary mixture (PCB 126 + PCB 153, 1000 ng/kg/day + 1000 μg/kg/day) or PCB 126 alone (1000 ng/kg/day),

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respectively.[7,68,69] Additionally, concentrations of PCB 126 and PCB 153 were determined in fat, liver, lung, and blood at the 14, 31, and 53 week interim evaluations and at the end of the 2-year study (105 weeks). PCB 126 was not detectable in vehicle control animals but increased with increasing dose of PCB 126 and duration of exposure in exposed animals with the highest concentrations being found in liver and fat and lower levels seen in lung and blood. Increasing the proportion of PCB 153 in the mixture relative to PCB 126 led to a general decrease in the amount of PCB 126 in liver and lung at the later time points, whereas in fat and blood, there was generally either no effect of PCB 153 on the disposition of PCB 126 or there was an increase in the amount of PCB 126 in the tissue. In vehicle control animals, PCB 153 was not detectable in the liver at any time points in the lung at the 14 wk interim evaluation or in the blood except for the 31 wk exposure group. PCB 153 was measurable in all examined tissues of treated animals with the highest concentrations found in fat at the end of the 2-year study in groups administered the highest doses. At 53 wks, the PCB 153 levels in the PCB 126 + PCB 153 (1000 ng/kg/day + 1000 μg/kg/day) group were 59450 ng/g, which is over 3.5× higher than those in PCB 153 alone (1000 μg/kg/day). Therefore, in the presence of PCB 126, there are much higher levels of PCB 153. In contrast, the presence of PCB 153 did not affect the amount of PCB 126.[7,68,69]

Oxidative DNA adducts may interfere with DNA sequence translation, ultimately resulting in modifications to gene expression that can lead to malignant cells if the DNA is not repaired prior to cell replication.[50–55] Many studies reported the diverse mutation spectrum induced by the oxidative DNA adducts listed in this paper.[50–55] The predominant mutation induced by 8-oxo-dG was G to T transversions in both bacterial and mammalian cells.[52] 1,N^6-εdA mainly induced A to G transition in *E. coli* and kidney cells, and N^2,3-εG produced specifically G to A transitions in vitro and in *E. coli*.[51] Although various mutations were induced by 1,N^2-εdG, G to T transversion was dominant in Chinese hamster ovary cells.[54] This mutation was also found to be induced by AcrdG and CrdG[49] as well as MDA in *E. coli* and 4-HNE in hamster cells.[53,55] Increased oxidative DNA adducts were also detected in animal studies exposed to various chemical carcinogens.[39,77–83] Significant increases in HNEdG, M_1G, and 8-oxo-dG were detected in the liver of rats after an intraperitoneal injection of a single dose of CCl_4,[77–79] a widely applied model toxicant for hepatic carcinogenesis studies in animals. Trimethylarsine oxide, an organic metabolite of inorganic arsenics, was found to significantly induce the formation of hepatic 8-oxo-dG and hepatocellular adenomas in male F344 rats after two-year exposure with the dose of 200 ppm.[80] A 2-fold significant increase of 8-oxo-dG was detected in mouse bone marrow DNA 1 h following oral administration of benzene (880 mg/kg).[81] The induction of hepatic 8-oxo-dG, cell proliferation, and enzyme-altered hepatocellular foci by TCDD were observed after 30 wk exposure in female rats with diethylnitrosamine initiation.[82,83] Significantly increased M_1G was observed in the liver of female *Sprague–Dawley* rats following chronic exposure (53 weeks) to PCB 126 or the binary mixture of PCB 126 and PCB 153 at the highest dose with an almost 2-fold increase compared with controls. The increased M_1G was accompanied by increased cell proliferation.[39] A significant increase in 1,N^6-εdA and 3,N^4-εdC were found in premalignant target organs affected by various chronic diseases, such as genetic metal storage disorders (Wilson's disease and hemochromatosis), chronic pancreatitis, and chronic hepatitis.[60] Elevated 8-oxo-dG has been detected in patients with

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diverse malignancies, acute leukemia, colorectal cancer, hepatic cancer, and breast cancer.[57] Higher M_1dG was detected in smoking patients with lung cancer compared with control smokers.[61] All of these studies consistently support the importance of these oxidative DNA adducts as critical biomarkers in carcinogenesis. Moreover, Lehmann et al. found a significant increase in G to T transversions in the livers of male Fisher rats after 4-monochlorobiphenyl exposure,[84] which agrees with the dominant mutation spectrum induced by several oxidative DNA adducts in this study and implies the possible importance of these adducts for the toxicity of PCBs as well.

