DNA Product Formation in Female Sprague–Dawley Rats Following Polyhalogenated Aromatic Hydrocarbon (PAAH) Exposure

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ABSTRACT: DNA oxidation damage has been regarded as one of the possible mechanisms for the hepatic carcinogenesis of dioxin-like compounds (DLCs). In this study, we evaluated the toxic equivalency factor (TEF) from the standpoint of induced DNA oxidation products and their relationship to toxicity and carcinogenicity. Nine DNA oxidation products were analyzed in the liver of female Sprague–Dawley rats exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) alone or the tertiary mixture of TCDD, 3,3′,4,4′,5-pentachlorodibenzofuran (PCB 126), and 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) by gavage for 14, 31, and 53 weeks (5 days/week) by LC–MS/MS: 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxo-dGuo); 1,N′-etheno-2′-deoxyadenosine (1,N′-edAdo); N₂,N₂-etheno-2′-deoxyguanosine (1,N₂-εdGuo); 7-(2-oxoethyl)guanine (7-OEG); 1,N²-etheno-2′-deoxyguanosine (1,N²-εdGuo); malondialdehyde (M₁dGuo); acrolein (AcrdGuo); crotonaldehyde (CrdGuo); and 4-hydroxynonenal (HNEdGuo) derived 2′-deoxyguanosine adducts. Exposure to TCDD (100 ng/kg/day) significantly induced 1,N²-εdAdo at 31 and 53 weeks, while no increase of 8-oxo-dGuo was observed. Significant increases were observed for 8-oxo-dGuo and 1,N²-εdAdo at all time points following exposure to the tertiary mixture (TEQ 100 ng/kg/day). Exposure to TCDD for 53 weeks only significantly increased 1,N²-εdAdo, while increases of N₂,3-εG and 7-OEG were only found in the highest dose group (100 ng/kg/day). Exposure to the tertiary mixture for 53 weeks had no effect on N₂,3-εG in any exposure group (TEQ 0, 22, 46, or 100 ng/kg/day), while significant increases were observed for 1,N²-εdAdo (all dose groups), 8-oxo-dGuo (46 and 100 ng/kg/day), and 7-OEG (100 ng/kg/day). While no significant increase was observed at 53 weeks for 1,N²-edGuo, M₁dGuo, AcrdGuo, or CrdGuo following exposure to TCDD (100 ng/kg/day), all of them were significantly induced in animals exposed to the tertiary mixture (TEQ 100 ng/kg/day). This oxidation DNA product data suggest that the simple TEF methodology cannot be applied to evaluate the diverse patterns of toxic effects induced by DLCs.

INTRODUCTION

Oxidative stress, a common state in pathophysiology, occurs when the number of reactive oxygen species (ROS) being formed is exceeded by those being detoxified. Many endogenous processes, as well as exogenous chemicals or their metabolites, are known to produce ROS. Besides ROS, reactive nitrogen species (RNS) are also generated by macrophages and neutrophils involved in chronic inflammation, which has been recognized as a risk factor in many human cancers.† These species or their active metabolites can interact with cellular constituents, especially lipids or nucleic acids, and further induce various DNA oxidation products.‡ These DNA lesions have been implicated in aging, neurodegeneration, and a myriad of diseases including cancer.† To date, there are several key ROS/RNS-induced DNA oxidation products: 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxo-dGuo); 1,N′-etheno-2′-deoxyadenosine (1,N′-edAdo); 3,N₂-etheno-2′-deoxycytidine (3,N₂-εdC); N₂,3-etheno-adenosine (N₂,3-εG); 1,N₂-etheno-2′-deoxyguanosine (1,N₂-εdGuo); and malondialdehyde (MDA), acrolein, crotonaldehyde, and 4-hydroxynonenal (HNE) derived 2′-deoxyguanosine (dGuo) adducts, designated as M₁dGuo, AcrdGuo, CrdGuo, and HNEdGuo, respectively.‡⊥ Among them, the most studied adduct, 8-oxo-dGuo, is formed in relatively high amounts in vivo with steady-state levels usually around 1/10⁶ guanine. It is formed directly by the reaction between dGuo and ROS or carbonate anion radical induced by RNS.‖‡ ROS and RNS metabolites can also abstract hydrogen atoms from polyunsaturated fatty acids producing lipid peroxides and many reactive byproducts such as MDA, HNE, crotonaldehyde, and acrolein. These compounds can further damage DNA and generate multiple oxidation DNA products, which include exocyclic products with either a five-member (etheno products) or a six-member (propano
products) ring attached to DNA bases, as depicted in Figure 1. Recently, 7-(2-oxoethyl)guanine (7-OEG) has been identified as a new DNA product formed by lipid peroxidation (LPO) with steady-state levels around 1–10 adduct/10⁶ guanine. In addition to these distinct formation pathways, site-directed mutagenicity studies found that most of these DNA products can induce specific transition or transversion point mutations in bacteria or mammalian cells. 7-OEG can induce apurinic/apyrimidinic sites (AP sites) in biological systems, although it has no miscoding properties. Considering the diverse metabolic pathways and the mutation spectrum induced by those important ROS/RNS-induced DNA products, evaluating their profile data could compensate for biased results induced by a single product. Growing evidence also indicates that these DNA lesions are significantly induced in patients and animals with various chronic inflammatory diseases including certain cancers. Although DNA oxidation product formation is generally regarded as a key event for the carcinogenesis of genotoxic chemicals, it may also be a significant contributor for the tumorigenesis of nongenotoxic chemicals, especially chemicals capable of enhancing the formation of endogenous active metabolites, RNS and ROS, such as polyhalogenated aromatic hydrocarbons (PHAHs).

PHAHs comprise a large class of compounds such as polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs). PCBs were commercially produced and widely used for various industrial purposes including heat transfer agents, dielectric insulating fluids for capacitors and transformers, plasticizers, and paint additives. Because of PHAHs’ resistance to degradation and persistence in environment, their ability to bioaccumulate in humans and wildlife animals may result in chronic lifetime exposure, possible toxicity, and carcinogenicity. Depending on the location and type of halogenations, some PHAHs induce a similar spectrum of biochemical and toxic responses in experimental animals. These responses are considered to be mediated through a common mechanism of action initiated by binding to a cytosolic receptor known as the aryl hydrocarbon receptor (AhR) and triggering the expression of a variety of genes, the so-called AhR gene battery.

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), commonly referred to as “dioxin”, is the prototype of these structurally related compounds and exhibits the highest potency of binding to the AhR. Hence, these structurally related compounds are commonly referred to as dioxin-like compounds (DLCs). Because of similarities in toxicity, the concept of the toxic equivalency factor (TEF) has been applied for the risk assessment and regulatory control for DLCs. Therefore, the toxicity of PHAH mixtures is expressed in terms of its total toxic equivalent quotient (TEQ), which is the amount of TCDD that would produce the equal toxic effect of all contributing congeners within the mixture. This allows for the estimation of the potential dioxin-like activity of PHAH mixtures in the environment.

