Toxicology and the biological role of methanol and ethanol: Current view

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Background. Alcohol variants such as ethanol and methanol are simple organic compounds widely used in foods, pharmaceuticals, chemical synthesis, etc. Both are becoming an emerging health problem; abuse of ethanol containing beverages can lead to disparate health problems and methanol is highly toxic and unfit for consumption.

Methods and Results. This review summarizes the basic knowledge about ethanol and methanol toxicity, the effect mechanism on the body, the current care of poisoned individuals and the implication of alcohols in the development of diseases. Alcohol related dementia, stroke, metabolic syndrome and hepatitis are discussed as well. Besides ethanol, methanol toxicity and its biodegradation pathways are addressed.

Conclusions. The impact of ethanol and methanol on the body is shown as case reports, along with a discussion on the possible implication of alcohol in Alzheimer's disease and antidotal therapy for methanol poisoning. The role of ethanol in cancer and degenerative disorders seems to be underestimated given the current knowledge. Treatment in case of poisoning is another issue that remains unresolved even though effective protocols and drugs exist.

Key words: ethanol, methanol, ethylene glycol, alcohol dehydrogenase, cancer, Alzheimer's disease, acetaldehyde dehydrogenase, gamma-aminobutyric acid receptor, fomepizole, alcohol, catalase, P450

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INTRODUCTION

Ethanol and methanol, the two very simple alcohols, play a significant role as precursors in chemical synthesis and/or as solvents. Additionally, ethanol, the less toxic compound of the two, is used in pharmacology for drug dissolution and in the food industry. Alcohol consumption varies considerably country-to-country around the globe^{1,2}, with disproportionate intake of significant volumes of alcohol among social groups³. The consumption of alcoholic beverages is known to be positively associated with lifestyle diseases, such as cancer⁴. These effects and the knowledge assembled thus far clearly emphasize the relevance of alcohol to health.

Despite the fact that the chemistry of ethanol and methanol is well known and their biological effects have been extensively investigated, they remain an emerging problem. This review is focused on the toxicology of these alcohols, including proposals and discussion pertaining to their regulatory and metabolic pathways. Particular attention is given to lifestyle diseases, metabolic disruptions and toxicology of cases where ethanol is inadvertently or criminally substituted by methanol.

Production and use of the alcohols

The production of alcoholic beverages containing ethanol from fermentation has been going on for centuries and it is very difficult to precisely ascertain when Man first obtained the ability to prepare such drinks. Kefir has been around for probably more than 2000 years and it still remains as one of the main fermented milk products⁵. Another alcoholic beverage, wine, was known to

ancient civilizations in the same period, or even earlier, as iron. The production, use and marketing of wine in Ancient Greek and Chinese civilizations is a testimony of this fact^{6,7}. However, wine planting can be traced back to earlier ancient times⁸ – wine growing in Georgia since the 6th millennium BC (ref.⁹).

The content of ethanol in alcoholic beverages varies considerably. Generally, we distinguish fermented and distilled alcoholic beverages. In fact, some alcohols with higher ethanol content are frequently prepared by dissolving tasty additives (flavourings) in ethanol water solution. In the European Union countries, rules regarding alcoholic beverages are given by the Council Directive 92/83/EEC. Alcoholic beverages have ethanol content higher than approximately 0.75 – 1% (v/v). Drinks having alcohol content below this level are not considered as alcoholic beverages. In fermented alcoholic beverages, non-alcoholic beer has alcohol content under 0.5% v/v (ref.^{10,11}) while alcoholic beer has ethanol typically up to 5.5% (ref.^{12,13}). Standard alcohol content in wine is 12.5 – 13.5% v/v (ref.¹⁴). However, the content of ethanol in beverages can be different in disparate countries and regions. In the Czech Republic, for example, the typical content of ethanol in beers is 3-4%, respectively 4-5% (beer strength 10°, respectively 12°) and 10 – 13% v/v for wines¹⁵⁻¹⁸. Fermented alcoholic beverages are mainly produced using the yeast *Saccharomyces cerevisiae*, *S. eubayanus* and *S. pastorianus* (formerly *S. carlsbergensis*), but other yeasts such as *Kluyveromyces* sp. can be involved in the fermentation process of some beverages such as tequila¹⁹. Kefir is an exception from this point of view because it is created by kefir grains containing inoculated

lactobacilli and yeast²⁰. It is not a true alcoholic beverage since the ethanol content is around 0.5% v/v for kefir prepared by modern technologies²¹.

