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Peer Review Report for "Toxicological Effects of Inhalation Exposure to Trichloroethylene on Serum Immunoglobulin and Electrolyte Levels in Rats"

Author(s): zahra zamanian, Saeed Yousefinejad, Mohammad Javad Khoshnood, F Golbabaii, Mohammad Zare, Aboutaleb Modaresi, Mohammad Reza Zarei, Abdolrasoul Rahmani

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Revision (0)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

Raheleh Hashemi Habybabady : Reviewer

4 Jul 2018

Dear Author:
The paper has a well-established theoretical framework and well-organized content. However, the following modifications are recommended:

In the abstract and method section, the total number of animals should be mentioned.

In the method section, further explanations are needed about the exposure chamber:

Since the system has been dynamically exposed, how much was the inflow and outlet flow of air and TCE? How the exhaust air was drawn from the chamber?

What measures were taken to ensure the uniformity of the pollutant concentration inside the chambers?

At which points of the chamber did you measure TCE concentrations?

How did you control the vapors resulting from the feces and urine of animals?

Did the animals have access to food and water during exposure?

Were animals in the control group also housed in the chamber during the test? Why?

In the discussion section, please list your study limitations.

In the references section, some references are not written according to journal instructions, such as refrences:3,5, 7, 9,22. The number of authors and the number of pages should be written in accordance with the

Nasser Keikha : Reviewer

8 Aug 2018

Dear Authors:
1. Correct and proper English writing.
2. Your conclusion section is not like a conclusion. It is mostly a kind of repeating results.
3. Plagiarism detection in the manuscript was found. As far as the reviewer could find, one sentence has apparently been copied from other sources.
4. All in all, minor revisions are needed.

Raheleh Hashemi Habybabady : Associate Editor

9 Aug 2018

Dear Author:
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In the discussion section, please list your study's limitations.

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Reviewer2:
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4. All in all, minor revisions are needed.

Ramazan Mirzaei : EIC

12 Aug 2018

Dear EIC:
The article has 33% similarity with other published articles.
There are some similarities in the text of this manuscript with other publications

The paper has a well-established theoretical framework and well-organized content. However, the following modifications are recommended:

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In the discussion section, please list your study limitations.

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OPEN PEER REVIEW

Revision (1)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

Abstract

Background: Trichloroethylene (TCE) is one of the most important environmental contaminants that can introduce both occupational and public health risks because of its widespread use.

Objectives: This study aimed to investigate the effects of inhalation exposure to TCE on serum immunoglobulin and electrolyte levels under controlled laboratory conditions.

Methods: Adult male Sprague–Dawley rats were randomly divided into five equal groups of 5 animals each. TCE vapors were generated by the dynamic evaporation method in the exposure chamber. The animals were exposed to TCE at concentrations of 0 (control), 10, 100, 250, and 400 ppm through inhalation for 8 h a day, for 10 consecutive days.

Results: Based on results, IgG, IgM, and IgA levels were significantly increased in rats following exposure to various levels of TCE≥100 ppm ($P<0.05$). Conversely, TCE exposure significantly caused a decrease in the levels of IgE in the case group, with those values compared to the control group ($P<0.05$). Contrariwise, serum levels of Ca were significantly increased in rats exposed to TCE levels above 250 ppm, the high and very high exposure levels ($P<0.05$). At a concentration of 100 and 400 ppm, changes were noted in serum P levels ($P<0.05$). A moderate positive relationship was found between serum P and the TCE concentration in sub-acute exposure ($R2 = 0.66$, $P<0.05$).

Conclusion: In conclusion, sub-acute inhalation exposure to TCE (≥ 100 PPM) is associated with electrolyte and immunoglobulin toxicity in the form of increased Ca, P, IgA, IgG, and IgM and decreased Cl, Na, and IgE.