Figure 1. Illustration of the major DNA adducts induced by ROS/RNS.
The association of oxidative stress and PHAHs, especially TCDD, has been studied for several decades. Substantial evidence has accumulated to support that TCDD can induce oxidative stress in mammalian cells in vitro and in rodents in vivo. Different biomarkers have been applied to assess the oxidative stress induced by TCDD in the liver of animals including hepatic LPO, DNA single strand breaks, hepatic membrane fluidity, glutathione, nonprotein sulfhydryl, and NADPH. Significantly increased LPO with large strain differences has also been detected in other organs of rats and mice exposed to TCDD including kidney, thymus, heart, testes, and brain. Similarly, PCB-induced oxidative stress was also observed in numerous studies. These studies indicate that oxidative stress is a ubiquitous side effect produced by these compounds. Although many PHAHs have been shown to have very weak initiating activity without direct genotoxic effects, it has been postulated that they may be indirectly genotoxic through the formation of DNA lesions induced by ROS and RNS. Because the TEF has been widely applied for the evaluation of the toxic effects of PHAHs and oxidative stress is universally induced in the animals exposed to these compounds, it is meaningful to evaluate the application of the TEF approach in the toxicity of PHAHs using DNA oxidation products, especially in chronic animal carcinogenesis studies.

Several PHAHs were chosen by the National Toxicology Program (NTP) as model compounds including TCDD, PCB 126, and 2,3,4,7,8-pentachlorodibenzofuran (PeCDF). PCB 126 is a non-ortho-substituted PCB with a TEF value of 0.1. As the most potent DLC in the environment, PCB 126 accounts for 40–90% of the toxic potency of dioxin-like PCBs. PeCDF, with a TEF value of 0.5, represents the most potent PCDF present in human tissues. The structures of these compounds are shown in Figure 2. An important assumption for the TEF methodology is that the toxicity of a mixture of DLCs is dose additive based on the TEF value of the individual components. This study has evaluated this assumption from the standpoint of the number of induced DNA oxidation products and their relationship to toxicity and carcinogenicity of PHAHs.

In this study, we collaborated with the NTP to understand the DNA oxidation product profile in hepatic DNA of female Sprague–Dawley rats that were exposed to TCDD and the tertiary mixture of TCDD, PCB 126, and PeCDF (Figure 2) for 14, 31, and 53 weeks. Nine DNA oxidation lesions (7-OEG; 8-oxo-dGuo; 1,N6-ε-dAdo; 1,N6-ε-dGuo; N2,N3-ε-dEtG; M5,dGuo; AcrdGuo; CrdGuo; HNEdGuo) were measured in hepatic DNA isolated from female Sprague–Dawley rats. Since each product has distinct metabolic pathways in vivo, our assessment of a battery of DNA oxidation lesions provides extensive information on DNA oxidation damage. This knowledge enables us to better estimate the toxicity of PHAHs and improve the scientific basis of human risk assessment of PHAHs 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 obtained from Thermo Fisher Scientific Company (Raleigh, NC). 7-N,N5-dGuo, 15N5-dGuo, and 13C10-dGuo were purchased from Cambridge Isotope Laboratories (Andover, MA, USA). Other chemical reagents were from Sigma-Aldrich Chemical Co. (St. Louis, MO). 15N5,N6-ε-dAdo standard was synthesized as described by Ham et al. 1,N6-ε-dGuo and 15N5,1,N6-ε-dGuo were synthesized as reported by Kusmierek et al. MDA modified 15N5 and 15N4 DNA were made using the method in Jeong’s study. AcrdGuo, CrdGuo, and HNEdGuo standards and their 15N4 labeled internal standards were synthesized according to previous studies. The 7-OEG, 15N5,7-OEG, and 15N5,3,4-ε-G were synthesized as described previously by Mutlu et al.

**Animal Exposure and DNA Isolation.** Rat liver tissues were provided by Battelle Laboratories (Columbus, OH) and State University of New York at Buffalo, which conducted the studies under NIEHS contract (NO1-ES-75411). Female Sprague–Dawley rats were exposed to either TCDD alone or the tertiary mixture by gavage 5 days per week for 14, 31, and 53 weeks. The doses used for TCDD were 0 and 100 ng/kg/day for 14, 31, 53 weeks; 0, 22, 46, and 100 ng/kg/day for 53 weeks. The TEQ doses used for the tertiary mixture were 0 and 100 ng/kg/day for 14, 31, 53 weeks; 0, 22, 46, and 100 ng/kg/day for 53 weeks. Further explanation of the TEQ doses can be found in the NTP technical report on the toxicology and carcinogenesis studies of a mixture of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (Cas No. 1746–01–6), 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) (Cas No. 57117–31–4), and 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) (Cas No. 57465–28–8) in female Harlan Sprague–Dawley rats (gavage studies). Liver tissues were collected from 4–8 female rats per group/day after the final exposure and stored frozen at −80 °C. DNA was isolated as described previously.

**8-Oxo-dGuo and 1,N6-ε-dAdo Assay.** The assay was performed as previously described by Pang et al. with minor modifications. A 100 μg sample of DNA in NaOAc buffer I (sodium acetate 30 mM, 0.2 mM ZnCl2, pH 5.6) was incubated with nuclease P1 (15 μg) at 37 °C for 1 h. Immediately after incubation, DNA samples were digested with 2,2,6,6-tetramethyl-piperidinedioxyl (TEMPO, 5 μL, 1 M), 15N5,8-oxo-dGuo (500 fmol), and 15N4-1,N6-ε-dAdo (20 fmol) followed by addition of NaOAc buffer II (sodium acetate 30 mM, pH 8.1), alkaline phosphatase (20 units), and phosphodiesterase (0.012 units) 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 using a SpeedVac.

