

DISSERTATION FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Health risks associated with consumption of recorded and unrecorded
spirits in Albania and Hungary

by Teuta Muhollari

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DOCTORAL SCHOOL OF HEALTH SCIENCES

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Supervisor: Dr. László Pál



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Table of Contents

List of abbreviations.....	3
List of figures	6
List of tables	8
1. Introduction	9
1.1. The origin of alcohol consumption.....	9
1.2. The definition and types of alcoholic beverages	10
1.2.1. Definition of alcoholic beverages	10
1.2.2. Most consumed types of alcoholic beverages.....	12
1.3. Level of alcohol consumption and drinking patterns	15
1.4. Recorded and unrecorded alcoholic beverages	17
1.5. Health consequences of alcohol consumption.....	19
1.6. Contaminants in alcoholic beverages	21
1.6.1. Aliphatic alcohols	21
1.6.2. Metalloids and metals	23
1.6.3. Ethyl-carbamate	24
1.6.4. Acetaldehyde.....	25
1.6.5. Other contaminants	26
2. Aims of the study	27
3. Materials and methods.....	29
3.1. Collection of spirit samples in Albania and Hungary.....	29
3.1.1. Gas chromatographic mass spectrometric analysis of spirit samples collected in Albania and Hungary	30
3.1.2. Inductively coupled plasma optical emission spectrometric analysis of spirit samples collected in Albania and Hungary.....	31
3.1.3. Comparison of heavy metal concentrations in spirits collected in Albania and Hungary with limit values.....	33
3.1.4. Estimation of health risks related to the intake of heavy metals in recorded and unrecorded spirits collected in Hungary	34
3.1.5. Statistical analysis of data on spirits collected in Albania and Hungary	38
3.2. Estimation of health risk from intake of methanol in unrecorded spirits	39
3.2.1. Sources of data on the concentration of methanol in unrecorded fruit spirits	39
3.2.2. Compliance of methanol concentrations with AMPHORA limits	43
3.2.3. Estimation of exposure to methanol and related blood methanol levels considering different levels of alcohol consumption.....	43

3.2.4. Statistical analysis	47
4. Results	48
4.1. Estimation of health risks associated with the consumption of recorded and unrecorded spirits collected in Hungary	48
4.1.1. Concentration of heavy metals in recorded and unrecorded spirits collected in Hungary.....	48
4.1.2. Compliance of levels of heavy metals in recorded and unrecorded spirits collected in Hungary with AMPHORA limits	50
4.1.3. Results of factor analysis	53
4.1.4. Health risks associated with the consumption of heavy metals in recorded and unrecorded spirits collected in Hungary	54
4.2. Composition of unrecorded rakia collected in Albania	59
4.2.1 Characteristics of unrecorded Albanian rakia samples	59
4.2.2. Results of comparison of reported and measured ethanol concentrations of the rakia samples.....	62
4.2.3. Concentration of elements in unrecorded Albanian rakia.....	62
4.3. Estimation of health risk from intake of methanol in unrecorded spirits	66
4.3.1. Characteristics of unrecorded fruit spirit samples analysed in the included studies	66
4.3.2. Estimated daily methanol and ethanol intake associated with the consumption of unrecorded fruit spirits	68
4.3.3. Estimated blood methanol and ethanol levels associated with the consumption of unrecorded fruit spirits	74
5. Discussion	79
6. Conclusions	86
7. Recommendations for future research	87
8. Limitations	89
9. Summary	90
10. References	93
Key words	119
Acknowledgements	120
List of publications.....	122
Appendix	124

List of abbreviations

AET	average exposure time
AFR	African Region
AIC	Akaike information criterion
AMPHORA	The Alcohol Measures for Public Health Research Alliance project
AMR	Region of Americas
APC	alcohol per capita consumption
A_e	amount of alcohol consumed
A_m	distribution of the amount of ethanol consumed
BCE	before common era
BEL	blood ethanol level
BMDL	the lower one-sided confidence limit of the benchmark dose
BML	blood methanol level
BW	body weight
C_{be}	distribution of blood ethanol level
C_{bm}	distribution of blood methanol concentration
C_e	the level of ethanol in 40% V/V unrecorded fruit spirits
CEE	Central and Eastern European
CF	conversion factor
CHD	coronary heart disease
CI	confidence interval
C_m	concentration of methanol
DALYs	Disability Adjusted Life Years
DBW	distribution of body weight
DCUFS	daily consumption of unrecorded fruit spirits
ED	exposure duration
EDEI	estimated daily ethanol intake
EDI	estimated daily intakes
EDMI	estimated daily methanol intake
EF	exposure frequency
EMR	East Mediterranean Region
EU	European Union

EUR	European Region
GC/MS	gas chromatography / mass spectrometry
H₂O₂	hydrogen-peroxide
HED	heavy episodic drinking
HFS	hypothetical fruit spirit
HNO₃	nitric acid
HP	Hewlett-Packard
IARC	International Agency for Research on Cancer
IBM	International Business Machines Corporation
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
IQR	interquartile ranges
ISTD	internal standard
LOD	limit of detection
MCS	the concentration of different heavy metals in spirits
MDI	mass of daily intake
MLs	maximum levels
MSD	mass selective detector
MTBML	maximum tolerable blood methanol level
OAAs	other aliphatic alcohols
p.a.	pure alcohol
R	the ratio of 1000 ml and the volume of 40% V/V unrecorded fruit spirit containing 10 g, 20 g, 40 g, 60 g, and 80 g of ethanol
RfD	reference dose
r_e	the Widmark's factor used for ethanol
r_m	the Widmark's factor used for methanol
RR	relative risk
SD	standard deviation
SEAR	South East Asian Region
SPSS	Statistical Package for the Social Science
THQ	target hazard quotient
THQ_c	combined target hazard quotient
V/V	volume per volume
WB	Western Balkan

WHO

World Health Organisation

WPR

Western Pacific Region

List of figures

Figure 1. Global differences in the ethanol content of drinks above which the national legislation classifies them as alcoholic beverages

Figure 2. The process of spirit production

Figure 3. Unrecorded alcohol consumption: country-level comparisons to estimated global average

Figure 4. Flowchart of computer-assisted literature search

Figure 5. Levels of heavy metals in recorded and unrecorded spirits collected in Hungary

Figure 6. Groups of related metals in recorded spirits collected in Hungary

Figure 7. Health risk from combined exposure to heavy metals in recorded and unrecorded spirits by average drinkers

Figure 8. Health risk from combined exposure to heavy metals in recorded and unrecorded spirits by regular drinkers

Figure 9. Health risk from combined exposure to heavy metals in recorded and unrecorded spirits by heavy drinkers (version A)

Figure 10. Health risk from combined exposure to heavy metals in recorded and unrecorded spirits by heavy drinkers (version B)

Figure 11. Ethanol concentration of rakia samples by person from whom the samples were obtained and by the GC/MS analysis

Figure 12. Estimated daily methanol and ethanol intakes by males and females when consuming unrecorded fruit spirits containing methanol

Figure 13. Estimated daily methanol and ethanol intakes by males and females when consuming unrecorded fruit spirits containing methanol

Figure 14. Estimated daily methanol and ethanol intakes by males and females when consuming unrecorded fruit spirits containing methanol

Figure 15. Estimated blood methanol and ethanol concentrations in males and females when consuming unrecorded fruit spirits containing methanol

Figure 16. Estimated blood methanol and ethanol concentrations in males and females when consuming unrecorded fruit spirits containing methanol

Figure 17. Estimated blood methanol and ethanol concentrations in males and females when consuming unrecorded fruit spirits containing methanol

List of tables

Table 1. Detection and quantitation limits of heavy metals

Table 2. AMPHORA limit values of heavy metals

Table 3. Default body weight values for adult subjects

Table 4. Volumes of per capita alcohol consumption and daily ethanol intake from recorded and unrecorded spirits considering average consumers, regular and chronic heavy drinkers

Table 5. Oral reference doses of the detected heavy metals

Table 6. Studies selected for data extraction

Table 7. Volume of 40% V/V unrecorded fruit spirits required for the ingestion of 10 g, 20 g, 40 g, 60 g, and 80 g of ethanol

Table 8. Mean levels of heavy metals in recorded spirit samples collected in Hungary and compliance with AMPHORA limits

Table 9. Mean levels of heavy metals in unrecorded spirit samples collected in Hungary and compliance with AMPHORA limits

Table 10. Characteristics of rakia samples analysed

Table 11. Concentration of metals detected in rakia samples collected in Albania

Table 12. Number and characteristics of unrecorded fruit samples analysed in the studies from which data were extracted

Table 13. Methanol concentrations in unrecorded fruit spirits required to reach the reference dose at different consumption levels

Table 14. Levels of methanol in unrecorded fruit spirits required to reach the maximum tolerable blood methanol level at different intake levels

1. Introduction

1.1. The origin of alcohol consumption

Throughout human history, people have regularly consumed ethanol in overripe fruit (1). Spontaneous alcoholic fermentation of glucose, fructose and sucrose in fruits is a natural process and carried out by yeast cells often found on the peel of fruits (2, 3). The exact time when humans began producing alcoholic beverages is uncertain, however there is evidence that beer was consumed in China as early as 9,000–8,700 Before Common Era (BCE) (4). The earliest written records of winemaking date back to 4,000 BCE in ancient Egypt (5, 6). In addition, historical evidence suggests that other ancient civilizations, including Sumerian, Mayan, Babylonian, Hebrew, Hellenic and Persian, developed their own methods of fermentation to produce wine and beer (5, 6). Later, the development of agriculture contributed to higher crop yields, providing the basis for the production of large volumes of alcoholic beverages (5). At the same time, the establishment of permanent settlements increased the demand for these products (5). Eventually, these processes led to alcoholic beverages being produced and consumed more widely around the world (5). Over the millennia, people have valued alcoholic drinks for their nutritional value and often consumed them at social occasions and events (7). These occasions included, but were not limited to, pledging allegiance before battles, celebrating victories and taking oaths (7). Alcohol was and still is consumed in many region of the world as part of ceremonies commemorating important events in life including births, marriages, reunions and funerals (7, 8). However, there has been increasing evidence that excessive alcohol consumption plays a major role in the development of a wide range of diseases and thus poses a risk to the health of the population in many countries (9).

1.2. The definition and types of alcoholic beverages

1.2.1. Definition of alcoholic beverages

According to the Global status report on alcohol and health of the World Health Organization (WHO), there is no universally accepted definition of alcoholic beverages (10). This may be due to the different views of lawmakers and health professionals on alcoholic beverages (10-15). For example, the legislature of 104 countries classifies drinks as alcoholic beverages if they contain at least 0.5% ethanol by volume (**Figure 1**) (10). In contrast, public health researchers in the European Union (EU) place more emphasis on the health effects of alcoholic beverages and define them as “drinkable liquids containing ethanol, a substance rapidly absorbed from the gastrointestinal tract and distributed throughout the body with psychoactive effects” (13). In this work, we define alcoholic beverages by combining these different perspectives (10-15). Thus, an “alcoholic beverage” is *any type of ethanol containing potable liquid of agricultural origin, produced through fermentation and then brewing or distillation of raw materials from fruits, vegetables or grains, acknowledged and recognized for having negative health impacts by public health authorities.*

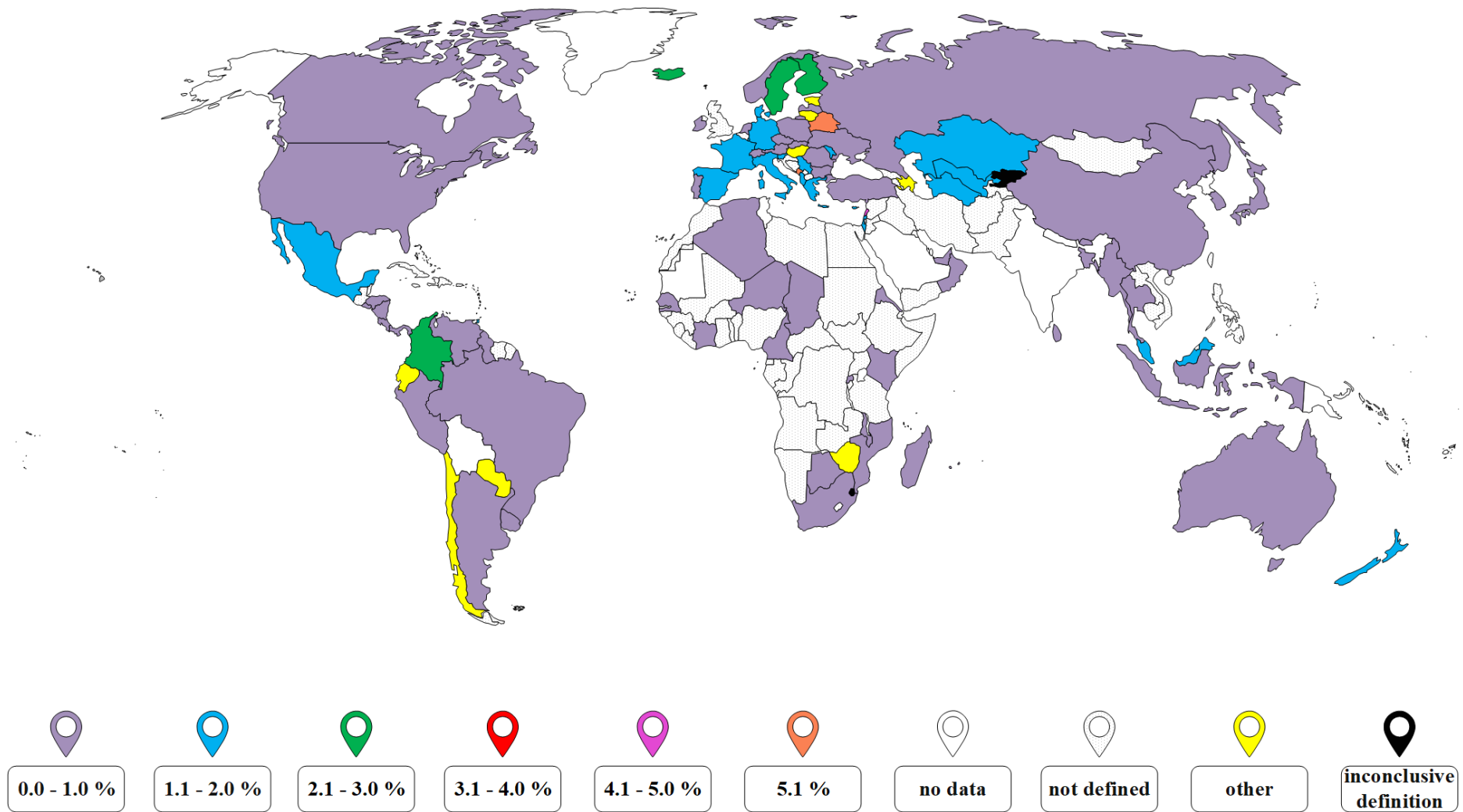


Figure 1. Global differences in the ethanol content of drinks above which the national legislation classifies them as alcoholic beverages [based on data from the World Health Organisation (10)]

1.2.2. Most consumed types of alcoholic beverages

Globally, 90.8% of legally sold alcohol is consumed in the form of wine (11.7%), beer (34.3%) and spirits (44.8%) (10). Wines are usually made from the fermentation of grapes, although they can also be produced from fruits such as apricots, bananas, peaches and plums (16-18). After harvesting, grapes are crushed to extract fruit juice essential for making both white and red wines (19). White grapes are pressed immediately, while red grapes ferment with their skins, seeds and juice (19). This fermentation lasts from days to weeks (20). Subsequently, the wine is clarified and matured, then cleaned and bottled (17, 21-28). The alcohol content of wines usually varies between 11% to 13% Volume/Volume (V/V) (29-31).

Beer is produced by brewing and fermenting the starch present in grains including wheat, rice, corn, and oats (32-36). In the initial stage, malt is produced by soaking the grains in water to induce germination (37). Then, the germinated grains are dried and roasted to produce malt (38). Subsequently, the malt is mixed with hot water to extract the sugars from the grains. The resulting liquid is the wort which is boiled with hops to add flavour and aroma to the beer (39, 40). Following boiling, the wort is cooled and yeast is added for fermentation (41). During the process of fermentation yeast breaks down sugars in the wort, producing alcohol and carbon dioxide (39, 40). Finally, the beer is conditioned and bottled with a typical ethanol content of 4-6% V/V (42, 43).