The concentrations of the oxidative DNA adducts that were measured in this study reflect several complex factors such as exposure time, exposure dose, chemical metabolism, adduct repair pathway, tissue, age, sex, species, and so forth. Thus, the background concentration of each hepatic DNA adduct (8-oxo-dG > AcrdG > M_1dG > 1,N^2-εdG > 1,N^6-εdA > N^2,3-εG > HNEdG > CrdG) in female rats showed different distributions with a range from ~2 adducts/10^6 parent bases to ~1 adduct/10^9 parent bases. Similar findings were reported by Pang et al.[58]

Several age-dependent studies showed that the background levels of 8-oxo-dG and 1,N^6-εdA increased with age.[85,86] These results might be explained by the decreased capability of DNA repair in aged animals.[87,88] Chung et al. also reported a variation of distribution of AcrdG and CrdG in several tissues (lung, kidney, colon, brain, etc.) and suggested that the significance of tissue specific and stereoselective patterns of the adducts plays a role in the background amounts of those DNA adducts.[89]

In summary, the present study consistently demonstrates an association between oxidative DNA damage and hepatic toxicity in female *Sprague–Dawley* rats exposed to PCB 126, PCB 153, and the binary mixture of these two compounds. The time-course study confirmed that accumulation of these lesions was time-dependent, which agrees with the tumor incidence and cell proliferation results reported by NTP.[7,68,69] The DNA adduct profile study further confirmed the enhanced toxicity of PCB 126 by PCB 153 cannot be evaluated by the simplified TEF methodology. However, measurement of DNA adduct profiles in comparison with their relative repair potential, the specific susceptibilities of different tissue types or regions of tissue to specific types of DNA damage, or to associate the toxicity of chemicals with specific formation patterns of adducts is still underdeveloped. Further mechanistic studies on PCB-induced oxidative DNA adducts can provide more valuable information.

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ABBREVIATIONS

1,N^2-εdG	1,N^2-ethenodeoxyguanosine
1,N^6-εdA	1,N^6-ethenodeoxyadenosine
8-oxo-dG	8-oxo-deoxyguanosine
AcrdG	acrolein-derived dG adducts
AHR	aryl hydrocarbon receptor
CrdG	crotonaldehyde-derived dG adducts
HNE	4-hydroxynonenal
HNEdG	4-hydroxynonenal-derived dG adducts
M_1dG	malondialdehyde-derived dG adducts
MDA	malondialdehyde
N^2,3-εG	N^2,3-ethenoguanine
NTP	National Toxicology Program
PCB 126	3,3′,4,4′,5-pentachlorobiphenyl
PCB 153	2,2′,4,4′,5,5′-hexachlorobiphenyl
PCB	polychlorinated biphenyls
ROM	reactive oxygenated metabolites
ROS	reactive oxygen species
TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
TEF	toxic equivalency factor
TEMPO	2,2,6,6-tetramethyl-1-piperidinyloxy

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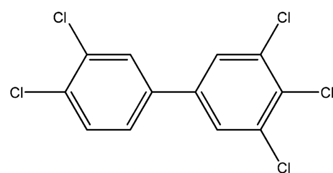

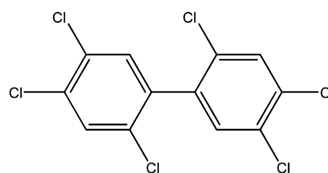

Figure 1.
Chemical structures of PCB 126 and PCB 153.