Samples were enriched for 8-oxo-dGuo and 1,N6-ε-dAdo using an Agilent 1200 HPLC system equipped with a 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% MeOH gradient in 10 mM ammonium acetate buffer as follows: hold at 5% MeOH for 5 min, 5–10% MeOH over 5 min, 10–20% MeOH over 10 min, 20–80% over 10 min; re-equilibrate at 5% MeOH.
**Table 1. Number of 8-Oxo-dGuo Adducts/10^6 dGuo and 1,Nε- edAdo Adducts/10^6 dAdo Measured in Female Sprague–Dawley Rat Hepatic DNA Following Exposure to TCDD (100 ng/kg/day) or the Tertiary Mixture of TCDD, PCB 126, and PeCDF (TEQ 100 ng/kg/day) for 14, 31, and 53 Weeks**

|                  | TCDD                        | Tertiary                     |
|------------------|-----------------------------|------------------------------|
|                  | 14 weeks                    | 31 weeks                     | 53 weeks                     |
|                  | 14 weeks                    | 31 weeks                     | 53 weeks                     |
| 8-oxo-dGuo add/10^6 dGuo |                             |                              |                              |
| control          | 2.41 ± 1.28                 | 2.44 ± 0.80                  | 3.20 ± 0.67                  |
| exposed          | 2.55 ± 0.91                 | 2.72 ± 0.88                  | 3.87 ± 0.47                  |
| 1,Nε-edAdo add/10^6 dAdo |                             |                              |                              |
| control          | 1.47 ± 0.65                 | 1.32 ± 0.44                  | 0.91 ± 0.48                  |
| exposed          | 1.56 ± 0.23                 | 2.76 ± 1.07                  | 2.13 ± 0.52                  |

- Indicates the exposures of TCDD, 100 ng/kg/day, and tertiary mixture (TCDD + PCB 126 + PeCDF), TEQ 100 ng/kg/day.
- Indicates p < 0.05 compared to corresponding control groups.
- Indicates p ≤ 0.01 compared to corresponding control groups.

**Table 2. Number of N^2,3-εG Adducts/10^8 G, 7-OEG Adducts/10^7 G, 8-Oxo-dGuo Adducts/10^6 dGuo, and 1,Nε- edAdo Adducts/10^6 dAdo Measured in Female Sprague–Dawley Rat Hepatic DNA Following Exposure to Multiple Concentrations of TCDD or the Tertiary Mixture of TCDD, PCB 126, and PeCDF for 53 Weeks**

|                  | N^2,3-εG add/10^8 G | 7-OEG add/10^7 G | 8-Oxo-dGuo add/10^6 dGuo | 1,Nε-edAdo add/10^6 dAdo |
|------------------|---------------------|-----------------|--------------------------|-------------------------|
| TCDD 33 weeks    |                     |                 |                          |                         |
| control          | 2.18 ± 0.44         | 4.92 ± 1.07     | 3.20 ± 0.67               | 1.19 ± 0.39             |
| 22 ng/kg         | 2.55 ± 0.37         | 6.96 ± 0.81     | 3.54 ± 0.62               | 2.37 ± 0.84             |
| 46 ng/kg         | 2.11 ± 0.56         | 7.47 ± 5.96     | 4.32 ± 0.14               | 2.51 ± 0.83             |
| 100 ng/kg        | 4.44 ± 2.04         | 29.8 ± 16.47    | 3.87 ± 0.47               | 2.13 ± 0.52             |
| female Sprague–Dawley 33 weeks |                     |                 |                          |                         |
| control          | 2.07 ± 1.15         | 7.88 ± 3.77     | 2.81 ± 0.49               | 0.93 ± 0.56             |
| 22 ng/kg         | 1.78 ± 0.19         | 7.81 ± 2.96     | 3.07 ± 0.11               | 3.93 ± 1.15             |
| 46 ng/kg         | 2.41 ± 1.04         | 9.92 ± 7.22     | 4.11 ± 0.60               | 4.94 ± 1.63             |
| 100 ng/kg        | 2.55 ± 0.37         | 22.4 ± 14.84    | 4.87 ± 0.80               | 4.13 ± 0.87             |

- Indicates p ≤ 0.05 compared to corresponding control groups.
- Indicates p ≤ 0.01 compared to corresponding control groups.
- Indicates TEQ dose.

AcrdGuo, 1,Nε-edGuo, M4dGuo, CrdGuo, and HNEdGuo Assay. An assay similar to 8-oxo-dGuo and 1,Nε-edAdo was applied to measure AcrdGuo, 1,Nε-edGuo, M4dGuo, CrdGuo, and HNEdGuo with minor modifications. Considering their similar chromatography behaviors, these oxidation products were analyzed simultaneously. A Waters Acquity UPLC coupled to a Thermo Finnigan TSQ Quantum Ultra triple-quadrupole mass spectrometer in positive SRM monitoring the signals m/z 276.0 → 160.0 for 1,Nε-edAdo, m/z 281.0 → 165.0 for 1,Nε-1,Nε-edAdo, m/z 304.0 → 180.0 for M4dGuo, m/z 309.0 → 193.0 for 1,Nε-M4dGuo, m/z 292.0 → 176.0 for 1,Nε-edGuo, m/z 302.0 → 181.0 for 1,Nε-1,Nε-edGuo, m/z 424.0 → 210.0 for HNEdGuo, m/z 240.0 → 224.0 for CrdGuo, m/z 332.0 → 227.0 for 1,Nε-CrdGuo, m/z 324.0 → 218.0 for M4dGuo, and m/z 329.0 → 213.0 for 1,Nε-AcrdGuo. Separation was performed on a UPLC BEH C18 column (1.7 μm, 100 μm x 100 mm) with a flow rate of 1 μL/min using gradient (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), the vaporizer temperature (250 °C), sheath and auxiliary gas pressures (35 and 30 arbitrary units), and collision energy (12 eV). 1,Nε-edAdo, AcrdGuo, 1,Nε-edGuo, M4dGuo, CrdGuo, and HNEdGuo 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,Nε-edAdo, m/z 281.0 → 165.0 for 1,Nε-1,Nε-edAdo, m/z 304.0 → 180.0 for M4dGuo, m/z 309.0 → 193.0 for 1,Nε-M4dGuo, m/z 292.0 → 176.0 for 1,Nε-edGuo, m/z 302.0 → 181.0 for 1,Nε-1,Nε-edGuo, m/z 424.0 → 210.0 for HNEdGuo, m/z 240.0 → 224.0 for CrdGuo, m/z 332.0 → 227.0 for 1,Nε-CrdGuo, m/z 324.0 → 218.0 for M4dGuo, and m/z 329.0 → 213.0 for 1,Nε-AcrdGuo. Separation was performed on a UPLC BEH C18 column (1.7 μm, 100 μm x 100 mm) with a flow rate of 1 μL/min using gradient (A) 5 mM ammonium formate in water and (B) 1% formic acid in acetonitrile for 1,Nε-edAdo, or (A) 0.1% formic acid in water and (B) 0.1% formic acid in methanol. MS settings were as follows: electrospray voltage (3000 V), ion transfer capillary temperature (285 °C), the vaporizer temperature (250 °C), sheath and auxiliary gas pressures (35 and 30 arbitrary units), and collision energy (12 eV). 