A variety of raw materials rich in starch and sugar, including fruits and grains, can be used to produce spirits (10, 14, 44-46). Following the fermentation of the mash, the resulting liquid with low level of alcohol is distilled in a still to increase its ethanol content (47). Spirits may then undergo further processes, including dilution, flavouring and aging, depending on the type of the spirit and the producer (**Figure 2**). Spirits are the most consumed alcoholic beverage in the WHO South-East Asia Region (SEAR, 87.9%), the Eastern Mediterranean Region (EMR, 48.3%) and Western Pacific Region (WPR, 58.8%) (10). However, beer is the most popular

drink in the WHO Region of the Americas (AMR, 53.8%) and in the European Region (EUR, 40.0%) (10). In addition, wine consumption accounts for 29.8% of the legally sold alcohol consumption in the WHO EUR (10). Although, other beverages, including fortified wines, rice wine and palm wine, account only for 9.3% of total recorded alcohol consumption worldwide, they are the most consumed beverage type in the African Region (AFR) of the WHO (65.1%) (10).

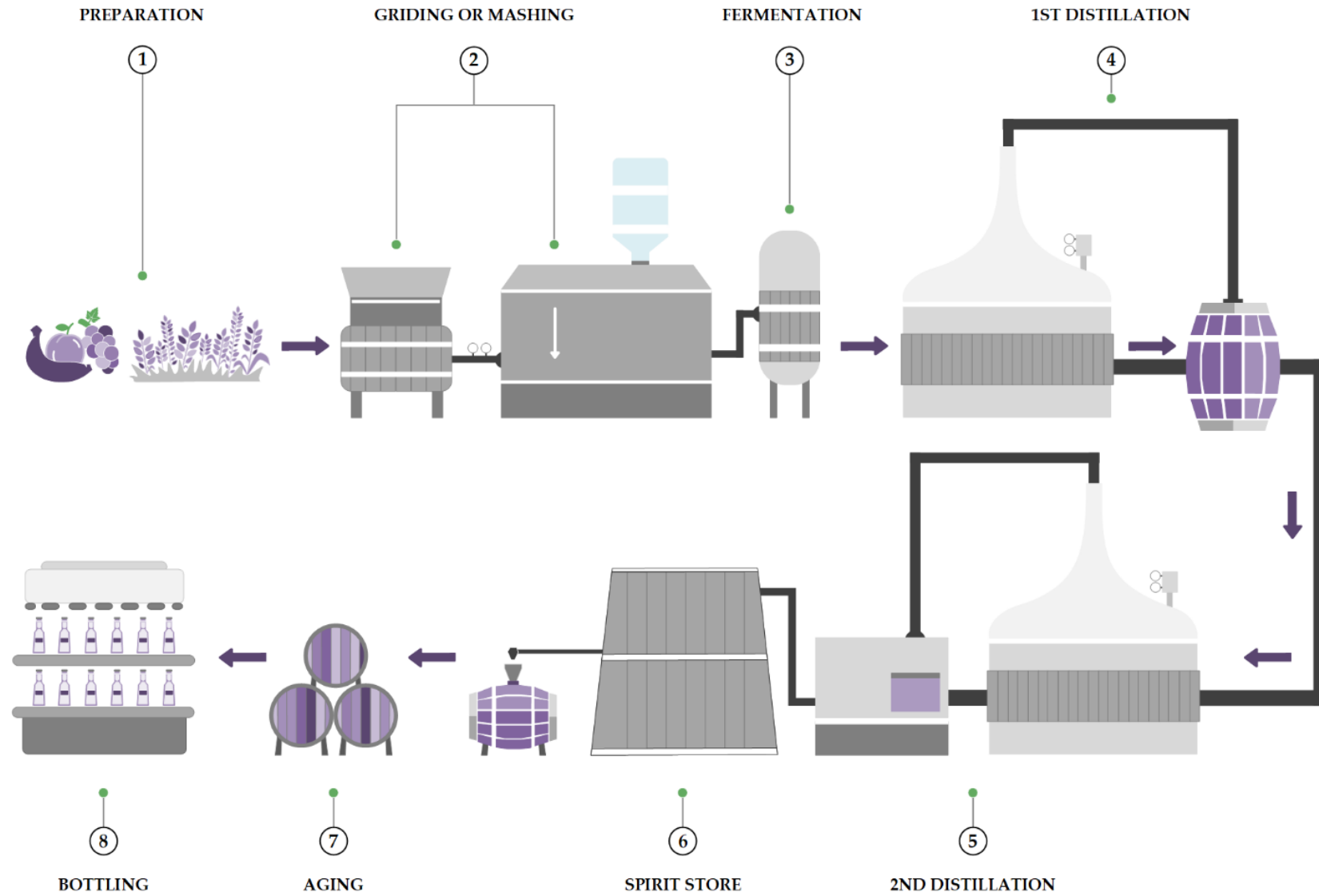


Figure 2. The process of spirit production

1.3. Level of alcohol consumption and drinking patterns

The level and pattern of ethanol intake are key indicators to assess alcohol-attributable morbidity and mortality (10, 48-50). Therefore, different measures have been developed to characterise alcohol consumption in a population. Among them, one of the most commonly used is the total alcohol per capita consumption (APC) (10). The WHO defines APC as the total alcohol consumption per capita in one year for persons aged 15 years and over (10). It is measured in litres of pure alcohol (p.a.) and adjusted for tourist consumption (10).

Globally, the average level of alcohol drinking was reported to be 6.4 litres of p.a. per capita/year in 2016 (10). This is equivalent to a daily intake of 13.9 grams of ethanol per person (10). However, large differences exist in the consumption levels across the Regions of the WHO (51). The highest APC was recorded in the EUR (9.8 litres of p.a. per capita/year), which is followed by the averages of the member states in the AMR (8.0 litres of p.a. per capita/year) and the WPR (7.3 litres of p.a. per capita/year) (10). Although the level of alcohol consumption has significantly decreased in the WHO EUR and AMR over the past decades, this trend is not universal. Ethanol intake have been recorded to increase from 4.6 to 7.3 litres of p.a. per capita/year in the WPR between 2005 and 2016 (10). A comparable change has been observed in the SEAR, where the mean alcohol consumption has grown from 2.1 litres of p.a. per capita/ year in 2005 to 4.5 litres of p.a. per capita/ year in 2016 (10). Among the regions of the WHO, the mean ethanol intake has been found to be the lowest in the EMR (0.6 litres of p.a. per capita/year), where the majority of the population refrains from alcohol consumption due to religious beliefs (10).

In addition to information on the volume of ethanol intake, the proportion of alcohol consumers and non-consumers in a population is also essential to better understand the association between drinking habits and alcohol-related diseases (52). The WHO defines current drinkers “as the individuals in the population who are aged 15 years or older and have consumed alcoholic

beverages in the past 12-months” (10). Among them, persons who “consume 60 or more grams of ethanol on at least one single occasion at least once per month fall into the category of heavy episodic drinkers” (HED) (10). Non-drinkers also known as abstainers can be divided into lifetime abstainers and former drinkers. Individuals in the first category have abstained from alcohol throughout their lives, whereas former drinkers are persons who had previously consumed alcohol but have not done so in the previous 12-month period (10). According to data from the WHO, 43% of the global population aged 15 years or older consumed alcohol in 2016 (10). In only three WHO regions, over half of the population is identified as current drinkers. These include the EUR (59.9%), the AMR (54.1%) and the WPR (53.8%) (10).

Considering the prevalence of abstinence, high-income countries of Western Europe, North and South America, as well as Australia and New Zealand reported the lowest share of non-drinking population in the world in 2016 (10). Globally, women are more likely to abstain from alcohol than men, while those who do drink generally consume less than men (10). However, there has been an increase in alcohol consumption among females in many countries in the past decades (53-55). When examining drinking patterns across age groups, the prevalence of abstainers and current drinkers among adolescents typically mirrors that of the adults (10). While younger consumers are less likely to consume alcohol than the corresponding general population aged 15 years and above, they become current drinkers at a similar rate as the general population as they grow older (10). In contrast, there is an increase in the proportion of HED from the age group of 15-19 years, reaching its peaks in the age group of 20-24 years, where it surpasses that of the general population (10).

1.4. Recorded and unrecorded alcoholic beverages

Previous epidemiological studies have shown that not only the volume of ethanol consumed, drinking patterns, but also the source and the quality of the beverage can play an important role in the onset of alcohol-related diseases (50, 56, 57). According to the classification system of the WHO, alcoholic beverages can be categorised as either recorded or unrecorded (10). Drinks that bear a tax stamp and meet specific quality criteria are legally permitted to be sold and referred to as recorded alcohol (10). Consumption of these alcoholic beverages is traceable via official production and sales statistics (58). On the other hand, unrecorded alcohol comprise “all alcoholic beverages that are not accounted for in official statistics on alcohol taxation or sales in the country where are consumed because usually are produced, distributed and sold outside the formal channels under government control” (10). This category comprises alcoholic beverages that can be purchased through cross-border shopping, home-made, informally produced, or smuggled and products containing ethanol originally intended for industrial or medical use (10, 59, 60). On average, alcohol consumed in unrecorded beverages accounts for 25.5% of total alcohol intake globally, resulting in an annual per capita consumption of 1.6 litres of ethanol from this source (10). However, there are considerable variations in unrecorded alcohol intake across the world. In many countries of the WHO EMR, including Afghanistan, Iran, Pakistan, Saudi Arabia, and Sudan, ethanol is consumed exclusively in the form of unrecorded beverages (**Figure 3**) (10). Although the share of unrecorded alcohol in the total alcohol intake is considerably lower in the member states of EUR (18.3%) than in EMR (100%) and SEAR (46.6%), the volume of ethanol consumed in this form is not negligible (10). This is particularly relevant in the countries of Western Balkan (WB) and Central and Eastern Europe (CEE) including Albania and Hungary where unrecorded alcohol consumption was 2.4 and 1.3 litres of p.a. per capita in 2016, respectively (10).

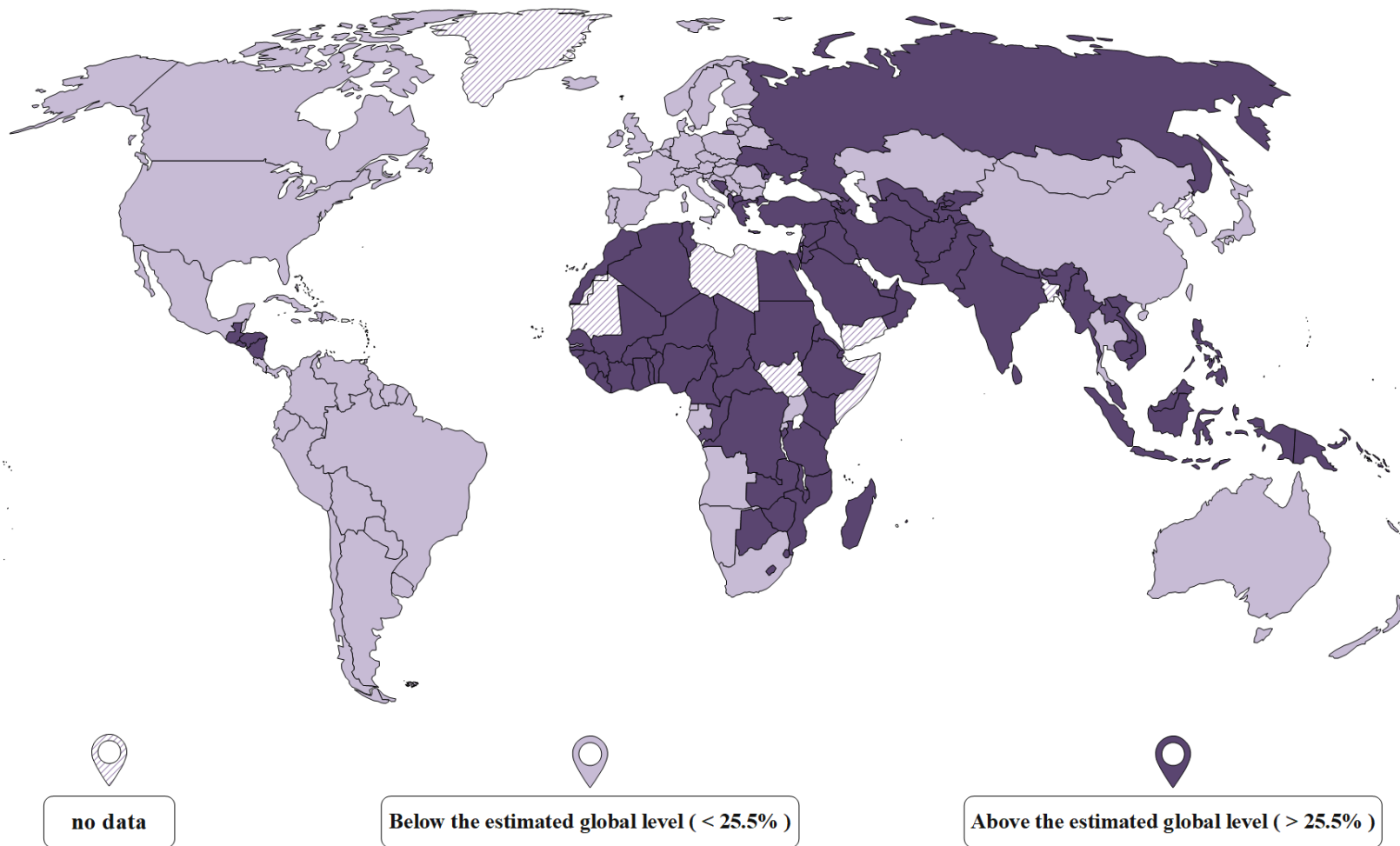


Figure 3. Unrecorded alcohol consumption: country-level comparisons to estimated global average (10)

1.5. Health consequences of alcohol consumption

Excessive alcohol consumption has been shown to be associated with 2.8 million deaths and 132.6 million disability-adjusted life years (DALYs) worldwide in 2016 (10, 61, 62). The alcohol-related disease burden and mortality were the highest in the WHO AFR with 3043.7 DALYs and 70.6 deaths per 100 000 population, respectively (10). However, the share of all deaths and DALYs attributable to alcohol drinking was the largest in the EUR, reaching 10.1% and 10.8%, respectively (10).

According to large-scale epidemiological studies, alcohol consumption is the primary cause for more than 30 diseases and health conditions (49). These include alcohol abuse, addiction, alcoholic liver diseases and alcoholic cardiomyopathy (63). Furthermore, ethanol intake has been identified as a component cause of more than 200 diseases and injuries (49). There is sufficient evidence that alcohol consumption is associated with unipolar major depression, hypertension, and haemorrhagic stroke (63). In addition, ethanol consumed in alcoholic beverages is recognized as a carcinogen and has been reported to increase the risk of mouth, oesophagus, liver, breast, and colon cancers (64, 65). For most alcohol-related health issues, there is a linear dose-response relationship between the amount of ethanol consumed and the risk of the disease (50). The greater the average daily dose of alcohol consumed by the population, the more frequently alcohol-related diseases develop (50). However, coronary heart disease (CHD) and type-2 diabetes mellitus are exceptions as abstainers have a higher risk of those illnesses than light drinkers resulting in a J shaped dose-response curve (62). Nevertheless, it is important to note that findings of recent studies seem to contradict the results of previous investigations (66, 67). A large cohort study showed that alcohol consumption at all levels was associated with increased risk of cardiovascular disease (66, 67). In addition, a research using Mendelian randomization found that there may not be protective effects of modest alcohol consumption on type-2 diabetes (67).

In addition to the amount of alcohol consumed, drinking pattern has been increasingly recognized as a factor influencing the development of alcohol-related adverse health conditions (48, 57, 68-70). For example, frequent HED has been found to elevate the risk of over 60 acute and chronic diseases (9). Daily intake of more than 60 g of ethanol has been shown to considerably worsen existing liver cirrhosis [relative risk (RR): 5.0 in men [95% confidence interval (CI): 3.9–6.4] and RR: 6.1 in women (95% CI: 4.6–8.0)] (49). HED has been also described to negatively affect functions of the immune system, thereby increasing the risk of death in patients with liver diseases [RR: 14.0 in men (95% CI: 11.7–16.7) and RR: 22.7 in women (95% CI: 17.3–30.1)] (49). In addition, heavy drinking women are at increased risk of mortality from haemorrhagic (RR: 1.69 [95% CI: 1.34-2.15]) and other non-ischaemic strokes (RR: 2.18 [95% CI: 1.48–3.20]) (49). There is sufficient evidence that chronic heavy drinking can reduce the effectiveness of treatment in patients with tuberculosis and human immunodeficiency virus infection by decreasing their adherence to therapeutic protocols. Non-compliance to treatment regimens was also reported among HED with epilepsy and depression (49).