Since ethanol is cytotoxic in high concentration, yeast is used in limited amounts as ethanol concentration of up to 16% v/v can be reached with fermentation alone. However, standard yeast has high mortality when ethanol concentration of 10% v/v is reached²². Alcoholic beverages with higher content of ethanol are prepared by distillation and some biotechnological processes are also proposed to have higher efficacy in ethanol production^{23,24} e.g. whisky has around 40% v/v (ref.²⁵), the same is true for ethanol in tequila²⁶, and the typical rum has around 40% (ref.²⁷). In the Czech Republic, the common local modification of rum has around 38% v/v. The approximate content of alcohol in alcoholic beverages is summarized in Table 1.

Alcohols are suitable for dissolving drugs that are not soluble in water. Both ethanol and methanol can be used for these purposes; nevertheless, ethanol should be preferred because of its low toxicity²⁸⁻³⁰. The alcohols are frequently applied in organic synthesis where it can serve for dissolving intermediates, extraction of intermediates or precursors^{31,32} and as anti-freeze liquids³³. Usage as biofuel and in fuel-cells is another application for the alcohols^{34,35}. Their role in organic synthesis and energy applications are, however, less important from the toxicological point of view when compared to alcoholic beverages and drugs since poisoning can only be accidental.

Metabolism and toxicity arising

Alcohols are sensitive to chemical or physical oxidation³⁶. Hence, the major pathway for ethanol and methanol detoxification is based on alcohol dehydrogenase and acetaldehyde dehydrogenase that oxidizes the alcohols to acids. However, other less specific biochemical pathways can play a role in the metabolism. In the brain, ethanol is dominantly oxidized by catalase (EC 1.11.1.6) with contemporary consumption of cytotoxic hydrogen peroxide leading to acetaldehyde formation³⁷. The catalase is inducible by ethanol³⁸ and is responsible for approximately 60% of ethanol metabolism in the brain; cytochrome P450 2E1 is the second most important enzyme for degrading ethanol in the brain³⁹. It should be emphasized that the aforementioned pathways based on catalase and cytochrome P450 are not specific to alcohols and many other xenobiotics can be detoxified as well⁴⁰. Cytochrome P450 2E1 is an important part of the microsomal ethanol oxidizing system (MEOS). The MEOS is located on the

endoplasmic reticulum of hepatocytes and it is involved in the conversion of ethanol to acetaldehyde⁴¹. After a period of heavy drinking, MEOS activity is significantly increased and may be responsible for increased ethanol metabolism⁴². Due to the chemical similarity and proximity, the metabolic pathways are common for ethanol and methanol, although the latter variant has serious negative consequences.

Alcohol dehydrogenase (EC 1.1.1.1) is an enzyme dominantly present in the liver. In humans, five types of alcohol dehydrogenase labelled as I - V can be found⁴³. Beside the major groups, some atypical alcohol dehydrogenases are known as well. As an example, atypical alcohol dehydrogenase from the liver of some Caucasians has been shown to have higher specificity and lower pH optimum than the common alcohol dehydrogenases^{44,45}. The enzyme uses NAD⁺ as a co-substrate and it contains Zn^{II+}. Zinc is necessary for stabilizing the alcohol dehydrogenase structure, the enzyme then can assume a stable homodimeric form that is the most common⁴⁶. During the catalysation reaction, alcohol is oxidized to aldehyde while NAD⁺ is reduced to NADH (ref.^{47,48}). Some bacteria use NADP⁺ rather than NAD⁺. The NADP⁺ dependent alcohol dehydrogenase from *Ralstonia* sp. can be cited here as an example⁴⁹.