**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 PHAH-treated rats or various control groups for the number of DNA oxidation products by Wilcoxon Rank Sum test. 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 exposure to either TCDD or the tertiary mixture of TCDD, PCB 126, and PeCDF and the formation of DNA oxidation products. Female Sprague–Dawley rats were exposed to 0 and 100 ng/
Measurements of 8-oxo-dGuo and 1, N²-εdAdo formation in the liver of the female rats exposed to TCDD (0 and 100 ng/kg/day) or the tertiary mixture (TEQ 0 and 100 ng/kg/day) at 14, 31, and 53 weeks were shown in Table 1. While no significant increase of 8-oxo-dGuo was detected after TCDD exposure for 14, 31, or 53 weeks, 1, N²-εdAdo concentrations for 31 (p = 0.03) and 53 (p = 0.003) weeks were higher in comparison to their respective control groups. Exposure to the tertiary mixture for 14 (p = 0.004 for 8-oxo-dGuo, and p = 0.004 for 1, N²-εdAdo), 31 (p = 0.0001 for 8-oxo-dGuo, and p = 0.0002 for 1, N²-εdAdo), and 53 weeks (p = 0.02 for 8-oxo-dGuo, and p = 0.0002 for 1, N²-εdAdo) all showed statistically significant increases in 8-oxo-dGuo and 1, N²-εdAdo. These increases correspond to a 1.5–1.8-fold increase in 8-oxo-dGuo and a 1.5–5.0-fold increase in 1, N²-εdAdo in the hepatic DNA of female rats.

The accumulations of 2,3-εG, 7-OEG, 8-oxo-dGuo, and 1, N²-εdAdo were evaluated in the hepatic DNA of the female Sprague–Dawley Rats exposed to TCDD at 0, 22, 46, 100 ng/kg/day or the tertiary mixture at TEQ doses of 0, 22, 46, 100 ng/kg/day for 53 weeks (Table 2). No accumulation of 8-oxo-dGuo was observed after TCDD exposure, but a significant increase of 8-oxo-dGuo was observed following exposure to the tertiary mixture at TEQ doses of 46 (p = 0.03) and 100 ng/kg/day (p = 0.02). 1, N²-εdAdo concentrations increased significantly at all doses (22, 46, 100 ng/kg/day) following either TCDD (p = 0.008, p = 0.008, and p = 0.02, respectively) or the tertiary (p = 0.003, p = 0.003, and p = 0.002, respectively) exposures. Table 2 shows the significant increase in the number of 7-OEG in the liver at the highest dose exposures of both TCDD (100 ng/kg, p = 0.0098) and the tertiary mixture (TEQ 100 ng/kg, p = 0.014). Endogenous 7-OEG was measured to be 4.92 ± 1.07 adducts/10⁷G in control samples, while exposure to 100 ng/kg/day TCDD induced 29.79 ± 16.47 adducts/10⁷G of 7-OEG. Additionally, a three-fold increase was observed in 7-OEG formation from exposure to the tertiary mixture. No significant increase was observed for N², 3-εG concentration after exposure to the tertiary mixture, including the highest TEQ dose group, while a two-fold increase in N², 3-εG was observed after 100 ng/kg/day TCDD exposure (p = 0.015).

Several other LPO-induced DNA products (1, N²-εdGuo; M₂dGuo; CrdGuo; HNEdGuo; AcrdGuo) were analyzed in the hepatic DNA of female rats exposed to TCDD (100 ng/kg/day) and the tertiary mixture (TEQ 100 ng/kg/day) for 53

![Figure 3](image-url) - (Top) Cell proliferation in the liver of female Sprague–Dawley rats exposed to TCDD (100 ng/kg/day) or the tertiary mixture of TCDD, PCB 126, and PeCDF (TEQ 100 ng/kg/day) at 14, 31, and 53 weeks. 21,24 (Bottom) Cell proliferation in the liver of female Sprague–Dawley rats exposed to TCDD (0, 22, 46, and 100 ng/kg/day) or the tertiary mixture of TCDD, PCB 126, and PeCDF (TEQ 0, 22, 46, and 100 ng/kg/day) at 53 weeks. 21,24 *Significantly different (p ≤ 0.05) from the vehicle control group by Shirley's test.

![Figure 4](image-url) - Tumor incidence from 2-year cancer bioassay of female Sprague–Dawley rat livers exposed to TCDD (0, 22, 46, and 100 ng/kg/day) or the tertiary mixture of TCDD, PCB 126, and PeCDF (TEQ 0, 22, 46, and 100 ng/kg/day). 21,24

Table 3. Number of 1, N²-εdGuo Adducts/10⁷ G, M₂dGuo adducts/10⁷ G, CrdGuo Adducts/10⁷ G, HNEdGuo Adducts/10⁷ G, and AcrdGuo Adducts/10⁷ G Measured in Female Sprague–Dawley Rat Hepatic DNA Following Exposure to TCDD (100 ng/kg/day) or the Tertiary Mixture of TCDD, PCB 126, and PeCDF (TEQ 100 ng/kg/day) for 53 Weeks

|              | TCDD add/10⁷ G       | tertiary add/10⁷ G   |
|--------------|----------------------|---------------------|
| 1, N²-εdGuo control | 1.61 ± 0.37          | 2.07 ± 0.43         |
| 1, N²-εdGuo exposed | 2.14 ± 0.51          | 3.80 ± 1.12         |
| M₂dGuo control    | 4.16 ± 1.02          | 4.71 ± 2.23         |
| M₂dGuo exposed    | 6.43 ± 1.68          | 12.4 ± 6.73         |
| CrdGuo control    | 0.24 ± 0.10          | 0.35 ± 0.07         |
| CrdGuo exposed    | 0.24 ± 0.06          | 0.50 ± 0.12         |
| HNEdGuo control   | 1.12 ± 0.05          | 0.90 ± 0.22         |
| HNEdGuo exposed   | 1.18 ± 0.38          | 1.40 ± 0.38         |
| AcrdGuo control   | 6.02 ± 1.30          | 8.26 ± 0.98         |
| AcrdGuo exposed   | 7.16 ± 0.84          | 42.9 ± 24.50        |

*Indicates the exposures of TCDD, 100 ng/kg/day, and tertiary mixture (TCDD + PCB 126 + PeCDF), TEQ 100 ng/kg/day, 53 weeks. 21 Indicates p ≤ 0.05 compared to corresponding control groups. 22 Indicates p ≤ 0.01 compared to corresponding control groups.
in diverse biological systems exposed to PHAHs, the TEF methodology may also be valuable for the evaluation of oxidative stress and oxidative damage induced by mixtures of PHAHs, especially DLCs. However, the DNA oxidation product findings in this study, and several other studies, indicate that a simple TEF value cannot be applied to evaluate oxidative stress and oxidative damage. 