Keywords: Trichloroethylene, Immunoglobulin, Electrolytes, Sub-acute exposure, Animal Model

List of Abbreviations

IgA: Immunoglobulin A

IgG: Immunoglobulin G

IgE: Immunoglobulin E

IgM: Immunoglobulin M

Ca: Calcium

P: phosphorus

Na: Sodium

K: Potassium

Mg: magnesium

Cl: Chlorine

1. Introduction

Trichloroethylene (TCE) is one of the best-known synthetic compounds that has been widely used in household and industrial applications, such as dry cleaning and degreasing, and microelectronic manufacturing. The most important group at risk has always been workers who engaged in the metal plating industry. In such situations, exposure occurs through inhalation of TCE vapors and, to a slight extent, through skin contamination with the liquid (1). Previous studies have shown that TCE has toxic effects on the kidney and blood, and is currently known as a carcinogenic compound by the International Agency for Research on Cancer (IARC)(2). In the past decade, immune-related effects of TCE have been studied with different attitudes, but most of these investigations are focused on immunosuppressive, autoimmune disease, immune dysfunction, and hypersensitivity disorders (3,4). Lavicoli et al. 2005) and Zhang et al. 2012) documented changes in immunoglobulin serum levels (i.e., IgA, IgG, IgM, and IgE) in workers exposed to TCE (5,6). Nevertheless, few studies have been conducted on the effects of inhalation exposure to TCE on serum immunoglobulin levels, especially under controlled laboratory conditions. Thus, further and comprehensive research should be performed to find the causes of the increase or decrease and the amount of change of immunoglobulin serum levels in exposure to TCE under controlled conditions.

A balance of different electrolytes is vital for healthy function. Because electrolytes have so many different effects within the cells and organisms, an interferer normally causes severe effects in how mammals feel pretty quickly. Some causes of electrolyte shortage, such as kidney disease, cannot be prevented. Inhalation exposure of TCE using animals has shown that this compound can introduce renal defects in the form of karyomegaly and cytomegaly of the renal tubules in tested rats. So, it can be concluded that kidney cells are one of the most important target organs for TCE effects (7). Studies on the effect of TCE on the electrolyte imbalance are usually focused on certain cardiac class disorders (8), but consensus on the impact of TCE on the electrolytes has not been established. To the best of our research, the present study is the first to investigate the effect of in vivo exposure to trichloroethylene on electrolyte levels. According to the above-mentioned descriptions, this research presents representative work that investigates the effect of TCE on serum immunoglobulin and electrolyte levels and highlights future opportunities.

2. Materials and methods

This case-control study was performed in the medical research center of Larestan, a city in the South of Iran, in 2018.

2.1. Animal

The present research was permitted by the Animal Ethics Experimentation Committee of Shiraz University in accordance with the principles of the National Health and Medical Research Council of Iran. Male Sprague-Dawley rats weighing 250–300 g at the beginning of the research

were held in groups of five with ad libitum access to water and food, a 12 h light–dark cycle, and a temperature of approximately 23±1°C.

2.2. Test Material

TCE was purchased from the Japan Association for Hygiene of Chlorinated Solvents. Purity of such solution was at least 99% and was examined by gas chromatography-mass spectrometry.

2.3. Exposure conditions

Each group of male rats (Groups 2-5) was exposed by inhalation of TCE for a total of 10 consecutive days for 8 hours/day in stainless-steel frame exposure chambers (35cm diameter, 40 cm height, and 80 cm length) covered with clear double glazing. Chamber airflow was maintained at ≈22.4 L/min (12 calculated air changes per hour). Five fans (RDM4010S1 40 × 40 × 10mm) were placed in the center and corners of the chamber at a 45" angle to thoroughly blend the vapors. In addition, perforated plates at the top and bottom of the chamber allowed the distribution of vapors throughout the entire chamber. Temperature with a small range of variation (23±1°C) and relative humidity were monitored by a digital thermometer and hygrometer (Super Digital Timer, DTB-8MA; Iran). A centrifugal fan on the outlet of the chamber provided approximately 12 air changes per hour within the chamber. Animals were individually placed in cages (15 cm length× 15cm diameter × 14 cm height) with wire-mesh floors. During the test, there was no access to food and water for the rats, and the waste produced was removed from the outlet at the bottom of the chamber.