Previous investigations have suggested that consuming unrecorded alcohol of questionable quality may contribute to alcohol-related diseases, particularly in the countries of WB and CEE in the WHO EUR, such as Albania and Hungary, where these products are consumed in significant volumes in the form of fruit spirits (10, 56, 68, 71). It has been shown that consumption of unrecorded spirits can increase the frequency of HED as their price is lower than that of recorded spirits (72, 73). Furthermore, the ethanol content of unrecorded fruit spirits is often higher than the typical 40% V/V alcohol concentration of legally-sold beverages (74). Therefore, consumption of large volumes of unrecorded fruit spirits can lead to alcohol poisoning and more frequent occurrence of alcohol attributable diseases in heavy drinkers (75, 76). Consumption of excessive volumes of unrecorded fruit spirits can not only harm the

consumers but also lead to negative social consequences, including increased domestic violence and reduced workplace productivity (77, 78). Therefore, in addition to consumption of recorded alcoholic beverages, excessive drinking of unrecorded alcohols can also pose a public health risk as it can significantly impact on individuals, communities, and society as a whole (56, 68, 71).

1.6. Contaminants in alcoholic beverages

In addition to ethanol and water, alcoholic beverages comprise hundreds of different chemicals (79-82). Many of these are non-toxic and essential for the characteristic smell and flavour of the beverage (44, 83). However, toxic substances can be also present in alcoholic drinks. They can be naturally occurring compounds such as methanol, ethyl carbamate, and acetaldehyde that can form during fermentation and are, consequently, present in almost all types of alcoholic drinks (81, 84). Other compounds, such as pesticides and lead, can be released from the raw materials and the equipment used to produce the beverage. Toxicologists and public health researchers have expressed concerns regarding the potential health risks associated with the consumption of alcoholic beverages containing high levels of these toxic substances (74, 85-87).

1.6.1. Aliphatic alcohols

Gas-chromatographic analyses of alcoholic beverages including beer, wine, cognac, whiskey, rum, and fruit-based spirits have shown that they contain not only ethanol but also methanol and other aliphatic alcohols (OAAs) comprising more than two carbon atoms including 1- and 2-propanol, 1- and 2-butanol, isobutanol, and isoamyl alcohol (88, 89). Methanol and OAAs are by-products of alcoholic fermentation and their concentration depends on the raw materials and methods used in the production of beverages (80, 90). Compared to ethanol, OAAs have

more pronounced hepatotoxic effects and it has been suggested that excessive consumption of home-made fruit spirits containing high concentrations of OAAs may be related to high rates of premature mortality from chronic liver diseases and cirrhosis in CEE countries including Hungary (91).

However, of the aliphatic alcohols present in alcoholic beverages, methanol poses the greatest risk to public health (92-99). Methanol, also known as methyl alcohol or wood alcohol, is the simplest alcohol. It is colourless, flammable and has a unique odour (100). Methanol is highly toxic to humans (92). It is metabolised differently than ethanol and its toxic by-products, including formaldehyde and formic acid, can cause blindness, organ damage and even death (92, 96). Symptoms of methanol poisoning may not become apparent until after an asymptomatic period of 1 to 72 hours (92, 96). This delayed onset of symptoms is due to its biological half-life in the human body ranging from 30 to 85 hours (92, 96).

Methanol can be produced in the chemical industry using a number of processes, including the catalytic conversion of carbon monoxide and natural gas (101-103). Fermentation of pectin containing fruits and vegetables can naturally produce small amounts of methanol (80, 90). This is the consequence of the enzymatic degradation of pectin to methanol by enzymes including pectin methylesterase by bacteria, fungi and yeast species (80, 90). The concentration of methanol present in alcoholic beverages can significantly vary depending on the raw materials used, the methods of fermentation and distillation, and the type of alcoholic beverage (80, 90). Research has indicated that methanol levels ranging from 6-27 mg/l of p.a. can be found in beer, whereas wine can contain as much as 96-321 mg/l of p.a. (104). Methanol has been found in legally manufactured vodka, rum, whiskey, and brandy at levels varying between 0–64 mg/l of p.a., 0–73 mg/l of p.a., 21–67 mg/l of p.a., and 128–388 mg/l of p.a., respectively (105). However, recorded and unrecorded fruit spirits typically contain the highest concentrations of methanol (79). Furthermore, Bujdosó et al. (2019) demonstrated that the level of methanol

present in unrecorded fruit spirits can be considerably greater [median: 6347.3 mg/l of p.a., interquartile range (IQR): 4095.9–8514.5 mg/l of p.a.] than that of in their recorded counterparts [median: 1086.6 mg/l of p.a., IQR: 204.3–4697.3 mg/l of p.a.] (56). In some cases, methanol is deliberately added to alcoholic beverages as a cheap substitute for ethanol due to its similar properties (106, 107).

These adulterated beverages are sold illegally and there are many accounts of methanol poisoning worldwide with numerous examples in African (Gambia, Kenya, Libya), American (Costa Rica, Ecuador, Mexico), Asian (India, Indonesia, Turkey) and European countries (Czech Republic, Estonia, Norway) (79, 93-99, 108-111). Due to its toxicity, several countries including Australia, Canada, China, New Zealand, and the United States of America have established maximum levels (MLs) for methanol in alcoholic beverages (112). In the EU, these limits are based on the raw material used for production and range from 50.0 to 15 000.0 mg/l of p.a. for London gin and fruit marc spirits, respectively (113). The maximum allowable limit for fruit spirits is 10 000.0 mg/l of p.a. (113). The Alcohol Measures for Public Health Research Alliance (AMPHORA) project has proposed an identical threshold for methanol in unrecorded fruit spirits (114). Generally, recorded fruit spirits in the EU are manufactured under controlled conditions and subjected to monitoring by spirit producers and official food control laboratories to ensure concentrations do not exceed the MLs for methanol (115, 116). Unrecorded fruit spirits, on the other hand, are typically made with low quality or rudimentary distillation equipment and under conditions that lack any quality control, thus their methanol content may exceed recommended limits, posing a potential risk to consumers (80, 85, 117-119).

1.6.2. Metalloids and metals

The presence of metalloids and metals including arsenic, chromium, cobalt, copper, iron, lead, manganese, nickel, tin, mercury and zinc in alcoholic beverages is of a growing public health

issue (56, 74, 85, 120-122). They may contaminate alcoholic beverages from a number of sources, including water, soil, raw materials and equipment used in brewing, distilling, bottling, ageing and storage (121, 123). Although, some metals such as iron, manganese and zinc are essential elements and play a key role in the function of several enzymes, others can be hazardous even at low levels (83, 124). For instance, research has demonstrated that chronic exposure to Pb or Cd can cause anaemia, kidney damage and neurological impairment (125-127). Epidemiological results have also shown that the inhalation of nickel-containing dust can increase the risk of lung and nasal cavity cancers (83, 128). Although less toxic heavy metals including Cu (spirits: 0.0-51.6 mg/l; wine: 0.0-55.09 mg/l; beer: 0.02-58 mg/l), Cr (III) (spirits: 0.0-0.071 mg/l; wine: 0.0-0.41 mg/l; beer: 0.01-0.045 mg/l), Fe (spirits: 0.0-34.63 mg/l; wine: 0.006-50 mg/l; beer: 0.022-25 mg/l), Mn (spirits: 0.0-5.5 mg/l; wine: 0.0-10 mg/l; beer: 0.004-0.008 mg/l), Sn (spirits: 0.0-4.1 mg/l), Zn (spirits: 0.0-20 mg/l; wine: 0.0-8.9 mg/l; beer: 0.1-68 mg/l) have been detected in all types of alcoholic beverages, Pb and Cd may be present in unrecorded alcohols due to the use of low-quality ingredients and inadequate metal equipment in their production (114, 129). Previous studies have reported that unrecorded fruit spirits can contain Pb (31-600 µg/l), Cd (6-40 µg/l) and other heavy metal at levels posing a public health concern (114, 118). Thus, individuals who consume these products may be at a higher risk of exposure to heavy metals than those who drink only recorded spirits. Among alcohol users heavy drinkers can be especially affected (130).

1.6.3. Ethyl-carbamate

Ethyl carbamate, also known as urethane, has been found in a variety of fermented foods and beverages, particularly in fruit spirits (131-133). Although it can be detected in all types of fruit spirits, its concentration is highest in spirits distilled from the mash of stone fruits including cherries (10 - 18 000 µg/l of p.a.), plums (10 - 8 800 µg/l of p.a.) and apricots (2156 µg/kg)

(132-136). Inadequate fermentation of stone fruits can result in the formation of cyanogenic glycosides leading to the generation of cyanides in the mash (137). These substances can react with ethanol and produce ethyl carbamate (137). Chronic exposure of high concentrations of ethyl carbamate has been linked to increased cancer risk (137-139). Therefore, the International Agency for Research on Cancer (IARC) classified this compound as a possible human carcinogen (Group 2A) (138, 139). Similarly to other contaminants detected in alcoholic beverages, the concentration of ethyl carbamate can be higher in unrecorded fruit spirits than in their recorded counterparts (140). As a result, ethyl-carbamate can present a risk to the health of consumers of these products.

1.6.4. Acetaldehyde

Acetaldehyde, a volatile aldehyde compound, can be present in both alcoholic and non-alcoholic beverages (141, 142). However, its concentration is much higher in alcoholic drinks (143). During fermentation, yeast cells and acetic acid bacteria naturally produce acetaldehyde (144-146). Acute exposure to high levels of acetaldehyde has been reported to induce adverse effects such as headache, nausea and tachycardia (147-149). Chronic consumption of this substance in alcoholic drinks has been demonstrated to be associated with an increased risk of developing breast and oropharyngeal cancer (143, 150). Therefore, acetaldehyde associated with the consumption of alcoholic drinks has been classified as carcinogenic to humans (Group 1) by the IARC (151). Previous studies have found that unrecorded alcohols, especially fruit spirits, contain higher levels of acetaldehyde (0.5 - 62.0 g/hl p.a.) than their recorded counterparts (0.6 – 0.9 g/hl p.a.), thus pose a larger risk to consumers (152).

1.6.5. Other contaminants

Among the pollutants found in alcoholic beverages, less attention has been paid to pesticides and mycotoxins (153-155). However, several recent studies raised concern about the potential public health risks of these emerging contaminants (87, 156). Of the alcoholic beverages, wine has often been reported to contain pesticide residues (157, 158). Wine producers often use large amounts of pesticides in their vineyards to increase yields (159). As these substances can persist in the fruits, they can find their way into the final product. Glyphosate, a commonly used herbicide classified as probably carcinogenic (Group 2A) by IARC (160), is one of the most frequently detected pesticides in alcoholic beverages (153). Chronic exposure to glyphosate has been linked to a range of adverse outcomes, such as cancer, developmental and neurological disorders, as well as disruption to the reproductive and endocrine systems (161).

In addition to pesticides, various mycotoxins have been found in alcoholic beverages (154, 155). Mycotoxins are toxic substances produced by certain types of fungi, such as *Aspergillus* and *Penicillium*, which can grow on crops such as cereals, nuts, and dried fruits (162-165). Exposure to mycotoxins has also been associated to a range of health problems, including liver damage, cancer, and immune system suppression (166, 167). Due to their high alcohol content, neither recorded nor unrecorded spirits are not susceptible to bacterial contamination. However, a few studies reported the presence of *Bacillus*, *Escherichia*, *Lactobacillus*, *Staphylococcus*, and *Streptococcus* genera in traditionally produced beer and wine (168-170). In addition, bacterial endotoxins have been detected in home-made beer collected in Nigeria and South-Africa (171). Therefore, consumers of these type of beverages may be exposed to pathogenic bacteria and their toxic products and adverse health effects of cannot be excluded.

2. Aims of the study

In addition to the health effects of ethanol, the potential presence of heavy metals in unrecorded alcoholic beverages is sufficient to cause a public health concern, given that a large share of the Hungarian population consumes home-made and informally produced spirits (114, 129). Although concentrations of heavy metals have been measured in recorded spirits, there is limited data on their concentration in unrecorded spirits (114, 172). Furthermore, detailed analysis of differences in concentrations of heavy metals between recorded and unrecorded spirits was not carried out and the levels of these elements in spirits were not compared with their toxicological thresholds. In addition, the health risks associated with the consumption of recorded and unrecorded spirits in Hungary have not been assessed comprehensively. We were also not aware of any published studies comparing the associated health risk of drinking recorded and unrecorded spirits in Hungary.

1. To fill this gap, our aim was to determine and compare the concentration of heavy metals in recorded and unrecorded spirits in Hungary.
2. Our additional objective was to use probabilistic simulations to calculate the daily intake of heavy metals of the Hungarian population from recorded and unrecorded spirits, analysing differences by sex and volumes of consumption.
3. We also intended to evaluate the health risk associated with the consumption of recorded and unrecorded spirits containing heavy metals in the Hungarian population.

Comprehensive analysis has not been performed to identify the types and concentrations of metals that may be present in rakia, a fruit spirit frequently consumed in Albania. To our knowledge, data on the chemical composition of only one unrecorded Albanian rakia were previously reported (114).

4. Therefore, we have measured the concentration of metals in rakia samples collected in Albania. The levels of metals in rakia samples were then compared to their threshold values.

Since unrecorded fruit spirits can contain higher levels of methanol, the intake of this toxic alcohol and related blood methanol levels (BMLs) in HED may exceed the reference dose (RfD) and the maximum tolerable blood methanol level (MTBML) of 2.0 mg/kg body weight kg/day and 5.0 mg/dl, respectively (173, 174). However, no studies have been found that estimated the daily intake of methanol and BMLs associated with consuming unrecorded fruit spirits at population level. Therefore, our study aimed:

5. to calculate the daily methanol intake and BMLs due to the consumption of unrecorded fruit spirits in CEE countries, a probabilistic simulation was conducted for both men and women taking into account the varying levels of alcohol drinking.
6. to evaluate the health risks associated with the intake of unrecorded fruit spirits, we compared the daily methanol intake and the associated BMLs with their limit.

3. Materials and methods

3.1. Collection of spirit samples in Albania and Hungary

Members of our research team carried out unrecorded spirit sample collection using the snowball sampling method (175, 176). Industrially produced spirits were considered recorded, whereas spirits manufactured in private distilleries or in households were classified as unrecorded. Ninety-seven spirit samples collected in Hungary were recorded comprising absinthe (n = 1), tequila (n = 2), gin (n = 3), artificially flavoured spirits (n = 5), rum (n = 6), brandy (n = 18), vodka (n = 16), whiskey (n = 21) and fruit spirits (pálinka, n = 25). All recorded samples collected in Hungary were obtained from grocery stores and had tax stamps. One hundred unrecorded spirits without tax stamps were purchased from 31 individuals in 19 municipalities located in Eastern Hungary. These persons produced spirits using home-fermented fruits and distilled the mash in their own stills or sent it to local distilleries. The criteria for inclusion were that the unrecorded spirits should be prepared from fruit mash from private homes and be untaxed. The same methodology was used for the collection of 30 samples of unrecorded Albanian rakia. After collection, each sample was labelled with a unique identification number to avoid mistakes. The spirits were then poured into glass containers and kept at 4 °C until gas chromatographic mass spectrometric (GC/MS) and inductively coupled plasma optical emission spectrometry (ICP-OES) analysis could be carried out. Source and raw materials of spirits, as well as the date of sampling were recorded. Recorded and unrecorded spirits collected in Hungary, manufactured from a variety of fruits, have been classified as follows: fruit spirits prepared by the distillation of fermented sour cherry, plum, pear, apple, grape, apricot; grain spirits distilled from fermented wheat, maize, barley; and other spirits distilled from unknown raw materials, fermented agave and molasses. Fermented plums,

cherries, grapes, mulberries, juniper and blackberries were used to produce unrecorded Albanian rakia samples.

3.1.1. Gas chromatographic mass spectrometric analysis of spirit samples collected in Albania and Hungary

3.1.1.1. Materials

Ethanol was obtained from Merck (Darmstadt, Germany) and used as a standard for the qualitative analysis of spirit samples. The internal standard (ISTD) was acetonitrile, which was also purchased from Merck. High-performance liquid chromatography grade chemicals were used in the analysis.

