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Accumulation of M_1dG DNA adducts after chronic exposure to PCBs, but not from acute exposure to polychlorinated aromatic hydrocarbons

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Abstract

Oxidative DNA damage is one of the key events thought to be involved in mutation and cancer. The present study examined the accumulation of M_1dG, 3-(2′-deoxy-β-D-*erythro*-pentofuranosyl)-pyrimido[1,2-*a*]-purin-10(3*H*)-one, DNA adducts after single dose or one-year exposure to polyhalogenated aromatic hydrocarbons (PHAH) in order to evaluate the potential role of oxidative DNA damage in PHAH toxicity and carcinogenicity. The effect of PHAH exposure on the number of M_1dG adducts was explored initially in female mice exposed to a single dose of either 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) or a PHAH mixture. This study demonstrated that a single exposure to PHAH had no significant effect on the number of M_1dG adducts compared to the corn oil control group. The role of M_1dG adducts in polychlorinated biphenyl (PCB) induced toxicity and carcinogenicity was further investigated in rats exposed for a year to PCB 153, PCB 126, or a mixture of the two. PCB 153, at doses up to 3000 μg/kg/d, had no significant effect on the number of M_1dG adducts in liver and brain tissues from the exposed rats compared to controls. However, 1000 ng/kg/d of PCB 126 resulted in M_1dG adduct accumulation in the liver. More importantly, co-administration of equal proportions of PCB 153 and PCB 126 resulted in dose-dependent increases in M_1dG adduct accumulation in the liver from 300-1000 ng/kg/d of PCB 126 with 300-1000 μg/kg/d of PCB 153. Interestingly, the co-administration of different amounts of PCB 153 with fixed amounts of PCB 126 demonstrated more M_1dG adduct accumulation with higher doses of PCB 153. These results are consistent with the results from cancer bioassays that demonstrated a synergistic effect between PCB 126 and PCB 153 on toxicity and tumor development. In summary, the results from the present study support the hypothesis that oxidative DNA damage plays a key role in toxicity and carcinogenicity following long-term PCB exposure.

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Introduction

Polyhalogenated aromatic hydrocarbons (PHAH) include polychlorinated biphenyls (PCB), polychlorinated dibenzo-p-dioxins (PCDD), and polychlorinated dibenzofurans (PCDF) (Fig. 1). These are industrial compounds or combustion byproducts that widely contaminate the environment. Therefore, humans, wildlife, and laboratory animals are exposed daily to a complex mixture of these chemicals via trace amounts present in food and the environment [1,2]. Due to their hydrophobic nature and resistance to metabolism, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related PHAH congeners accumulate in tissues of exposed populations [1-5]. Therefore, repeated exposures to small amounts of PHAH may evoke adverse health effects due to chronic accumulation in target tissues [6].

TCDD has been shown to act as a multi-site rodent carcinogen with potent tumor promoter activity [7]. Furthermore, increased cancer rates in humans have also been associated with exposures to dioxin-like chemicals [8-11]. Although dioxin-like compounds do not covalently bind to DNA, nor induce direct genotoxic effects, it has been suggested that DLC may be indirectly genotoxic by increasing the formation of reactive oxygen species (ROS) (Fig. 2). This increased ROS formation may result from CYP1A1 induction by PHAH [12] or from the formation of catechol estrogens [13-15].

Seven of the 75 PCDD congeners, 10 of the 135 PCDF congeners, and 13 of the 209 PCB congeners induce dioxin-like toxicity [3,16]. Due to the similarity in toxicity and mechanisms, the concept of toxic equivalency factors (TEF) has been used for risk assessment and regulatory control for DLC [17,18]. The TEF is the relative potency of biological effects assigned to each congener relative to that of TCDD on the basis of available *in vivo* and *in vitro* data [17,18]. This allows for the estimation of the potential dioxin-like activity of DLC mixtures in the environment. Recently, as an effort to evaluate the TEF methodology for chronic toxicity and carcinogenicity of DLC and structurally related PCB, the National Toxicology Program (NTP) conducted a series of 2-year bioassays in female Harlan Sprague-Dawley rats [19-22]. The most potent dioxin-like PCB congener, 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126), caused dose-dependent increases in hepatic, lung, and oral mucosal neoplasms as well as in the activity of CYP 1A1 and 2B1. Interestingly, PCB 153, while it does not have dioxin-like activity, significantly enhanced carcinogenicity associated with PCB 126.

As an effort to elucidate molecular pathways involved in carcinogenesis from PHAH exposure and to develop biomarkers, this study investigated the correlation between PCB exposure and the numbers of oxidative DNA lesions. Such oxidative DNA lesions are suspected to be increased with non-DNA reactive chemicals, resulting in the induction of mutations followed by tumor formation. While base oxidation and abasic sites are the most frequent insults to DNA, substantial evidence supports that secondary DNA damage produced from byproducts of primary DNA damage or lipid membrane damage may play an important role in mutation [23,24]. Recently, we have developed ultra sensitive and highly specific mass spectrometric methods for the quantitation of M_1dG adducts. In the follow up studies, it was established that M_1dG adducts are important indirect DNA lesions primarily resulting from ROS attack on deoxyribose as well as on lipid membrane [25,26] (Fig. 2). Furthermore, M_1dG adducts appear to be less prone to artifactual formation while most primary oxidative DNA lesions are significantly increased from artifactual oxidation of normal DNA nucleotides [27]. These facts support that M_1dG adducts are an excellent biomarker for monitoring oxidative DNA damage from *in vitro* and *in vivo* samples. The present study investigated the numbers of M_1dG adducts in tissues of rats or mice after exposures to PHAH in order to improve our understanding of the role of oxidative DNA damage in the toxicity and carcinogenicity of PHAH.

