## Letter to the Editor

# Trichloroethylene Induces Biphasic Concentration-dependent Changes in Cell Proliferation and the Expression of SET-Associated Proteins in Human Hepatic L-02 Cells*

HONG Wen Xu[1,^], YE Jin Bo[1,2,^], CHEN Mou Tong[1,^], YAN Yan[3], ZHOU Gui Feng[2], YANG Xi Fei[1], YANG Liang[1], REN Xiao Hu[1], HUANG Hai Yan[1], ZHOU Li[1], HUANG Xin Feng[1], ZHUANG Zhi Xiong[1], and LIU Jian Jun[1,#]

Trichloroethylene (TCE) is a major pollutant that affects both occupational and general environments[1]. The liver is an important target organ of TCE[1]. Substantial efforts and remarkable progress into understanding TCE cytotoxicity have been made in cultured liver cells. However, the molecular mechanisms by which TCE induces hepatotoxicity are not well understood. SET (also known as protein phosphatase 2A inhibitor, I2PP2A, or template-activating factor-I, TAF-I) is a nuclear protein that regulates histone modification, gene transcription, DNA replication, nucleosome assembly, phosphatase activity and kinase activity[2]. Previously, we observed a significant TCE-induced up-regulation of SET in human hepatic L-02 cells[3]. We further reported that the knockdown of SET significantly attenuated TCE-induced cytotoxicity, rescued the inhibition of PP2A activity, and suppressed the activation of caspase-3 mediated by TCE treatment in L-02 cells. Recently, we identified 42 novel Gene Ontology (GO) co-annotated SET-binding proteins and revealed the effects of TCE on the distribution and interactions of SET and its binding partners, eEF1A1 and eEF1A2, in L-02 cells[4]. Herein, we describe a biphasic concentration-response in both cell proliferation and the expression of SET-associated proteins to TCE treatment of L-02 cells. Our results suggest that the TCE-induced alterations in the expression of SET and its binding partners may be involved in the biphasic effect induced by TCE and partially constitute the mechanisms leading to TCE hepatotoxicity.

In our previous study, we investigated the concentration-response in the viability of L-02 cells to treatment with TCE for 24 h and determined an $IC_{50}$ value of approximately 16 mmol/L[4]. We therefore chose the $IC_{25}$ value (8 mmol/L) as the maximum concentration for studying cell proliferation and protein expression. Briefly, L-02 cells were cultured in medium containing various concentrations of TCE (0.031, 0.063, 0.125, 0.25, 0.50, 1.0, 2.0, 4.0, 8.0 mmol/L) without serum for 12, 24, and 48 h. The control group was treated with the solvent (DMSO, 0.5% v/v) only. A 10-μL aliquot of the Counting Kit-8 (CCK-8) solution was added to each well of the plate. The absorbance was corrected with cell-free controls. At least five replicate experiments were performed. The effect of TCE treatment on the levels of SET, eEF1A1, eEF1A2, CAPZB, and DDB1 was analyzed by western blot analysis. After treatment with TCE (0, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0 mmol/L) for 24 h, whole cell lysates were collected and probed with specific antibodies against each protein. The bands were visualized by incubation with a chemiluminescent substrate. At least three replicate experiments were performed. The data were analyzed using the statistical program, SigmaStat® (Jandel Scientific, Chicago, IL, USA). To compare the differences of two means, Student's *t*-test was used. To compare the differences of three means or more, one-way ANOVA followed by a Student-Newman-Keuls post-hoc test was used. The level of significance was set as $P<0.05$.

The protein interaction network was established using Osprey Network System Version 1.2.0 (Human GRID), a data integrating visual framework for biological networks and modules[5]. Five proteins in this study (SET, eEF1A1, eEF1A2, CAPZB, and DDB1) were chosen as origin nodes. P53 and c-myc were specifically included to analyze potential interactions with these two important molecules related to tumorigenesis. The node colors indicate the protein functions. The localized PICASSO (protein interaction

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1.Key Laboratory of Modern Toxicology of Shenzhen, Medical Key Laboratory of Guangdong Province, Medical Key Laboratory of Health Toxicology of Shenzhen, Shenzhen Center for Disease Control and Prevention, Shenzhen 518055, Guangdong, China; 2.Medical College, Hunan Normal University, Changsha 410013, Hunan, China; 3.Shenzhen Nanshan Center for Disease Control and Prevention, Shenzhen 518054, Guang Dong, China

confidence assessment system with multiple sources) program (http:/61.50.138.118//PICASSO/) was used to analyze the Gene Ontology (GO) Coannotation of CAPZB, DDB1, eEF1A1, and eEF1A2 with the SET gene[6].