Hassoun et al. showed that following 13-weeks exposure, higher levels of superoxide anions and LPO were induced in the liver of female rats exposed to the tertiary mixture (TEQ 100 ng/kg/day) than TCDD alone (100 ng/kg/day). With similar background concentrations in control samples, the concentrations of superoxide anion were ~1.3 and 0.4 nmol cytochrome c reduced/mg in the animals treated with the tertiary mixture or TCDD alone, respectively. The concentrations of LPO were ~3 and 2 nmol 2-thiobarbituric acid substances (TBARS) formed/mg protein detected in these two groups, respectively. Additionally, synergistic effects of TCDD, PeCDF, and PCB126 were indicated in the production of superoxide anion in hepatic tissues, which required only 0.25–0.5% of the doses of the three individual congeners within the tertiary mixture to produce similar effects for each congener alone. The interactions of these three congeners were also seen in the process of superoxide anion production in female rats exposed to the tertiary mixture for 30 weeks. A significant difference was observed when comparing the dose–response curves of superoxide anion production in the hepatic tissues exposed to the tertiary mixture versus TCDD, PeCDF or PCB126 alone, with larger effects produced by an equivalent dose of the tertiary mixture than the three congeners alone.

In our study, we compared oxidation DNA product formation induced by either TCDD (100 ng/kg/day) or the tertiary mixture (TEQ 100 ng/kg/day) for 14, 31, and 53 weeks. A significant production of hepatic 8-oxo-dGuo and 1,N6-ε-dAdo was observed following exposure to the tertiary mixture at all time points, while TCDD-exposed animals showed a significant increase in 1,N6-ε-dAdo (31 and 53 weeks) but no significant difference in 8-oxo-dGuo. After 33-week exposure, significant induction of 1,N6-ε-dGuo, M7-dGuo, AcrdGuo, and CrdGuo was observed in the tertiary mixture-treated rat liver DNA; however, none of the products showed significant changes in TCDD-treated animals. Conversely, a significant induction of 7-OEG and N7,3-ε-G was observed in the liver following 53-week exposure to TCDD, while only 7-OEG was significantly induced in tertiary mixture-exposed animals, as indicated in Table 2.

Complex results were also reported in the toxicogenomic analysis by Vezina et al. and Ovando et al. Vezina et al. examined the gene expression in the hepatic DNA of female rats exposed to TCDD, PCB 126, and PeCDF for 14 weeks. With the same TEF dose used in this study (100 ng/kg/day), a limited subset of genes, which included CAT, cytochrome b5 (CYPB5), and COX oxidative stress response genes, was activated by PeCDF and PCB 126 alone, but not TCDD. PeCDF and PCB 126 also induced growth arrest and a DNA-damage-inducible gene product, Gadd45, indicating oxidative DNA damage in the liver from those animals exposed to these chemicals. Interestingly, Gadd45 was not induced in TCDD-treated animals. Therefore, PeCDF and PCB126 were more effective in activating the expression of oxidative stress response genes than TCDD in the liver of these animals after a 14-week exposure. Similar genomic studies were conducted in the hepatic tissue of female rats following 53-week exposure to TCDD.
Multiple toxicity pathways, modes, or mechanisms of action can occur simultaneously.

Recent advances in scientific understanding of cancer biology support the view that environmental chemicals can act through multiple toxicity pathways, modes, or mechanisms of action to induce cancer. 64, 65 As depicted in Figure 3, hepatic cells from female rats showed a high rate of proliferation following exposure to TCDD alone (100 ng/kg/day) or the tertiary mixture (TEQ 100 ng/kg/day) for 53 weeks, which is consistent with our DNA oxidation product induction results in Tables 2 and 3. No significant enhancement of cell proliferation was observed in 14-week TCDD or tertiary mixture exposed animals, but it was observed in 31- and 53-week TCDD and tertiary mixture exposed animals. 21, 22 Low-dose exposure of TCDD or the tertiary mixture for 53 weeks failed to induce significant production of cell proliferation, which agreed with the observation of dose-dependent accumulation of DNA oxidation products following 53-week exposure. Dose- and time-dependent induction of CYP1A1 and CYP1A2 was detected in the livers of female rats exposed to TCDD or the tertiary mixture, which is also in accordance with our results of DNA oxidation products. 21, 22 Additionally, increased incidences and severities of hepatocyte inflammation were observed at 31 and 53 weeks in TCDD or the tertiary mixture exposed animals. All these factors are potential players, which can be combined to induce the dose-dependent incidence of hepatocellular adenoma and cholangiocarcinoma in the 2-year cancer bioassay by the NTP. 21, 22

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TCDD and PCB126 by Ovando et al. 49 A dose-dependent increase in the number of differentially expressed genes was observed in animals exposed to PC126 for 53 weeks with 30, 300, and 1000 ng/kg/day. While fewer genes were differentially expressed in animals exposed to PC126 for 53 weeks (216) than 13 weeks (371), many more genes showed differential expression in animals exposed to TCDD for 53 weeks (299) than 13 weeks (103) with the same TEQ dose. More ROS or their active metabolite-related detoxification genes were upregulated or downregulated in the chronic TCDD exposed animals including glutathione-S-transferase (GST), glutathione peroxidase, aldehyde dehydrogenase (Aldh), and aldo-keto reductase (Akr).