Materials and Methods

Materials and Instrumentation

Unless stated otherwise, all chemicals and enzymes were purchased from Sigma-Aldrich Chemical Company (St Louis, MO). Proteinase K, lysis buffer, and 70% phenol solutions were purchased from Applied Biosystems (Foster City, CA). Absolute ethanol was purchased from Aaper ethanol (Shelbyville, KY). Other solvents were HPLC grade and were purchased from Fisher Scientific (Raleigh, NC). Quantification of M_1dG conjugates with t-butylhydroxylamine (tBHA) and its internal standard were performed using a Finnigan Quantum (Thermo, Woburn, MA) triple-quadrupole mass spectrometer connected to the Finnigan surveyor Micro-LC (Thermo, Woburn, MA).

Exposures and tissues isolated from PHAH-exposed animals

Female C57BL/6J mice (10 weeks old; average weight 25.6 ± 0.4 g) received a single treatment with either corn oil (i.e., vehicle) or one of the three PHAH mixtures [28]. These chemical mixtures are listed in Table 1. The ratios for Mix B were selected based on the TEF value of chemicals used and their profile detected in foods [29]. The doses used were 0, 1, 10 ng toxic equivalents (TEQ)/kg/d body weight (BW) for Mixes A, B, and C. For TCDD exposures, the doses were 0, 1, 100 ng/kg/d BW. Seven days after treatment, mice were euthanized by carbon dioxide anoxia and liver tissues were snap-frozen and stored in a -80 °C freezer.

For the chronic exposure study, rat liver and brain tissues were provided by Battelle Laboratories (Columbus, OH) which conducted the studies according to an NIEHS contract (N01-ES-75411) [19,30,31]. Female Harlan Sprague-Dawley rats received one of the following exposures by gavage 5 days per week for 53 weeks: PCB 153 alone; PCB 126 alone; or a mixture of PCB 153 and PCB 126 in corn oil:acetone (99:1). The doses used were 0, 10, 100, 300, and 1000 μg/kg/d BW for PCB 153, and 0, 30, 100, 300, 1000 ng/kg/d BW for PCB 126. The PCB mixtures were prepared in fixed proportions of PCB 153 (μg/kg/d BW) and PCB 126 (ng/kg/d BW): 0 + 0; 10 +10; 100 + 100; 300 + 300; or 1000 + 1000. In addition, the following three mixture groups were used to examine the interaction between PCB 153 (μg/kg/d BW) and PCB 126 (ng/kg/d BW): 100 + 300; 1000 + 300; or 3000+ 300. Liver and brain tissue samples were collected from 9 or 10 female rats per group a day after the final exposure and stored frozen at -80 °C.

DNA isolation from tissues

DNA was extracted by a procedure described in the previous study [27]. Briefly, frozen liver or brain tissue (1 g) was thawed in 7 ml ice-cold homogenization buffer consisting of phosphate buffered saline (PBS, pH 7.4) with 10 mM 2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPO). The tissue samples were homogenized with a Tehran homogenizer (Wheaton Instruments, Millville, NJ). After centrifugation at 1,000 × g for 15 min, the precipitate was washed with 7 ml homogenization buffer. The nuclear fraction was collected by centrifugation and was reconstituted in 6 ml lysis buffer (Applied Biosystems). Proteinase K (400 U/ml, 60 μl) was added to the sample and incubated overnight at 4 °C. Hydrolyzed protein was then extracted twice with 6 ml 70% phenol solution (Applied Biosystems) and once with 6 ml chloroform:isoamyl alcohol (49:1, Sevag solution). Nucleic acids were precipitated from the aqueous layer by sequential addition of 300 μl 3 M NaCl and 12 ml cold ethanol. The nucleic acids were collected by centrifugation and were rinsed with 6 ml of 70% ethanol. The nucleic acid pellet was reconstituted in 2 ml RNA digestion buffer consisting of RNase A (0.8 KeU/ml), RNase T1 (3 mU/ml), and 1 mM TEMPO in 10 mM HEPES buffer (pH 7.8). After 1 hour incubation at 37°C, DNA was precipitated by the sequential addition of 100 μl 3 M NaCl and 4 ml cold ethanol. The DNA was collected by centrifugation and rinsed with 70% ethanol. The DNA pellet was resuspended in 1 ml HPLC grade water. DNA samples (25 μl) were mixed

with 975 μl 20 mM Tris-EDTA buffer (TE, pH 8.0) to measure DNA concentration and purity by UV. The DNA solution was stored at -80°C until M_1dG adduct analysis.

Analysis of M_1dG adducts in DNA

The number of M_1dG adducts in sample DNA was determined by the derivatization of DNA adducts with tBHA and LC-MS/MS analysis for the M_1dG-tBHA conjugate similar to the protocol described elsewhere [32]. Briefly, sample DNA (200 μg) was mixed with internal standard (IS) DNA corresponding to 400 fmol $^{15}N_5$-M_1G-dR. The DNA mix was incubated with 2 mM tBHA in 10 mM $KHPO_4$ (pH 5.5) solution for 30 min at 50°C. Free tBHA in the sample solution was removed by DNA precipitation after sequential additions of 1/20 volume of 3 M sodium acetate and 1 volume of isopropanol. The sample DNA was reconstituted in 570 μL of 20 mM HEPES buffer (pH 7.4) containing 1 mM $MgCl_2$ and 100 U/mL DNase I. The DNA was hydrolyzed to nucleosides for 20 min at 37°C, followed by the addition of 30 μl solution containing alkaline phosphatase and phosphodiesterase I to final concentrations of 5 and 0.06 U/mL, respectively. The DNA and enzyme mixture was further incubated at 37 °C for 1 h with mild agitation. The hydrolyzed DNA was placed on an SPE column (HLB-60, Waters, Milford, MA) that had been preactivated with methanol and equilibrated with water. The SPE column was washed with 1 mL water and with 3 mL 40% methanol. M_1dG-tBHA and the internal standard were eluted with 1 mL of 90% methanol in water. The solvent was removed by vacuum evaporation. The samples were reconstituted in 50 μL of 10% methanol in water and stored at -80°C until analysis.