3.1.1.2. Determination of ethanol concentration in spirit samples collected in Albania and Hungary

The samples were analysed using a Hewlett-Packard (HP) GC/MS system (Palo Alto, USA) (56, 177). The system consisted of a HP 6890 GC, a HP 5973 mass selective detector (MSD) and an Agilent 7683 automatic liquid sampler (Agilent Technologies, Palo Alto, CA, USA). A HP Free Fatty Acid Phase capillary column (Hewlett-Packard, Palo Alto, CA, USA) with a length of 50 m, an internal diameter of 0.2 mm and a film thickness of 0.33 μm was used for the separation of ethanol. Helium was employed as the carrier gas at a constant pressure of 3.0 bar (56, 177). One μl of spirit samples was injected into the GC/MS in split mode at an inlet temperature of 200 $^{\circ}\text{C}$. The oven was programmed as follows: 4.0 minutes at 60 $^{\circ}\text{C}$, followed by a gradual increase to 110 $^{\circ}\text{C}$ at a rate of 5 $^{\circ}\text{C}$ per minute. The temperature of the GC/MS interface, the MSD ion source and the MSD quadrupole were set at 280 $^{\circ}\text{C}$, 230 $^{\circ}\text{C}$ and 150 $^{\circ}\text{C}$, respectively. The ionization energy was 70 eV. Calibration of the GC/MS system was

performed before the quantitative analysis of spirit samples. One μl of mixtures containing ethanol at levels of 237.0, 276.2, 315.6, 355.1, 394.5, 394.5, 433.1, 473.4, and 512.1 g/l was injected to determine the calibration curve for ethanol. To measure the amount of ethanol in our samples, acetonitrile was used as an ISTD at a concentration of 118 g/l. Standard mixtures had a volume of 1.0 ml. 850 μl from each sample and 150 μl from the ISTD were pipetted into Eppendorf tubes. Then, 1.0 μl of these mixtures was injected into the GC/MS system to determine the level of ethanol in spirit samples collected in Albania and Hungary. The HP G1701BA MSD Productivity Chemstation software (Hewlett-Packard, Palo Alto, CA, USA) was used for system control, data acquisition and processing. The same software was used to compute the concentration of ethanol in the samples.

3.1.2. Inductively coupled plasma optical emission spectrometric analysis of spirit samples collected in Albania and Hungary

3.1.2.1. Materials

Scharlab (Debrecen, Hungary) supplied monoelement standard solutions and nitric acid (HNO_3) for the determination. Hydrogen-peroxide (H_2O_2) and multielement standard solutions were purchased from Merck (Darmstadt, Germany). Reagent and spectroscopic grade chemicals were used in the analysis.

3.1.2.2. Sample preparation

Samples were prepared according to the methodology published by Simon and co-authors (178). Briefly, five millilitres from each spirit sample were pipetted into glass beakers and dried on a laboratory heating plate. Then, the dried samples were subjected to chemical degradation by adding a mixture of 5.0 ml of 65% HNO_3 and 1.0 ml of 30% H_2O_2 . Subsequently, the

samples were dried again. The chemically degraded samples were then solubilised and decanted into volume-calibrated tubes and diluted to 10.0 ml with ultrapure water. The samples prepared were stored in the dark at 4 °C before the ICP-OES analysis.

3.1.2.3. ICP-OES analysis

Samples were analysed by an Agilent ICP-OES system (5100 SVDV model, Agilent Technologies, Santa Clara, USA) (178, 179). Monoelement spectroscopic standards with a concentration of 1000 mg/l were used to prepare standard solutions of arsenic and tin. The multielement standard solution used in our analysis contained Ag, Al, B, Ba, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga, In, K, Li, Mg, Mn, Na, Ni, Pb, Sr, Tl, and Zn at a concentration of 1000 mg/l (ICP, IV, Merck, Darmstadt, Germany). Subsequently, mono- and multielement standard solutions were used to prepare calibration series for 5-point calibration curves. Samples were introduced to ICP-OES system using a Meinhard type nebulizer with a concentric spray chamber. Argon gas was applied to generate plasma while nitrogen gas was used to introduce samples and optically purge the system. To verify the measurement method, control samples were prepared to test the purity of the water and equipment used. To assess the effect of ethanol on the detection of heavy metals, mixtures containing ethanol (Merck, Darmstadt, Germany) at concentrations of 0.0%, 5.0%, 10.0%, 25.0% and 40.0% V/V were spiked with the standard solution. The final concentration of the elements was 0.1 mg/l. The measurement accuracy exceeded 95%. Three parallel measurements were performed for each sample. **Table 1** shows the detection and quantitation limits for analysed heavy metals.

Table 1. Detection and quantitation limits of heavy metals

no.	heavy metal	limit of detection [$\mu\text{g/l}$]	limit of quantitation [$\mu\text{g/l}$]
1	cadmium	0.1	0.5
2	chromium (III)	0.2	1.0
3	cobalt	0.3	1.5
4	copper	0.1	0.5
5	iron	0.2	1.0
6	lead	2.2	11.0
7	manganese	0.02	0.1
8	nickel	0.5	2.5
9	zinc	0.1	0.5
10	tin	1.0	5.0

3.1.3. Comparison of heavy metal concentrations in spirits collected in Albania and Hungary with limit values

The AMPHORA project has published limits for heavy metals in spirits (114). The level of heavy metals determined in our samples were compared to the AMPHORA limits presented in **Table 2.**

Table 2. AMPHORA limit values of heavy metals

no.	heavy metal	AMPHORA limits of heavy metal [mg/l]
1	copper	2.0
2	cobalt	-
3	chromium (III)	0.5
4	iron	2.0
5	manganese	0.5
6	nickel	0.2
7	zinc	5.0
8	tin	1.0

3.1.4. Estimation of health risks related to the intake of heavy metals in recorded and unrecorded spirits collected in Hungary

To calculate the health risks related to consumption of recorded and unrecorded spirits collected in Hungary, we used a probabilistic risk assessment method with three scenarios (described in details in Supplement 1, Appendix) (56). First, the distribution of estimated daily intakes (EDI) of heavy metals were determined (180-182) as follows:

$$EDI = \frac{MDI \times MCS}{BW}$$

where, the mass of daily alcohol intake in g/day is referred to as MDI, MCS indicates the concentration of different heavy metals in spirits collected in Hungary in mg/g, and BW is the average body weight. **Table 3.** shows the mean default body weight for both sexes (73.9 ± 14.9 kg), males (82.0 ± 13.1 kg) and females (67.2 ± 12.8 kg) (183, 184). Body weight was assumed to follow a normal distribution in our risk estimation.

Table 3. Default body weight values for adult subjects

category	mean body weight \pm standard deviation [kg]*
both sexes	73.9 \pm 14.9
males	82.0 \pm 13.1
females	67.2 \pm 12.8

* Default values were obtained from report of the Scientific Committee of European Food Safety Authority

Probabilistic simulations were performed with the @Risk for Excel software, version 7.6 (Palisade Corporation, Ithaca, NY, USA). The simulations were carried out with 10,000 iterations, Latin Hypercube sampling, and Mersenne Twister random number generator (56). To determine the probability density functions for the levels of heavy metals (mg/g), we selected the best fit distributions by the Akaike information criterion (AIC) with a minimum set at 0.0 mg/g. Our model incorporated these probability density functions and the mass alcohol intake in grams per day. To obtain the distribution of EDI at population level, the probabilities were combined using Monte Carlo simulations. The following three exposure scenarios were used in our estimation. The 1st scenario was referred to as “average”, using data based on per capita alcohol intake averaged across the total population aged 15 years and above. The 2nd scenario was "regular", using information on consumers (those who drink alcohol, not including abstainers) who are aged 15 years and above. Two categories were used in the 3rd scenario “chronic heavy drinkers, version A” (referring to individuals who consume 60 g/day of ethanol for men and 40 g/day of ethanol for women, taking into account the proportion of recorded and unrecorded alcohol intake) and “chronic heavy drinkers version B” (referring to individuals who consume 60 g/day of ethanol for men and 40 g/day of ethanol for women exclusively from spirits). The data on alcohol consumption in each scenario are presented in **Table 4**. For all scenarios, the daily metal intake was in mg/kg body weight/day.

Table 4. Volumes of per capita alcohol consumption and daily ethanol intake from recorded and unrecorded spirits considering average consumers, regular and chronic heavy drinkers

sex	average consumption on a population level [litres of absolute ethanol per capita]		regular drinkers only [litres of absolute ethanol per capita]		chronic heavy drinkers version A [litres of absolute ethanol per capita]		chronic heavy drinkers version B [litres of absolute ethanol per capita]	
	recorded spirits	unrecorded spirits	recorded spirits	unrecorded spirits	recorded spirits	unrecorded spirits	recorded spirits	unrecorded spirits
males	5.35 (11.5 g/day)	2.52 (5.4 g/day)	6.74 (14.5 g/day)	3.16 (6.8 g/day)	7.77 (16.8 g/day)	3.66 (7.9 g/day)	27.75 (60 g/day)	27.75 (60 g/day)
females	1.26 (2.7 g/day)	0.59 (1.2 g/day)	2.33 (5.0 g/day)	1.09 (2.3 g/day)	5.18 (11.2 g/day)	2.44 (5.2 g/day)	18.50 (40 g/day)	18.50 (40 g/day)
both sexes	3.2 (6.9 g/day)	1.5 (3.2 g/day)	4.80 (10.3 g/day)	2.25 (4.8 g/day)	-	-	-	-

Subsequently, target hazard quotient (THQ) values were calculated. THQ is a method used to assess non-cancer health risks related to chronic exposure to chemicals (180-182). In our study, the THQ was defined as “the ratio of the oral dose of a heavy metal and its reference concentration” (180-182). If the ratio is 1.0 or less, the hazard is considered to be low. However, ratios greater than 1.0 imply an elevated health risk (180-182).

To calculate THQ values, we considered the distribution of EDI, as well as individual values of exposure frequency (EF, days/year), exposure duration (ED, years), reference dose of the heavy metal (RfD, mg/kg/day) and the average exposure time (AET) for non-carcinogens (365 days/year x ED) (180-182). **Table 5** shows the RfD values of heavy metals applied in our investigation (182). The EF was defined as 365 days per year. For the ED, the average life expectancy in Hungary at the age of 15 years was used for both sexes (61.8 years), men (58.1 years) and women (65.2 years) (185). The THQ was calculated using the following formula (180-182):

$$THQ = \frac{MCS \times MDI \times EF \times ED}{RfD \times BW \times AET}$$

Next, Monte Carlo simulations were used to combine the distributions of EDI with the individual values described earlier and to obtain the distributions of THQ values in the exposed population in Hungary. To assess the overall health risk resulting from combined exposure to heavy metals present in recorded and unrecorded spirits collected in Hungary, the sum of individual THQ values was calculated to obtain the combined THQ (THQ_c) (180-182). This method is valid only under the assumption of an additive effect.

Table 5. Oral reference doses of the detected heavy metals

no.	heavy metal	oral reference dose [mg/kg/day]
1	chromium (III)	1.5
2	cobalt	0.0003
3	copper	0.04
4	iron	0.7
5	manganese	0.024
6	nickel	0.011
7	zinc	0.3
8	tin	0.6

3.1.5. Statistical analysis of data on spirits collected in Albania and Hungary

To ensure the comparability of concentrations of heavy metals in spirits collected in Albania and Hungary containing different levels of ethanol, the heavy metal content of the samples was expressed in milligrams per litre of p.a. When the level of a heavy metal in spirits was below the limit of quantification of the ICP-OES system, it was considered to be zero. International Business Machines (IBM), Statistical Package for the Social Sciences (SPSS) statistics 25.0 software (IBM Inc, Armonk, New York, USA) was used to perform statistical analyses. Values of p equal to or less than 0.05 were considered statistically significant.

3.1.5.1. Statistical analysis of data on the concentration of ethanol in Albanian spirits

The concentrations of ethanol reported by sample providers (reported ethanol concentration) were compared to those of determined by GC/MS analyses (measured ethanol concentration). Mean differences between the reported and measured ethanol concentrations were compared using the Wilcoxon signed-rank test.

3.1.5.2. Statistical analysis of data on the concentration of heavy metals in spirits collected in Hungary

To carry out statistical analyses, samples obtained from Hungary were categorised as recorded (n = 97) and unrecorded (n = 100). Four subgroups were created within recorded spirits including vodka (n = 16), brandy (n = 18), whiskey (n = 21) and pálinka (n = 25). Subcategories were not created for unrecorded spirits, because all of them were pálinka (n=100). Due to limited sample size, absinth (n = 1), tequila (n= 2), gin (n = 3), artificially flavoured spirits (n = 5) and rum (n = 6) were not categorised. Data on the concentrations of heavy metals were found to be non-normally distributed based on the Shapiro-Wilk test. Therefore, the Mann-Whitney U tests were employed to determine whether there are differences between levels of heavy metals in recorded and unrecorded spirits. Descriptive statistics were computed including mean \pm standard deviation (SD), minimum and maximum values, percentage of samples with heavy metals, and the share of spirits containing metals above the AMPHORA limits. To compare the distributions of THQ_c values, we used Kruskal-Wallis test with Dunn-Bonferroni post hoc method. A factor analysis was carried out using principal component analysis to identify clusters of heavy metals. When eigenvalues greater than 1 were obtained, groups of heavy metals were considered to be related.

3.2. Estimation of health risk from intake of methanol in unrecorded spirits

3.2.1. Sources of data on the concentration of methanol in unrecorded fruit spirits

Two sources, including our previous research (56, 177) and literature searches conducted in PubMed (US National Library of Medicine, Bethesda, MD, USA), Web of Science (Thompson Reuters, Philadelphia, PA, USA) and Scopus (Elsevier B.V., Amsterdam, the Netherlands) databases, were used to obtain data on methanol levels in unrecorded fruit spirits.

Combination of the following search terms was used during literature research: home-made, illegal, illicit, traditional, unrecorded, fruit, distillate, spirits and methanol. Two researchers independently reviewed papers obtained from scientific databases. Data on the concentration of methanol were extracted from articles meeting the following criteria: papers published after 2000 that include data on the level of methanol in at least 10 samples of unrecorded fruit spirit collected in the EU and written in English or German. To identify additional articles, the references of each selected paper were also manually searched. **Figure 4** and **Table 6** show the flow chart of the literature search and the list of studies meeting the criteria for inclusion, respectively.

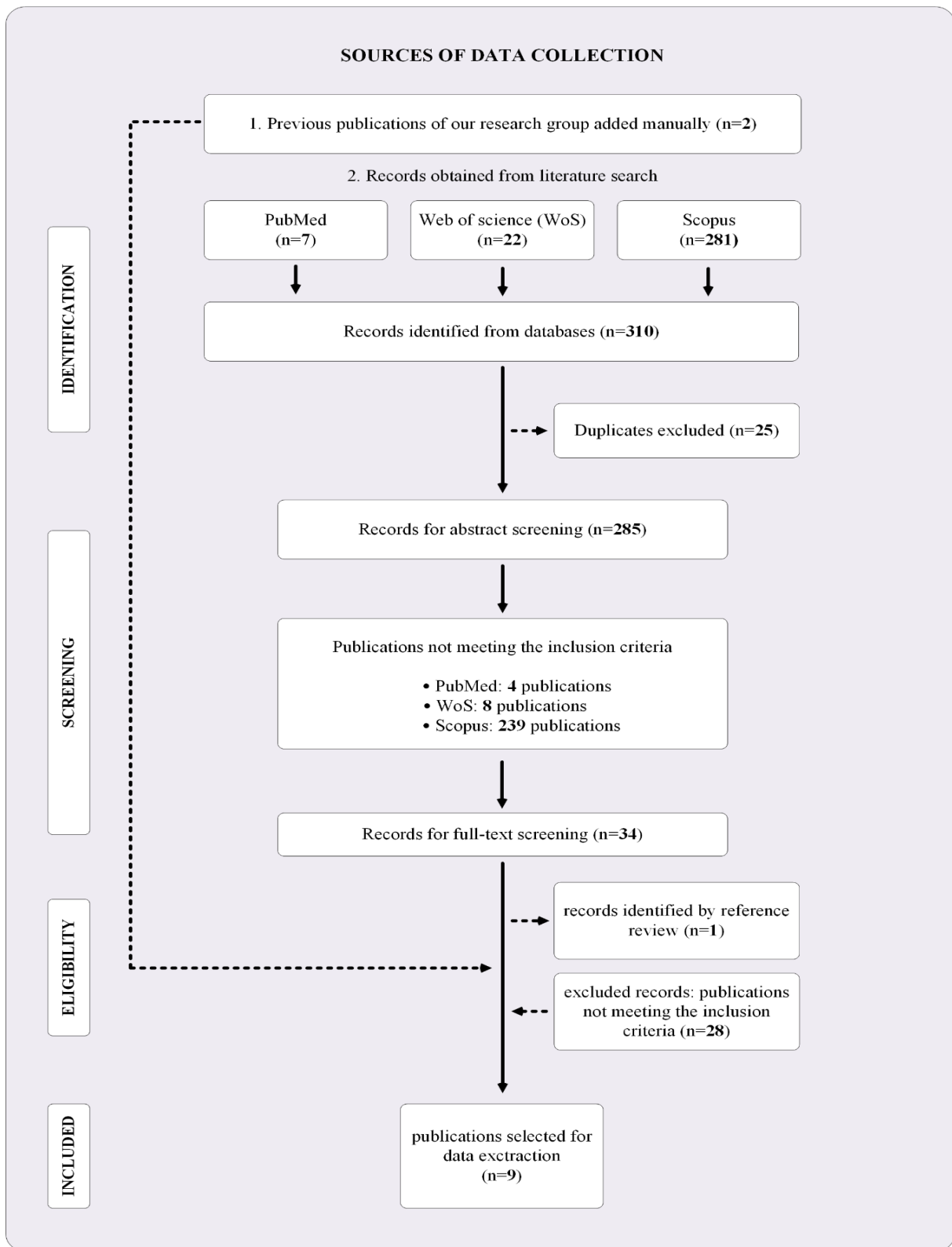


Figure 4. Flowchart of computer-assisted literature search (186)

Table 6. Studies selected for data extraction

studies selected	name of fruit spirits	raw materials	place of sample collection	number of samples analysed
Hanousek-Čiča et al., 2019	wine-derived spirits	grape	Croatia	13
Huckenbeck et al., 2003*	schnapps	apple, apricot, cherry, peach, pear, plum, quinces	Germany	50
Soufleros et al., 2004	mouro	mulberry tree fruit	Greece	10
Soufleros et al., 2005	koumaro	strawberry tree fruit	Greece	19
Huckenbeck et al., 2003*	pálinka	apple, apricot, cherry, peach, pear, plum	Hungary	25
Szűcs et al., 2005	pálinka	apple, apricot, grape, pear, plum	Hungary	34
Lachenmeier et al., 2009	pálinka	apricot, cherry, plum	Hungary	11
Bujdosó et al., 2019	pálinka	apple, apricot, grape, pear, plum, sour cherry	Hungary	87
Levy et al., 2003	țuică	apple, apricots, cherry, orange, pears, plum	Romania	26
Rusu Coldea et al., 2011	brandy	apple, pear, plum	Romania	25

*The article published in 2004 by Huckenbeck et al, includes both, schnapps samples collected in Germany and pálinka samples obtained in Hungary.