DNA oxidation damage in rodents exposed to DLCs is probably induced through upregulation of cytochrome P450 superfamily of enzymes, mediated by AhR-dependent pathways. 37, 38 The dose-response and different time course induction of CYP1A1 is well characterized in the liver of female Sprague-Dawley rats following exposure to TCDD and the tertiary mixture. 21, 24, 50 CYP1A1 induction occurs in virtually every tissue of the body following exposure to either TCDD or the tertiary mixture. 21, 24 By using the continuous nonlinear Hill model, the induction of hepatic CYP1A1 was compared in female rats following exposure to TCDD, PCB126, and PeCDF alone or their tertiary mixture with doses or TEQ doses ranging from 0–100 ng/kg/day for 14, 31, or 53 weeks. 20 The estimated parameters indicated that congenener-specific dose-response shapes were significantly different, and the additivity of TEF methodology failed for these individual congeners and their mixture. Six of the 12 equiv time-dose combinations failed to agree between the tertiary mixture and TCDD alone. The same results were found when liver concentrations of TCDD or TEQ dose for the tertiary mixture were used. The maximum activity of 7-ethoxyresorufin-o-deethylase (EROD) is substantially higher (1.2–3.0-fold) for PeCDF than TCDD with the same TEQ dose. Toyoshiba et al. observed significant, nonadditive interaction for EROD activity at 31 and 53 weeks in the tertiary mixture exposed samples but not at 14 weeks. 20 In summary, although the importance of CYP1A1 in the induction of oxidative DNA damage was implied previously, the complicated association between DNA oxidation damage and induction of CYP1A1 by DLCs or their mixtures cannot be completely described in our study and still requires further detailed exploration. DNA oxidation products, oxidative stress biomarkers, CYP1A1 induction, and genomic studies consistently suggest that the simple TEF methodology cannot be applied to evaluate the diverse patterns of toxic effects induced by DLCs. Mutation studies have suggested that oxidative stress and DNA oxidation damage, especially from chronic inflammation, are associated with carcinogenesis. 2, 3 At present, more than one hundred DNA oxidation products are reported in model studies, but less than 20 of them are measured in cellular studies. 24, 51 Among them, 8-oxo-dGuo is the most extensively studied in vivo adduct with G to T transversions as the dominant mutation pattern. 2 Many assays have been developed to detect this product in animal or human tissues, but artificial biomarker during sample preparation has hampered its application as a good biomarker. 52, 53 1, N2-ε-dAdo is another popular biomarker, widely applied to evaluate chronic inflammation and LPO in animal or human tissues. 5 1, N2-ε-dAdo, 1, N2-ε-dGuo, N2, 3-ε-G, M2dGuo, AcrdGuo, CrdGuo, and HNEdGuo can also induce distinct mutation spectra, similar to 8-oxo-dGuo. 10–12 However, compared with 1, N2-ε-dAdo and 8-oxo-dGuo, the other products examined in our study are less widely applied in risk assessment of carcinogens. 7-OEG, the predominant product formed by vinyl chloride in rodents, was also recently established as an LPO-induced product. 24 Although it is not a direct promutagenic adduct, 7-OEG can cause AP sites and further induce mutations if they cannot be repaired before cell replication. 54 In addition to promutagenic properties, 8-oxo-dGuo, 1, N2-ε-dAdo, 1, N2-ε-dGuo, N2, 3-ε-G, M2dGuo, AcrdGuo, CrdGuo, and HNEdGuo also have distinct metabolic pathways, as shown in Figure 1. Furthermore, the primary repair pathways for these products are also different. 11, 55–58 For small products (8-oxo-dGuo, 1, N2-ε-dAdo; 1, N2-ε-dGuo; N2, 3-ε-G), base excision repair (BER) is the dominant repair pathway, with different glycosylases involved to repair different products. 55–57, 59 For the medium and bulky products (AcrdGuo; CrdGuo; HNEdGuo; M2dGuo), nuclear excision repair (NER) is the primary repair pathway. 11, 58 Therefore, the simple concentration of a DNA oxidation product in animals reflects the complex interactions between environmental and biological systems. The potential adverse effect of a product is dynamically controlled by its formation and repair pathways, which could be determined by numerous factors including, but not limited to, exposure dose, exposure time, exposure pathway, chemical metabolism, tissue, age, sex, and species. A corresponding mutation spectrum study is still necessary for further validation of the biological significance of certain DNA oxidation products in hepatic toxicity of TCDD and the tertiary mixture exposed animals examined in our study.

Additionally, increased incidences and severities of hepatocyte inflammation were observed at 31 and 53 weeks in TCDD or the tertiary mixture exposed animals, which is also in accordance with our results of DNA oxidation products. 21, 22
in female rats may be a good case in point. Although the 2-year tumor incidence data (Figure 4) support the application of the TEF approach in the risk assessment of DLCs based on the generation of similar dose–response curves in response to TCDD and the tertiary mixture exposed female rats, the TEF approach cannot be applied to evaluate the DNA oxidation products in this study, CYP1A1 or CYP1A2 induction, or oxidative biomarkers: Such inconsistency further indicates the complexity of the formation of DNA oxidation damage and carcinogenesis of DLCs in the liver of female rats.

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■ ABBREVIATIONS
1,N2-εdGuo, 1,N2-etheno-2′-deoxyguanosine; 1,N6-εdAdo, 1,N6-etheno-2′-deoxyadenosine; 7-OEG, 7-(2-oxyethoxy)-guanine; 8-oxo-dGuo, 8-oxo-7,8-dihydro-2′-deoxyguanosine; AcrdGuo, acrolein derived dGuo adducts; AhR, aryl hydrocarbon receptor; AlkB, alpha-ketoglutarate-dependent dioxygenase; ANPG, alkyl-N-purine-DNA glycosylase; CrdGuo, crotonaldehyde derived dGuo adducts; DLCs, dioxin-like compounds; HNEdG, 4-hydroxynonenal derived dG adducts; LPO, lipid peroxidation; M1dGuo, malondialdehyde derived dGuo adducts; MDA, malondialdehyde; N2,3-εG, N3,3′ethenoguanine; PCB 126, 3,3′,4,4′,5-pentachlorobiphenyl; PeCDF, 2,3,4,7,8-pentachlorodibenzofuran; PHAHs, polyhalogenated aromatic hydrocarbons; RNS, reactive nitrogen species; ROS, reactive oxygen species; SRM, selected reaction mode; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; TEF, toxic equivalent factor; TEMPO, 2,2,6,6-tetramethyl-1-piperidinolox; TEQ, toxic equivalent quotient

