Synergistic effect of trichloroethylene and Cu(II) on DNA-adduct 8-hydroxy deoxyguanosine (8-OHdG) formation as a biomarker of cancer risk

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Abstract: Identification of the synergistic effect of trichloroethylene and Cu (II) in the formation of DNA adduct 8-hydroxy deoxyguanosine (8-OHdG) as a cancer biomarker were carried out. TCE and CuCl₂ were incubated with deoxyguanosine with variations in temperature (37ºC and 60ºC) and pH (7.4 and 8.4) for 6 hours. The 8-OHdG levels formed were analyzed using high performance liquid chromatography (HPLC). The formation of 8-OHdG from exposure to TCE seems to require high concentrations, because of its difficulty to oxidize. The addition of Cu (II) was shown to have a synergistic effect with TCE in the formation of DNA adducts which increased up to 65.8%. Temperature did not have a significant effect on DNA adduct formation. Furthermore, it is found that alkaline pH produces more DNA adducts than neutral pH.

1. Introduction
Trichloroethylene (TCE) is a volatile organic solvent that is widely used as a fat removal agent, polymer precursor, and cleaning agent in the dry cleaning process. Chlorinated ethylenes were known to produce toxic effects and induce lung, liver, and kidney tumors in mice [1]. TCE is rapidly absorbed by environmental exposure routes, especially oral consumption and inhalation [2]. TCE is distributed throughout the body, where it can accumulate in fat and other tissues [3].

TCE is known to form DNA adducts through the biotransformation process. TCE metabolism can occur in two different mechanisms, oxidation by Cytochrome P-450 [4] or conjugation with glutathione [5]. Both of these pathways are known to produce DNA adducts and trigger the formation of cancer.

8-hydroxy-2’-deoxyguanosine (8-OHdG) is a DNA adduct that is found in much xenobiotic exposures [6]. The formation of 8-OHdG is triggered by the presence of oxidative stress, which is a condition of excessive concentration of reactive oxygen species (ROS) in cells. The xenobiotic oxidation process is known to produce ROS through the fenton- reaction. Cu(II) is known to influence this process [7]. The presence of 8-OHdG in urine or blood indicates the formation of DNA adducts that can lead to the formation of cancer [8]. Analysis method of 8-OHdG has been carried out through 32P post-labeling [9], enzyme-linked immunoabsorbent assay (ELISA) [10], gas chromatography-mass spectrometry (GC-MS) [11], and High-Performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) [12].

TCE has been sown to produce 8-OHdG and trigger oxidative DNA damage when exposed to HepG2 cells [13]. However, existing studies are limited to a single exposure to TCE. In this study, studies on DNA adducts formation from TCE and Cu (II) had been carried out. The results of this study are expected to provide a further understanding of the formation of DNA adducts and can be used in the risk assessment of TCE.

2. Materials and Method
2.1. Materials
Trichlorethylene (TCE), 2-deoxyguanosine monophosphate, CuCl$_2$ were purchased from sigma-aldrich. Reagents for incubation were all pro analyst grade. Methanol (HPLC grade) was used as eluent in analysis.

2.2. In vitro Study
Deoxyguanosine monophosphate (dG) was diluted to phosphate buffer (pH 7.4 and pH 8.4). Two incubation solutions were prepared. The First mixture was 100ul dG (25 ppm), 100ul TCE (200 ppm), and 100ul CuCl$_2$ (25 ppm). The second mixture was 100ul dG (25 ppm) and 100ul CuCl$_2$ (25 ppm). dG was also incubated as control. aquabidest was then added to all mixture until volume reach 500ul. all mixtures were then incubated for 6 hours. After both results were obtained, a temperature variation of 37°C and 60°C was applied to the pH resulting in higher 8-OHdG concentration.

2.3. DNA adduct analysis using HPLC
Analysis of 8-OHdG was performed by injecting an incubation solution of 50 µL into High-performance Liquid Chromatography (HPLC). The column was C-18 reverse phase. The flow rate was 1.5 ml/s with The mobile phase was phosphate buffer pH 6.7 and methanol (85:15). External standards were applied to quantify the concentration of 8-OHdG. Analysis of Standard linearity, Limit of Detection (LoD), and Limit of Quantification (LoQ) was performed.

3. Results and Discussion

3.1. Analysis DNA adduct 8-OHdG using HPLC
Determination of 8-OHdG retention time was done by injecting 50 µL of standard solution into the HPLC instrument. dG was also injected into the HPLC instrument to determine its retention time. chromatogram peak of dG was shown at retention time of 4.8 minutes, while the standard 8-OHdG solution showed chromatogram peak 6.2 minutes after injection. Chromatograms of the two standard solutions are shown in Figure 1 and figure 2.