M_1dG-tBHA and IS were separated on an Aquasile column (5 μm, 1 mm × 150 mm, Thermo) at a flow rate of 100 μL/min. The analysis was performed by positive ion electrospray using SRM. Nitrogen gas was used for the sheath and auxiliary gas. Fragmentation was accomplished at 38 V using argon gas at 1.5 mTorr. Two ion transitions, *m/z* 635→ *m/z* 188 and *m/z* 640→ *m/z* 193, were used to monitor M_1dG and $^{15}N_5$-M_1dG.

Statistical Analyses

Statistical analyses were performed using SAS Ver. 8.02 (Cary, NC) and nonlinear equations were solved numerically using Matlab Ver. 7.3 (The Mathworks, Natick, MA). The effect of chemical exposures on the accumulation of M_1dG DNA adducts was assessed with one-way ANOVA after the homogeneity of variances was verified with O'Brien's test and normality of residuals with the Kolmogorov-Smirnov test [33]. Plots for M_1dG DNA adducts in rat liver were fitted using linear regression to test PCB-dose dependency in the accumulation of M_1dG adducts. Differences between control and PCB-treated rats in the number of M_1dG adducts were assessed by planned pairwise comparisons after the means were adjusted by Dunnett's method for multiple comparisons [34]. In cases where the sample size was limited, the non-parametric Jonckheere-Terpstra exact test was used to assess the trend in the number of M_1dG adducts over different doses [35]. Two-sided and one-sided *p*-values were considered significant if they were less than 0.05.

Results and Discussion

The major goal of this research was to test the hypothesis that PHAH exposures increase ROS generation, which in turn results in higher numbers of M_1dG adducts in genomic DNA. As an initial approach to test the hypothesis, the effect of TCDD on the number of M_1dG adducts was examined using mice treated with single doses of different amounts of TCDD. In a parallel experiment, different combinations of PHAH mixtures were given to mice to evaluate the TEF methodology in their toxicities, including oxidative DNA damage. Mix A consisted of DLC including TCDD. Mix B consisted of non-dioxin like PCB that are major PCB congeners found in the environment. To emulate environmental exposures which include a mixture of PHAH,

mix C consisted of a combination of mix A and mix B. The toxicity and the change in the number of M_1dG adducts were examined a week after PHAH treatment.

A previous study [28] had shown that TCDD treatment resulted in a dose-dependent increase in the concentration of malondialdehyde (i.e., a major precursor to M_1dG adduct formation) in the livers of these mice. Cytochrome C reduction (i.e., an indicator of superoxide anion radical production) showed statistically significant increases in mice that received 10 or 100 μg/kg/d of TCDD [28]. However, as described in Fig. 3, there was no trend in the numbers of M_1dG DNA adducts in response to the different doses of TCDD or one of the PHAH mixtures (non-parametric Jonckheere-Terpstra exact test, $p > 0.18$). This result is consistent with the findings from other investigators who reported no significant changes in the numbers of 8-OH-dG adducts after a single exposure to DLC [36,37]. In contrast, subchronic or chronic exposures to the chemicals resulted in an accumulation of 8-OH-dG adducts in animals [36,37].

It has been hypothesized by a number of investigators [12,37] that DNA repair in healthy control animals is sufficient to repair DNA adducts formed by an acute exposure to PHAH. Unlike acute exposures, however, long-term exposures to PHAH may change the capacity of DNA repair by modifying the expression of proteins involved in DNA repair, analogous to how PHAH changes the expression of metabolic enzymes [38-40]. Additionally, it has been proposed that DNA repair proteins can be impaired by mutations caused by the slow accumulation of DNA adducts over a long period of time [37]. This argument is supported by the findings from previous studies that reported sustained DNA adduct (i.e., 8-OH-dG) accumulation, toxicity, and carcinogenesis [12,36,37,41].

To determine the role of oxidative DNA damage in the toxicity and carcinogenicity of PCBs, the accumulation of M_1dG adducts in livers and brain was examined in rats that had been exposed for a year to PCB 153, PCB 126, or a mixture of PCB 153 and PCB 126. As shown in Fig. 4, PCB exposure produced statistically significant changes in the accumulation of hepatic M_1dG adducts in the rats exposed to PCB 126 ($F(4,35)=13.31$, $p < .0001$) or to the mixture of PCB 126 and PCB 153 in a 1:1000 ratio ($F(4,35)=19.44$, $p < .0001$). There was no significant effect of PCB 153 exposure on M_1dG DNA adduct accumulation in rat liver ($F(4,33)=1.32$, $p =.2820$). Linear regression confirmed dose-dependent increases in the numbers of M_1dG adducts in the rats exposed to PCB 126 ($R^2 = .5851$, $p < .0001$) or to the mixture of PCB 126 and PCB 153 ($R^2 = .6880$, $p < .0001$). Interestingly, analyses of the linear regression model revealed that the mixture of PCB 126 and PCB 153 had a stronger effect on the accumulation of hepatic M_1dG adducts than PCB 126 alone ($p = .0013$). The response to the mixture of PCB 126 and PCB 153 in the accumulation of hepatic M_1dG adducts was even stronger than the sum of the individual response to PCB 126 and PCB 153 ($p = .0027$). Compared to the control, PCB 126 resulted in significantly increased accumulation of M_1dG adducts in rat liver at 1000 ng/kg. However, by co-administrating a 1000-fold excess of PCB 153, PCB 126 produced significantly higher numbers of M_1dG adduct in rat liver at 300 ng/kg/ as well as at 1000 ng/kg/d when the numbers were compared to that of control rats. ($p = .0274$, and $p < .0001$, respectively). These results provide strong evidence in support of the hypothesis that long-term exposure to PCB causes the accumulation of oxidative DNA lesions that may play a role in toxicity, mutation and cancer development. Furthermore, the results from this study support the hypothesis that PCB 153 which is a non-dioxin like PCB potentiates the PCB 126 mediated DNA damage.

