DE GRUYTER OPEN

*J Biomed Clin Res* Volume 9 Number 1, 2016

*DOI: 10.1515/jbcr-2016-0007*

*Original Article*

# TOXICOLOGICAL CHEMICAL ANALYSIS OF METHANOL IN BLOOD OF PATIENTS WITH ACUTE ETHANOL INTOXICATION FOR DETERMINING DETECTABLE QUANTITIES OF METHANOL AND ANALYSIS OF THE CORRELATION BETWEEN INGESTED ALCOHOL

**Georgi D. Bonchev, Snezha Z. Zlateva[1], Marieta I. Yovcheva[1]**

*Laboratory of Analytical Toxicology, Military Medical Academy, Navy Hospital - Varna*
*[1]Department of Intensive Treatment of Acute Intoxications and Toxicoallergies, Military Medical Academy, Navy Hospital - Varna*

## Summary

The aim of the study was to carry out a toxicological chemical analysis of methanol in detectable quantities in the blood of patients with acute alcohol intoxication. Blood samples from 85 patients with acute alcohol intoxication were analysed for the presence of methanol. All patients with acute methanol intoxication were excluded from the study. The methods of gas chromatography with vapor-phase analysis (head-space) and flame ionization detection (FID) were used. The limit of detection (LOD=0.015 g/L) and the limit of quantification (LOQ=0.025 g/L) of methanol in whole blood were evaluated. In 30% of the cases, methanol was found in the blood in detectable quantities. The levels of methanol were on the average 5 to 6 times lower than the toxic methanol level (0.200 g/L) and they were not due to natural metabolic processes (ingestion of fruit, fruit juices or vegetables). No reliable statistically linear correlation between the concentration of ethanol and methanol was found. Methanol subintoxications are major factors in alcohol intoxications, in which the quantity of the alcohol ingested is not as important as its quality. Chronic methanol subintoxication of people who often consume alcohol of poor quality is discussed.

**Key words:** ethanol, methanol

## Introduction

Methanol (methyl alcohol, methyl spirit or wood alcohol) has molecular formula $CH_3OH$ (often written as MeOH) and is the simplest aliphatic alcohol. Under normal conditions, it is a colorless, mobile, volatile liquid with a characteristic odor, similar to that of ethanol. Its molar mass is 32.04 g/mol, the density is 0.792 g/cm$^3$ and the boiling point - 64.7°C.

The lethal dose $LD_{50}$ for rats is 5628 mg/kg [1]. Often, the history of the quantity of methanol ingested by human patients is not reliable. Cases of toxic blindness caused by ingesting 4 ml of methanol, and death from ingesting 30 ml of methanol have been reported, as well as a case of survival without organ damages after ingesting 500 ml methanol, with hemodialysis in the early stage. Most handbooks consider plasma concentration of

**Corresponding author:**
Georgi Bonchev
Military Medical Academy,
Navy Hospital - Varna
3, Christo Smirnenski Str.
Varna, 9000
Bulgaria
*e-mail: toxilab.varna@abv.bg*

**Received:** March 01, 2016
**Revision received:** May 10, 2016
**Accepted:** May 30, 2016

methanol as a more reliable indicator of the stage of methanol intoxication [2]. However, according to Seyffart, this indicator is also not fully reliable since very high plasma concentrations of methanol (from 4 to 8 g/L) can be found at the early stage of intoxication, with light or medium acidosis and initial damage of vision. Although at the subsequent stage the concentration of methanol can be not so high (0.5 g/L or less), patients present with severe acidosis and blindness. Methanol has to be metabolized to formaldehyde and then to formic acid and its salts, before its full toxic effects are manifested [3]. At present, the following evaluations of plasma concentrations of methanol are accepted: toxic – 0.2 g/L and comatose – 0.9 g/L [4].

Evidence exists that the Ancient Egyptians extracted methanol by the process of "dry distillation" (pyrolysis) of wood. The modern technologies of methanol production are based mainly on catalytic synthesis directly from CO, $CO_2$ and $H_2$, as well as on extraction from natural gas. It is one of the most traded compounds in the world, because it has multiple applications in industry. A considerable number of other compounds used in consumer goods production and in industry are synthesized from its main metabolite – formaldehyde.

In nature, methanol is present in very small quantities in the air. It gets into the atmosphere as a side product of anaerobic metabolism of different bacteria. Within several days the atmospheric methanol is oxidized to $CO_2$ and water under the influence of sunlight without accumulation in soil or water [5]. Large amounts of methanol were found in space in 2006 [6].