3.2.2. Compliance of methanol concentrations with AMPHORA limits

For methanol, 10 000 mg/l of p.a. has been determined by the AMPHORA project as the maximum level in unrecorded fruit spirits (114). The concentrations of methanol reported in the articles selected have been compared to this threshold value. The number and proportion of spirit samples containing methanol exceeding the AMPHORA limit of 10 000 mg/l of p.a. was calculated for each study selected.

3.2.3. Estimation of exposure to methanol and related blood methanol levels considering different levels of alcohol consumption

To assess the daily consumption of methanol from unrecorded fruit spirits and the related BMLs at population level, we performed Monte Carlo simulations using the @Risk for Excel software, version 8.1 (Palisade Corporation, Ithaca, NY, USA) with 10 000 iterations, Latin hypercube sampling and Mersenne Twister random number generator (56).

To estimate the daily methanol intakes, the volume of 40% V/V unrecorded fruit spirits consumed and its methanol and ethanol content was considered. The first step was to determine the probability density functions separately for the levels of methanol obtained from the articles identified in the literature search. The AIC test was used with a lower limit set at zero to determine the best fit distributions. To compare the estimated daily methanol intakes (EDMIs) with the RfD of 2.0 mg/kg body weight/day, we defined chronic exposure to methanol based on the assumption that unrecorded fruit spirits contain methanol at concentrations reported in selected studies, and also that alcohol drinkers consume 10 g, 20 g, 40 g, 60 g, and 80 g of ethanol per day over their lifetime (173). RfD is “an estimate of a daily oral exposure to an agent that is assumed to confer no appreciable risk of adverse health effects over a human lifetime” (187). The corresponding volumes of unrecorded fruit spirits containing 40% V/V ethanol are shown in **Table 7**. The table illustrates the weights and volumes of ethanol as well

as the volumes of unrecorded fruit spirits (40% V/V) that are required for the consumption of 10 g, 20 g, 40 g, 60 g, and 80 g of ethanol. To calculate the volumes of ethanol (ml), the weight of ethanol (10 g, 20 g, 40 g, 60 g, 80 g) was divided with its density of 0.789 g/ml. To compute the volumes of 40% V/V unrecorded fruit spirits (ml), the volume of ethanol was multiplied with 2.5 (100/40). The daily methanol intake and related blood methanol levels were calculated by considering the volumes of 40% V/V unrecorded fruit spirits required to ingest 10 g, 20 g, 40 g, 60 g, and 80 g of ethanol.

Table 7. Volume of 40% V/V unrecorded fruit spirits required for the ingestion of 10 g, 20 g, 40 g, 60 g, and 80 g of ethanol

ethanol consumption [g]	ethanol consumption [ml]	volume of unrecorded fruit spirit containing 40% V/V ethanol [ml]
10	12.7	31.7
20	25.4	63.4
40	50.7	126.7
60	76.1	190.1
80	101.4	253.5

The distributions of EDMIs (mg/day) were obtained by taking into account the probability density functions of methanol at different intake levels. In addition, the body weight of 82.0 ± 13.1 kg for males and 67.2 ± 12.8 kg for females, assuming a normal distribution, were also considered in the estimation. The following formula was used to calculate the distribution of EDMIs at each consumption level:

$$EDMI = \frac{C_m \times DCUFS}{DBW}$$

where C_m is the probability density functions of methanol levels obtained from the articles retrieved from the literature search (mg/ml), DCUFS is the daily consumption of 40% V/V unrecorded fruit spirits containing 10 g, 20 g, 40 g, 60 g, and 80 g of ethanol (ml/day; see the volumes of unrecorded fruit spirits containing 40 % V/V ethanol in **Table 7**), DBW is the distribution of body weight (kg). The unit of EDMIs was mg/kg body weight/day. The same approach was used to calculate the distributions of the corresponding estimated daily ethanol intakes (EDEIs):

$$EDEI = \frac{C_e \times DCUFS}{DBW}$$

where C_e is the level of ethanol in 40% V/V unrecorded fruit spirits (mg/ml), DCUFS is the daily consumption of 40% V/V unrecorded fruit spirits containing 10 g, 20 g, 40 g, 60 g, and 80 g of ethanol (ml/day; see the volumes of unrecorded fruit spirits containing 40 % ethanol in **Table 7**), DBW is the distribution of body weight (kg). EDEIs were expressed in mg/kg body weight/day.

Methanol levels in unrecorded fruit spirits required to reach the RfD at varying consumption levels were calculated in the following manner: Firstly, to determine the quantity of methanol that needed to be ingested to reach the RfD, the RfD of methanol was multiplied by the distribution of body weights. Next, to calculate the concentration of methanol in unrecorded fruit spirits in mg/l, we multiplied the distribution of the amount of methanol with the ratio of 1000 ml/x ml where x is the volume of 40% V/V unrecorded fruit spirit containing 10 g, 20 g, 40 g, 60 g, and 80 g of ethanol. Finally, the levels of methanol obtained were converted to mg/l of p.a. by applying a conversion factor of 2.5.

Consumption of a single drink of unrecorded fruit spirits containing methanol at concentrations obtained from the studies selected and also 10 g, 20 g, 40 g, 60 g, and 80 g of ethanol was defined as acute exposure to methanol. The corresponding volumes of unrecorded fruit spirits containing 40 % V/V ethanol are presented in **Table 7**. The probability density functions of

methanol at different consumption levels were taken into account to calculate the distributions of estimated intakes of methanol (grams). The distributions of BMLs were determined using Widmark's equation as follows:

$$C_{bm} = \frac{A_m}{r_m \times BW}$$

where, A_m is the distribution of the amount of methanol consumed (gram), C_{bm} is the distribution of blood methanol concentration (g/l), BW is the body weight (kg), distributed as described above, r_m is Widmark's factor replaced with volume of distribution of methanol (0.6-0.7 l/kg for males and females supposing an uniform distribution) (104, 183, 188). For each consumption level, BMLs were converted to mg/dl. The corresponding distributions of blood ethanol levels (BELs) were also calculated via Widmark's equation as follows:

$$C_{be} = \frac{A_e}{r_e \times BW}$$

where A_e is the amount of ethanol consumed (10 g, 20 g, 40 g, 60 g, and 80 g on a single occasion), C_{be} is the distribution of BELs (g/l), BW is the body weight (kg), distributed as described above, r_e is Widmark's factor (0.68 ± 0.085 l/kg for males and 0.55 ± 0.050 l/kg for females supposing a uniform distribution) (183, 188, 189). For each consumption level, BELs were also expressed in mg/dl.

Methanol concentrations in unrecorded fruit spirits (C_m) needed to reach MTBML of 0.05 g/litre at different intake levels were computed as follows:

$$C_m = \text{MTBML} \times r \times BW \times R \times CF$$

where r is Widmark's factor and BW is the body weight (kg), 82.0 ± 13.1 kg for males and 67.2 ± 12.8 kg for females, supposing a normal distribution, R is the ratio of 1000 ml x ml where x is the volume of 40% V/V unrecorded fruit spirit containing 10 g, 20 g, 40 g, 60 g, and 80 g of ethanol, and the CF is the conversion factor of 2.5 to p.a. The methanol concentrations were obtained in g/l and then converted to mg/l of p.a.

3.2.4. Statistical analysis

The distributions on the levels of methanol in unrecorded fruit spirits were assessed for normality using the Shapiro-Wilk test. The distributions of BMLs and EDMIs related to the consumption of unrecorded fruit spirits were compared to those of BMLs and EDMIs, respectively, when drinking a hypothetical fruit spirit (HFS) containing methanol at the maximum level of 10 000.0 mg/l of p.a. as specified by the AMPHORA project. Differences between distributions were assessed using the Kruskal-Wallis test with Dunn-Bonferroni post hoc method. IBM SPSS statistics 25.0 software (IBM Inc, Armonk, New York, USA) was used to perform statistical analyses. Differences were considered statistically significant when p was less than 0.05.

4. Results

4.1. Estimation of health risks associated with the consumption of recorded and unrecorded spirits collected in Hungary

4.1.1. Concentration of heavy metals in recorded and unrecorded spirits collected in Hungary

As demonstrated in Panels A-F of **Figure 5**, Cu, Fe, Mn, Ni, Sn, and Zn were present in both recorded and unrecorded spirits obtained from Hungary. However, Co and Cr were detected only in recorded spirit samples (Panel G of **Figure 5**). Unrecorded spirits contained significantly higher levels ($p < 0.001$) of Cu (Panel A of **Figure 5**), Zn (Panel F of **Figure 5**) and Sn (Panel E of **Figure 5**) than their recorded counterparts. Significantly greater concentrations of Fe, Mn and Ni were detected in recorded than in unrecorded spirits [shown in Panel B ($p < 0.001$), Panel C ($p < 0.05$) and Panel D ($p < 0.001$) of **Figure 5**, respectively]. The concentration of Pb was below the limit of quantitation in all spirit samples collected in Hungary. Cd was not present in our samples.

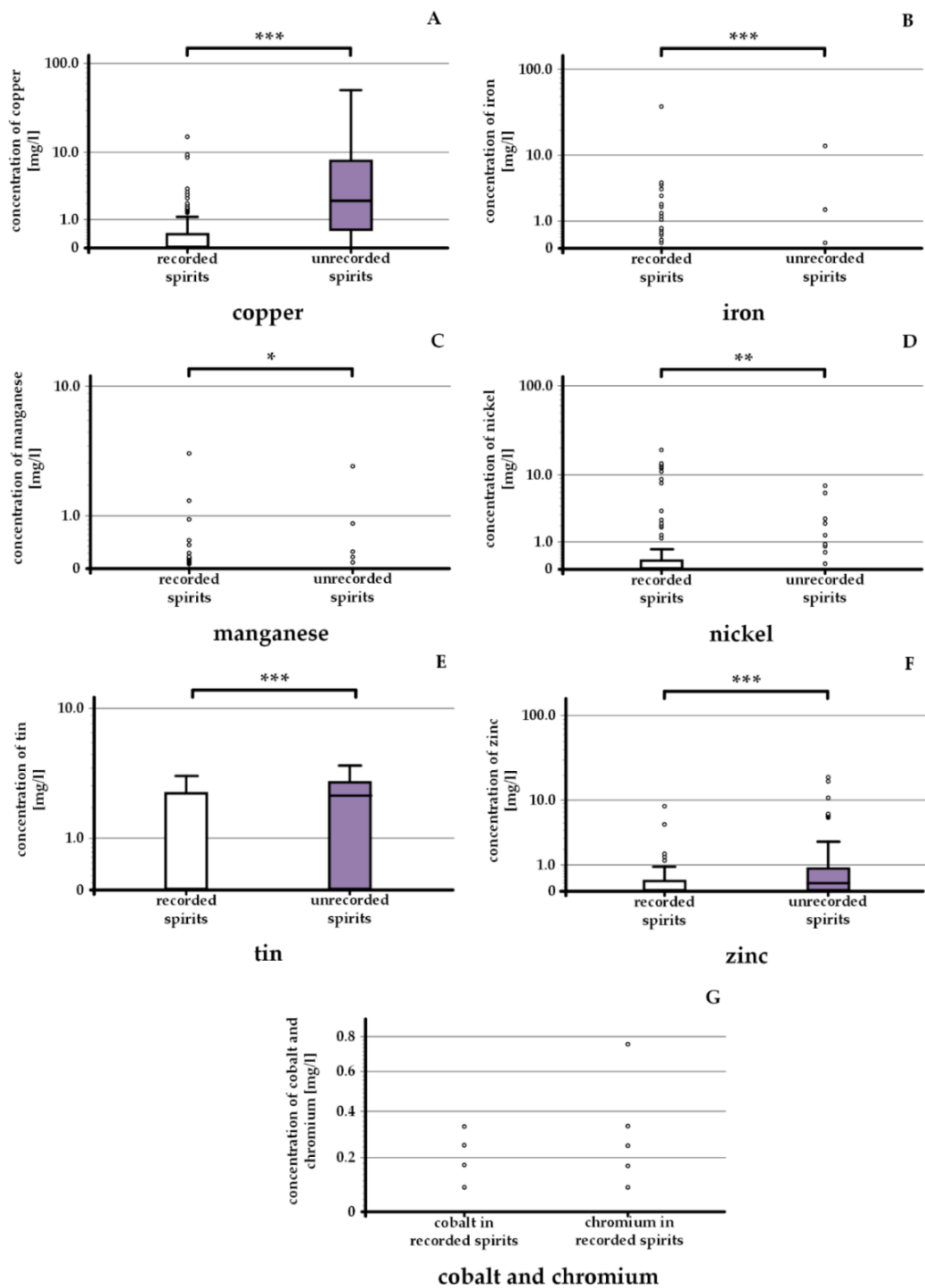


Figure 5. Levels of heavy metals in recorded and unrecorded spirits collected in Hungary

Level of copper (Panel A), iron (Panel B), manganese (Panel C), nickel (Panel D), tin (Panel E), and zinc (Panel F) in recorded and unrecorded spirits. Level of cobalt and chromium (Panel G) in recorded spirits. Median levels of heavy metals, their interquartile ranges (IQRs), and 1.5 times of IQRs as whiskers are depicted. Outliers are shown by open circles. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

4.1.2. Compliance of levels of heavy metals in recorded and unrecorded spirits collected in Hungary with AMPHORA limits

Copper was detected in 99% and 62%, zinc in 95% and 76%, and tin in 52% and 33% of the unrecorded and recorded spirits samples (**Table 8** and **Table 9**), respectively. **Table 8** and **Table 9** show that Fe, Mn, and Ni were present more frequently in recorded spirits when compared with their unrecorded counterparts, with Fe detected in 23% and 44%, Mn in 18% and 13%, and Ni in 26% and 11% of the samples, respectively. When compared to the AMPHORA limits, 33 % and 26 % of the recorded spirits contained Sn and Ni above the suggested thresholds, respectively (see **Table 8**). 52% and 51% of unrecorded spirit samples contained Sn and Cu at levels higher than the AMPHORA limits, respectively (**Table 9**).