■ REFERENCES
(1) Cadet, J., Loft, S., Olinski, R., Evans, M. D., Bialkowski, K., Richard Wagner, J., Dedon, P. C., Møller, P., Greenberg, M. M., and Cooke, M. S. (2012) Biologically relevant oxidants and terminology, classification and nomenclature of oxidatively generated damage to nucleobases and 2-deoxyribose in nucleic acids. Free Radical Res. 46, 367–381.
(2) Rasmussen, J. L. (2006) Oxidative damage to DNA and its repair, in Oxidative Stress, Disease, and Cancer (Singh, K. K., Ed.), Imperial College Press.
(3) Nair, U., Bartsch, H., and Nair, J. (2007) Lipid peroxidation-induced DNA damage in cancer-prone inflammatory diseases: a review of published adduct types and levels in humans. Free Radical Biol. Med. 43, 1109–1120.
(4) Zhang, S., Villalta, P. W., Wang, M., and Hecht, S. S. (2007) Detection and quantitation of acrolein-derived 1,N2-propanodeoxyguanosine adducts in human lung by liquid chromatography-electrospray ionization-tandem mass spectrometry. Chem. Res. Toxicol. 20, 565–571.
(5) Chung, F. L., Zhang, L., Ocando, J. E., and Nath, R. G. (1999) Role of 1,N2-propanodeoxyguanosine adducts as endogenous DNA lesions in rodents and humans. JARC Sci. Publ., 45–54.
(6) Liu, X., Lovell, M. A., and Lynn, B. C. (2006) Detection and quantification of endogenous cyclic DNA adducts derived from trans-4-hydroxy-2-nonenal in human brain tissue by isotope dilution capillary liquid chromatography nanoelectrospray tandem mass spectrometry. Chem. Res. Toxicol. 19, 710–718.
(7) Pang, B., Zhou, X., Yu, H., Dong, M., Taghizadeh, K., Wishnok, J. S., Tannenbaum, S. R., and Deden, P. C. (2007) Lipid peroxidation dominates the chemistry of DNA adduct formation in a mouse model of inflammation. Carcinosgenesis 28, 1807–1813.
(8) Valavanidis, A., Vlachogianni, T., and Fiotakis, C. (2009) 8-hydroxy-2′-deoxyguanosine (8-OHdG): A critical biomarker of oxidative stress and carcinogenesis. J. Environ. Sci. Health C Environ. Carcinog. Ecotoxicol. Rev. 27, 120–139.
(9) Mutlu, E., Jeong, Y. C., Collins, L. B., Ham, A. J., Upton, P. B., Hatch, G., Winsett, D., Evansky, P., and Swenberg, J. A. (2012) A new LC–MS/MS method for the quantification of endogenous and vinyl chloride-induced 7-(2-Oxoethyl)guanine in Sprague–Dawley rats. Chem. Res. Toxicol. 25, 391–399.
(10) Niedermhofer, L. J., Daniels, J. S., Rouzer, C. A., Greene, R. E., and Marnett, L. J. (2003) Malondialdehyde, a product of lipid peroxidation, is mutagenic in human cells. J. Biol. Chem. 278, 31426–31433.
(11) Minko, I. G., Kozekov, I. D., Harris, T. M., Rizzo, C. J., Lloyd, R. S., and Stone, M. P. (2009) Chemistry and biology of DNA containing 1,N2-deoxyguanosine adducts of the alpha,beta-unsaturated aldehydes acrolein, crotonaldehyde, and 4-hydroxynonenal. Chem. Res. Toxicol. 22, 759–778.
(12) Moriya, M., Zhang, W., Johnson, F., and Grollman, A. P. (1994) Mutagenic potency of exocyclic DNA adducts: marked differences between Escherichia coli and simian kidney cells. Proc. Natl. Acad. Sci. U. S. A. 91, 11899–11903.
(13) Munnia, A., Bonassi, S., Verna, A., Quaglia, R., Peluccho, D., Ceppi, M., Neri, M., Buratti, M., Taioli, E., Garte, S., and Peluso, M. (2006) Bronchial malondialdehyde DNA adducts, tobacco smoking, and lung cancer. Free Radical Biol. Med. 41, 1499–1505.
(14) Mangerich, A., Knuston, C. G., Parry, N. M., Muthupalani, S., Ye, W., Prestwich, E., Cui, L., McFaline, J. L., Mobley, M., Ge, Z., Taghizadeh, K., Wishnok, J. S., Wogan, G. N., Fox, J. G., Tannenbaum, S. R., and Deden, P. C. (2012) Infection-induced colitis in mice causes dynamic and tissue-specific changes in stress response and DNA damage leading to colon cancer. Proc. Natl. Acad. Sci. U. S. A. 109, E1820–E1829.
(15) Melnick, R. L., Kohn, M. C., and Portier, C. J. (1996) Implications for risk assessment of suggested nongenotoxic mechanisms of chemical carcinogenesis. Environ. Health Perspect. 104 (Suppl 1), 123–134.
(16) Jarabek, A. M., Pottenger, L. H., Andrews, L. S., Casciano, D., Embry, M. R., Kim, J. H., Preston, R. J., Reddy, M. V., Schoeny, R., Shuker, D., Skare, J., Swenberg, J., Williams, G. M., and Zeiger, E. (2009) Creating context for the use of DNA adduct data in cancer risk assessment: I. Data organization. Crit. Rev. Toxicol. 39, 659–678.
(17) Spencer, W. A., Lehmier, H. J., Robertson, L. W., and Gupta, R. C. (2009) Oxidative DNA adducts after Cu2+-mediated activation of dihydroxy PCBs: role of reactive oxygen species. Free Radical Biol. Med. 46, 1346–1352.
(18) Wyde, M. E., Wong, V. A., Kim, A. H., Lucier, G. W., and Walker, N. J. (2001) Induction of hepatic 8-oxo-deoxyguanosine adducts by 2,3,7,8-tetrachlorodibenzo-p-dioxin in Sprague–Dawley rats is female-specific and estrogen-dependent. Chem. Res. Toxicol. 14, 849–855.
(19) Tritscher, A. M., Seacat, A. M., Yager, J. D., Groopman, J. D., Miller, B. D., Bell, D., Sutter, T. R., and Lucier, G. W. (1996) Increased oxidative DNA damage in livers of 2,3,7,8-tetrachlorodibenzo-p-dioxin treated intact but not ovariec-tomized rats. Cancer Lett. 98, 219–225.
(20) Jeong, Y. C., Walker, N., J. Burgin, D. E., Kissling, G., Gupta, M., Kupper, L., Birnbaum, L. S., and Swenberg, J. A. (2008) Accumulation of M1dG DNA adducts after chronic exposure to PCBs, but not from acute exposure to polychlorinated aromatic hydrocarbons. Free Radical Biol. Med. 45, 585–591.
(21) NTP. (2006) in NTP Technical Report on the Toxicology and Carcinogenesis Studies of 3,3′,4,4′,5-Pentachlorobiphenyl (PCB 126) (CAS No. 57465−28−8) in Female Harlan Sprague–Dawley Rats (Gavage Studies), U.S. Department of Health and Human Services, Washington, DC.
(22) NTP. (2006) NTP Technical Report on the Toxicology and Carcinogenesis Studies of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) (CAS No. 1746−01−6) in Female Harlan Sprague–Dawley Rats (Gavage Studies), pp 4–232, U.S. Department of Health and Human Services, Washington, DC.
(23) NTP. (2006) NTP Technical Report on the Toxicology and Carcinogenesis Studies of 2,3,4,7,8-Pentachlorodibenzofuran (PeCDF) (CAS No. 57117−31−4) in Female Harlan Sprague–Dawley Rats (Gavage Studies), pp 1–198, U.S. Department of Health and Human Services, Washington, DC.
(24) NTP. (2006) NTP Technical Report on the Toxicology and Carcinogenesis Studies of a Mixture of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) (Cas No. 1746–01–6), 2,3,4,7,8-Pentachlorodibenzofuran (PCDF) (Cas No. 57117–31–4), and 3,3′,4′,5′-Pentachlorobiphenyl (PCB 126) (Cas No. 57465–28–8) in Female Harlan Sprague–Dawley Rats (Gavage Studies), pp 1–198, U.S. Department of Health and Human Services, Washington, DC.
(25) Van den Berg, M., Birnbaum, L. S., Denison, M., De Vito, M., Farland, W., Feeley, M., Fielder, H., Hakansson, H., Hanberg, A., Haws, L., Rose, M., Safe, S., Schrenk, D., Tohyama, C., Tritscher, A., Tuomisto, J., Tyskland, M., Walker, N., and Peterson, R. E. (2006) The 2005 World Health Organization reevaluation of human and mammalian tumor equivalence factors for dioxins and dioxin-like compounds. Toxicol. Sci. 93, 223–241.
(26) Hassoun, E. A., Li, F., Abushaban, A., and Stohs, S. J. (2001) Production of superoxide anion, lipid peroxidation and DNA damage in the hepatic and brain tissues of rats after subchronic exposure to mixtures of TCDD and its congeners. J. Appl. Toxicol. 21, 211–219.
(27) Hassoun, E. A., Li, F., Abushaban, A., and Stohs, S. J. (2000) The relative abilities of TCDD and its congeners to induce oxidative stress in the hepatic and brain tissues of rats after subchronic exposure. Toxicology 145, 103–113.
(28) Hassoun, E. A., and Periandri-Steinberg, S. (2010) Assessment of the roles of antioxidant enzymes and glutathione in 3,3′,4,4′,5′-Pentachlorobiphenyl (PCB 126)-induced oxidative stress in the brain tissues of rats after subchronic exposure. Toxicol. Environ. Chem. 92, 301.
(29) Hassoun, E. A., Wang, H., Abushaban, A., and Stohs, S. J. (2002) Induction of oxidative stress in the tissues of rats after chronic exposure to TCDD, 2,3,4,7,8-pentachlorodibenzofuran, and 3,3′,4,4′,5′-pentachlorobiphenyl. J. Toxicol. Environ. Health, Part A 65, 825–842.
(30) Stohs, S. (1990) Oxidative stress induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Free Radical Biol. Med. 9, 79–90.
(31) Stohs, S. J., Hassan, M. Q., and Murray, W. J. (1983) Lipid peroxidation as a possible cause of TCDD toxicity. Biochem. Biophys. Res. Commun. 111, 854–859.
(32) Dreiem, A., Rytkonen, S., Lehmler, H. J., Robertson, L. W., and Fonnun, F. (2009) Hydroxylated polychlorinated biphenyls increase reactive oxygen species formation and induce cell death in cultured cerebellar granule cells. Toxicol. Appl. Pharmacol. 240, 306–313.
(33) Fadhel, Z., Lu, Z., Robertson, L. W., and Glauert, H. P. (2002) Effect of 3,3′,4′,5′-tetrachlorobiphenyl and 2,2′,4,4′,5,5′-hexachlorobi-phenyl on the induction of hepatic lipid peroxidation and cytochrome P450 associated enzyme activities in rats. Toxicology 175, 15–25.
cytochromes P450 CYP1A1 and CYP1A2 enzyme activity by dioxin-like compounds. *Toxicol. Appl. Pharmacol.* 194, 156–168.