Control samples (Group 1) were tested using conditioned air filtered through high-efficiency particulate air (HEPA) and charcoal filters. The control chambers received the equal volumetric flow of pure air as the TCE exposure chambers. Male rats in Groups 2-5 were exposed to TCE vapor mixed with this air. TCE vapor was introduced into the rectangular chamber atmosphere by the evaporation method. Evaporation of TCE is one of the most important phases applied for the preparation of fixed concentrations of vapors using dynamic methods (9). All generation flasks were seated in water baths agitated with air and maintained at room temperature or heated at different temperatures with tank-type heaters. In many cases, it was necessary to heat the generation flasks to ensure saturation of the outgoing stream with the vapor produced. The air flows through the injection system and the exposure chamber were continuously monitored. By adjusting the flow rates of bypassed streams and temperature, various fixed concentrations of TCE vapors could be produced and conveyed to the exposure chamber. In the present experiment, we selected 10, 100, 250, and 400 ppm of TCE, displaying the Threshold Limit Values of American Conference of Governmental Industrial Hygienists (ACGIH) (10), Permissible Exposure Limits of Occupational Safety and Health Administration (OSHA)(11), high and very high, respectively, which were the basis for choosing these. The concentrations of TCE were analytically determined at least every 10 minutes by gas chromatography (SCION 456-GC; United Kingdom), which sampled from the breathing zone of rats in the exposure chamber. The estimated uncertainty of the measurement is ± 1%. After 10 days of exposure to TCE, the male rats were relocated to different cages to obtain a blood sample from each rat.

2.4. Determination of serum immunoglobulin and electrolyte

Blood samples were collected from the five groups (4 test groups and a control group) in the afternoon (at 16 o'clock) after a period of 8 h at the end of the 10th day of exposure. Serum was

separated after clot retraction by centrifugation for 5 min. at 3000 rpm, then collected by means of micropipette in a tightly sealed container and stored at -15 °C. An automated analyzer (Cobas Mira; Roche Diagnostics, Basel, Switzerland) was applied to determine serum immunoglobulins (IgG, IgA, IgE, and IgM), and also mineral electrolytes such as calcium (Ca), phosphorus (P), and magnesium (Mg) according to the manufacturer's protocol. Furthermore, serum sodium (Na), potassium (K), and chloride (Cl) concentrations were measured by an electrolyte analyzer (Caretium Medical Instruments Co).

2.5. Statistical analysis

The data analyses were performed using SPSS version 18 (SPSS Inc., Chicago, IL, USA). Data was analyzed statistically by an independent t-test for significant differences in the data from exposed rats as compared to control rats. Statistical comparison between different samples was tested using one-way analysis of variance (ANOVA). Results of analysis are registered as means ± SD. Bivariate correlation was performed to determine the relationships between the TCE exposure and the immunoglobulin and electrolyte parameters. All relationships were considered statistically significant when p-values were less than 0.05.

2.6. Ethics

The present research was permitted by the Animal Ethics Experimentation Committee of Shiraz University in accordance with the principles of the National Health and Medical Research Council of Iran.

3. Results

Rats (n = 5 per group) were exposed to TCE in concentrations of 10, 100, 250, and 400 ppm. Table 1 and 2 shows the changes in the immunoglobulin and electrolyte levels after TCE exposure. Significant differences in immunoglobulin levels were seen between the non-exposed control group and the exposed group of rats. IgG, IgM, and IgA levels were significantly increased in rats exposed to 100 and 250, and 400 ppm TCE ($P<0.05$). Conversely, TCE exposure significantly caused a decrease in the levels of IgE in the case group, with those values compared to the control group ($P<0.05$), suggesting that TCE influenced the immunoglobulin levels in rats (see Tables 1&2).

Statistical analysis by one-way analysis of variance (ANOVA) showed a significant difference between TCE exposures in various concentrations for parameters of IgG and IgA ($P<0.05$). The significant differences were also indicated for IgM and IgE levels after exposure to various TCE concentrations ($P<0.05$), except between 100 and 250 ppm TCE for IgM and 250 and 400 ppm TCE for IgE ($P>0.05$).

The relationship between TCE exposure with the immunoglobulin levels was evaluated using the bivariate correlation (Table 3). Among the immunoglobulins, a moderate positive relationship was found between IgA levels and the TCE exposures ($R2 = 0.59$, $P<0.05$) for sub-acute durations.