In the present study, the proliferation of L-02 cells exposed to TCE exhibited a biphasic concentration-dependent response. Figure 1 shows the concentration-responses in cell proliferation mediated by TCE treatment for 12, 24, and 48 h, as determined by the CCK-8 assay. The plots illustrate that the rates of cell proliferation increased at low TCE concentrations

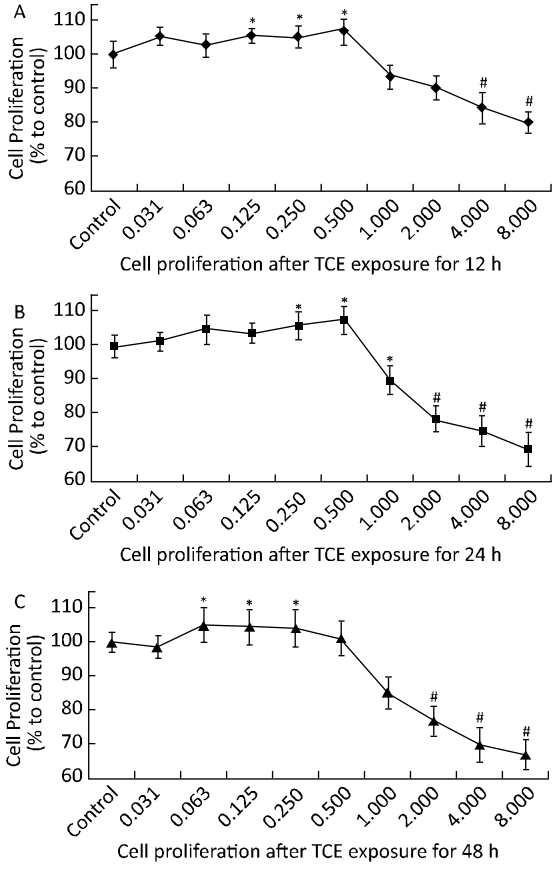


**Figure 1.** Proliferation of L-02 cells following treatment with TCE for 12, 24, and 48 h. L-02 cells were treated with various concentrations of TCE for 12 h (A), 24 h (B), and 48 h (C). The highest rates of cell proliferation were 106%, 108%, and 105% following TCE exposure for 12 h (at 0.5 mmol/L), 24 h (at 0.5 mmol/L), and 48 h (at 0.063 mmol/L), respectively. The data represent the means±SEM of at least five independent experiments. *$P$<0.05 compared with the control cells, #$P$<0.01 compared with the control cells.

(from 0.031 mmol/L to 0.5 mmol/L) upon treatment of cells for 12, 24, and 48 h. The highest rates of cell proliferation were 106%, 108% and 105% at 12, 24, and 48 h, respectively. The rates of cell proliferation decreased at high concentrations of TCE (from 1.0 to 8.0 mmol/L). The TCE concentration-response curves exhibited a typical inverted U-shape. By comparing cell proliferation rates at the different exposure times, we chose a 24-h exposure to TCE (which gave the highest rate of 108%) for further analysis of the effects of TCE on the levels of SET-associated proteins.

We observed a similar concentration-dependent increase in the expression levels of SET-associated proteins, including two isoforms of SET, eEF1A1, eEF1A2, and CAPZB, in TCE-treated L-02 cells, while the level of DDB1 decreased. We also observed that TCE exposure significantly altered the levels of the mRNAs encoding these proteins (data not shown). Figure 2 shows the changes in the levels of SET, eEF1A1, eEF1A2, CAPZB, and DDB1 proteins following treatment with various concentrations of TCE (0, 0.25, 0.5, 1.0, 2.0, 4.0, and 8.0 mmol/L) for 24 h, as determined by western blot analysis. The levels of two isoforms of SET (TAF-Iα and TAF-Iβ), two isoforms of eEF1A (eEF1A1 and eEF1A2) and CAPZB dramatically decreased at low TCE concentrations (0.25 and 0.5 mmol/L), but markedly increased at high TCE concentrations (2.0, 4.0, 8.0 mmol/L). Conversely, an inverted U-shape concentration-response curve was observed with respect to the level of DDB1.