(51) Cadet, J., and Wagner, J. R. (2013) DNA Base Damage by Reactive Oxygen Species, Oxidizing Agents, and UV Radiation. *Cold Spring Harbor Perspect. Biol.* 5, a012559.

(52) ESCODD (2003) Measurement of DNA oxidation in human cells by chromatographic and enzymic methods. *Free Radical Biol. Med.* 34, 1089–1099.

(53) Cadet, J., Douki, T., and Ravanat, J.-L. (2011) Measurement of oxidatively generated base damage in cellular DNA. *Mutat. Res., Fundam. Mol. Mech. Mutagen.* 711, 3–12.

(54) Barbin, A., Laib, R. J., and Bartsch, H. (1985) Lack of miscoding properties of 7-(2-oxoethyl)guanine, the major vinyl chloride-DNA adduct. *Cancer Res.* 45, 2440–2444.

(55) Maynard, S., Schurman, S. H., Harboe, C., de Souza-Pinto, N. C., and Bohr, V. A. (2008) Base excision repair of oxidative DNA damage and association with cancer and aging. *Carcinogenesis* 30, 2–10.

(56) Slupphaug, G., Kavl, B., and Krokan, H. E. (2003) The interacting pathways for prevention and repair of oxidative DNA damage. *Mutat. Res., Fundam. Mol. Mech. Mutagen.* 531, 231–251.

(57) Ringvoll, J., Moen, M. N., Nordstrand, L. M., Meira, L. B., Pang, B., Bekkelund, A., Dedon, P. C., Bjelland, S., Samson, L. D., Falnes, P. O., and Klungland, A. (2008) AlkB homologue 2-mediated repair of ethenoadenine lesions in mammalian DNA. *Cancer Res.* 68, 4142–4149.

(58) Jianowska, B., Komisarski, M., Prorok, P., Sokolowska, B., Kusnierek, J., Janion, C., and Tudek, B. (2009) Nucleotide excision repair and recombination are engaged in repair of trans-4-hydroxy-2-nonenal adducts to DNA bases in Escherichia coli. *Int. J. Biol. Sci.* 5, 611–620.

(59) Tudek, B., and Speina, E. (2012) Oxidatively damaged DNA and its repair in colon carcinogenesis. *Mutat. Res., Fundam. Mol. Mech. Mutagen.* 736, 82–92.

(60) Safe, S., and Hutzinger, O. (1984) Polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs): biochemistry, toxicology, and mechanism of action. *Crit. Rev. Toxicol.* 13, 319–395.

(61) Safe, S. H. (1986) Comparative toxicology and mechanism of action of polychlorinated dibenzo-p-dioxins and dibenzofurans. *Annu. Rev. Pharmacol. Toxicol.* 26, 371–399.

(62) Whysner, J., and Williams, G. M. (1996) 2,3,7,8-Tetrachlorodibenzo-p-dioxin mechanistic data and risk assessment: gene regulation, cytotoxicity, enhanced cell proliferation, and tumor promotion. *Pharmacol. Ther.* 71, 193–223.

(63) Huff, J., Lucier, G., and Tritscher, A. (1994) Carcinogenicity of TCDD: experimental, mechanistic, and epidemiologic evidence. *Annu. Rev. Pharmacol. Toxicol.* 34, 343–372.

(64) Hernandez, L. G., van Steeg, H., Luijten, M., and van Benthem, J. (2009) Mechanisms of non-genotoxic carcinogens and importance of a weight of evidence approach. *Mutat. Res., Rev. Mutat. Res.* 682, 94–109.

(65) Guyton, K. Z., Kyle, A. D., Aubrecht, J., Cogliano, V. J., Eastmond, D. A., Jackson, M., Keshava, N., Sandy, M. S., Sonawane, B., Zhang, L., Waters, M. D., and Smith, M. T. (2009) Improving prediction of chemical carcinogenicity by considering multiple mechanisms and applying toxicogenomic approaches. *Mutat. Res., Rev. Mutat. Res.* 681, 230–240.

(66) Walker, N. J., Crockett, P. W., Nyska, A., Brix, A. E., Jokinen, M. P., Sells, D. M., Hailey, J. R., Easterling, M., Haseman, J. K., Yin, M., Wyde, M. E., Bucher, J. R., and Portier, C. J. (2004) Dose-additive carcinogenicity of a defined mixture of “dioxin-like compounds. *Environ. Health Perspect.* 113, 43–48.