Aldehyde dehydrogenase (EC 1.2.1.3) is the second enzyme that is involved in alcohol oxidation. It oxidizes an aldehyde (formaldehyde and/or acetaldehyde) produced by alcohol dehydrogenase in the first step. NAD⁺ serves as a coenzyme in the reaction again⁵⁰. However, the specificity is much broader and many other aldehydes can be oxidized to carboxylic acids. In humans, 19 isoforms of aldehyde dehydrogenase can be found and the isoforms are organized into three groups of isoenzymes indicated as 1, 2 and 3 (ref.⁵¹). The isoenzyme 1 of aldehyde dehydrogenase can be revealed in cancer cells indicating its significance in the diagnosis; however, recent research suggests that aldehyde dehydrogenase 1 can be involved in cancer development^{52,53}. The role of aldehyde dehydrogenase in cancer development is not understood in detail. Besides aldehyde dehydrogenase, formaldehyde dehydrogenase (EC 1.2.1.46) can be involved in the oxidation of formaldehyde to formate with simultaneous reduction of NAD⁺ to NADH (ref.⁵⁴).

Alcohol dehydrogenase oxidizes alcohols to aldehyde form and the resulting aldehydes are further oxidized to carboxylic acids⁵⁵. Ethanol, methanol and ethylene glycol can be introduced as common alcohols. Ethanol is oxi-

Table 1. Content of ethanol in exemplified beverages.

Beverage	Approximate concentration	Ethanol per volume (g/100 mL)	Ref.
Kefir	0.5% v/v	0.4	21
Non-alcoholic beer	0.5% v/v	0.4	10,11
Beer	up to 5.5% v/v (3–5 % v/v in the Czech Rep.)	4.3	12,13
Wine	13% v/v (10–13 % v/v in the Czech Rep.)	10	14
Whisky	40% v/v	32	25
Tequila	40% v/v	32	26
Rum	40% v/v (35 % in the Czech Rep.)	32	27

Fig. 1. Biotransformation of methanol and ethanol on alcohol and aldehyde dehydrogenases.

dized to acetaldehyde by alcohol dehydrogenase and then to acetic acid by aldehyde dehydrogenase. Acetic acid is then degraded via acetylcoenzyme A and the Krebs cycle. The pathway is reversible and some microorganisms can produce acetaldehyde and ethanol in the same way in in reverse direction⁵⁶. Methanol is oxidized to formaldehyde and formic acid (formate in physiological pH) (ref.⁵⁷). The metabolism of ethanol and methanol is shown in figure 1. Ethylene glycol is degraded to glycolaldehyde (by alcohol dehydrogenase) and glycolic acid (by aldehyde dehydrogenase) (ref.⁵⁸). Glycolic acid further undergoes the process to become oxalic acid.

The metabolism of these alcohols is not actual detoxification in the true sense of the word. Aldehydes are quite reactive and toxic compounds and their toxicity, in most cases, is higher than the toxicity of original alcohols. As an example, let's consider acetaldehyde that is a mutagenic substance and considered as a carcinogen^{59,60}. Oxidation of methanol to formaldehyde is a more potent way how alcohols can be activated. Methanol toxicity would be limited if no metabolic activation occurs. On the other hand, the original alcohols including methanol are not completely safe even without the metabolic activation because many pathways and processes in the body can be regulated by them (see next chapter). Alterations in mRNA synthesis was revealed as well⁶¹. Formaldehyde created from methanol or accepted in another manner (pollution, evaporation from man-made resins, etc.) is suspected of having the potential of causing cancer⁶². In a clinical test, formaldehyde caused significant oxidative damage to cells represented by the malondialdehyde level and increase in protein p53, which indicates serious impact on the cell cycle regulation⁶³.