The comparison of the average values of serum electrolytes between rats of case and control groups after TCE exposure at various concentrations has been presented in Tables 1and 2. The most striking result to emerge from the data is that the level of Ca and P was higher in the rats of the case group after TCE exposure. Statistical analysis showed a significant difference between

serum Ca levels in rats in the control group with those values in rats of the case group after TCE exposure to a concentration≥250 ppm. According to Table 3, sub-acute exposure to TCE can lead to an increase in the serum Ca levels (R2 = 0.54, P<0.05).

The level of P in rats of the case group was significantly greater than that of the control group after TCE exposure to concentrations of 100 and 400 ppm (P<0.05). A moderate positive relationship was found between serum P and the TCE concentration in sub-acute exposure (R2 = 0.66, P<0.05).

This study found that a critical change in Na and Cl values occurs during exposure of rats to TCE. The levels of Na and Cl in rats of the case group were significantly greater than those of the control group after TCE exposure at low concentrations (100 ppm) (P<0.05); conversely, lower at higher exposure concentrations (P<0.05). A moderate negative relationship was found between serum Na and Cl and the TCE exposure (R2 = -0.46, P<0.05). Table 1. Variation of immunoglobulin and electrolyte levels of studied animals (mean ± SD) in different concentration of TCE (10 & 100 ppm)

Parameters	Control group (n=5)	Case group (n=5)			
		10 ppm	P-values	100 ppm	P-values
IgG (g/l)	2.81 ± 0.06	2.79 ± 0.04	0.23	11.00 ± 0.69	0.001
IgM (g/l)	0.45 ± 0.02	0.47 ± 0.03	0.14	1.89 ± 0.07	0.001
IgA (g/l)	0.44 ± 0.04	0.45 ± 0.02	0.17	2.58 ± 0.31	0.001
IgE (IU/ml)	3.38 ± 0.34	3.32 ± 0.32	0.31	0.8 ± 0.69	0.001
Ca (mg/dl)	9.22 ± 0.41	9.31 ± 0.34	0.18	10.06 ± 0.84	0.22
P (mg/dl)	6.38 ± 0.69	6.44 ± 0.23	0.29	7.84 ± 1.18	0.04
Na (mEq/L)	140.6 ± 0.54	139.86 ± 0.39	0.22	145.0 ± 3.08	0.001
K (mEq/L)	4.42 ± 0.08	4.51 ± 0.04	0.19	4.96 ± 0.79	0.22
Mg (mEq/L)	2.70 ± 1.10	2.63 ± 0.11	0.11	3.06 ± 0.79	0.50
Cl (mEq/L)	96.40 ± 1.14	96.49 ± 0.89	0.27	99.44 ± 1.99	0.003

Note: The list of abbreviations and their corresponding definitions can be seen at first page.
Note: Statistical test: independent sample t-test; the mean difference between case and control group is significant at the 0.05 level; Significant values are in bold.

Table 2. Variation of immunoglobulin and electrolyte levels of studied animals (mean ± SD) in different concentration of TCE (250 & 400 ppm)

Parameters	Control group (n=5)	Case group (n=5)			
		250 ppm	P-values	400 ppm	P-values
IgG (g/l)	2.81 ± 0.06	8.96 ± 1.16	0.001	7.90 ± 0.58	0.001
IgM (g/l)	0.45 ± 0.02	1.76 ± 0.15	0.001	0.92 ± 0.27	0.001
IgA (g/l)	0.44 ± 0.04	2.94 ± 0.11	0.001	2.26 ± 0.27	0.001
IgE (IU/ml)	3.38 ± 0.34	2.02 ± 0.51	0.001	2.42 ± 0.40	0.008
Ca (mg/dl)	9.22 ± 0.41	11.84 ± 1.62	0.001	10.90 ± 0.89	0.02
P (mg/dl)	6.38 ± 0.69	7.34 ± 0.55	0.17	9.52 ± 1.53	0.001
Na (mEq/L)	140.6 ± 0.54	134.8 ± 0.44	0.001	139.4 ± 1.67	0.30
K (mEq/L)	4.42 ± 0.08	4.36 ± 0.08	0.89	4.56 ± 1.09	0.75
Mg (mEq/L)	2.70 ± 1.10	2.26 ± 0.50	0.40	2.50 ± 0.79	0.70
Cl (mEq/L)	96.40 ± 1.14	94.60 ± 1.14	0.04	95.22 ± 0.83	0.18

Note: The list of abbreviations and their corresponding definitions can be seen at first page.
Note: Statistical test: independent sample t-test; the mean difference between case and control group is significant at the 0.05 level; Significant values are in bold.