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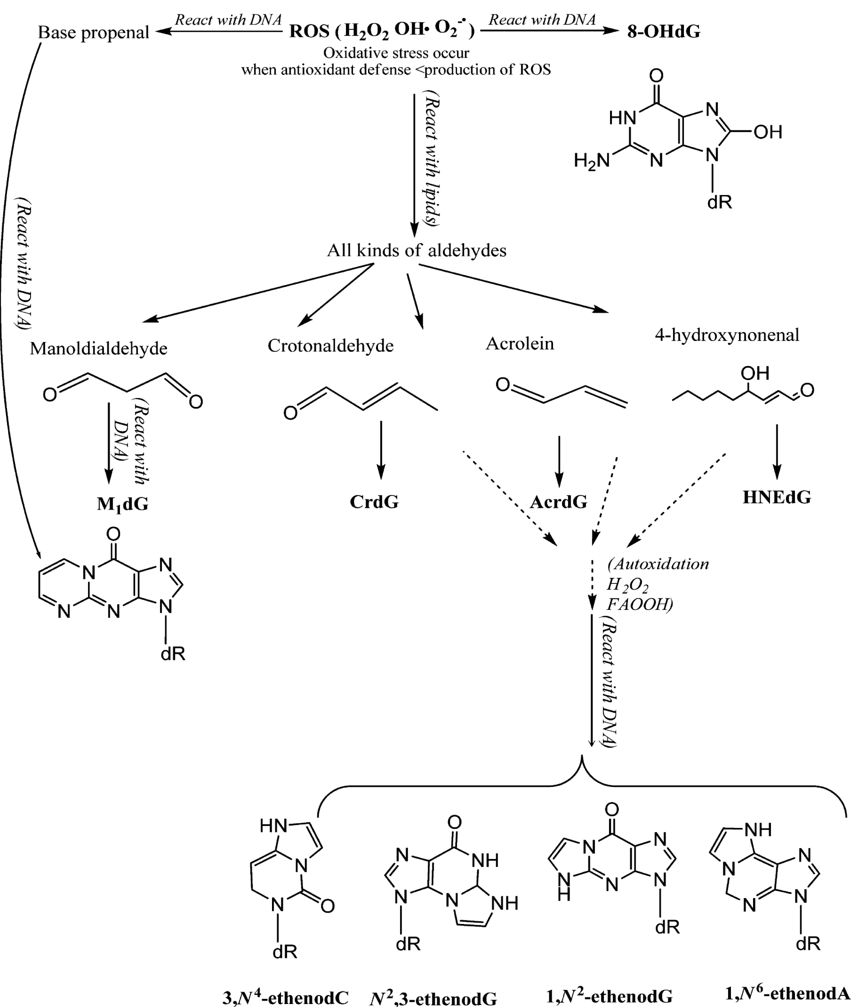

Figure 2.

An illustration of the major DNA adducts induced by ROS.

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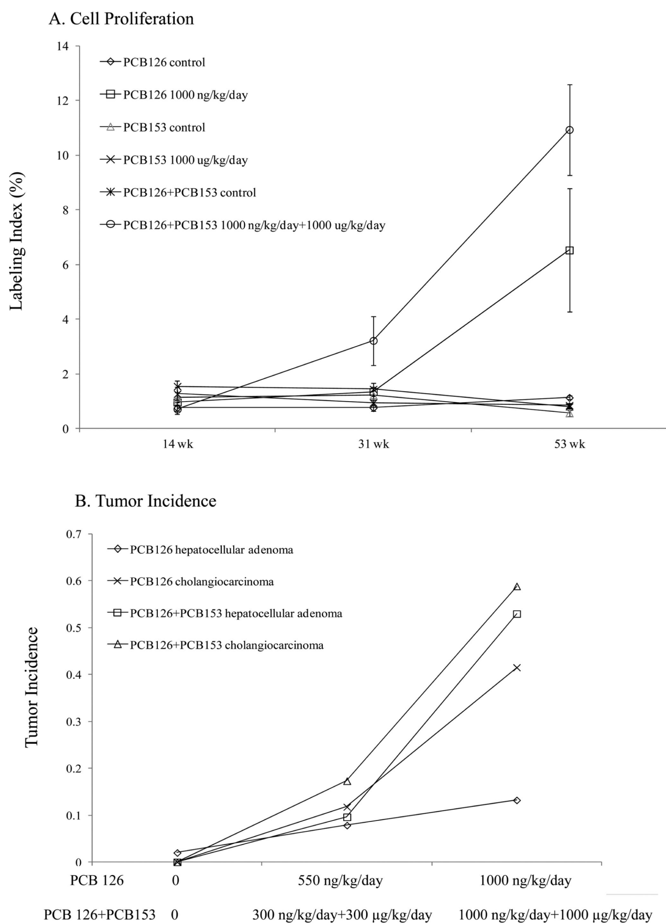

Figure 3.

Cell proliferation (A) in the liver of female *Sprague–Dawley* rats exposed to PCB 126 (1000 ng/kg/day), PCB 153 (1000 μg/kg/day), or the binary mixture of PCB 126 and PCB 153 (1000 ng/kg/day +1000 μg/kg/day) for 14, 31, and 53 weeks. Tumor incidence (B) in the liver of female *Sprague–Dawley* rats exposed to PCB 126 (0, 550, and 1000 ng/kg/day) or the binary mixture of PCB 126 and PCB 153 (0, 300 ng/kg/day + 300 μg/kg/day, and 1000 ng/kg/day + 1000 μg/kg/day);[7] $n = 8–10$.[7,68,69]