Under normal conditions very small quantities of methanol can be found in a healthy individual. Diet and natural metabolic processes are also sources of methanol (Figure 1). Methanol enters the organism through diet, mainly as a metabolite of the artificial sweetener aspartame [7], which is a methyl ester and is hydrolyzed in the small intestine. The concentration of methanol in exhaled air is about 4.5 ppm [8], and in whole blood – about 0.007 g/L [9]. The average amount of endogenous methanol, metabolized from the pectin in fruit is about 0.45 g a day.

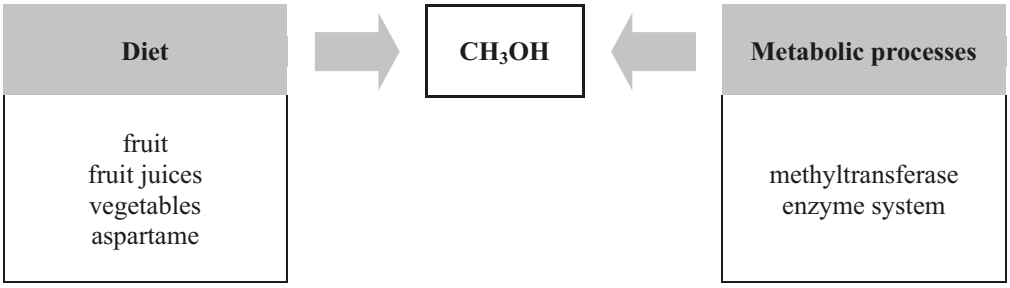


**Figure 1.** Main natural sources of methanol

The presence of methanol in alcoholic drinks is of greatest significance for toxicology. It is an inseparable part of these drinks as it is formed in some amounts during fermentation – a process in which ethanol is produced from different fruits. Normally, methanol is found in beer, wine and beverages with high levels of alcohol concentration (Table 1). The presence of methanol in these drinks makes them potentially hazardous for health because methanol is much more toxic than ethanol itself.

From the widespread commercial household products, the highest methanol content is found in the so called "blue spirit" (blue colored spirit), "spirit for burning" or "wood spirit" (up to 100% methanol), wind-screen wiper liquid (from 4% to 90%) and antifreeze liquid (up to 90%) [9]. They are of toxicological importance in cases of intentional or accidental ingestion.

The alcohol, produced for commercial purposes is much safer because the producers use specific technologies for steady division of the methanol from ethanol. People who consume mainly home-made alcohol (domestic distillation), in which the methanol can not be thoroughly removed, are the group in risk. Most frequently, these drinks are domestic rakia, domestic gin, rum or whisky. Domestic systems for producing alcohol do not use modern technologies which makes the separation of methanol and ethanol much more difficult. Specialists in this field claim that there is no safe domestic method of removing the methanol from ethanol [10, 11]. It is supposed that the presence of methanol in commercial trade mark alcohol is due to mixing of commercial ethanol (without

methanol) with methanol (sold without excise) or with home-made distillate (containing significant amount of methanol) [2]. In both cases the aim is a commercial profit, as the mixed drink is sold as cheap bottled rakia, whisky and other hard drinks.

**Table 1.** Contents of methanol in alcohol drinks according to Bonte W. (1987) [10]

| Alcohol drink | Methanol (mg/L ) |
|---|---|
| Beer | 4 - 50 |
| Rum | 6 - 70 |
| Vodka | 5 - 170 |
| White wine | 15 - 45 |
| Whisky | 10 - 110 |
| Liquor | 10 - 560 |
| Gin | 10 - 1350 |
| Red wine | 70 - 130 |
| Scotch | 100 - 130 |
| Burbon | 200 - 300 |
| Brandy | 200 - 350 |
| Cognac | 180 - 370 |
| Rakia | 1 500 - 4 000 |
| Sherry brandy | 1 900 - 2 500 |
| Plum brandy | 3 000 - 4 500 |

The current clinical practice of the Department of Intensive Treatment of Acute Intoxications and Toxicoallergies in Navy Hospital - Varna has shown an alarmingly frequent presence of small quantities of methanol in the blood of patients with acute ethanol alcohol intoxication. However, as the average content of methanol in whole blood of a healthy man does not exceed an exemplary average value of 0.007 g/L [9], every actually measured concentration level above the indicated limit of detection (LOD, 0.015 g/L) should be explained using another mechanism.

