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Toxicological Effects of Inhalation Exposure to Trichloroethylene on Serum Immunoglobulin and Electrolyte Levels in Rats

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Abstract

Background: Trichloroethylene (TCE) is one of the most important environmental contaminants that can introduce both occupational and public health because of its widespread use.
Objectives: This study was aimed to investigate effects of inhalation exposure to TCE on serum immunoglobulin and electrolyte levels under controlled laboratory conditions.
Methods: Five equal groups (5 animals each) were randomly selected from a total of 25 adult male Sprague-Dawley rats. TCE vapor was generated by dynamic evaporation method in exposure chamber. The animals were exposed to TCE at five different concentration levels (0 ppm as control, 10, 100, 250, and 400 ppm through 8 hours inhalation during 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 $\geq$ 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 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 level ($P < 0.05$). 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 ($R^2 = 0.66$, $P < 0.05$).
Conclusions: In conclusion, sub-acute inhalation exposure to TCE ($\geq$ 100 ppm) is related to the 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

1. Background

Trichloroethylene (TCE) is one of the best known synthesized compounds that have been used widely in household and industrial applications, such as dry cleaning and degreasing and microelectronic manufacture. The most important group at risk has always been workers that are engaged in the metal plating industry. In such situations, exposure occurs through inhalation of TCE vapors, and to a slighter extent through skin contamination with the liquid (1). Previous studies have shown that TCE has a toxic effect on kidney and blood and currently is 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 attitude, but most of these investigations are focused on immunosuppressive, autoimmune disease, immune dysfunction and hypersensitivity disorders (3, 4). Lavicoli et al., and Zhang et al., 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 effects of inhalation exposure to TCE on serum immunoglobulin levels, especially under controlled laboratory conditions. Thus, further and comprehensive researches should be performed to find causes of 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 interference 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. Thus, 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 is generally discussed on specific cardiac class disorders (8), but consensus on the effect of TCE on the electrolytes has not been reported. To the best of our research, the current work is the first to investigate the effect of *in vivo* exposure of trichloroethylene on electrolyte levels.

2. Objectives

According to the above-mentioned descriptions, this work deals with the effect of TCE on serum immunoglobulin and electrolyte levels and detects future opportunities.

3. Methods

The current case control research was done in medical research center of Larestan, a city in the South of Iran, in 2018.

3.1. 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 (n = 5 per group) with ad libitum access to food and water, a 12 hours light-dark cycle and temperature of approximately 23 $\pm$ 1°C.

3.2. Test Material

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

3.3. Exposure Conditions

Each group of male rats (groups of 2 - 5) was exposed to TCE by inhalation for 8 hours/day and for a total of 10 consecutive days in stainless-steel frame chambers (35cm diameter, 40 cm height, and 80 cm length) covered with clear double glazing. Chamber airflow was stabilized around 22.4 L/min (12 calculated air changes per hour). Five fans (RDM4010S1 40 $\times$ 40 $\times$ 10 mm) were used in the center and corners of the chamber at a 45" angle to thoroughly blend the vapors. In addition, perforated plates at the bottom and top of the chamber allowed the vapors be distributed throughout the entire chamber. Temperatures with a small range of variation (23 $\pm$ 1°C) and relative humidity were monitored by digital thermometer and hygrometer (Super Digital Timer, DTB-8MA; Iran). A centrifugal fan was applied on the outlet of the chamber which provided approximately 12 air changes per hour inside of the chamber. Animals were individually placed in cages (15 cm length $\times$ 15 cm diameter $\times$ 14 cm height) with wire-mesh floors. There was no access to water and food for the investigated rats during the tests and produced waste was removed from the outlet in 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 chamber received the equal volumetric flow of pure air as the TCE exposure chamber. Male rats in groups of 2 - 5 were exposed to TCE vapor mixed with such air. TCE vapor was introduced into a rectangular chamber atmosphere by evaporation method. Evaporation of TCE is one of the most important phases applied for the preparation of fixed concentration 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 in order to assure saturation of outgoing stream is maintained with the vapor. The air flows through the injection system and exposure chambers 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, 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 was the basis for choosing these concentrations. The adjusted concentrations of TCE were analytically identified at least in 10-minute increments by a SCION gas chromatography (SCION Inc., 456-GC; United Kingdom). This identification was done on the sampled air from the breathing zone of exposed rats in the exposure chamber. The estimated uncertainty of the TCE measurement is $\pm$ 1%. After 10 days exposed to TCE, the male rats were located in different cages to obtain blood samples from each of them.