Table 8. Mean levels of heavy metals in recorded spirit samples collected in Hungary and compliance with AMPHORA limits

		heavy metals detected in recorded spirits samples							
		copper	cobalt	chromium	iron	manganese	nickel	zinc	tin
Recorded spirits (n = 97)	AMPHORA limits of heavy metals [mg/l]	2.0	-	0.5	2.0	0.5	0.2	5.0	1.0
	mean concentration of heavy metals ± SD [mg/l]	0.71 ± 2.05	0.01 ± 0.05	0.03 ± 0.13	0.65 ± 3.57	0.07 ± 0.37	4.29 ± 13.04	0.33 ± 0.90	0.93 ± 1.34
	minimum – maximum concentration of heavy metals [mg/l]	0.0 – 15.3	0.0 – 0.31	0.0 – 0.71	0.0 – 34.63	0.0 – 3.38	0.0 – 77.16	0.0 – 7.49	0.0 – 3.47
	share of samples in which heavy metals were detected [%]	62	6	12	23	18	26	76	33
	share of samples in which level of heavy metals were above the AMPHORA limit [%]	7	-	3	6	3	26	1	33

Table 9. Mean levels of heavy metals in unrecorded spirit samples collected in Hungary and compliance with AMPHORA limits

		heavy metals detected in unrecorded spirits samples					
		copper	iron	manganese	nickel	zinc	tin
Unrecorded spirits (n = 100)	AMPHORA limits of heavy metals [mg/l]	2.0	2.0	0.5	0.2	5.0	1.0
	mean concentration of heavy metals \pm SD [mg/l]	5.51 \pm 8.06	0.13 \pm 1.19	0.01 \pm 0.08	0.21 \pm 0.93	1.08 \pm 2.62	1.59 \pm 1.56
	minimum – maximum concentration of heavy metals [mg/l]	0.0 – 51.60	0.0 – 11.91	0.0 – 0.79	0.0 – 6.86	0.0 – 16.96	0.0 – 4.10
	share of samples in which heavy metals were detected [%]	99	44	13	11	95	52
	share of samples in which level of heavy metals were above the AMPHORA threshold values [%]	51	1	1	10	7	52

4.1.3. Results of factor analysis

Three groups (factors) of heavy metals present in recorded spirits collected in Hungary were identified by factor analysis, accounting for 75.6% of the total variance (**Figure 6**). Mn, Zn, and Cu (accounting for 36.6% of total variance); Co, Cr, and Ni (accounting for 24.7% of total variance); and Fe and Sn (accounting for 14.3% of total variance) were included in group (factor) 1, 2 and 3, respectively. Data on the levels of heavy metals detected in unrecorded spirits obtained in Hungary did not meet the assumptions of factor analysis. Therefore, it was not carried out for those spirits.

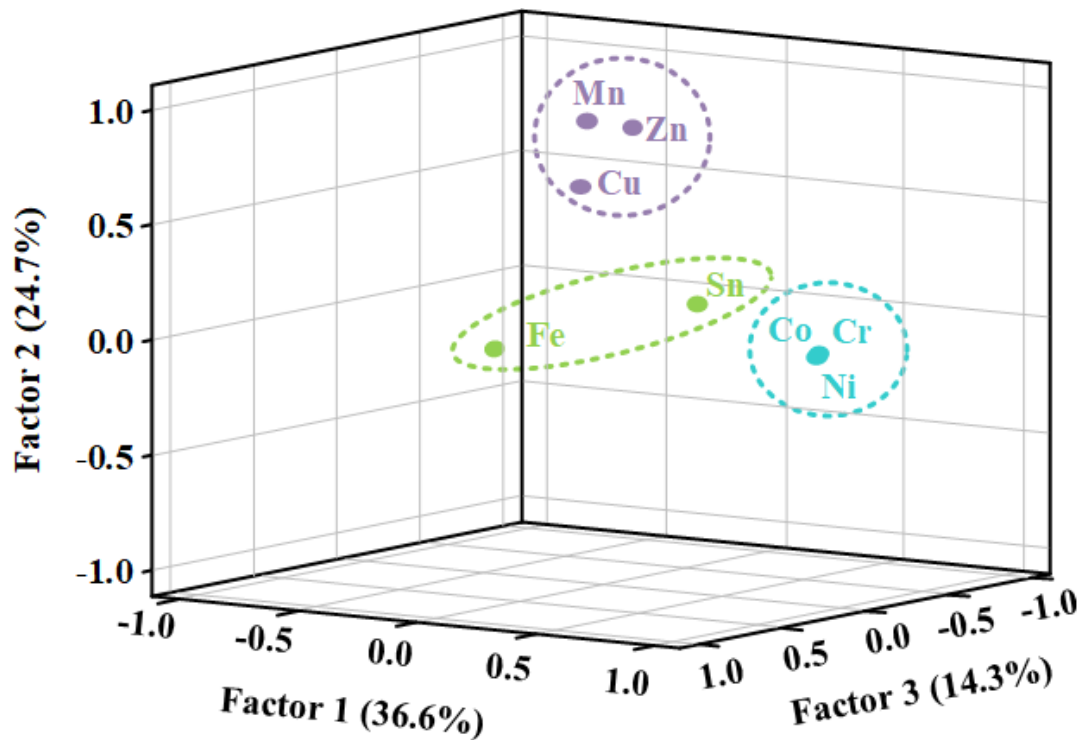


Figure 6. Groups of related metals in recorded spirits collected in Hungary

Groups of related heavy metals (factors) determined in recorded spirits by factor analysis are shown. The effect of individual heavy metals on the groups of related heavy metals and the variance accounted for by each factor are presented by the loading plot. Factor 1: Mn, Zn, Cu; Factor 2: Co, Cr, Ni; Factor 3: Sn, Fe.

4.1.4. Health risks associated with the consumption of heavy metals in recorded and unrecorded spirits collected in Hungary

The findings of our health risk assessment are shown in **Figures 7-10**, using scenarios of population average, regular drinkers, and chronic heavy drinkers (version A and B). **Figures 7-9** depict that the distributions of THQ_c values associated with the consumption of recorded spirits, brandy, pálinka, whiskey, and vodka containing heavy metals were less than 1.0 for both sexes, and separately for men and women. Average (Panels A-C of **Figure 7**), and regular consumers (Panels A-C of **Figure 8**), as well as chronic heavy drinkers (version A, Panels A and B of **Figure 9**) consuming recorded pálinka ($p < 0.001$), whiskey ($p < 0.001$), and vodka ($p < 0.001$) collected in Hungary had significantly lower THQ_c values than those who drink recorded brandy.

Panel D of **Figure 7** and **8** as well as Panel C of **Figure 9** and **10** demonstrate that THQ_c values were also less than 1.0 at each drinking level, when consuming unrecorded spirits. The distributions of THQ_c values for men consuming recorded spirits, brandy and pálinka reached above 1.0 (Panel A of **Figure 10**) and were significantly lower for chronic heavy drinker males (version B) consuming recorded vodka ($p < 0.001$). Panel B of **Figure 10** indicates that distributions of THQ_c values reached above 1.0 for women consuming recorded spirits, brandy and pálinka and were significantly lower for chronic heavy drinker females (version B) consuming recorded whiskey ($p < 0.001$) and vodka ($p < 0.001$) collected in Hungary. In addition, Panel C of **Figure 10** demonstrates that compared to chronic heavy drinker males and females (version B) consuming recorded spirits and recorded pálinka, THQ_c values were significantly lower for those who drink unrecorded spirits ($p < 0.001$).

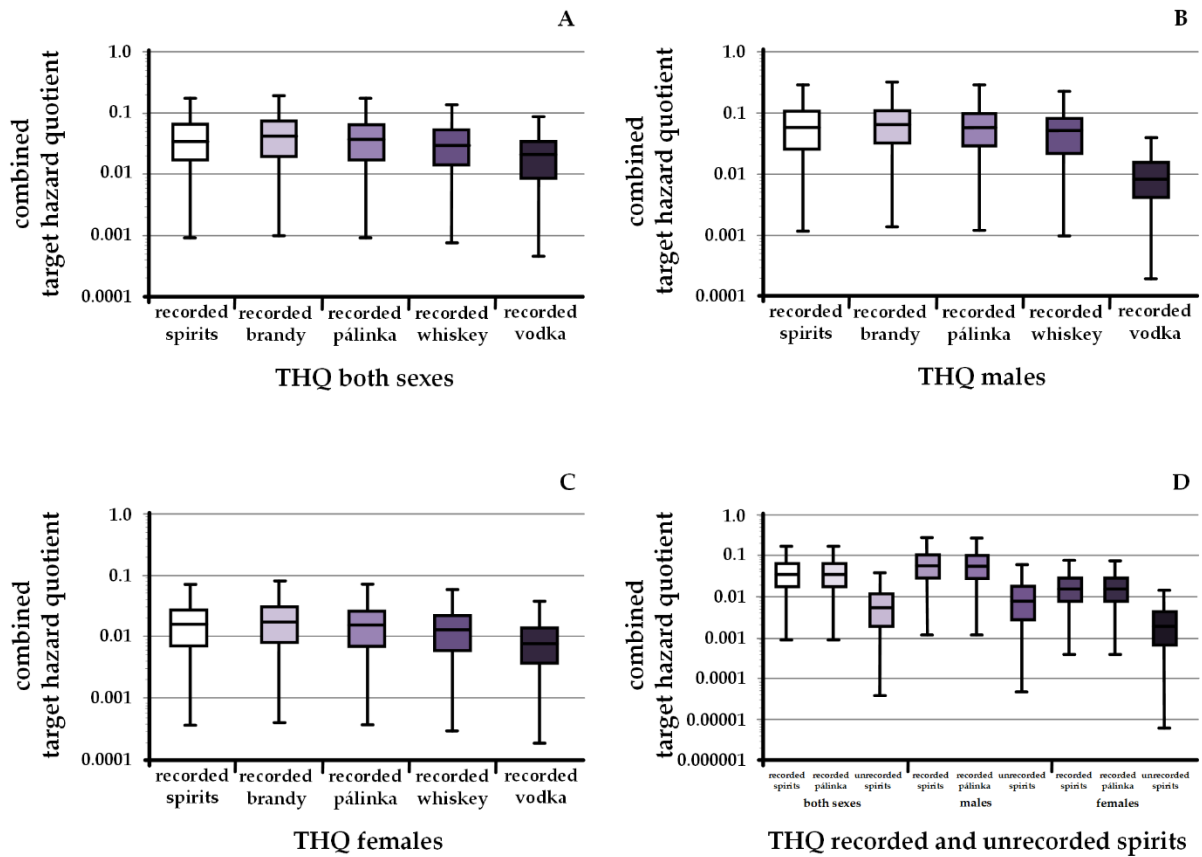


Figure 7. Health risk from combined exposure to heavy metals in recorded and unrecorded spirits by average drinkers

Distributions of combined target hazard quotient (THQ_c) values for both sexes, men and women drinking recorded spirits are presented in Panels A, B and C, respectively. Panel D demonstrates distributions of THQ_c values for both sexes, men and women consuming recorded and unrecorded spirits. Median values of THQ_c, their interquartile ranges, 1st and 99th percentiles are shown.

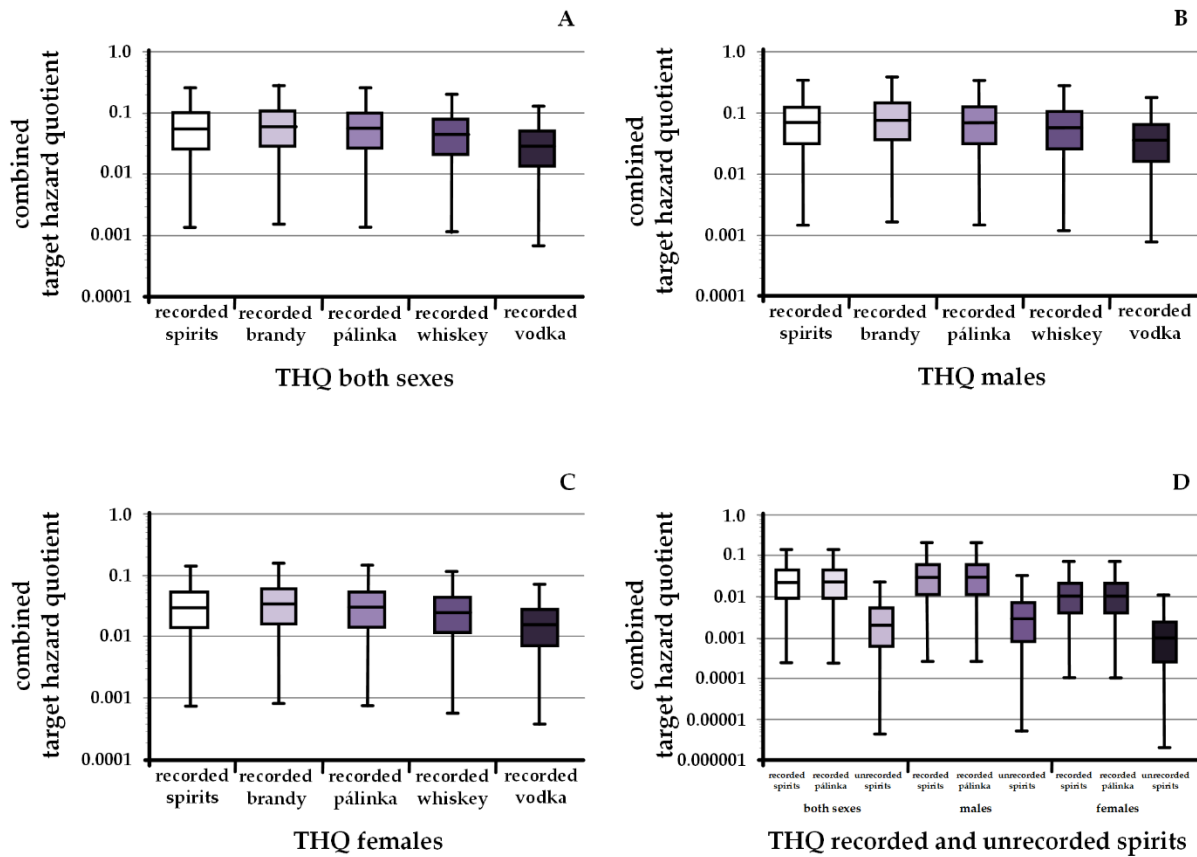


Figure 8. Health risk from combined exposure to heavy metals in recorded and unrecorded spirits by regular drinkers

Distributions of combined target hazard quotient (THQ_c) values for both sexes, men and women drinking recorded spirits are presented in Panels A, B and C, respectively. Panel D demonstrates distributions of THQ_c values for both sexes, men and women consuming recorded and unrecorded spirits. Median values of THQ_c, their interquartile ranges, 1st and 99th percentiles are shown.



Figure 9. Health risk from combined exposure to heavy metals in recorded and unrecorded spirits by heavy drinkers (version A)

The distributions of combined target hazard quotient (THQ_c) values for men and women drinking recorded spirits are depicted in Panels A and B, respectively. Panel C shows the distributions of THQ_c values for men and women consuming recorded and unrecorded spirits. Median values of THQ_c, their interquartile ranges, 1st and 99th percentiles are demonstrated.