This hypothesis was further tested in the experiments with rats that were dosed with fixed amounts of PCB 126 (300 ng/kg/d) in combination of different amounts of PCB 153. The effect of PCB treatments on hepatic M_1dG adducts is shown in Fig. 5. The number of M_1dG adducts in livers for the mixture of PCB 126/PCB 153 at 300/3000 was significantly higher compared to both 300/0 ($p < .0001$) and 0/3000 ($p < .0001$) (Fig. 5A. A positive linear association (R^2

= .603, $p < .0001$) was confirmed between the number of M_1dG adducts in liver and the amount of PCB 153 when the rats were co-administered 300 ng/kg/d PCB 126 (Fig 5B). Overall, these results support the hypothesis that non-dioxin like PHAH can significantly increase DNA damage resulting from simultaneous exposures to DLC.

The effect of PCB exposure on the accumulation of M_1dG adducts in the brain was examined and the results are described in Fig. 6. There was no trend in the number of M_1dG adducts in response to the different doses of either PCB 153 alone or the mixture of PCB 153 and PCB 126 (non-parametric Jonckheere-Terpstra exact test, $p > .28$). The effect of PCB 126 exposure on M_1dG accumulation was not examined because tissue samples were not available.

Finally, the results from this study were compared to those from the NTP cancer bioassays [30]. The NTP reported that increasing the proportion of PCB 153 in the mixtures of PCB 126 resulted in increases in cell proliferation in rat liver. Furthermore, there was a positive effect of PCB 153 in the PCB 153/ PCB 126 mixture on liver toxicity, including hepatocyte hypertrophy, cholangiofibrosis, eosinophilic foci, clear cell foci, basophilic foci, diffuse and focal fatty change, bile duct hyperplasia, and hematopoietic cell proliferation. Carcinogenesis was also enhanced after a two-year exposure. A positive effect of increasing the amount of PCB 153 in the mixture was evident in increased incidences of hepatocellular adenoma and cholangiocarcinoma. As described in Fig. 7, the dose response for M_1dG adduct accumulation after PCB exposure is very similar to that of developing neoplastic lesions. The number of M_1dG adducts in liver showed positive correlations with the incidence of hepatocellular adenoma and cholangiocarcinoma. It is noteworthy that the change in the number of hepatic M_1dG adducts was detected after a one-year exposure to PCBs while the increase in cancer incidence was observed after a two-year exposure to PCBs. This result supports the hypothesis that oxidative DNA damage plays an important role in cancer development associated with chronic exposure to PHAHs. Compared to other biomarkers such as cell proliferation and cytochrome P450 expression that have been used in the TEF evaluation for PHAHs, the numbers of hepatic M_1dG adducts showed strong positive correlations to the incidence of neoplastic lesions in the rats treated with the mixture of PCBs.

PCB153, which does not have a TEF value since it has no dioxin like effects, enhances the accumulation of oxidative DNA lesions in genomic DNA, thus increasing chances for toxicity, mutation and cancer development. Similar to these results, the synergistic effect of PCB 153 with DLC other than PCB 126 on the toxicity of DLC has been previously reported. Van Birgelen [42] demonstrated a synergistic effect of co-administration of PCB 153 and TCDD on hepatic porphyrin accumulation in female Sprague-Dawley rats fed diets containing these chemicals for 13 weeks. To explore this synergistic effect of PCB 153 on the toxicity of DLC, a number of researchers investigated the role of PCB 153 and found that it increased the hepatic concentrations of DLC [43,44]. PCB 153 was shown to enhance the retention of DLC in the cell by inducing both AhR and CYP1A2, the two major binding proteins for DLC [45,46]. However, analysis of hepatic PCB 126 concentrations by the NTP as a part of the cancer bioassay, showed that the proportion of PCB 153 in the mixture relative to PCB 126 had no significant effect on the amounts of PCB 126 in liver and lung tissues [30]. Consistent with this result, hepatic CYP1A1 and CYP1A2 enzyme activities were not affected by the proportion of PCB 153 in mixtures with constant amounts of PCB 126. Therefore, the molecular mechanism involved in this synergistic effect between PCB 126 and PCB 153 on M_1dG adduct accumulation in the liver requires additional research.

In summary, the present study supports the hypothesis that oxidative DNA damage plays an important role in the toxicity and carcinogenicity associated with chronic exposure to PHAHs. The accumulation of M_1dG adducts in the tissues were dose- and time-dependent on PHAH exposure, which is similar to the incidence of pre-neoplastic lesions and subsequent cancer

development. Enhanced DLC toxicities (i.e., the accumulation of M_1dG adducts and pre-neoplastic lesions in liver) by non-dioxin like PHAH suggest that the combination of different types of PHAH can result in greater toxicity than predicted by TEFs. Overall, the findings from the present study provide additional information that may be important in the scientific assessment of risk for complex PHAH mixtures present in the environment.