Table 3. Relationship between TCE exposure and immunoglobulin and electrolyte levels (n=25)

Parameters	Correlation Coefficient	P-values
IgG (g/l)	0.40	0.08
IgM (g/l)	0.14	0.53
IgA (g/l)	0.59	0.005
IgE (IU/ml)	-0.10	0.67
Ca (mg/dl)	0.54	0.01
P (mg/dl)	0.66	0.001
Na (mEq/L)	-0.47	0.04
K (mEq/L)	-0.05	0.82
Mg (mEq/L)	-0.21	0.36
Cl (mEq/L)	-0.46	0.03

Note: The list of abbreviations and their corresponding definitions can be seen at first page.
Note: Statistical test: Bivariate correlations; the mean difference is significant at the 0.05 level; Significant values are in bold.

4. Discussion

In this study, the effect of in vivo TCE exposure on serum immunoglobulin and electrolyte levels was investigated for adult male Sprague–Dawley rats. The comparison of the immunoglobulin and electrolyte parameters between case and control groups can represent the sub-acute effects of TCE exposure. Considering the above assumption, the most significant finding of the study is that TCE exposure to concentrations≥100 ppm can significantly increase the levels of IgG, IgM, and IgA and decrease the levels of IgE. This study found that a critical change in some electrolyte parameters occurs during exposure of rats to TCE.

The most abundant type of immunoglobulin in the blood is IgG (approximately 75% of serum antibodies), followed by smaller quantities of IgA and IgM. The parameters of IgG, IgM, and IgA are often measured together. In this way, they can provide investigators with useful information about immune system performance, especially relating to autoimmune or infection. The present study found that a critical change in immunoglobulin values occurs when rats are exposed to TCE. In particular, a considerable increase was noted in the levels of serum IgA, IgG, and IgM of rats after sub-acute exposure to TCE concentrations≥100 ppm. It was also observed that a cumulative positive association between increasing TCE concentrations and the level of serum IgA, IgG, and IgM, although it did not reach a level of statistical significance for IgG and IgM. Serum immunoglobulin levels in different creatures are determined in clinical and experimental research because such information provides important information on the humoral immune system. In previous studies, high immunoglobulin levels have been detected in infections, hematological disorders, chronic inflammatory disorders, and liver diseases. Moreover, immunoglobulin levels aid in the diagnosis of some diseases, particularly liver disorders (12). Researchers showed that inhalation exposure to TCE can produce several forms of liver disease, such as fatty liver, cirrhosis, hepatic necrosis (13), and hematological disorders (14). Animal exposure to TCE using a specific mouse model of systemic autoimmunity, is induces an autoimmune hepatitis-like syndrome (15). Therefore, one of the causes of an increase in immunoglobulins is the effect of TCE on the liver or blood.

Another in vivo study by Kaneko et al. 2000) surveyed the effects of TCE on the serum immunoglobulin (IgG, IgM, IgA) levels and T-cell function. The animals were exposed to TCE at dosages of 0, 500, 1000, and 2000 ppm through inhalation for 4 h a day, 6 days a week, for 8 weeks. A significant dose-dependent reduction in IgG levels in serum obtained from the caudal vein was observed after the fourth week of exposure. There were no significant reductions in IgA and IgM levels up to 8 weeks of exposure, and there was a decline in IgM level in the 2000ppm groups after 8 weeks of exposure (16). Zhang et al. 2012) conducted an epidemiological cross-sectional study on 80 industry workers exposed to TCE levels by measuring their blood serum immunoglobulin levels (i.e., IgG, IgM). The mean of TCE was 38.4 ppm in the high-exposure workers and 22.2

ppm in middle middle-exposed group. This study reported a 17.5% and 38% decrease in IgG and IgM levels, respectively, in the exposed group compared to the unexposed control (6). The incorporation of confounding variables, especially lifestyle factors and the presence of co-contaminants, needs to be considered to further advance the studies using animal models. The differences between findings of studies can be associated with the age, sex, solvent concentration, length of the exposure period, and type of species used, or due to the experimental methodology applied. Also, Gonzalez-Quintela et al. 2008) concluded that serum concentrations of serum immunoglobulins can be affected by common metabolic abnormalities and habits (12).