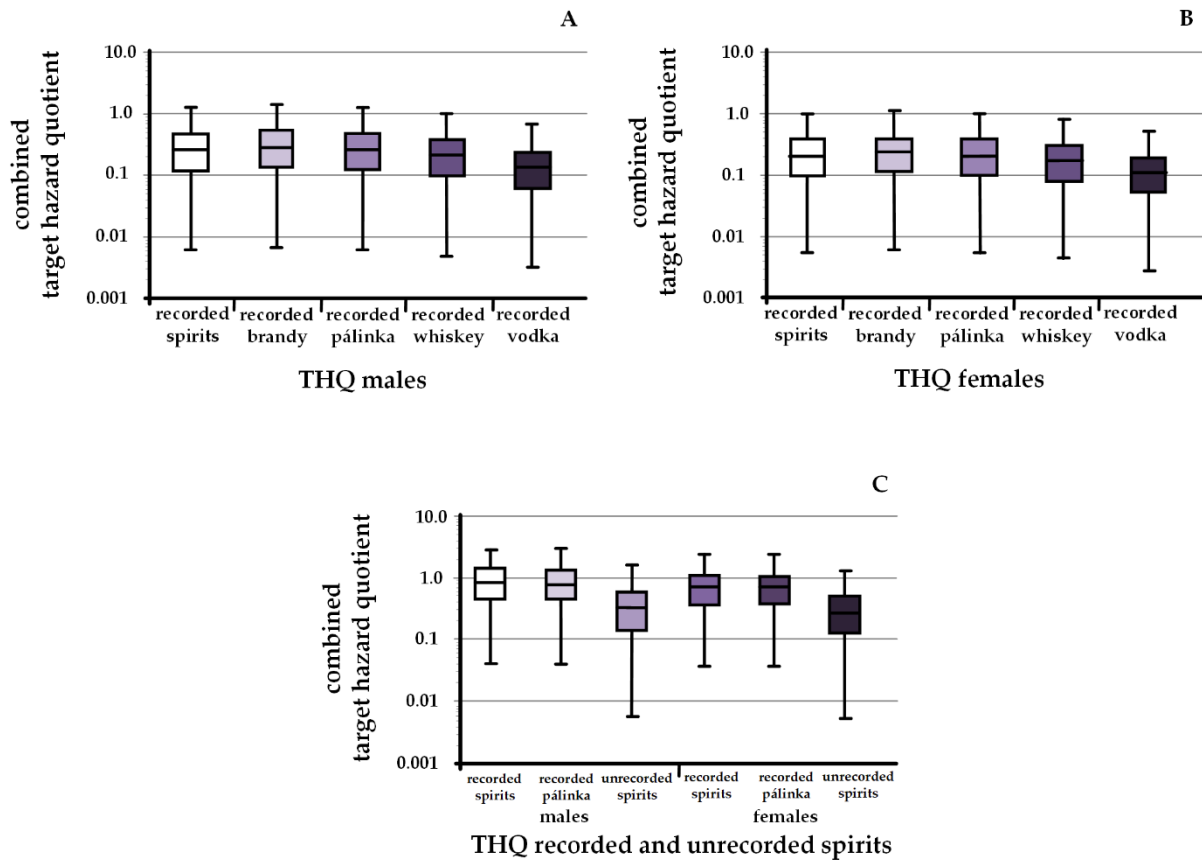


Figure 10. Health risk from combined exposure to heavy metals in recorded and unrecorded spirits by heavy drinkers (version B)

The distributions of combined target hazard quotient (THQ_c) values for men and women drinking recorded spirits are depicted in Panels A and B, respectively. Panel C shows the distributions of THQ_c values for men and women consuming recorded and unrecorded spirits. Median values of THQ_c, their interquartile ranges, 1st and 99th percentiles are demonstrated.

4.2. Composition of unrecorded rakia collected in Albania

4.2.1 Characteristics of unrecorded Albanian rakia samples

Table 10 illustrates that fermented blackberries, juniper, plums, cornelian cherries, mulberries and grapes were used to produce 1 (3.3%), 1 (3.3%), 4 (13.3%), 1 (3.3%), 1 (3.3%) and 22 (73.3%) of the 30 unrecorded rakia samples collected, respectively. This table also shows that 3 of the spirit samples were distilled in 2010, 2014, and 2015, whereas 27 of them were produced in the period from 2017 to 2019. During sample collection, the alcohol content of only 15 rakia (50% of the samples) were reported by sample providers. According to information obtained from the persons providing unrecorded rakia, the ethanol concentration of the samples varied between 16.0 and 22.0% V/V (**Table 10**).

Table 10. Characteristics of rakia samples analysed

sample number	place of sample collection	raw material	year of distillation	reported ethanol concentration (% V/V) ^a	measured ethanol concentration (% V/V) ^b	difference between measured and reported ethanol concentration (%V/V)
sample 1	producer's home	grape	2017	20.0	53.4	33.4
sample 2	producer's home	grape	2017	20.0	43.4	23.4
sample 3	producer's home	grape	2010	20.0	47.1	27.1
sample 4	producer's home	mulberry	2014	22.0	57.6	37.6
sample 5	producer's home	grape	2015	21.0	47.7	26.7
sample 6	producer's home	grape	2018	19.0	49.7	30.7
sample 7	producer's home	grape	2018	19.0	46.5	27.5
sample 8	restaurant	grape	2018	17.0	45.3	28.3
sample 9	restaurant	grape	2018	18.0	47.8	29.8
sample 10	producer's home	cornelian cherry	2017	19.0	52.1	33.1
sample 11	producer's home	plum	2017	20.0	52.5	32.5
sample 12	bar	grape	2019	unknown	52.1	not applicable ^c
sample 13	bar	juniper	2018	unknown	34.2	not applicable
sample 14	bar	grape	2019	unknown	41.6	not applicable
sample 15	bar	plum	2018	unknown	34.4	not applicable
sample 16	bar	grape	2019	unknown	32.0	not applicable
sample 17	bar	grape	2019	17.0	36.7	19.7
sample 18	bar	grape	2018	unknown	46.8	not applicable
sample 19	bar	grape	2018	17.0	43.2	26.2
sample 20	bar	grape	2019	19.0	45.0	26.0
sample 21	bar	grape	2019	unknown	47.4	not applicable
sample 22	bar	blackberry	2017	unknown	33.1	not applicable
sample 23	bar	plum	2018	unknown	42.4	not applicable
sample 24	bar	grape	2019	unknown	41.4	not applicable
sample 25	bar	grape	2019	16.0	32.2	16.2

Table 10. continued: Characteristics of rakia samples analysed

sample number	place of sample collection	raw material	year of distillation	reported ethanol concentration (%V/V) ^a	measured ethanol concentration (%V/V) ^b	difference between measured and reported ethanol concentration (%V/V)
sample 26	bar	grape	2019	unknown	33.0	not applicable
sample 27	bar	grape	2019	unknown	28.6	not applicable
sample 28	bar	grape	2019	unknown	29.5	not applicable
sample 29	bar	plum	2018	unknown	29.6	not applicable
sample 30	bar	grape	2018	unknown	29.1	not applicable

^a Information on ethanol concentration were reported by the producer/seller of the rakia sample. All sample providers used hydrometer to determine the alcohol content of their spirit.

^b The concentration of ethanol was determined by GC/MS analysis.

^c The difference between the measured and reported ethanol concentration cannot be calculated.

4.2.2. Results of comparison of reported and measured ethanol concentrations of the rakia samples

The measured ethanol concentrations were between 28.6 and 57.6% V/V. They (mean: 46.7% V/V, IQR: 43.4–52.1% V/V) were significantly higher than reported ones (mean: 18.9% V/V, IQR: 17.0–20.0% V/V) (**Figure 11**, $p < 0.001$).

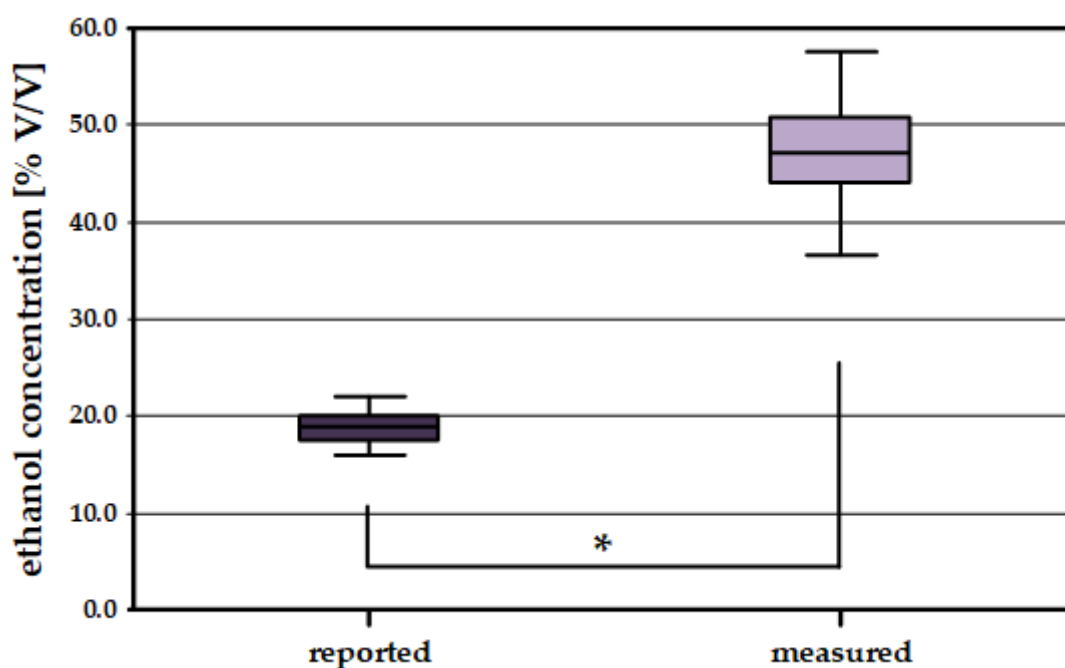


Figure 11. Ethanol concentration of rakia samples by person from whom the samples were obtained and by the GC/MS analysis

4.2.3. Concentration of elements in unrecorded Albanian rakia

Table 11 presents the levels of the elements detected in rakia samples by ICP-OES measurements. Al was detected in 97% ($n = 29$) of the samples. Its concentrations ranged from 0.013 to 0.866 mg/l of p.a., and remained below the AMPHORA limit of 2.0 mg/l of p.a. in all rakia samples. Cu was detected in all of the rakia samples at concentrations varying between 0.025 and 31.629 mg/l of p.a. and its level was above the recommended threshold of 2.0 mg/l

of p.a. in 90% (n = 27) of samples. Although, Fe was present in 93.3% (n = 28) of rakia samples, none of them contained Fe at concentrations exceeding the AMPHORA limit of 2.0 mg/l of p.a. Mg was present in all samples at concentrations varying between 0.185 and 45.244 mg/l of p.a. but its level exceeded the AMPHORA limit of 0.5 mg/l of p.a. only in one sample. Pb was found in 60% (n = 18) of the samples, with ten (33%) of them containing levels of this heavy metal exceeding the 0.2 mg/l of p.a. threshold of the AMPHORA project. With the exception of one sample, which contained twice the limit of Zn (10.156 mg/l of p.a.), all of the Zn levels were below the threshold of 5.0 mg/l of p.a.

Table 11. Concentration of metals detected in rakia samples collected in Albania

	concentration of detected metals [mg/l] ^a					
	Al	Cu	Fe	Mn	Pb	Zn
AMPHORA threshold values of metals	2.0	2.0	2.0	0.5	0.2	5.0
sample 1	0.054	4.729^b	0.071	0.004	<LOD ^c	1.415
sample 2	0.037	1.416	0.060	0.009	<LOD	0.092
sample 3	0.013	9.216	0.021	0.013	<LOD	0.004
sample 4	0.427	8.039	<LOD	0.010	0.066	1.493
sample 5	0.038	25.498	0.117	0.520	0.134	<LOD
sample 6	0.805	21.917	0.076	0.012	<LOD	<LOD
sample 7	0.082	23.386	0.073	0.004	0.052	0.219
sample 8	0.212	4.831	0.141	0.009	0.044	<LOD
sample 9	0.042	3.446	0.028	0.005	<LOD	0.084
sample 10	0.017	2.134	0.004	0.004	<LOD	<LOD
sample 11	0.053	10.824	0.030	0.011	1.337	1.524
sample 12	0.223	25.721	0.092	0.012	1.010	1.255
sample 13	0.047	0.025	0.018	<LOD	<LOD	0.018
sample 14	0.135	20.405	0.284	0.005	0.135	0.317
sample 15	0.058	0.043	0.070	<LOD	<LOD	<LOD
sample 16	0.044	6.415	0.225	<LOD	<LOD	<LOD
sample 17	0.065	25.555	0.125	0.011	0.065	0.610
sample 18	0.060	5.078	0.068	0.004	0.124	<LOD
sample 19	0.171	13.090	0.593	0.005	0.083	0.273
sample 20	0.027	5.481	0.031	<LOD	0.351	0.982
sample 21	<LOD	15.411	<LOD	0.004	0.599	0.717
sample 22	0.018	19.736	0.677	0.006	0.236	0.036
sample 23	0.104	9.233	0.024	0.009	0.217	1.948
sample 24	0.106	17.050	0.097	<LOD	<LOD	0.097
sample 25	0.056	19.511	0.068	<LOD	0.261	0.298

Table 11. continued: Concentration of metals detected in rakia samples collected in Albania

	concentration of detected metals [mg/l] ^a					
	Al	Cu	Fe	Mn	Pb	Zn
AMPHORA threshold values of metals	2.0	2.0	2.0	0.5	0.2	5.0
sample 26	0.091	26.451	0.085	0.006	<LOD	0.582
sample 27	0.287	10.932	0.126	<LOD	<LOD	<LOD
sample 28	0.108	31.629	1.173	0.014	0.468	10.156
sample 29	0.703	13.941	0.027	<LOD	0.527	<LOD
sample 30	0.866	14.422	0.144	<LOD	0.543	0.137

^a Concentrations of metals were determined by inductively coupled plasma optical emission spectrometric analysis (ICP-OES) and expressed in mg/l of pure alcohol

^b Bold values indicate spirit samples that contained metals at levels above the AMPHORA threshold values

^c <LOD: The concentration of metals was below the limit of detection (LOD) of the ICP-OES

4.3. Estimation of health risk from intake of methanol in unrecorded spirits

4.3.1. Characteristics of unrecorded fruit spirit samples analysed in the included studies

As demonstrated in **Table 12**, the median level of methanol in unrecorded fruit spirits ranged from 539.0 to 9542.2 mg/l of p.a. In addition, the concentration of methanol exceeded the AMPHORA limit of 10 000 mg/l of p.a. in 2%, 4%, 9%, 11%, 11%, 12%, and 35% of the schnapps (Huckenbeck et al., 2003), țuică (Levy et al., 2003), pálinka (Szűcs et al., 2005), pálinka (Bujdosó et al., 2019), koumaro (Soufleros et al., 2005), pálinka (Huckenbeck et al., 2003), and brandy samples (Rusu Coldea et al., 2011), respectively.

Table 12. Number and characteristics of unrecorded fruit samples analysed in the studies from which data were extracted

reference	number of samples analysed	median concentration of methanol and IQR* [mg/l of pure alcohol]		share of samples containing methanol above the AMPHORA limit [%]
Bujdosó et al., 2019	87	6347.3	4166.3-8435.2	11
Hanousek Čiča et al., 2019	13	539	426-1046	0
Huckenbeck et al., 2003	50**	4674.6	2343.9-6348.3	2
	25***	6408	3087.1-8178.3	12
Lachenmeier et al., 2009	11	3075	475-4950	0
Levy et al., 2003	26	2000	1000-4000	4
Rusu Coldea et al., 2011	25	9542.2	8634.5-10766.1	35
Soufleros et al., 2004	10	1323	1102.7-1848.6	0
Soufleros et al., 2005	19	8436	7682-9504.5	11
Szűcs et al., 2005	34	4994.4	1267.7-6740.1	9

* interquartile range

** place of sample collection: Germany

*** place of sample collection: Hungary

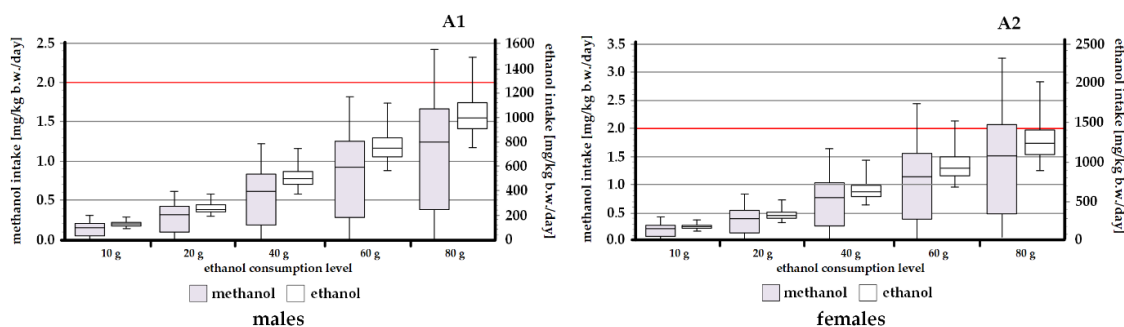
4.3.2. Estimated daily methanol and ethanol intake associated with the consumption of unrecorded fruit spirits