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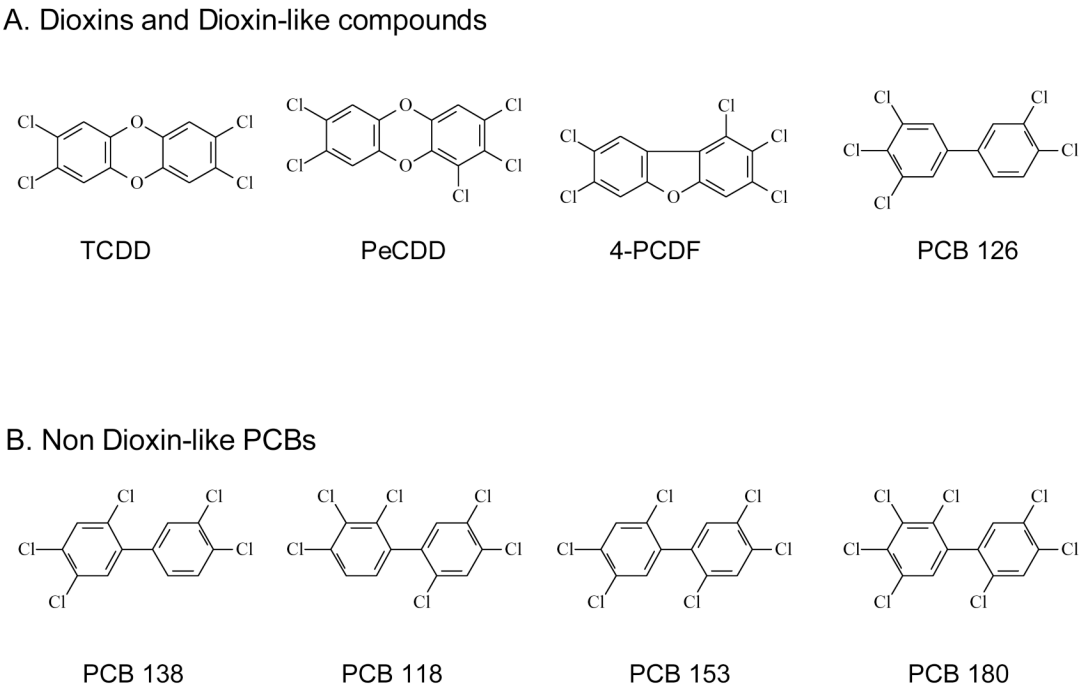

Fig. 1. Polyhalogenated Aromatic Hydrocarbons (PHAH)

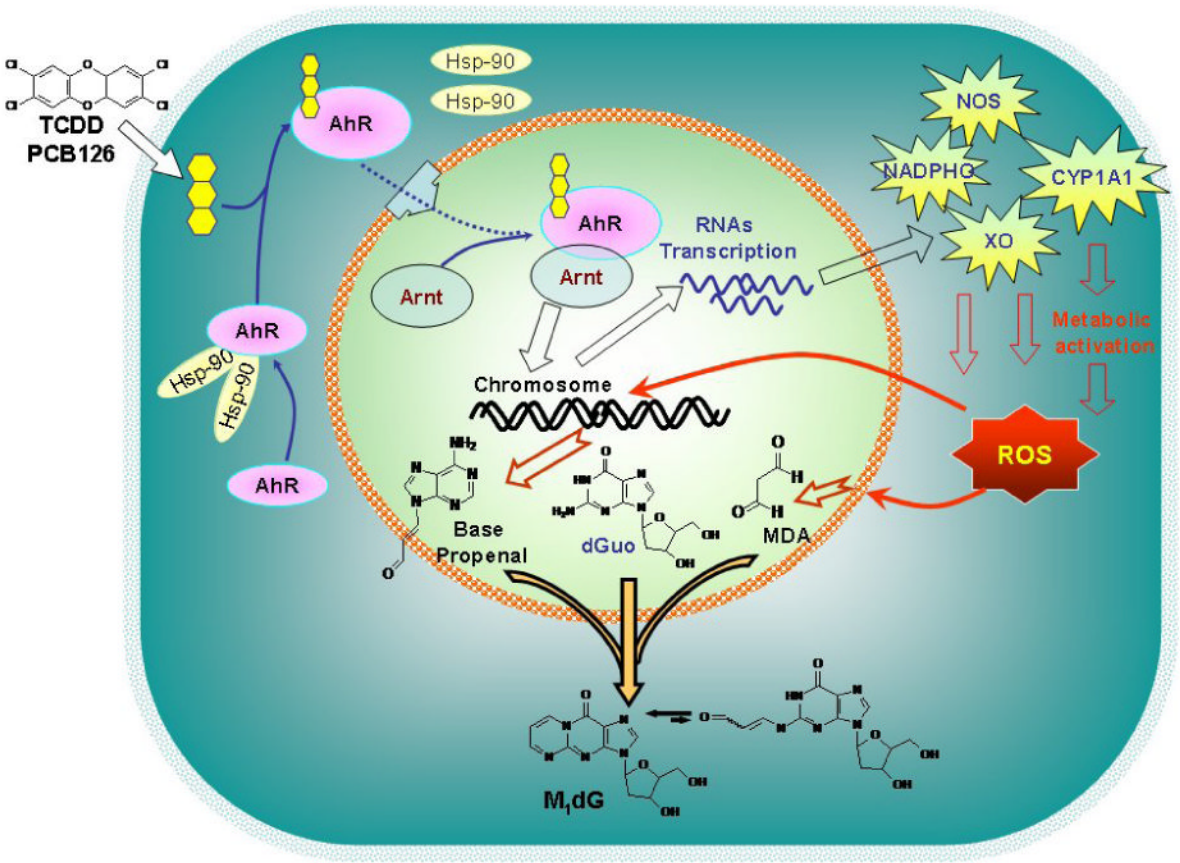

Fig. 2. Pathways leading to production of ROS from DLC exposure, and to formation of M_1dG DNA adducts from ROS attack on lipid membranes and on DNA backbone

AhR: aryl hydrocarbon receptor, HSP-90: heat-shock protein -90, Arnt: aryl hydrocarbon receptor nuclear translocator, NADPHO: nicotinamide adenosine dinucleotide phosphate oxidase, NOS: nitric oxide synthase, XO: xanthine oxidase, MDA: malondialdehyde

