# DNA ADDUCT FORMATION BY CO-EXPOSURE OF TCDD AND BENZO[A]PYRENE IN BALB AND CBA MICE

Qing Wu[1, 2], Junko S. Suzuki[2], Hiroko Zaha[2], Tien-Min Lin[3], Richard E. Peterson[3], Chiharu Tohyama[4, 2] and Seiichiroh Ohsako[4, 2]

[1]School of Public Health, Fudan University, 130 Dongan Road, Shanghai 200032, China; [2]Research Center for Environmental Risk, National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba 305-8506, Japan; [3]School of Pharmacy and Molecular and Environmental Toxicology Center, University of Wisconsin, Madison, Wisconsin, USA; [4]Division of Environmental Health Sciences, Center for Disease Biology and Integrative Medicine, Graduate School of Medicine, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

## Introduction

Halogenated and polycyclic aromatic hydrocarbons, like 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and benzo[a]pyrene (BaP), are ubiquitous environmental pollutants that cause a broad spectrum of toxicity in mammals, including carcinogenesis, teratogenesis, and immune dysfunction[1]. Most toxic effects of TCDD and BaP are mediated by the aryl hydrocarbon receptor (AhR). Activation of the AhR in the liver can result in the detoxification or activation of a parent xenobiotic by phase I enzymes such as cytochrome P450 1A1 (CYP1A1). AhR-mediated CYP family gene activation is considered to be one of the causal factors of TCDD and BaP induced oncogenesis [2]. Induction of cytochrome P450 is involved in the activation of BaP to the ultimate carcinogens, which may subsequently modify DNA covalently to form DNA adducts [3]. It is considered that ligand-binding affinity of the AhR is the main cause for susceptibility to carcinogens such as TCDD and BaP. However, in our previous study[4], it was found that TCDD induced higher glutathione-S-transferase (GST) mu family gene expression in BALB/cAnNCrj (BALB) than in CBA/JNCrj (CBA) mice, although both two strains have identical AhR. It is unclear yet whether the different induction may result in different toxic endpoint. Since BaP is detoxified by both phase I and phase II enzymes. Therefore, in the present study, we co-exposed TCDD and BaP to compare the DNA adduct formation between co-exposure of TCDD and BaP and exposure of BaP alone. The goal of present study is to determine the effect of TCDD treatment on BaP-induced DNA-adduct formation in the liver of female mice of BALB and CBA that have the $AhR^{b2}$ genotype.

## Materials and Methods

### Mice

Female BALB and CBA strain mice were purchased from Charles River Japan, Inc. (Tokyo, Japan). The mice were provided food and water ad libitum and maintained in a controlled environment at a temperature of 24±1ºC, a humidity of 45±5%, and a 12-h light/12-h dark cycle, and given free access to a solid diet (certified diet MF: Oriental Yeast Co., Tokyo, Japan) and distilled water. Animals were treated in a humane manner according to the National Institute for Environmental Studies' guidelines for animal experiments.

### Exposure to TCDD and BaP

TCDD (>99.5% pure, 50 μg/ml in n-nonane) was purchased from Cambridge Isotope Laboratory (Andover,

MA) and BaP was purchased from Wako Pure Chemicals (Osaka, Japan). Three females per group were given a single oral dose of TCDD (0 or 40 μg/kg), followed 24 hr later by an intraperitoneal injection of BaP (50 or 200 mg/kg). Mice were killed by cervical dislocation 24 hr after BaP administration, and livers were collected and kept at -80°C for determination of DNA adducts.

**$32^P$-Postlabeling**

BaP DNA adduct formation was determined by the $32^P$-postlabeling method [5]. In brief, DNA was extracted from liver samples by using a Wizard genome DNA isolation kit (Promega, Madison, WI). The DNA sample (8 μg) was digested with micrococcal endonuclease and spleen phosphodiesterase (Sigma-Aldrich) at 37°C for 3 hr, followed by a further digestion with nuclease P1 at 37°C for 45 min. Tris-base was added to stop the reaction. The adduct nucleotides were labeled with [$\gamma$-$32^P$] ATP using T4 polynucleoside kinase at 37°C for 30 min. Potato apyrase (Sigma-Aldrich) was added and the solution was cultured at 37°C for 30 min to destroy excess ATP. After collecting a 1 μl aliquot of the $32^P$-labeled solution for the determination of total nucleotides and ATP, the remaining $32^P$ labeled sample was applied to thin-layer chromatography using a solvent system of 1M sodium phosphate, pH6.5 for developing direction 1; 3.5M lithium formate, 8.5M urea, pH3.5 for direction 2; 0.8M LiCl, 0.5M Tris-HCl, 8.5M urea, pH 8.0 for direction 3, 1.7M sodium phosphate, pH 6.0 also for direction 3, on polyethylenimine (PEI) cellulose sheets (EM Science, Gibbstown, NJ). Autoradiography was used to detect DNA adducts, and their amounts were estimated as relative adduct level (RAL) = intensity of adduct nucleotides / (intensity of total nucleotides x dilution factor).