![Figure 1](image1.png)

Calibration curve was plotted from standard 8-OHdG solutions with various concentrations of 5, 10, 25, 50, and 100 ppb. The calibration curve equation was $y = 0.0166x + 0.0895$ with $R^2 = 0.999$. Furthermore, Limit of Detection (LoD) and Limit of Quantification (LoQ) were determined statistically from the standard curve. The LoD and LoQ were 2.75 ppb and 9.19 ppb, respectively.
3.2. In Vitro Study

3.2.1. DNA adduct formation from TCE exposure. TCE, deoxyguanosine, and Cu (II) were incubated and 8-OHdG levels were measured by HPLC. The control solution was dG which was incubated like the other mixtures. 8-OHdG peak was not shown in control solution. Incubation of Deoxyguanosine and TCE showed that 9.89 ppb (pH 7.4) and 11.5 ppb (pH 8.4) of 8-OHdG was formed after 6 hours incubation at 37°C. The concentration of 8-OHdG was very low, confirming that TCE concentration required to exhibit mutagenicity effects is much higher than other chemicals [14][15]. Studies of DNA damage in TCE metabolites such as chloral hydrate and dichloroacetate show that TCE needs oxidative metabolism to cause DNA damage. The low yield may be due to the structure of TCE which is difficult to oxidize.

3.2.2. DNA adduct formation from TCE exposure Mediated by Cu(II). As TCE needs oxidative metabolism to produce DNA damage, Metal ions are known to influence the TCE oxidation process in aqueous systems. In this study, 25 ppm of CuCl₂ was added to the incubation mixtures. All solutions were incubated for 6 hours and 8-OHdG was analyzed using HPLC.

Incubation of dG and CuCl₂ did not show 8-OHdG chromatogram peak. However, 8-OHdG concentrations from incubation of dG, TCE, and CuCl₂ were 14.57 ppb (pH 7.4) and 15.51 ppb (pH 8.4). Concentration of 8-OHdG formed was increased up to 65.8% by the addition of Cu(II). Comparison of 8-OHdG from TCE only and TCE mediated by Cu(II) is shown in figure 3.

![Figure 2. Chromatogram standard solution of 8-OHdG](image_url)

![Figure 3. Concentration of 8-OHdG formed from incubation of dG, TCE and Cu(II)](image_url)
Cu (II) acts as a mediator in the oxidation of TCE. This redox cycle of Cu(II)/Cu(I) involves transfer electrons which promote the oxidation of TCE. Cu(II) also has the potential to produce reactive oxygen species (ROS) like OH•, O2•− and H2O2 from Haber-Weiss or Fenton reaction [16].

3.2.3. Effect of pH and Temperature on DNA adduct Formation. The results showed that the 8-OHdG level was higher at a pH of 8.4 compared to 7.4 (figure 2). Although the degradation of TCE is lower in alkaline conditions and the most optimal at neutral pH [17], 8-OHdG is possible to be produced more in alkaline conditions due to increased OH• production [18]. This phenomenon is also related to the reduction of Cu. Reduction of Cu(II) to Cu(I) is known to occur more at higher pH [19]. TCE oxidation was also increased at incubation with Cu due to this redox process.

![Figure 4](image-url) Concentration of 8-OHdG formed from incubation at 37°C and 60°C

To see the effect of temperature on 8-OHdG formation, incubation was carried out at 60°C at pH 8.4. The result shown in figure 4. incubation of dG and TCE yielded 13.24 ppb of 8-OHdG, whereas incubation of dG, TCE, and CuCl2 yielded 15.37 ppb of 8-OHdG. No significant difference was seen from incubation at higher temperatures. The increase in temperature is known to increase the kinetic energy of the molecules so that the reaction products are greater. However, it seems like the volatile nature of TCE also has an influence. The TCE vapor which is formed due to heating will be more difficult to interact with dG in the solution phase.

4. Conclusion
TCE exposure to dG has been shown to increase radical species as indicated by the formation of DNA adduct 8-OHdG. This indicates that exposure to TCE in the human body has the potential to cause DNA changes that lead to the formation of cancer cells. A synergistic effect can be seen from the exposure to TCE coupled with Cu (II) metal to increase the formation of 8-OHdG. However, because free dG and dG in DNA are not the same, to ascertain the effect of TCE exposure in humans, additional studies are needed either using cells or in vivo experiments.

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