Table 1

Number of 8-oxo-dG Adducts/10^6 dG and 1,N^6-εdA Adducts/10^8 dA Measured in *Sprague-Dawley* Rat Hepatic DNA Following Exposure to PCB 126 (1000 ng/kg/day), PCB 153 (1000 μg/kg/day), or the Binary Mixture of PCB 126 + PCB 153 (1000 ng/kg/day + 1000 μg/kg/day) for 14, 31, or 53 weeks

		PCB 126			**PCB 153**			**binary (PCB 126 + 153)**		
		14 wks	**31 wks**	**53 wks**	**14 wks**	**31 wks**	**53 wks**	**14 wks**	**31 wks**	**53 wks**
8-oxo-dG add/10^6 dG	control	2.65 ± 1.84	2.46 ± 0.82	3.61 ± 0.70	3.28 ± 0.82	2.67 ± 0.84	2.37 ± 0.28	3.45 ± 1.15	4.88 ± 1.06	4.96 ± 0.66
	exposed[a]	3.82 ± 2.39	3.63 ± 1.71	6.80 ± 0.31[b]	3.65 ± 0.64	3.21 ± 0.49	4.27 ± 1.71[b]	6.01 ± 1.14[b]	7.08 ± 1.38[b]	12.50 ± 2.52[b]
1,N^6-εdA add/10^8 dA	control	2.24 ± 1.11	1.47 ± 0.17	1.90 ± 0.37	1.28 ± 0.23	1.56 ± 0.26	2.19 ± 0.71	0.56 ± 0.24	1.67 ± 0.74	2.89 ± 0.79
	exposed[a]	1.47 ± 0.18	1.81 ± 0.50	4.36 ± 1.34[b]	1.33 ± 0.41	1.43 ± 0.31	3.96 ± 0.98[b]	11.30 ± 2.75[b]	18.00 ± 7.10[c]	18.30 ± 8.00[c]

[a] Indicates exposure to PCB 126 (1000 ng/kg/day), PCB 153 (1000 μg/kg/day), or binary (PCB 126 + 153) (1000 ng/kg/day + 1000 μg/kg/day).

[b] Indicates p 0.05.

[c] Indicates p 0.01.

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Table 2

Number of N^2-3εG Adducts/10^8 G Measured in *Sprague-Dawley* Rat Hepatic DNA Following Exposure to Multiple Concentrations of PBC 126, PCB 153, or the Binary Mixture of PCB 126 + PCB 153 for 53 Weeks

		N^2,3εG add/10^8 G
PCB 126 53 wks	control	1.67 ± 0.30
	550 ng/kg	1.44 ± 0.70
	1000 ng/kg	2.55 ± 0.56[a]
PCB 153 53 wks	control	1.48 ± 0.37
	300 μg/kg	1.59 ± 0.63
	1000 μg/kg	2.55 ± 0.44[b]
binary (PCB 126 + 153) 53 wks	control	1.18 ± 0.26
	300 ng/kg + 300 μg/kg	1.70 ± 0.41
	1000 ng/kg + 1000 μg/kg	2.59 ± 0.78[b]

[a]Indicates p 0.05.

[b]Indicates p 0.01.

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Table 3

Number of 1,N^2-εdG Adducts/10^8 dG, M_1dG Adducts/10^8 dG, CrdG Adducts/10^8 dG, HNEdG Adducts/10^8 dG, and AcrdG Adducts/10^8 dG Measured in *Sprague-Dawley* Rat Hepatic DNA Following Exposure to PCB 126 (1000 ng/kg/day) or the Binary Mixture of PCB 126 + PCB 153 (1000 ng/kg/day + 1000 μg/kg/day) for 53 weeks

	PCB 126 add/10^8 dG	**binary add/10^8 dG**
1,N^2-εdG control	2.26 ± 0.15	2.59 ± 0.36
1,N^2-εdG exposed	2.74 ± 0.43	4.19 ± 0.98[b]
M_1dG control	4.02 ± 0.73	3.03 ± 0.48
M_1dG exposed[a]	5.65 ± 1.86[b]	5.80 ± 1.65
CrdG control	0.60 ± 0.14	0.34 ± 0.06
CrdG exposed[a]	0.68 ± 0.18	0.66 ± 0.20
HNEdG control	1.01 ± 0.11	0.93 ± 0.36
HNEdG exposed[a]	1.17 ± 0.25	2.27 ± 1.66
AcrdG control	9.32 ± 0.74	12.70 ± 2.93
AcrdG exposed[a]	11.90 ± 1.91[c]	24.30 ± 4.03[b]

[a]Indicates exposure to PCB 126 (1000 ng/kg/day) and binary (PCB 126 + 153) (1000 ng/kg/day + 1000 μg/kg/day) for 53 wks.

[b]Indicates p 0.05.

[c]Indicates p 0.01.