**Statistical analysis**

StatView software for Windows version 5.0 (SAS Institute, Cary, NC) was used for statistical analyses. Relative DNA-adduct levels were compared by one-way analysis of variance (ANOVA), followed by the Fisher PLSD test as a post-hoc test and Student's t-test. Significance was set at $p < 0.05$.

**Results and Discussion**

Direct sequence analyses demonstrated that both mouse strains BALB and CBA had an identical nucleotide sequence in the entire open reading frame of AhR gene. The BALB and CBA mice were pretreated with TCDD (0 or 40 μg/kg). BaP (50 or 200 mg/kg) was administrated 24 hr later after TCDD exposure. The BaP-DNA adduct formation level of liver was measured by the $32^P$-postlabeling method. Administration of TCDD alone failed to induce DNA adduct formation in both BALB and CBA strain mouse livers. Without exposure to TCDD, BaP alone (50 mg/kg) produced DNA adduct in the liver of both strains to a similar extent. However, when TCDD was preadministered to the mice, the amount of DNA adducts formed by BaP 50 mg/kg were decreased to 20% and 36% of BaP treatment alone for both the BALB and CBA strains, respectively, and there was a significantly larger decrease for the BALB strain. On the other hand, when the BaP dose was increased to 200 mg/kg and given 24 hr after TCDD exposure, DNA adduct formation was increased approximately 2.3 and 2.0-fold over that observed in mice treated with the lower dose of BaP (50 mg/kg). There was no significant difference between the two strains (Fig.1).

BaP is a substrate for CYP1A1 and CYP1B1, and is metabolized by these phase I enzymes to form reactive

intermediates that bind covalently to nucleic acids and proteins. BaP is detoxified by both phase I and phase II enzymes. BaP-DNA adduct formation depends on the balance between the activity of epoxidation and the activity of GST conjugation, which may be associated with CYP1A1 induction and GST family genes induction. In our previous study [6], we used microarray to determine the gene profile of BALB and CBA after TCDD exposure. It was found that several genes including GST mu class gene increased higher in BALB than in CBA, while phase I enzymes such as CYP1A1, CYP1A2 and CYP1B1, were up-regulated by TCDD in both strains to similar level. Therefore, the different formation of DNA-adduct in BALB and CBA may be due to different induction of GST family in BALB and CBA. GST family comprises two distinct superfamilies. The larger superfamily consists of cytosolic enzymes that are principally involved in biotransformation of toxic xenobiotics while the other family is composed of microsomal enzymes primarily involved in arachdonic acid metabolism [6]. The GSTmu (GSTm)-class, is belonging to the former superfamily. It has been reported that GST mu (GSTm) and pi (GSTp) are efficient in the conjugation of intermediate metabolites of polycyclic aromatic hydrocarbons (PAHs), including BaP diolepoxide [7-9]. Since GSTm genes were more markedly induced by TCDD in the liver of female mice in the BALB strain than in CBA strains, the strain difference in formation of BaP-DNA adduct may be due to differential induction of GST family genes by TCDD. However, the reduced formation of BaP-DNA adducts was only observed at the lowest dose of BaP tested (50 mg/kg) following TCDD exposure. At the higher BaP dose (200 mg/kg) following TCDD exposure there was not any significant strain difference in BaP-DNA adduct formation. The lack of a strain difference in response to TCDD treatment may have occurred because of the overwhelming high amount of BaP-induced DNA adduct formation in both mouse strains given the higher dose of BaP. Interestingly, in the present study, administration of TCDD followed 24 hr later by BaP (50 mg/kg) produced significantly less DNA adducts than after treatment with BaP alone. Although TCDD acts as a cancer promoter, Holcomb and Safe [10] reported that TCDD inhibited 7,12-dimethylbenz[a]anthracene-induced tumorigenesis possibly by effects of TCDD exerted during the initial exposure period to 7,12-dimethylbenz[a]anthracene.