When comparing ethanol and methanol, major toxicological differences can be seen in the terminal product of oxidation. While ethanol is oxidized to harmless acetate in

Fig. 2. Ethylene glycol metabolism.

the process involving conversion to acetylcoenzyme A and further oxidation via the Krebs cycle, formate is the terminal product of methanol oxidation. Formate is a toxin that can react with many target structures, the mitochondrial cytochrome oxidase being the most relevant⁶⁴. Only formate alone is responsible for the typical manifestation of methanol poisoning (dysfunction of retina followed by irreversible damage); however, other manifestations such as metabolic acidosis is also typical^{65,66}.

Interaction with receptors and regulatory pathways

In the body, ethanol acts as a modulator of several receptors, including receptors in the central nervous system. γ -aminobutyric acid receptor (GABA) is responsible for excitation caused by ethanol⁶⁷. In standard conditions, GABA receptors are Cl^-/K^+ channels (GABA_A) or G protein coupled protein (GABA_B) involved in the reduction of neuronal excitability in the both the central and peripheral nervous systems⁶⁸. Ethanol acts as a positive allosteric modulator of GABA receptor⁶⁹ and the evidence of modulation of both GABA_A and GABA_B can be derived from literature⁷⁰⁻⁷².

N-methyl-D-aspartate (NMDA) receptor is another target for ethanol. It is a glutamate receptor acting as a non-selective ion channel in neurosynaptic clefts and elsewhere, and it plays an important role in memory^{73,74}. Sympathoexcitation is another effect evoked by ethanol via the NMDA receptor⁷⁵. Ethanol can also interact with the NMDA receptor as a negative allosteric modulator^{76,77}. Long term cognitive dysfunction such as memory impairment, mental disability, etc., in alcoholism can be attributed to the effect on GABA and MNDA receptors^{78,79}.

Besides the aforementioned receptors, ethanol interacts with some other receptors as a positive allosteric modulator. **Nicotinic acetylcholine receptor (nAChR)**, **glycine receptor** and **serotonin receptor** can be introduced as

the most relevant ones. nAChR and glycine receptor are both pentameric ion channels with similar mechanism of interaction with alcohols⁸⁰. Both receptors play an important role in the nervous system and they can be found in the central and peripheral parts of the nervous system and their presence in effector cells such as the cells of immune system for nAChR or retina for glycine receptor are known as well⁸¹⁻⁸³. The neuronal nAChR probably plays a significant role in the development of alcoholism when considering indirect evidence. Alcohol is frequently co-abused with nicotine accepted via smoking and the role of ethanol in the mesolimbic pathway is also discussed⁸⁴. Abuse of alcohol can be reduced by the application of another nAChR modulator, as proven with varenicline⁸⁵.

Ethanol effect via glycine receptors has a significant impact on alcohol abuse because of the intermediation by dopamine activating effects⁸⁶. Motor impairment and sexual function alteration by glycine receptors via stria terminalis is a significant action of ethanol as well^{87,88}. The function of other receptors in the both the central and peripheral nervous systems can be influenced by the serotonin receptor and ethanol can have an effect when mediated in this manner^{89,90}.

Methanol is structurally very similar to ethanol, sharing many similarities also in interaction with the receptors. As an example, methanol acts as a negative allosteric modulator of NMDA and the ability to block NMDA by methanol is very similar to ethanol and butanol⁹¹. Similar findings can be ascertained for the GABA receptor that has binding sites for low molecular weight alcohols, including methanol and ethanol⁹². From the global point of view, the both methanol and ethanol are able to bind to pentameric ligand-gate ion channels, such as the glycine receptor, nAChR, etc.⁹³. No significant difference in the ability to bind to the receptors can be revealed when methanol and ethanol are compared. Considering the aforementioned text, methanol and ethanol are nearly the same compounds from the biological point of view. The major difference between the two alcohols is in the toxicity of their metabolic products. Up to the creation of respective metabolic products, methanol and ethanol have the same impact on the body and the same target structures. The relevant interactions of these alcohols with the receptors are summarized in table 2. Apart from the significant target structures in the body, these alcohols can interact with other biomolecules - the inhibition of acetylcholinesterase enzyme by organic solvents including methanol and ethanol is an example^{94,95}. This inhibition is

apparent when the concentration of the alcohols exceeds approximately 5% (w/w). Such a dose is, however, too high to have biological relevance.