SET has two isoforms: a longer isoform, TAF-Iα, and a shorter isoform, TAF-Iβ. It is thought that the expression levels of TAF-Iα and TAF-Iβ regulate the chromatin-related activity of SET[2]. The PICASSO scores, a bioinformatics index to evaluate the biological relevance between two proteins[6], for CAPZB, DDB1, eEF1A1, and eEF1A2 with SET were 106.6, 8.8, 4.6, and 4.6, respectively. A true interaction is predicted if the PICASSO score is higher than 2.0[6]. CAPZB, a member of the F-actin capping protein family[7], exhibited a PICASSO score of more than 100. CAPZB has been demonstrated to be a key coordinator of the assembly of F-actin and microtubules, the two major cytoskeletal components that determine the morphology and dynamics of growth cones. Eukaryotic translation elongation factor 1 alpha (eEF1A1) and eEF1A2 are two SET-binding proteins identified in our previous study[4], for which the PICASSO score with SET of both was 4.6. The ability of both eEF1A1 and eEF1A2 to modulate apoptosis and cell growth is strictly linked

with cancer development. Damage-specific DNA binding protein 1 (DDB1) functions in nucleotide-excision repair, and its PICASSO score with SET is 8.8. Deletion of DDB1 has been demonstrated to induce liver regeneration and tumorigenesis[8]. The common biological approach to achieve a biphasic dose response has been to use two receptor subtypes that bind to the same agonist, one leading to a stimulatory pathway and the other to an inhibitory pathway. In general, the agonist may bind one receptor subtype with greater efficiency than the other receptor, resulting in the activation of its pathway at low doses. If the receptor with lower binding affinity has greater binding capacity, it would become dominant at higher agonist doses and induce an inhibitory response[9]. Our data in the present study suggest that SET and its binding partners may be involved in the biphasic effect induced by TCE.

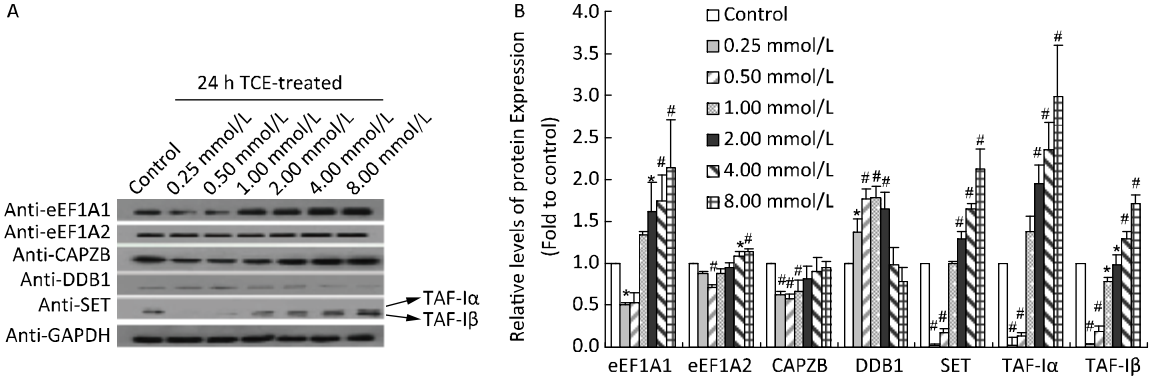


**Figure 2.** Levels of SET (TAF-Iα and TAF-Iβ), eEF1A1, eEF1A2, CAPZB, and DDB1 proteins following treatment of L-02 cells with TCE for 24 h. (A) The level of each protein in L-02 cells treated with TCE was determined by western blotting using specific antibodies. GAPDH was included as an internal control. (B) The expression levels were normalized against GAPDH and the fold differences were compared with controls. *$P$<0.05 compared with the control cells, #$P$<0.01 compared with the control cells.