In summary, the present study shows that co-exposure of TCDD and BaP induces different DNA adduct formations comparing with exposure of BaP alone. The DNA adducts formation by co-exposure of TCDD and BaP is associated with dose of BaP, and may be associated with GST family induction. The present results suggest that expression of GST family genes, in particular GSTmu-family, may reflect a distinct susceptibility to carcinogenicity by polycyclic aromatic hydrocarbons.

**Acknowledgements**

This work was supported in part by grants from the Environmental Technology Development Fund (S.O.) and Environmental Risk Office Fund (C.T.) of the Ministry of the Environment, Japan, and was supported in part by grants from the National Natural Science Foundation of China to Q. Wu (30671728 ).

**References:**

1. Schecter A., Birnbaum L., Ryan, J.J., Constable J.D. *Environ Res* 2006; 101: 419-428.
2. Shimizu Y., Nakatsuru Y., Ichinose M., Takahashi Y., Kume H., Mimura J., Fujii-Kuriyama Y., and Ishikawa T. *Proc Natl Acad Sci U S A* 2000; 97: 779-782.

3. Sims P., Grover PL., Swaisland A., Pal K., and Hewer A. *Nature* 1974; 252: 326-328.
4. Wu Q., Suzuki J.S., Tohyama C., Lin T.M., Peterson R.E., Ohsako S. *Organohalogen Comp* 2004; 66: Tox5-Recent Advance in TCDD Toxicology：7.
5. Gupta RC., Reddy MV., Randerath K. *Carcinogenesis* 1982; 3: 1081-1092.
6. Hayes, J.D., Pulford, D.J. *Crit. Rev. Biochem. Mol. Biol.* 1995; 30: 445-600.
7. Robertson IG., Guthenberg C., Mannervik B., Jernstrom B. *Cancer Res* 1986; 46: 2220-2224.
8. Sundberg K., Widersten M., Seidel A., Mannervik B., Jernstrom B. *Chem Res Toxicol* 1997; 10: 1221-1227.
9. Parkinson A. 2001. Biotransformation of xenobiotics. In: Klaassen, C.D. (Eds.), *Casarett and Doull's Toxicology: The Basic Science of Poisons.* McGraw-Hill, pp. 215-218.
10. Holcomb M., Safe S. *Cancer Lett.* 1994; 82: 43-47.

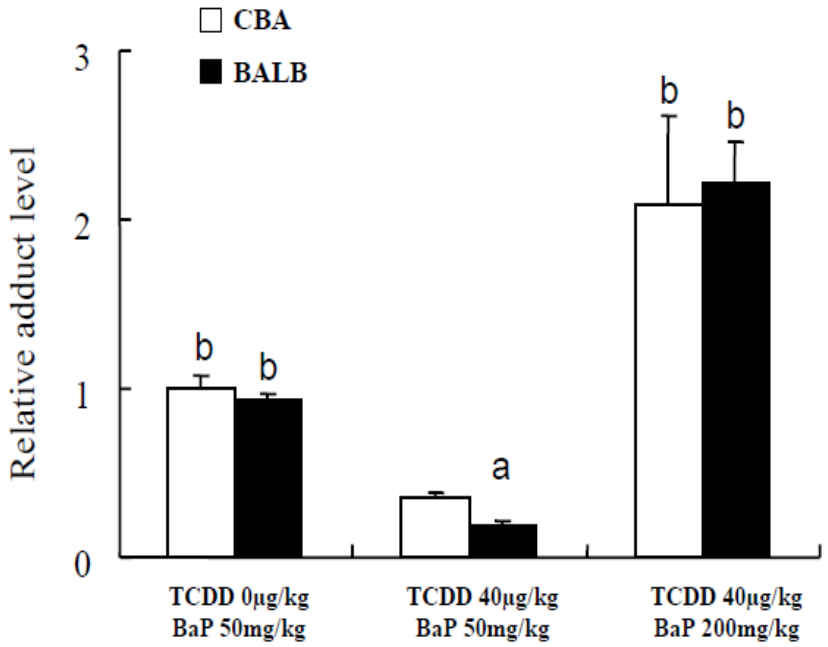


Fig.1 DNA adduct formation. a: $p<0.05$,compare with CBA group; b: $p < 0.05$, compared with the TCDD(40 μg/kg) and BaP (50 mg/kg) group.