In the present study, serum IgE levels were not statistically different between low-exposed rats exposed to TCE (10 ppm) and unexposed rats. These findings are in line with the data reported by Zhang et al. 2012, who suggest that the IgE levels were not significantly different between exposed and control subjects at relatively low exposure levels (6). Our results showed that the levels of serum IgE were significantly lower in the rats after exposure to TCE concentrations≥100 ppm. Also, a negative relationship was suggested between the TCE concentration and serum IgE (R2 = -0.10). Inconsistent with our findings in exposed rats is the fact that a straight relationship between TCE inhalation and serum IgE levels has not been established, with studies either reporting no effect or conflicting findings based on the concentration and length of the exposure period (17,18).

In addition to building bones, calcium enables muscles to contract, the heart to beat, and the blood to clot. The results of this study showed that the Ca levels were significantly higher in the rats after TCE exposure at concentrations≥250 ppm. Present research was the first to report those effects, but several studies have demonstrated the relationship between Ca levels and exposure to TCE in certain heart diseases. Previous studies concluded that TCE disrupted sarcolemmal Ca influx in neonatal rat cardiomyocytes (19). Inhalation of TCE has also been showed to be able to be genotoxic, especially those genes involved in the regulation of intracellular Ca (20). These findings are consistent with the study by Selmin et al. 2008, who noted that TCE disrupted the expression levels of ryanodine receptor 2 (RYR2), a Ca2+ release channel that is vital in the normal activity of the heart, in P19 mouse embryonal carcinoma cells (21). Based on the mentioned results, the disturbance of serum Ca is closely related to the “intensity” of TCE concentration during sub-acute exposure.

P is an essential mineral primarily used for the normal activity of tissues and body cells. It is also required for a variety of intercellular processes, including pH regulation and energy production. Based on findings, the serum concentration of P was significantly higher in the rats after exposure to TCE≥100 ppm. Also, it was observed a positive association was observed between TCE exposure increase and the level of P (R2 = 0.66). Kidneys help remove extra phosphate from the body to keep the levels in balance. When kidneys are damaged, the body can’t remove phosphate from the blood quickly enough. This can lead to chronically elevated levels of phosphate. Generally, epidemiology investigations and human case-control studies showed that TCE causes kidney disease and increases the risk of cancer (1). Histopathological research has shown that TCE inhalation caused morphological disorders in the kidney, leading to their necrosis and

renal tubular damage (7). However, little is discussed about the mechanism by which TCE increases of P level in blood; with these interpretations, one of the reasons for its increase may be the damage to the kidney. Thus, further research should be performed to find the causes of the increase in P level in exposure to TCE.

Na and Cl are necessary for mammals to maintain the proper balance of body fluids and blood pressure, as well as their pH (acid-alkali / acid-base) balance in the process. This study was performed to find the TCE-induced toxic effects on Na and Cl in rat blood. Based on the study findings, the level of those values in rats of the case group was significantly greater than that of the control group after TCE exposure at 100 ppm concentrations; conversely, lowered at higher exposure concentrations (250 ppm). However, this effect was not significant at very high TCE concentrations. Under normal circumstances, more than two-thirds of filtered NaCl and water are reabsorbed in the proximal tubule, which makes up a significant portion of the kidneys and carries out diverse regulatory and endocrine functions. The proximal tubule in the kidney was shown to be the prime TCE target (7). However, biochemical mechanisms and tissue response to toxicity are not completely understood, and hence need for further study to understand the mechanism of TCE on the increase or decrease of NaCl concentration in blood. Because of the very controlled conditions in this study that are very different from real ones, the responses of the tested subjects may not truly indicate the worker behaviors in work environments. There are a variety of workplaces, such as oil and gas industries and foundry and metal forming manufacturers, predisposing workers to the combination of heat, sound, and chemicals like trichloroethylene; therefore, further research should be performed to find factors influencing the variability of serum immunoglobulin and electrolyte levels of workers in these workplaces.