The aim of the study was to carry out a toxicological chemical analysis for methanol in detectable quantities in the blood of patients with acute alcohol intoxication, admitted to the Department of Intensive Treatment of Acute Intoxications and Toxicoallergies, Navy Hospital - Varna.

## Materials and Methods

Eighty-five blood tests of patients with acute alcohol intoxication during the period January-August 2015 were analyzed. The analysis was made using a gas chromatograph 7890 GC System (Agilent Technologies), supplied with an authomatised vapor-phase analyzer 7697A HS Sampler (Agilent Technologies) and a flame-ionization detector. The column was HP-INNOWAX 30 m × 0.250 mm Narrowbore, 0.25 µm. All the reagents were of p.f.a. grade or better. Acetone and methanol were purchased from Riedel-de Haën AG, ethanol (absolute) – from Chimtex Ltd, 1-propanol – from Ferak Berlin GmbH. Purified deionised water (0.067-0.10 µS/cm, TKA™ Pacific water purification system) was used. Statistical analysis was done with specialized software (Origin®, OriginLab Corp.).

## Results

At present, the method of gas chromatography with vapor-phase analysis (Head-space) and flame-ionization detection (FID) is considered a "gold standard" for quantitative determination of alcohols in biological samples in clinical toxicology. The detection of low concentrations (<0.1 g/L) of methanol in whole blood, however, is accompanied by difficulties of experimental nature. Firstly, these concentrations are close to the detection limit of the method, and secondly, they are far from the usual calibration curves,

expected for detection of toxic methanol levels.

That is why, as a stage of the procedure of method validation for methanol detection in the Laboratory of Analytical Toxicology, the two main limits were evaluated that estimate the possibilities of the applied routine methods for measurements in the low concentration range.

For the routine method used, the following value of limit of detection was evaluated:

LOD = 0.015 g/L $CH_3OH$

Samples that contain methanol concentration below 0.015 g/L can be neither qualitatively nor quantitatively reliably analyzed by this method, because measurements show zero value in an unsatisfactory large number of cases.

For the method routinely used, the following value of limit of quantification (LOQ) was evaluated:

LOQ = 0.025 g/L $CH_3OH$

Samples that contain methanol concentration above 0.025 g/L s are almost always positive for methanol. Besides, methanol concentration is determined with relative uncertainty not worse than 20% (at 90% confidence level).

Within the intermediate interval 0.015-0.025 g/L are samples, for which the presence of methanol can be proven qualitatively, but the methanol concentration can not be determined with a satisfactory precision (Figure 2).

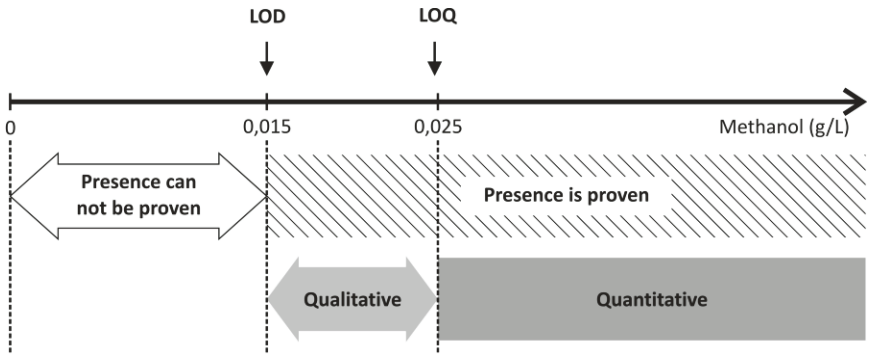


**Figure 2.** Visual interpretation of the limiting values LOD and LOQ for methanol determination by gas chromatography method in Laboratory of Analytical Toxicology

The study includes 85 patients (Table 2) with a certain consumption of alcohol, i.e., concentration of ethanol in whole blood was determined to exceed 0.200 g/L (subclinical phase of intoxication) [12]. The authors determine that part of the same group of patients, in whom methanol could be found as well, with a concentration lower than the toxic methanol concentration (0.200 g/L [4]), thus making an attempt to exclude all the accidents, accidental household intoxications and suicidal attempts from the statistics.