Treatment of poisoning with the alcohols

Poisoning with ethanol is typically manifested by aberration in behaviour, confusion and unpredictable mental reactions. Life threatening states such as respiratory depression, seizures, etc., are the most serious consequences of overdose⁹⁶. Methanol poisoning has some manifestations similar to ethanol; however, serious acidosis⁹⁷ and damage to some tissues such as retina leading to blindness can be expected⁹⁸. Neurological disability with quite highly frequent extrapyramidal disorders can arise in serious cases⁹⁹. Poisoning with alcohols can be ameliorated by the application of hemodialysis¹⁰⁰. In cases of methanol poisoning, hemodialysis significantly reduces the half-life formate: 1.8 h comparing to 6.0 h in untreated case¹⁰¹. Intermittent hemodialysis, continuous veno-venous hemodialysis and hemodiafiltration can be exemplified as other effective techniques¹⁰². Hyperventilation is presented as another available supporting therapy during severe poisoning¹⁰³.

Drinking of ethanol or ethanol containing beverage is the simplest antidotal therapy during poisoning with the most toxic alcohols such as methanol or ethylene glycol. Ethanol competes with the more toxic alcohols on alcohol dehydrogenase. Methanol and/or ethylene glycol do not undergo to the more toxic forms and are eliminated via the kidneys without causing irreversible effect on the human body¹⁰⁴. It is noteworthy that the use of ethanol as an antidote remains, in combination with hemodialysis, the most frequently used protocol in methanol poisoning therapy¹⁰⁵. Pharmacotherapy of alcohols poisoning is available and highly effective if administered promptly. Fomepizole (Fig. 3) is the drug of first choice for poisoning with methanol or ethylene glycol¹⁰⁶ and it is effective and well tolerated even by children^{107,108}. The mechanism of fomepizole action is very similar to that of ethanol. Fomepizole, like the other pyrazoles, is a competitive inhibitor of alcohol dehydrogenase and prevents the formation of toxic inter-metabolites¹⁰⁹. The efficacy of fomepizole in serious poisoning can be perceived from a case report where a poisoned man was rescued even despite initial acidosis with pH under 6.5 (ref.¹¹⁰). The half-life of methanol and formate is quite long when fomepizole administered. In a clinical report encompassing seven cases, the half-life of methanol was 71 h in fomepizole treated patients, decreas-

Table 2. Relevant target receptors for ethanol and methanol.

Receptor type	Effect of ethanol and methanol	Ref.
GABA receptor	positive allosteric modulator	69-72,92
NMDA receptor	negative allosteric modulator	75-79 91
nAChR	positive allosteric modulator	80,84,93
serotonin receptor	positive allosteric modulator	89,90
glycine receptor	positive allosteric modulator	80,86-88 93
acetylcholinesterase	inhibitor	80, 81

ing to 2.5 h because of hemodialysis¹¹¹. The elimination half-life of formate is longer than methanol. In a fomepizole treated patient, the half-life of formate was 77 h when no dialysis was applied and 2.9 h when the combination of fomepizole and hemodialysis was chosen¹¹². Due to the fact that fomepizole is highly effective, no or delayed hemodialysis is necessary in cases of methanol poisoning treated early just by fomepizole after alcohol intake¹¹³.

In the early 1980s, use of folic acid as an antidote to methanol poisoning was proposed and tested on a monkey¹¹⁴. Consequently, the mechanism of methanol detoxification was proposed and steps leading to oxidation to carbon dioxide were revealed. In the first step of the detoxification, formate reacts with tetrahydrofolate to 10-formyltetrahydrofolate by formate-tetrahydrofolate ligase (EC 6.3.4.3) (ref.¹¹⁵). One molecule of ATP per one molecule of formaldehyde is oxidized in the reaction. Reduction of 10-formyltetrahydrofolate by formyltetrahydrofolate dehydrogenase (EC 1.5.1.6) to tetrahydrofolate and carbon dioxide in the liver is the final reaction^{116,117}. One molecule of NADP⁺ per one molecule of 10-formyltetrahydrofolate is reduced. The principle of the detoxification is depicted in figure 4. Currently, folic acid is recommended as an antidote in cases of methanol poisoning in human medicine^{118,119}. Additionally, it was proved that formic acid is suitable for mitigating neurotoxicity pursuant to chronic ethanol intake¹²⁰.