3.4. 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 an exposure period of 8 hours at the end of the 10th day of exposure. Serum was separated after clot retraction by centrifugation for 5 minutes 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 manufacturers' protocol. Furthermore, serum sodium (Na), potassium (K), and chloride (Cl) concentrations were measured by electrolyte analyzer (Caretium Medical Instruments Co.).

3.5. Statistical Analysis

SPSS version 18 (SPSS Inc., Chicago, IL, USA) was applied for data analyses. Data were analyzed statistically by independent *t*-test for significant differences in the data from exposed rats as compared to control. Statistical comparison between different samples was tested using one-way analysis of variance (ANOVA). Results of analysis are registered as means $\pm$ 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.

3.6. 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.

4. Results

Rats (n = 5 group) were exposed to TCE in concentrations of 10, 100, 250, and 400 ppm. Tables 1 and 2 show 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 exposed group of rats. IgG, IgM and IgA levels were significantly increased in rats exposed to 100, 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 control group ($P < 0.05$), suggesting that TCE influenced the immunoglobulin levels in rats (see Tables 1 and 2).

Statistical analysis by one-way analysis of variance (ANOVA) represented a significant difference between TCE exposures in various applied 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 correlation between TCE exposures 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 ($R^2 = 0.59$, $P < 0.05$) for sub-acute durations.

The comparison of the average values of serum electrolytes between rats of case and control groups after TCE exposure in various concentrations has been presented in Tables 1 and 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 concentrations $\geq$ 250 ppm. According to Table 3, sub-acute exposure to TCE can lead to increasing serum Ca levels ($R^2 = 0.54$, $P < 0.05$).

Level of P in rats of the case group was significantly greater than that of the control group after TCE exposure to concentration 100 and 400 ppm ($P < 0.05$). A moderate positive relationship was found between serum P and the TCE concentration in sub-acute exposure ($R^2 = 0.66$, $P < 0.05$).

This study found that critical change in Na and Cl values occurs during exposure of rats to TCE. Level of Na and Cl in rats of the case group was significantly greater than that of the control group after TCE exposure at low concentrations (100 ppm) ($P < 0.05$); conversely, lowered at higher exposure concentrations ($P < 0.05$). A moderately negative relationship was found between serum Na and Cl and the TCE exposure ($R^2 = -0.46$, $P < 0.05$).

5. 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. According to which was assumed above, the main considerable result of the study is that exposure to concentrations $\geq$ 100 ppm of TCE can significantly increase the levels of IgG, IgM, and IgA and decrease the levels

Table 1. Variation of Immunoglobulin and Electrolyte Levels of Studied Rats (Mean ± SD) in Different Concentrations of TCE (10 and 100 ppm)[a]

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*

Abbreviations: Ca, Calcium; Cl, Chlorine; IgA, Immunoglobulin A; IgE, Immunoglobulin E; IgG, Immunoglobulin G; IgM, Immunoglobulin M; K, Potassium; Mg, magnesium; Na, Sodium; P, phosphorus.
[a] Statistical test: Independent Sample *t*-test; the mean difference between case and control group is significant at the 0.05 level.

Table 2. Variation of Immunoglobulin and Electrolyte Levels of Studied Rats (Mean ± SD) in Different Concentrations of TCE (250 and 400 ppm)[a]

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

Abbreviations: Ca, Calcium; Cl, Chlorine; IgA, Immunoglobulin A; IgE, Immunoglobulin E; IgG, Immunoglobulin G; IgM, Immunoglobulin M; K, Potassium; Mg, magnesium; Na, Sodium; P, phosphorus.
[a] Statistical test: Independent Sample *t*-test; the mean difference between case and control group is significant at the 0.05 level.

of IgE. This study found that 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 useful information about immune system performance, especially relating to autoimmune or infection. The present study discovered that critical change in immunoglobulin values occurs when rats are exposed to TCE. In particular, considerable increase was noted in the levels of serum IgA, IgG, and IgM of rats after sub-acute exposure to TCE concentrations $\geq$ 100 ppm. A cumulative positive association between increasing of TCE concentrations and the level of serum IgA, IgG, and IgM was also observed; although the statistical level was not significant for IgG and IgM. Serum immunoglobulin levels in different creatures are determined in clinical and experimental researches because such information provides important information on the humoral immune system. In previous studies, high immunoglobulin levels are detected in infections, hematological disorders, chronic inflammatory disorders, and liver diseases. Moreover, immunoglobulin levels aid in the diagnosis of

Table 3. Relationship Between TCE Exposure and Immunoglobulin and Electrolyte Levels (N = 25)[a]

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*

Abbreviations: Ca, Calcium; Cl, Chlorine; IgA, Immunoglobulin A; IgE, Immunoglobulin E; IgG, Immunoglobulin G; IgM, Immunoglobulin M; K, Potassium; Mg, magnesium; Na, Sodium; P, phosphorus.
[a]Statistical test: Bivariate correlations; the mean difference between case and control group is significant at the 0.05 level.