**Table 2.** Criteria for selection of target group of patients and preliminary statistics

| Patients | Alcohol | Concentration (g/L) | Number |
|---|---|---|---|
| With verified consumption of alcohol | ethanol | - | 85 |
| With verified consumption of alcohol, including presence of methanol | methanol | 0.015-0.200 | 25 |

From these data we can conclude that the presence of methanol in detectable quantities can be seen in an alarmingly great part - about 30%, of all the cases of alcohol ingestion (Figure 3).

A possible dependence between the concentration of the detected methanol and the ingested ethanol was also investigated. For this purpose, the data about the concentrations of ethanol and methanol in whole blood found by the chemical analyses were compared (Table 3). Such a reliable comparison was possible because the determination of the two types of alcohols using the applied method took place simultaneously in the original samples.

Statistically, data set collected is a representative one, because from a clinical point

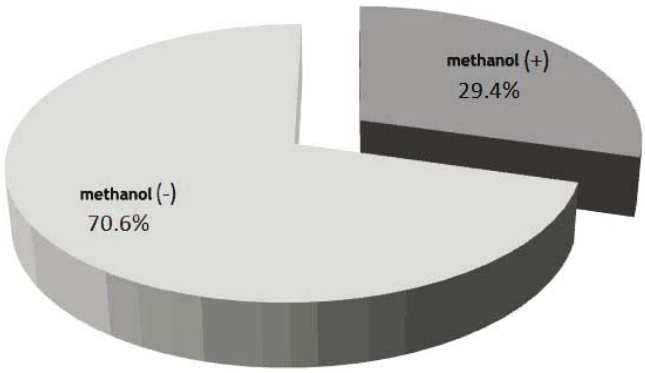


**Figure 3.** Relative part of proven positive methanol content among overall alcohol intoxications

of view a considerable range of ethanol concentrations has been covered (Table 4).

The statistical analysis of the compared data practically showed the absence of connection between the ethanol and methanol concentrations in the blood of the patients from the studied group. Really, a slightly expressed trend (i.e., a tendency higher levels of ethanol to correspond to higher levels of methanol) exists, but the Pearson's correlation coefficient of linear regression has an insignificant value (r=0.1315), thus indicating a questionable statistical reliability of such a conclusion.

The absence of a direct connection between the concentrations of ethanol and methanol in the blood of the patients from the target group becomes intuitively clear at a visual estimation of the same data, presented in graphic mode (Figure 4). It can be easily seen that levels of methanol above the average (>0.035 g/L) could often be seen in patients with a very low content of ethanol and, on the contrary, patients with a very high plasma concentration of ethanol demonstrate methanol content below the average (<0.035 g/L).

**Table 3.** Comparison between the ethanol and methanol concentrations in blood samples

| Patient № | Ethanol (g/L) | Methanol (g/L) | Patient № | Ethanol (g/L) | Methanol (g/L) |
|---|---|---|---|---|---|
| 1 | 3.60 | 0.034 | 14 | 4.13 | 0.020 |
| 2 | 0.82 | 0.040 | 15 | 4.40 | 0.020 |
| 3 | 3.85 | 0.052 | 16 | 2.14 | 0.032 |
| 4 | 3.57 | 0.052 | 17 | 0.65 | 0.043 |
| 5 | 3.26 | 0.040 | 18 | 1.02 | 0.020 |
| 6 | 4.24 | 0.044 | 19 | 3.08 | 0.022 |
| 7 | 3.27 | 0.022 | 20 | 3.23 | 0.028 |
| 8 | 4.13 | 0.027 | 21 | 3.01 | 0.065 |
| 9 | 5.26 | 0.034 | 22 | 4.41 | 0.076 |
| 10 | 0.30 | 0.020 | 23 | 1.57 | 0.020 |
| 11 | 2.97 | 0.034 | 24 | 1.79 | 0.040 |
| 12 | 4.87 | 0.037 | 25 | 5.74 | 0.020 |
| 13 | 0.66 | 0.030 | | | |

**Table 4.** Range and mean value of the data set

| Alcohol type | Concentration range min-max (g/L) | Average concentration (g/L) | Standard deviation |
|---|---|---|---|
| Ethanol | 0.30-5.74 | 3.04 | 1.54 |
| Methanol | 0.020-0.076 | 0.035 | 0.015 |