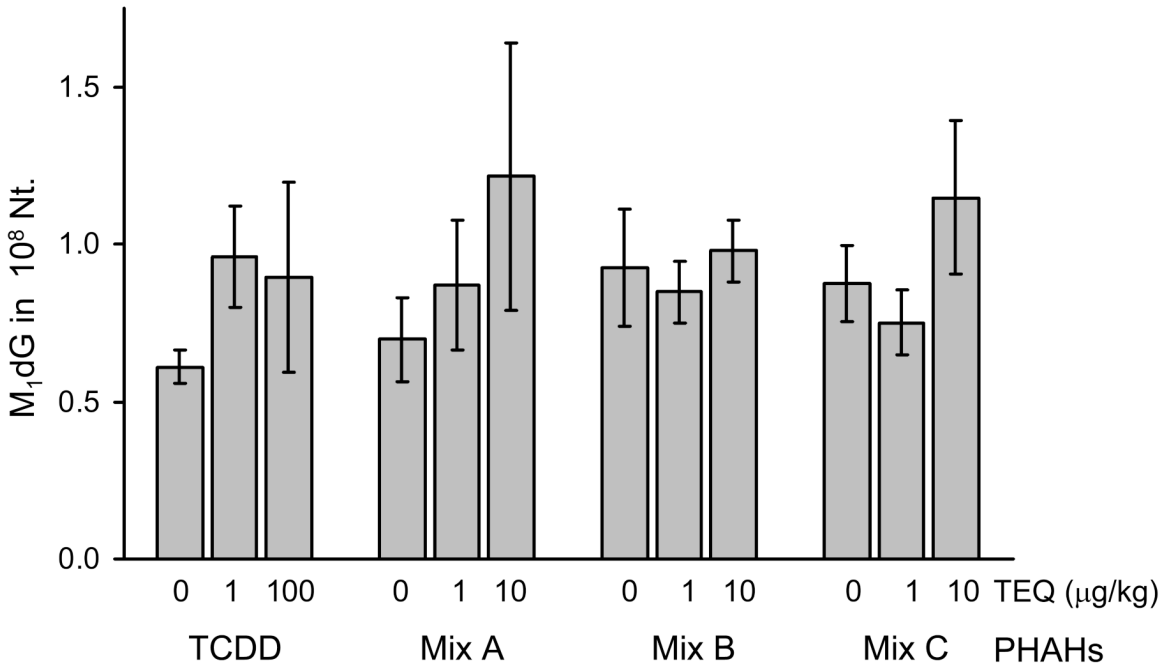

Fig. 3. Effect of single dose of TCDD or PHAH mixture on the background level of hepatic M_1dG adducts

Female C57BL/6J mice received a single treatment with corn oil containing either TCDD or one of the PHAH mixtures; Mix A(TCDD, PeCDD, 4-PCDF, PCB 126); Mix B(PCB 118, PCB 138, PCB 153, PCB 180); or Mix C(Mix A + Mix B). The ratios for the mixtures were selected based on the profile observed in foods. Liver tissues were collected a week after the treatment and subjected to DNA isolation. Each bar represents the mean + S.E. from 4-5 samples for each group.

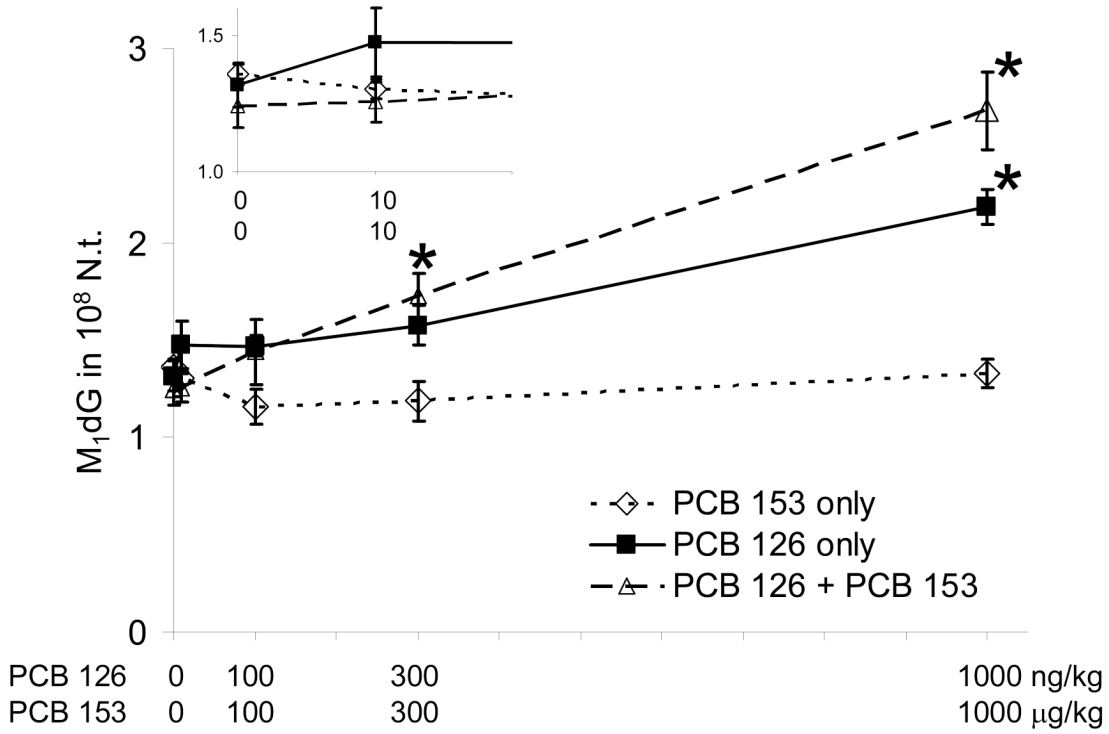

Fig. 4. Accumulation of M_1dG DNA adducts in rat liver tissues after chronic exposure to PCBs

Female Harlan Sprague-Dawley rats were treated with an aliquot of corn oil:acetone (99:1) containing PCB 153, PCB 126, or a mixture of PCB 126 and PCB 153 (1: 1000 mix, i.e., 100 ng/kg/d PCB 126 + 100 μg/kg/d PCB 153) by gavage 5 days per week for 53 weeks. DNA was isolated from liver tissue and processed for M_1dG analysis. B: The mixtures were prepared with a fixed amount of PCB 126 (300ng/kg/d) and with different amounts of PCB 153. Each point or bar represents the mean + S.E. from 7-8 samples for each group.