4.1. Conclusion

This study investigated alterations of immunoglobulin as well as electrolyte responses following exposure to various levels of TCE in adult male Sprague–Dawley rats. The present study provides evidence that sub-acute inhalation exposure to TCE (≥ 100 PPM) is associated with electrolyte and immunoglobulin toxicity in the form of increased Ca, P, IgA, IgG, and IgM and decreased Cl, Na, and IgE. Further study should be conducted to study the concentration and duration of exposure leading to electrolyte and immunoglobulin toxicity, and find the causes of change in humans when they are exposed to TCE.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

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OPEN PEER REVIEW

Revision (1)

Raheleh Hashemi Habybabady : Reviewer — 25 Aug 2018

Dear author:
Please make the necessary corrections in the following comments
Regarding the volume of the chamber, it seems that, using the airflow of 22.4 liters/min, air exchange is more than 12 times per hour (300 times per hour), and maintaining low concentrations seems very unlikely. Please provide more details on this section.

The total number of animals in the animal section should be written clearly.

Nasser Keikha : Reviewer — 5 Sep 2018

Dear EIC:
Some revisions in the methods and discussion sections of the study are needed.

Raheleh Hashemi Habybabady : Associate Editor — 9 Sep 2018

Dear authors:
Some minor revisions must be made by the author
Reviewer:
Regarding the volume of the chamber, it seems that, using the airflow of 22.4 liters/min, air exchange is more than 12 times per hour (300 times per hour), and maintaining low concentrations seems very unlikely. Please provide more details on this section.
The total number of animals in the animal section should be written clearly.
Reviewer:
Some revisions in the methods and discussion sections of the study are needed.

Ramazan Mirzaei : EIC — 10 Sep 2018

Dear authors:
Some comments in revision 1 need to be re-reviewed and the necessary corrections made by the author.

Regarding the volume of the chamber, it seems that, using the airflow of 22.4 liters/min, air exchange is more than 12 times per hour (300 times per hour), and maintaining low concentrations seems very unlikely. Please provide more details on

this section.

The total number of animals in the animal section should be written clearly.

OPEN PEER REVIEW

Revision (2)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

Dear Editor,

I have attached the revised version of our manuscript (corrected text in yellow highlight) with a point-by-point response to the reviewers' comments (below). We have addressed all of the reviewers 'concerns. We are very grateful to the reviewers for their positive and helpful suggestions.

Yours sincerely

A point-by-point response has been provided below.

Reviewer 1:

1. Regarding the volume of the chamber, it seems that, using the airflow of 22.4 liters/min, air exchange is more than 12 times per hour (300 times per hour), and maintaining low concentrations seems very unlikely. Please provide more details on this section.

Answer: Dear reviewer, calculations are as follows:

$0.35 \times 0.4 \times 0.8 = 0.112$ m3

$0.112 \times 12 = 1.344$ m3/h (air changes per hour)

volume	time
1.344	60
x	1

X= 0.0224 m^3/min

lit	m^3
1000	1
x	0.0224

X= 22. 4 lit/min

The air should be evacuated at a rate of 22.4 liters per minute

1. The total number of animals in the animal section should be written clearly.

Answer: Changes were made in the article

a) In the text (Abstract, material, and method): 25 adult male Sprague–Dawley rats were randomly divided into five equal groups of 5 animals each.

b) In the text (material and method, Animal): Male Sprague-Dawley rats weighing 250–300 g at the beginning of the research were held in five-number groups (n = 5 per group) with ad libitum access to water and food

Reviewer 2:

1. Some revisions in the methods and discussion sections of the study are needed.

Answer: Changes were made in the article

OPEN PEER REVIEW

Revision (2)

Raheleh Hashemi Habybabady : Associate Editor — 24 Sep 2018

Dear author:
I am writing to confirm that the authors of the following manuscript have carefully and meticulously revised their manuscript in line with the points and comments in the process of reviewing the paper.

Therefore, in my view, the manuscript is acceptable in its current format and could be published in this journal.

Regards,

Ramazan Mirzaei : EIC — 25 Sep 2018

Dear Authors:
My decision on this study is to accept, and the article is suitable for publication in the Journal of Health Scope.
Best Regards,