Alcoholism and consequent disorders

Chronic ethanol toxicity is an emerging problem. Comparing to ethanol, the other alcohols are less important regarding chronic toxicity and a serious problem can arise when one high dose of such compounds is consumed. Acute methanol toxicity was the subject of the lengthy discussion above; regarding ethanol, its role in the development of some degenerative disorders and cancer is considered in drinkers but full understanding of the issue is rather illusive when taking moderate and occasional drinkers into account.

Alcoholic beverage is currently evidenced as a risk factor in cancer even though no control is established on it¹²¹ and the risk because of alcohol intake is not fully rated and the association is plausibly evidenced for regular drinkers rather than for moderate consumers¹²². Ethanol containing beverages are a risk because of ethanol metabolizing to acetaldehyde and the consequent reaction between acetaldehyde and DNA (ref.¹²³). The fact that cancer is linked to acetaldehyde produced from ethanol is supported by the proven association between cancer risk and activity of alcohol dehydrogenase in drinkers^{110,124-126}. However, other pathological pathways may be incorporated into the mechanism, such as depletion of low molecular weight antioxidants followed by oxidative stress¹²⁷. More work to prove the link between alcohol and cancer should be done prior to making a plausible conclusion.

Fig. 3. Fomepizole.

Fig. 4. Formate detoxification based on tetrahydrofolate.

For the moment, ethanol can be considered rather as a risk factor than a causative agent.

Despite the expectation that alcohol consumption, as the other unhealthy lifestyles, can lead to the development of some neurodegenerative conditions, recent findings are not so convincing. The assumption that alcohol can play a negative role in neurodegenerative disorders is supported by the fact that oxidative stress, following alcohol intake, is significantly implicated in pathological processes¹²⁸. However, no association between ethanol, acetaldehyde and Alzheimer disease has been established¹²⁹. On the contrary, ethanol is able to mitigate the deposition of amyloid plaque *in vitro*¹³⁰. Formation of amyloid plaque is necessary for the development of Alzheimer disease and vascular dementias manifestation¹³¹. It is questionable whether the inhibition of amyloid plaque deposition can be of significance in the human body. On the other hand, the effect is visible even in ethanol concentrations of 0.02 – 0.08% (w/w); so, some degree of biological relevance can be expected¹³². If the effect is successfully proved in viable organisms, then ethanol could serve as a protective measure in the early stages of dementia and as a drug slowing down disease progression. Chronic intake of alcohol is typically followed by neurodegeneration of unknown aetiology known as alcohol related brain dementia^{133,134}. The effect is probably caused by a combination of neuro-inflammation, chronic activation of microglia, and imbalances in glutamate and dopamine signalling pathways^{135,136}.

Alcohol drinking is a risk factor for stroke and cardiovascular diseases¹³⁷. The aetiology of stroke is not clearly known but besides alcohol, diabetes, smoking, hyperlipidemia, hypertension and other are documented risk factors as well¹³⁸. The risk of cardiovascular diseases can be enhanced in metabolic syndrome. The metabolic syndrome alone is another possible consequence of heavy alcohol consumption even though the mechanism of onset of the syndrome remains unclear^{139,140}. The association between liver diseases and alcoholism is also known¹⁴¹ and in alcohol liver diseases the activation of ethanol to acetaldehyde and alcohol induced fibrosis and inflammation takes place resulting in hepatitis manifestation after a period of heavy drinking¹⁴²⁻¹⁴⁴.

Example of case reports

Alcoholism is both a phenomenon and an issue of global significance with serious consequences for the population's health and for healthcare costs¹⁴⁵⁻¹⁴⁸. Serious overdosing with ethanol is not an exceptional event and it ranks among overall abuse of alcohol. Though adults are quite resistant to ethanol and most cases can be successfully treated, children are highly vulnerable and ethanol intake can have serious consequences including life threatening states. This fact is evidenced in the stated case reports^{149,150}.