*: Statistical significance compared to corresponding control (Dunnett's test, $p < 0.05$)

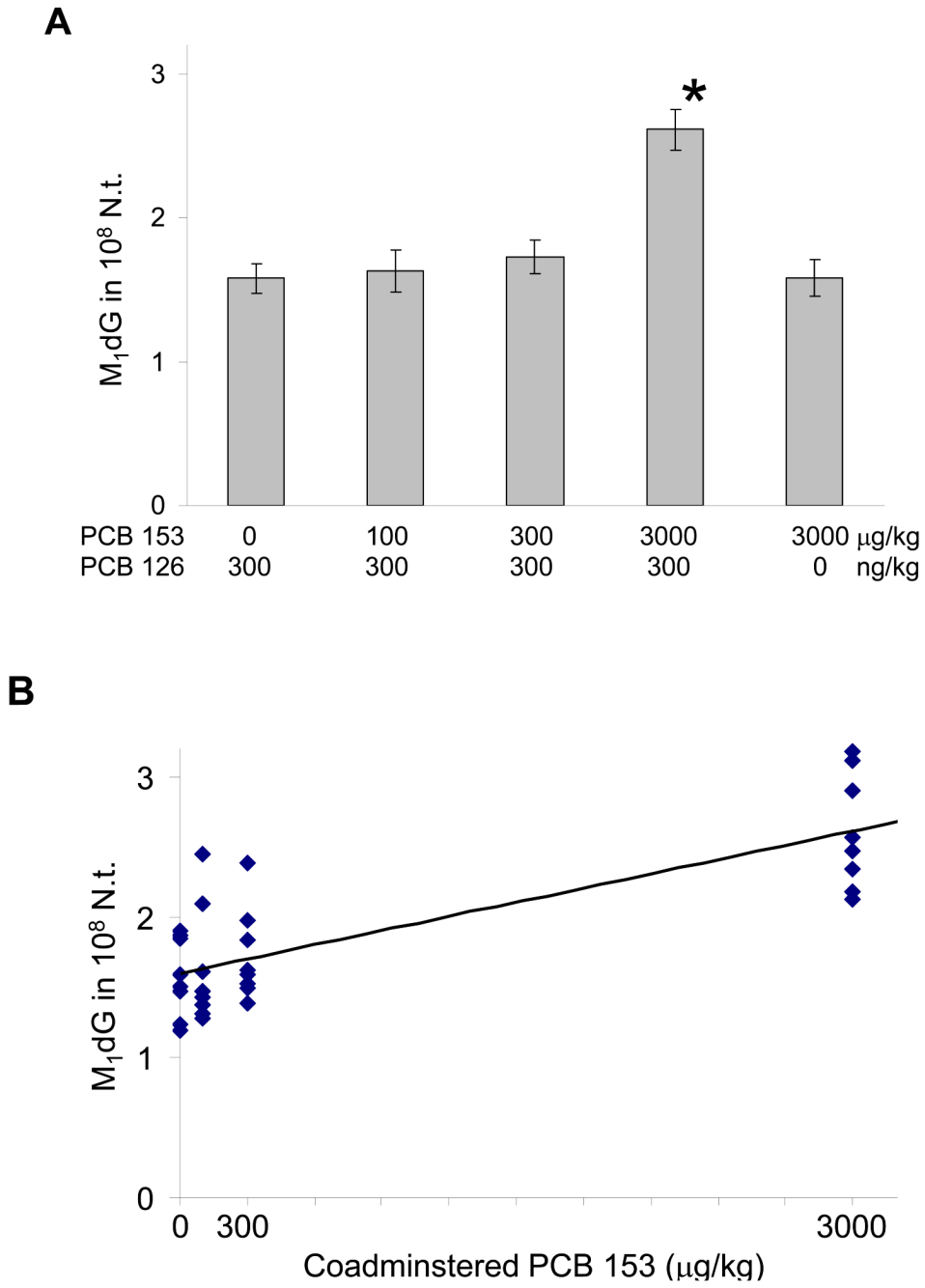

Fig. 5. Accumulation of M_1dG DNA adducts in rat liver tissues after chronic exposure to mixtures of PCB 126 and PCB 153

Female Harlan Sprague-Dawley rats were treated with an aliquot of corn oil:acetone (99:1) containing either 3000 μg/kg/d PCB 153 or a mixture consisting of fixed amounts of PCB 126 (300ng /kg/d) and different amounts of PCB 153 (0 – 3000 μg/kg/d). M_1dG adducts in liver DNA were analyzed with LC-MS/MS after selective labeling with tBHA (A). Each point or bar represents the mean + S.E. from 7-8 samples for each group. Correlation between the number of M_1dG adducts and the amount of PCB 153 coadminstered with 300 ng/kg/d PCB 126 was tested by linear regression (B).