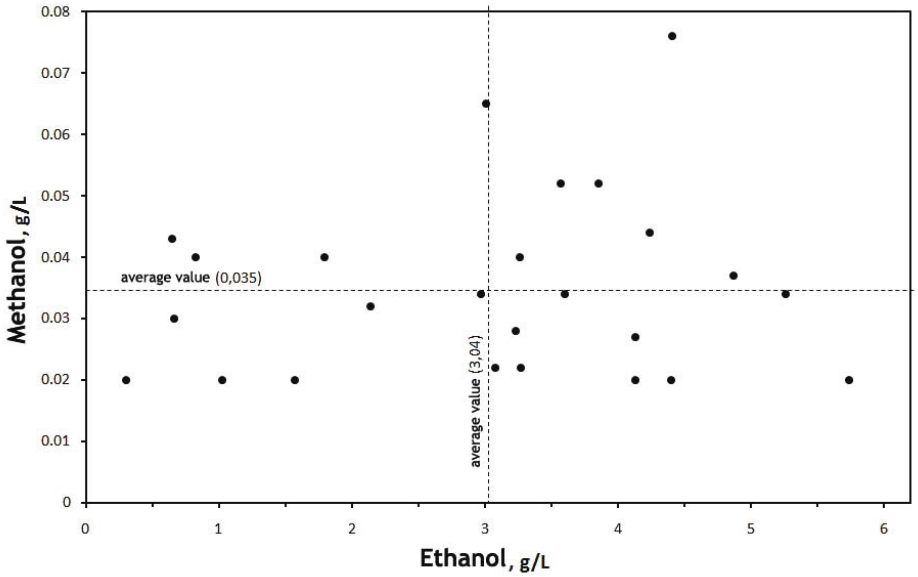


**Figure 4.** Comparison of ethanol and methanol concentrations in blood samples

## Discussion

One of the possible reasons for the presence of methanol in the studied group of patients could be that methanol enters as a concomitant toxin with the consumed alcohol drink. The history of the patients revealed a variety of sources of alcoholic drinks - from shops and stalls to disco clubs and luxury hotels. Possibly, the presence of methanol was due to mechanical admixture with commercial purposes or to domestic distillates with poor division of ethanol from the methanol.

The discussion about the harm of low concentrations of methanol on human organisms goes beyond the scope of clinical toxicology.

In toxicology practice, chronic consumption of alcohol drinks containing methanol is associated with the risk group of alcohol addicts, the so-called chronic drinkers. We consider that their prolonged methanol subintoxication puts them at risk because the increased plasma levels of methanol are directly related to the pathogenesis of the chronic alcoholism and the organ damages it causes [13, 14].

The significance of subclinic methanol intoxication for public health has increased after the publications on the pathogenetic role of methanol in connection with the so-called "diseases of civilization". They are connected not so much with incidental or periodical consumption of methanol with alcohol drinks but with the small quantities methanol produced by the metabolism of aspartame [15] and with the chronic inhalation of methanol vapors [16, 17]. Formic acid, generated by the metabolism of methanol, can induce multiple sclerosis ("methanol hypothesis") [16-18] and parkinsonism [19]. The publications about the eventual connection between methanol and cancerogenesis are not less alarming [20]. In his survey Monte has emphasized that the outbreaks of the disease of Alzheimer, multiple sclerosis, atherosclerotic cardiocirculatory diseases, lupus, autism and other diseases of civilization can be directly connected to the legally permitted aspartame since 1981 [15].

## Conclusions

Methanol in blood was found in an alarmingly large number (about 30%) of cases after consumption of alcoholic drinks. The blood methanol concentration of the studied cases was below the toxic value (0.200 g/L) but the

difference was not comfortingly wide – the average measured value (0.035 g/L) was only 5 to 6 times lower than the toxic, and in single cases it was even closer to the toxic value. The established data can not be explained with the natural content of methanol in blood, because all the measured values were greater than the normally expected value (0.007 g/L). The obvious explanation for the presence of methanol in the blood of the studied patients is that it enters the organism as a concomitant toxin together with ethanol after consumption of alcoholic drinks. It appears that the major factor in the studied methanol subintoxication was not the quantity of the consumed alcoholic drink, but its quality and the contents. This is an especially insidious factor as it is essentially unpredictable.

The fact that the indicated toxic concentration of methanol (0.200 g/L) was relative and corresponded explicitly with acute intoxication, should be taken into consideration. Therefore, the question of chronic action of methanol in the indicated doses (0.01-0.2 g/L) arises as a natural continuation of the study.