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 induced 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. surveyed the effects of TCE on the levels of serum immunoglobulin (IgG, IgA, IgM) and T-cell function. The rats were exposed to TCE at levels of 0, 500, 1000, and 2000 ppm through inhalation for 4 hours a day, 6 days a week, and for 8 weeks. It was observed that the reduction in IgG amounts in serum from the caudal vein was significantly dose-dependent after the 4th week of TCE exposure. No significant decrease in IgA and IgM levels was observed up to 8 weeks of exposure, and there was a decline in the level of IgM in the 2000 ppm groups after 8 weeks of TCE exposure (16). An epidemiologic cross-sectional study was done by Zhang et al. on 80 industry workers exposed to TCE levels by determination of serum immunoglobulin levels in their blood (i.e. IgM and IgG). The mean of TCE was 38.4 ppm in the high exposure workers and 22.2 ppm in average exposed group. By comparing the exposed group with unexposed controls, this research observed a 38% and 17.5% decrease in the levels of IgM and IgG, respectively (6). The incorporation of confounding variables, especially lifestyle parameters and the presence of co-contaminants requires studies using animal models in further advance works. 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 can be due to the applied experimental methodology. Also, Gonzalez-Quintela et al. concluded that concentrations of serum immunoglobulins can be affected with common metabolic abnormalities and habits (12).

In the present study, serum IgE levels were not statistically different between low exposed of TCE (10 ppm) and unexposed rats. These results are in agreement with the findings reported by Zhang et al., 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 $\geq$ 100 ppm. Also, a negative relationship was suggested between the TCE concentration and serum IgE (R^2 = -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 approved, 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, heart to beat, and blood to clot. The results of this study showed that the Ca levels were significantly higher in the rats after TCE exposure to concentration $\geq$ 250 ppm. Present research was the first to report those effects, but a number of studies have demonstrated the relationship between Ca levels and exposure to TCE in certain heart disease. Previous studies concluded that disruption of sarcolemmal Ca influx can be done during exposure to TCE in neonatal rat cardiomyocytes (19). Inhalation of TCE has also been shown to be able to be genotoxic especially those genes involved in 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 Ca^{2+} release channel which is highly required in the normal activity of heart, in P19 mouse embryonal carcinoma cells (21). Based on mentioned results, the disturbance of serum Ca is closely related to the "intensity" of TCE concentration during subacute exposure.

Phosphorus (P) is an essential mineral primarily used for 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 exposure $\geq$ 100 ppm. In addition, a positive association was observed between TCE exposure increase and the level of P (R^2 = 0.66). Kidneys help remove extra phosphate from body to keep the levels in balance. When kidneys are damaged, the body can't re-

move phosphate from blood quickly enough. This can lead to chronically elevated levels of phosphate. Generally, epidemiologic investigations and human case-control studies showed that TCE causes kidney disease and increases the risk of cancer (1). It was illustrated by histopathological researches that TCE inhalation can lead to 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 causes of increase of 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. During normal circumstances, the re-absorbtion of more than two thirds of filtered NaCl and water is done 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 illustrated to be the prime TCE target (7). However, the tissue response to toxicity and biochemical mechanisms are not completely understood and hence the need for further studies to understand the mechanism of TCE on increase or decrease of NaCl concentration in blood exists.

It is noteworthy that the experimental conditions in this research was completely controlled which are not very similar to real situations, the observed responses in the investigated subjects may not be truly reflecting the worker behaviors in the real work positions. On the other hand, very different workplaces such as gas and oil industries/foundry and metal-forming companies predisposing workers to the combination of sound, heat, and chemical compounds like TCE; make it necessary to conduct further researches to clarify effective parameters on variability of serum immunoglobulin and electrolyte levels of workers in such work-positions.

5.1. Conclusions

This study investigated alterations of immunoglobulin as well as electrolyte responses following exposure to different concentrations of TCE in adult male Sprague-Dawley rats. It was observed in the current research that sub-acute inhalation exposure to TCE ($\geq$ 100 ppm) is related to electrolyte and immunoglobulin toxicity in the form of increased Ca, P, IgA, IgG, and IgM and decreased Cl, Na, and IgE. Further studies should be performed to study the concentration and duration of exposure leading to electrolyte and immunoglobulin toxicity and find causes of change in humans when they are exposed to TCE.

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Footnotes

Conflict of Interests: The authors declare that they have no competing interests.

Ethical Approval: 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.

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