With accidental methanol poisoning, the effects are much more serious and grave than with ethanol. Sporadic cases of methanol poisoning can occur; however, incidents involving a higher number of victims are not rare: the paper by Celik and coworkers¹⁵¹. The authors analysed legal medical autopsies conducted in the Ankara Branch of the Council of Forensic Medicine (Turkey) for the period 2001-2011. In total, 10,720 fatal cases were analysed of which 35 cases were due to methanol and 39 due to ethanol poisoning. The median blood concentration in fatal cases of alcohol poisoning was 2.63 g/L for methanol and 2.36 g/L for ethanol. While poisoning by ethanol is a result of voluntary drinking, methanol can get into alcohol beverages through inadvertent substitution of ethanol. The resulting effect can be judged from the discussed affairs.

Very serious mass poisoning occurred in Parnu, Estonia, in 2001. Canisters with methanol were stolen from a local company and the offenders, thinking it to be ethanol, prepared alcohol that was promptly sold on the black market¹⁵². This unfortunate incident culminated in a high toll of 154 cases with 68 deaths¹⁵³.

In Europe, another methanol tragedy happened in the Czech Republic. Besides the Czech Republic, fatal cases of poisoning from alcohol made in the Czech Republic occurred in Poland and Slovakia resulting in 51 fatalities and more than one hundred poisoned since the affair began in September 2012 (ref.^{154,155}). In the Czech methanol affair, alcohol was distributed throughout the black market; however, poisoned alcohol was discovered in regular shops as well. Comparing to the tragedy in Estonia, the Czech methanol affair was more systematic involving a wide network of small companies and individual persons. From

Table 3. Role of alcoholism in disorders.

Disorder	Effect of ethanol	Pathway	Ref.
cancer	risk factor; suspected as a causative agent	via acetaldehyde	122-125
Alzheimer disease and vascular dementia	no effect or positive effect	inhibition of amyloid plaque deposition	129-132
Alcohol related brain dementia	causative agent	not known, probably via neuroinflammation and/or changes in glutamate and dopamine pathways	133-136
Stroke	risk factor	not known	137,138
Metabolic syndrome	risk factor	not known	139,140
Alcoholic liver diseases	causative agent	damage to functional tissue and causing inflammation, replacement of the tissue by fibroblasts	142-144

the wider perspective, the methanol affair stemmed from the introduction of the European Directive 1272/2008 of 16 December 2008. Before the directive came into force, the sale of methanol was strongly regulated in the Czech Republic. Following implementation of the directive, it was allowed for use and methanol was sold in final products such as windshield washer liquids. Companies and individuals deeply involved in the affair legally bought methanol for these purposes and only some of them had the trade certificate for windshield washer liquids production. However, they used it illegally to produce cheap alcohol. Initially, relatively low toxic mixture of ethanol and methanol was produced; however, prior to their arrests, the perpetrators produced alcohols such as rum containing methanol only. The Czech authorities remained oblivious to the situation because of no past experience and no knowledge of the measures to be taken when the first cases of methanol poisoning surfaced. It is, however, noteworthy that the orchestrators of the crime were sentenced to life imprisonment as an exceptional punishment. These affairs, thankfully, did not involve exhausting numbers of affectees when compared to similar cases worldwide¹⁵⁶⁻¹⁵⁸. However, they illustrate the significance and seriousness of incidental poisoning by methanol.

Conflict of interest: None declared.

CONCLUSIONS

Ethanol and methanol are not highly toxic in the true sense of the word. However, easy accessibility to these variants combined with their legal sale makes them an emerging concern for human health. Additionally, the role of ethanol in cancer and degenerative disorders seems to be underestimated. Comparing to ethanol, methanol is a toxic compound with high incidence of poisoning worldwide. Treatment of poisoning by these alcohol variants is another problem that remains unresolved even though effective protocols and drugs exist.

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