*: Statistical significance compared to 300 ng/kg/d PCB 126 without PCB 153 (Dunnett's test, $p < 0.05$)

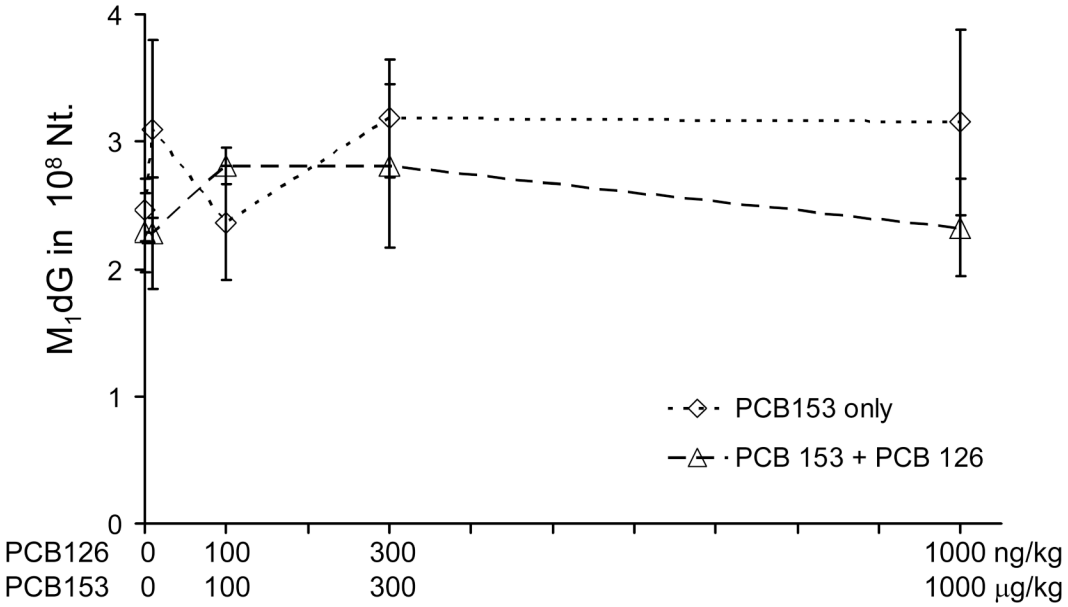

Fig. 6. Analyses of M_1dG DNA adducts in brain tissues after chronic exposure to PCBs

Female Harlan Sprague-Dawley rats were dosed with an aliquot of corn oil:acetone (99:1) containing PCB 153 with or without PCB 126 by gavage 5 days per week for 53 weeks. DNA was isolated from brain tissue of the animals and processed for M_1dG analysis. Each point represents the mean + S.E. from 4-5 samples for each group.

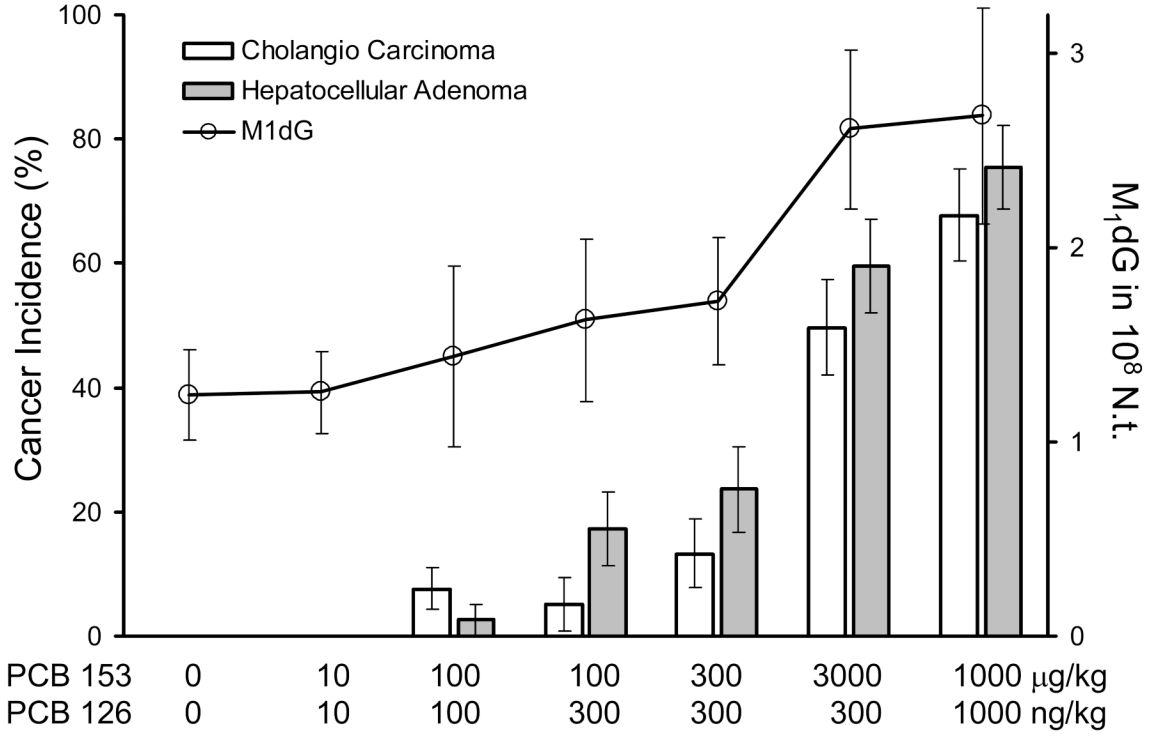

Fig. 7. Dose response for the formation of neoplastic lesions and for M_1dG adduct accumulation in female rats treated with mixtures consisting of PCB 126 and PCB 153

The incidence of neoplastic lesions is Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality [30]. Each point represents the mean + S.E.

Table 1

Composition of PHAH mixtures

Mixture	PHAH	TEF	Mass Ratio
Mix A	TCDD	1	1
	1,2,3,7,8-pentachlorodibenzo-p-dioxin (PeCDD)	1	1
	2,3,4,7,8-Pentachlorodibenzofuran (4-PCDF)	0.1	2
	3,3′,4,4′,5′-pentachlorobiphenyl (PCB 126)	0.1	45
	2,3′,4,4′,5′-pentachlorobiphenyl (PCB 118)	0.0001	10,000
Mix B	2,2′,3,4,4′,5′-hexachlorobiphenyl (PCB 138)	N/A	20,000
	2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB 153)	N/A	30,000
	2,2′,3,4,4′,5,5′-hexachlorobiphenyl (PCB 180)	N/A	20,000
Mix C	Mix A + Mix B		

Adapted from Burgin [28].