## References


1. National Institute for Occupational Safety and Health (NIOSH): Methyl alcohol [Internet]. Atlanta (GA): NIOSH; 1994 [updated 2014 Dec 4; cited 2015 Nov 15]. Available from: http://www.cdc.gov/niosh/idlh/67561.html.
2. Seyffart G. Poyson index: The treatment of acute intoxication. Lengerich: Pabst Science; 1997.671 p.
3. Barceloux DG, Bond GR, Krenzelok EP, Cooper H, Vale JA, American Academy of Clinical Toxicology Ad Hoc Committee on the Treatment Guidelines for Methanol Poisoning. American Academy of Clinical Toxicology practice guidelines on the treatment of methanol poisoning. J Toxicol Clin Toxicol. 2002;40(4):415-46.
4. Schulz M, Schmoldt A. Therapeutic and toxic blood concentrations of more than 800 drugs and other xenobiotics. Pharmazie. 2003;58(7):447-74.
5. Malcolm Pirnie, Inc. Evaluation of the fate and transport of methanol in the environment [Internet]. Washington (DC): American Methanol Institute; 1999 [cited 2015 Dec 15]. 57 p. Available from:http://www.methanol.org/Environment/Resources/Environment/MP-Methanol-Fate.aspx.
6. Heward A. PN 06/14 (NAM7): Upgraded MERLIN spies cloud of alcohol spanning 288 billion miles [Internet]. London: Royal Astronomical Society; c1996-2015 [cited 2015 Nov 15]. Available from: https://www.ras.org.uk/search/article-archive/973-pn-0614-nam7-upgraded-merlin-spies-cloud-of-alcohol-spanning-288-billion-miles.
7. Monte WC. Aspartame; methanol and the public health. J Appl Nutr. 1984;36(1):42-58.
8. Turner C, Spanel P, Smith D. A longitudinal study of methanol in the exhaled breath of 30 healthy volunteers using selected ion flow tube mass spectrometry, SIFT-MS. Physiol Meas. 2006;27(7):637-48.
9. Sedived V, Mraz M, Flek J. Biological monitoring of persons exposed to methanol vapours. Int Arch Occup Environ Health. 1981;48(3):257-71.
10. Lesch OM, Walter H, Wetschka CH, Hesselbrock M, Hesselbrock V. [Alcohol and tobacco: Medical and sociological aspects of use, abuse and addiction]. Sofia: Tempora; 2011. Bulgarian.
11. Schmidtke LM, Blackman JW, Agboola SO. Production technologies for reduced alcoholic wines. J Food Sci. 2012;77(1):R25-41.
12. Radanov S. [Forensic medicine]. Sofia: Siela; 2001. Bulgarian.
13. Gilg T. Methanol and congeners as markers of alcohol use and abuse. In: Wurst MF, editor. New and upcoming markers of alcohol consumption. Darmstadt: Steinkopff-Verlag; 2001. p. 35-52.
14. Mechkarska B, Hubenova A, Stankova E, Gesheva M, Loukova A. The role of methanol, isopropanol and acetone as possible markers for chronic ethanol consumption – preliminary results. In: Programme and abstracts EAPCCT 26th international congress; 2006 Apr 19-22; Prague, Czech Republic. Prague: European association of poisons centres and clinical toxicologists. p. 165.
15. Monte WC. Methanol: a chemical Trojan horse as the root of the inscrutable U. Med Hypotheses. 2010;74(3):493-6.
16. Kavet R, Nauss KM. The toxicity of inhaled methanol vapors. Crit Rev Toxicol. 1990;21(1):21-50.
17. Andrews LS, Clary JJ, Terrill JB, Bolte HF. Subchronic inhalation toxicity of methanol. J Toxicol Environ Health. 1987;20:117-24.
18. Henzi H. Chronic methanol poisoning with the clinical and pathologic-anatomical features of multiple sclerosis. Med Hypotheses. 1984;13(1):63-75.
19. Hageman G, van der Hoek JAF, van Hout MSE, van der Laan G, Steur EJ, de Bruin WI. Parkinsonism, pyramidal signs, polyneuropathy, and cognitive decline after long-term occupational solvent exposure. J Neurol. 1999;246(3):198-206.
20. Bailey LA, Prueitt RL, Rhomberg LR. Hypothesis-Based Weight-of-Evidence evaluation of methanol as a human carcinogen. Regul Toxicol Pharmacol. 2012;62(2):278-91.