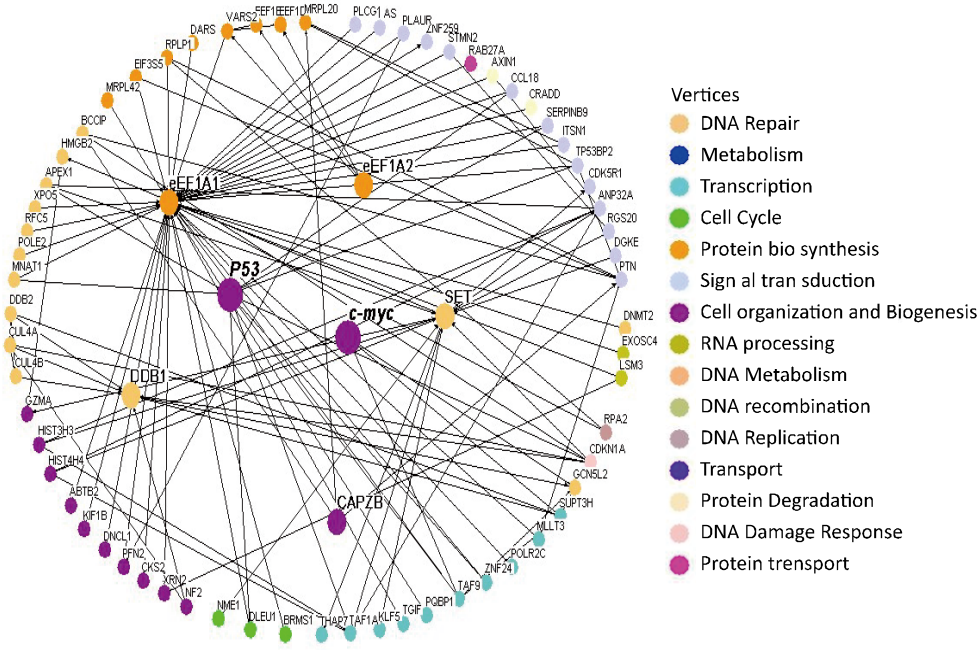


**Figure 3.** A protein-protein interaction network generated by Osprey software. Five proteins (SET, eEF1A1, eEF1A2, DDB1, CAPZB) were chosen as origin nodes. The database used was Human GRID and interactions were chosen for all five origin nodes. P53 and c-myc were specifically included to analyze their potential interactions with the origin nodes. The node colors indicated the protein functions.

Osprey software not only provides network drawing capabilities, but also supports the creation, visualization and analysis of mixed networks, i.e., networks containing both directed and undirected protein-protein interactions[5]. In the present study, we generated a protein-protein interaction network using Osprey software. As shown in Figure 3, SET and its binding partners can directly interact with proteins involved in various biological functions. The functions of most of these proteins can be classified into signal transduction, cell organization, transcription and protein biosynthesis. Therefore, the TCE-induced alterations in SET, eEF1A1, eEF1A2, DDB1, and CAPZB expression may affect many essential biological processes to induce hepatotoxicity. Furthermore, the tumor suppressor protein, p53, and the transcription factor, c-Myc[10], can interact with eEF1As and SET indirectly. p53 regulates the cell cycle and functions as a tumor suppressor to prevent cancer cell proliferation. c-Myc functions to regulate global chromatin structure and it is assumed to regulate the expression of 15% of all genes. The indirect interaction of p53 and c-Myc with SET-associated proteins provides new insights into the relationship between TCE exposure and tumorigenesis.

In summary, our study indicates that TCE induces cell proliferation in L-02 cells in a biphasic concentration-dependent manner. SET-associated proteins may play an important role in this TCE-induced biphasic response. Our findings in this study will be useful for further understanding the molecular mechanisms of TCE-induced hepatotoxicity and tumorigenesis.

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[#]Correspondence should be addressed to LIU Jian Jun. Tel: 86-755-25508584; Fax: 86-755- 25508584; E-mail: bio-research@hotmail.com

[^]These authors contributed equally to this work.

Biographical note of the first authors: HONG Wen Xu, male, born in 1997, Ph.D/Associate Professor, Toxicoproteomics; YE Jin Bo, male, 1987, Master, Epidemiology and health statistics; CHEN Mou Tong, male, 1984, Ph.D candidate, Molecular toxicology.

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## REFERENCES

1. Lash LH, Fisher JW, Lipscomb JC, et al. Metabolism of trichloroethylene. Environ. Health Perspect, 2000; 108, 177-200.
2. Asaka MN, Murano K, and Nagata K. Sp1-meidated transcription regulation of TAF-Iα gene encode a histone chaperone. Biochem Biophys Res Commun, 2008; 376, 665-70.
3. Liu J, Huang H, Xing X, et al. Comparative proteomic analysis on human L-02 liver cells treated with varying concentrations of trichloroethylene. Toxicol Ind Health, 2007; 23, 91-101.
4. Hong WH, Yang L, Chen M, et al. Proteomic analysis of trichloroethylene-induced alterations in expression, distribution, and interactions of SET/TAF-Iα and two SET/TAF-Iα-Binding proteins, eEF1A1 and eEF1A2, in Hepatic L-02 cells. Toxicol Appl Pharm, 2012; 263,259-72.
5. Breitkreutz BJ, Stark C, Tyers M. Osprey: a network visualization system. Genome Biol, 2003; 4, R22.
6. Li D, Liu W, Liu Z, et al. PRINCESS, a protein interaction confidence evaluation system with multiple data sources. Mol Cell Proteomics, 2008; 7, 1043-52.
7. Yamashita A, Maeda K, Maéda Y. Crystal structure of CapZ: structural basis for actin filament barbed end capping. EMBO J, 2003; 22, 1529-38.
8. Yamaji S, Zhang M, Zhang J, et al. Hepatocyte-specific deletion of DDB1 induces liver regeneration and tumorigenesis. Proc Natl Acad Sci USA, 2010; 107, 22237-42.
9. Calabrese EJ. Hormesis: Why it is important to toxicology and toxicologists. Environ Toxicol Chemistry, 2008; 27, 1451-74.
10. Croce CM. Oncogenes and cancer. N Engl J Med, 2008; 358, 502-11.