Panels A1-A2, B1-B2, C1-C2, and D1-D2 of **Figure 12** show the distributions of the EDMIs separately for males and females using the methanol levels detected in wine-derived spirits, schnapps, mouro, and koumaro, respectively. **Figure 12** demonstrates that the distributions of EDMIs in men exceeded the RfD when drinking koumaro (Panel D1), schnapps (Panel B1), mouro (Panel C1), or wine-derived spirits (Panel A1) containing methanol and also at least 10 g, 20 g, 60 g, and 80 g ethanol, respectively. Consumption of schnapps (Panel B2), koumaro (Panel D2), mouro (Panel C2), and wine-derived spirit (Panel A2) containing methanol, as well as 10 g, 10 g, 40 g, and 60 g of ethanol, respectively, resulted in distributions of EDMIs exceeding the RfD in female drinkers. Panels E1-H2 of **Figure 13** illustrate the distributions of EDMIs based on the levels of methanol in pálinka samples separately for men and women. For males, the distribution of EDMIs were above the RfD when drinking pálinka containing methanol, as well as 10 g (Panels E1, F1, and H1 of **Figure 13**), and 20 g of ethanol (Panel G1 of **Figure 13**). As illustrated, the distribution of EDMIs in women exceeded the RfD when consuming unrecorded fruit spirits that contain methanol and at least 10 g (Panels E2, F2, and H2), and 20 g of ethanol (Panel G2). Panels I1-I2, J1-J2 and K1-K2 of **Figure 14** demonstrate the distributions of EDMIs for males and females applying methanol levels found in țuică and brandy, and assuming the consumption of a HFS containing methanol at the maximum concentration determined by AMPHORA study (10 000 mg/l of p.a.), respectively. The figure depicts that the distribution of EDMIs reached the RfD when drinking țuică, brandy, and HFS containing methanol, as well as 10 g of ethanol (Panels I1, J1, and K1 for men and Panels I2, J2, and K2 for women). There was a significant difference between the distributions of EDMI values associated with drinking of unrecorded fruit spirits included in our study and those

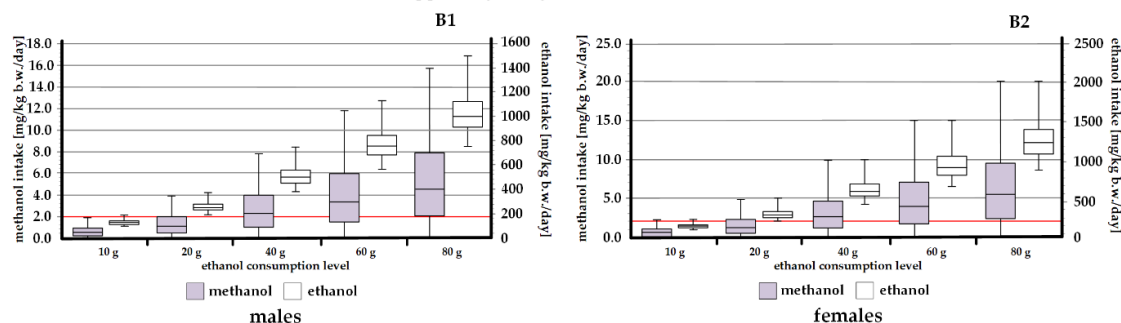
attributable to drinking an HFS at each consumption category both for men and women ($p < 0.001$).

The methanol levels in unrecorded fruit spirits required to reach the reference dose at different consumption levels are shown in **Table 13**. It is demonstrated that the consumption of unrecorded fruit spirits containing methanol at levels of 8598.1 or 6382.1 mg/l of p.a. and 10 g ethanol (equivalent to 31.5 ml of unrecorded fruit spirits containing 40 % V/V ethanol) can lead to a methanol intake that can reach the RfD in males and females, respectively.

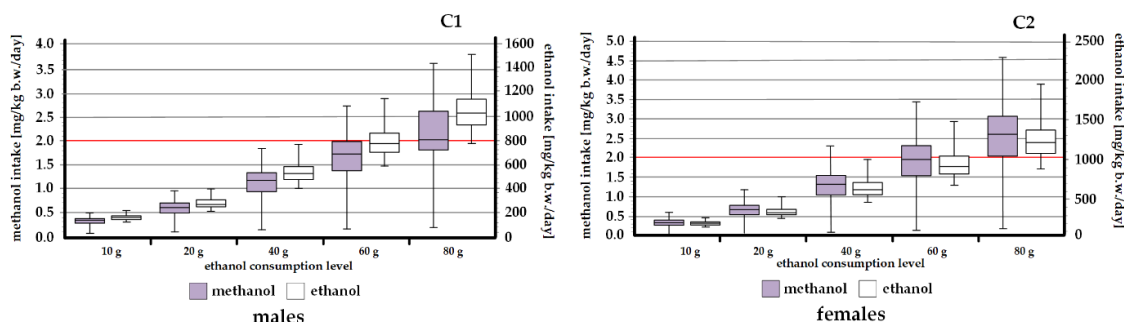
wine-derived spirits analysed by Hanousek-Čiča et al., 2019



schnapps analysed by Huckenbeck et al., 2003



mouro analysed by Soufleros et al., 2004



koumaro analysed by Soufleros et al., 2005

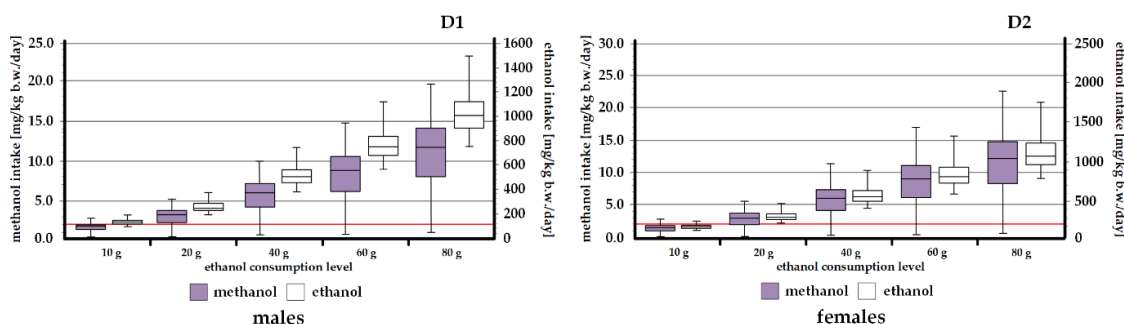


Figure 12. Estimated daily methanol and ethanol intakes by males and females when consuming unrecorded fruit spirits containing methanol

Methanol and ethanol intakes (mg/kg body weight/day) are illustrated on the left and right y-axes, respectively. Panels: A1-A2 wine-derived spirits, B1-B2 schnapps, C1-C2 mouro, D1-D2 koumaro. Median values, their interquartile ranges, 1st and 99th percentiles are presented. The red line shows the reference dose of methanol.

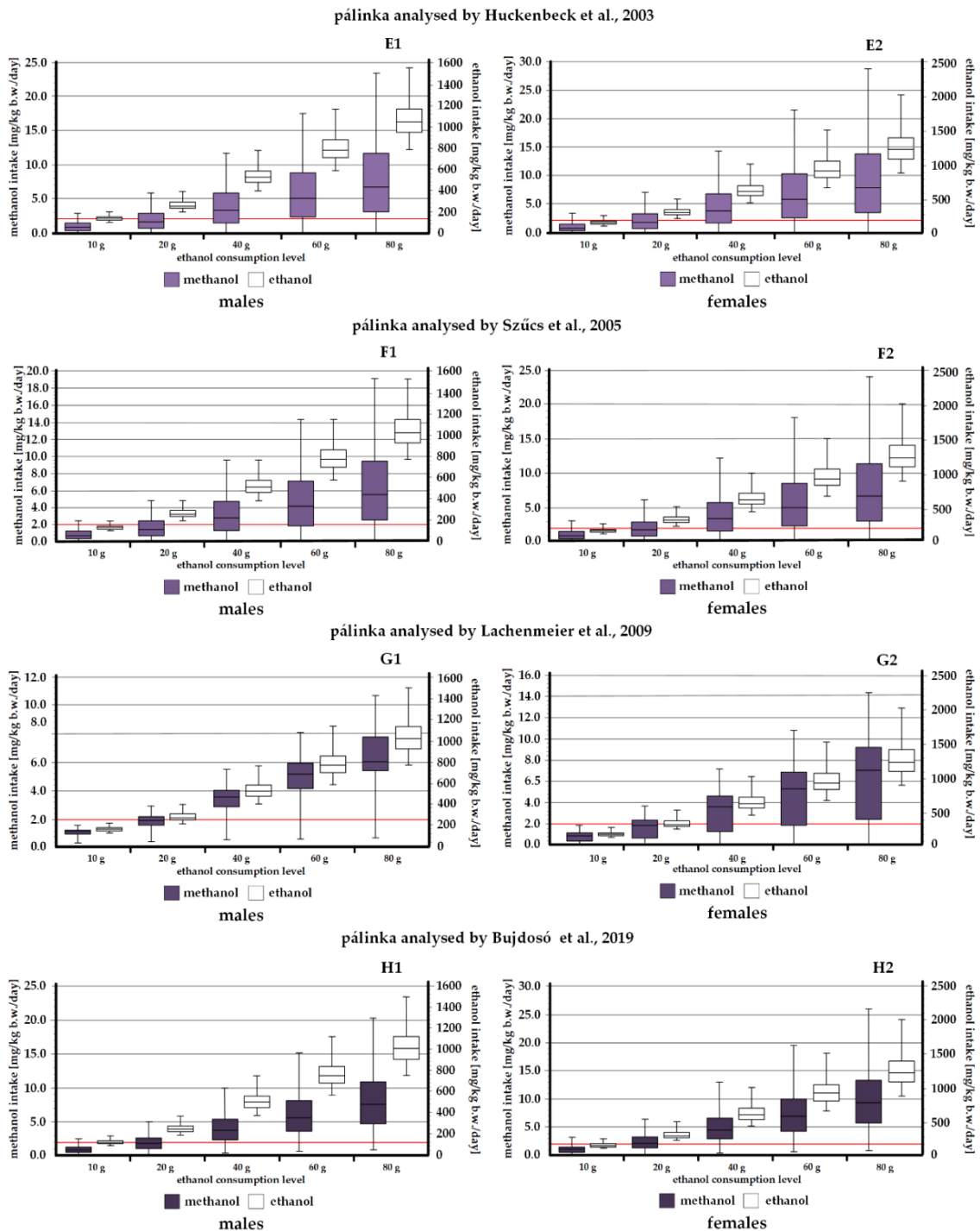


Figure 13. Estimated daily methanol and ethanol intakes by males and females when consuming unrecorded fruit spirits containing methanol

Methanol and ethanol intakes (mg/kg body weight/day) are illustrated on the left and right y-axes, respectively. Panels: E1-E2 pálinka, F1-F2 pálinka, G1-G2 pálinka, H1-H2 pálinka. Median values, their interquartile ranges, 1st and 99th percentiles are presented. The red line shows the reference dose of methanol.

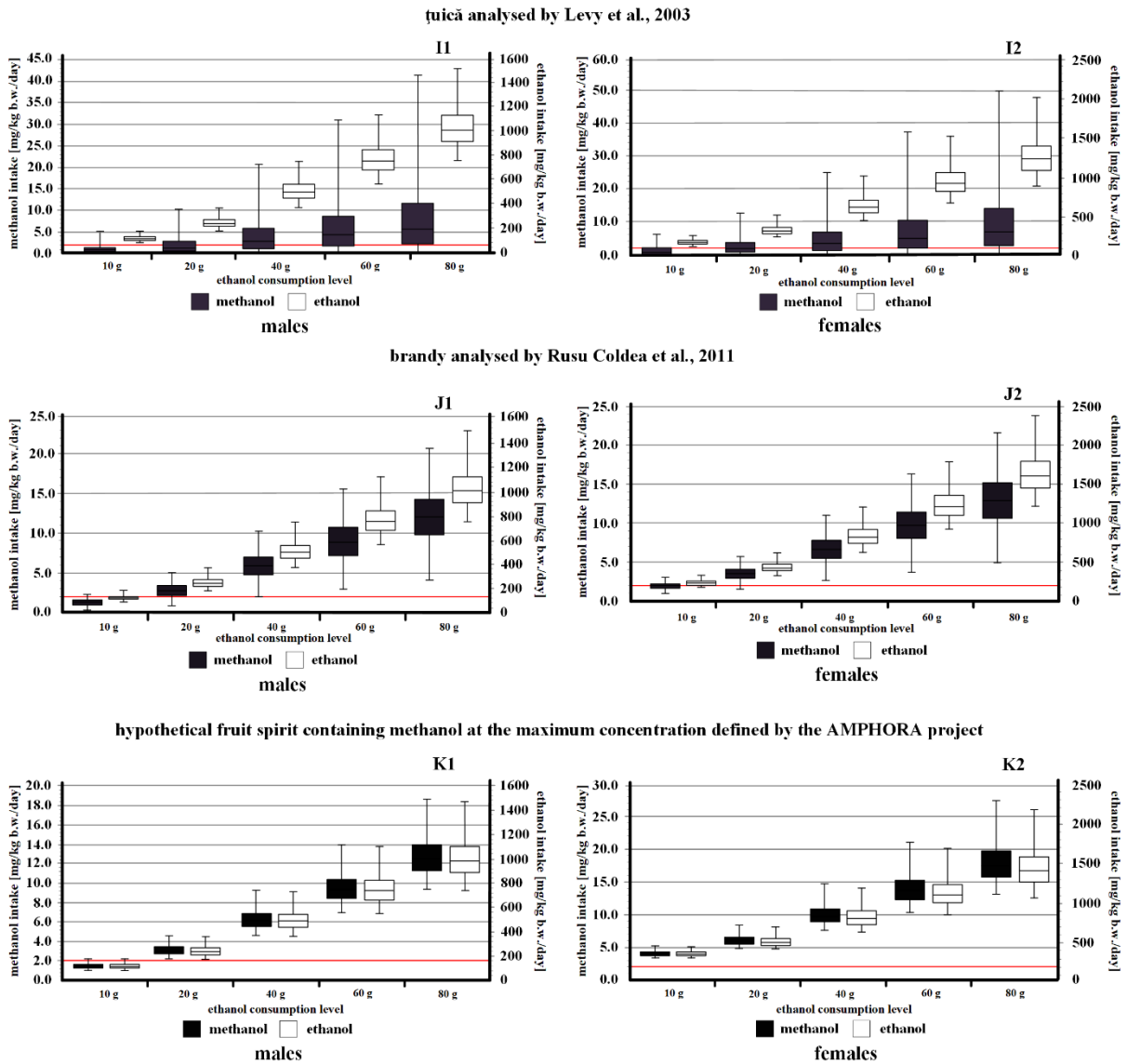


Figure 14. Estimated daily methanol and ethanol intakes by males and females when consuming unrecorded fruit spirits containing methanol

Methanol and ethanol intakes (mg/kg body weight/day) are illustrated on the left and right y-axes, respectively. Panels: I1-I2 țuică, J1-J2 brandy, K1-K2 hypothetical fruit spirit with a methanol concentration of 10 000 mg/l of p.a. Median values, their interquartile ranges, 1st and 99th percentiles are presented. The red line shows the reference dose of methanol.

Table 13. Methanol concentrations in unrecorded fruit spirits required to reach the reference dose at different consumption levels

percentiles	body weight distribution of males	methanol concentration* in unrecorded fruit spirits required to reach the reference dose of 2.0 mg/kg body weight/day at different ethanol consumption levels					body weight distribution of females	methanol concentration* in unrecorded fruit spirits required to reach the reference dose of 2.0 mg/kg body weight/day at different ethanol consumption levels				
		10 g ethanol (31.7 ml ⁺)	20 g ethanol (63.4 ml ⁺)	40 g ethanol (126.7 ml ⁺)	60 g ethanol (190.1 ml ⁺)	80 g ethanol (253.5 ml ⁺)		10 g ethanol (31.7 ml ⁺)	20 g ethanol (63.4 ml ⁺)	40 g ethanol (126.7 ml ⁺)	60 g ethanol (190.1 ml ⁺)	80 g ethanol (253.5 ml ⁺)
1%	55.0 kg	8 598.10	4 367.29	2 166.45	1 448.10	1 087.51	40.8 kg	6 382.12	3 241.71	1 608.09	1 074.88	807.22
25%	73.4 kg	11 463.66	5 822.81	2 888.48	1 930.72	1 449.95	58.8 kg	9 182.05	4 663.90	2 313.59	1 546.45	1 161.37
50%	82.0 kg	12 812.16	6 507.76	3 228.26	2 157.84	1 620.51	67.2 kg	10 499.67	5 333.16	2 645.59	1 768.37	1 328.02
75%	90.6 kg	14 160.57	7 192.67	3 568.02	2 384.94	1 791.06	75.6 kg	11 817.19	6 002.38	2 977.56	1 990.26	1 494.66
99%	108.9 kg	17 022.89	8 646.55	4 289.23	2 867.01	2 153.09	93.6 kg	14 613.97	7 422.97	3 682.26	2 461.30	1 848.41

* in mg/l of pure alcohol

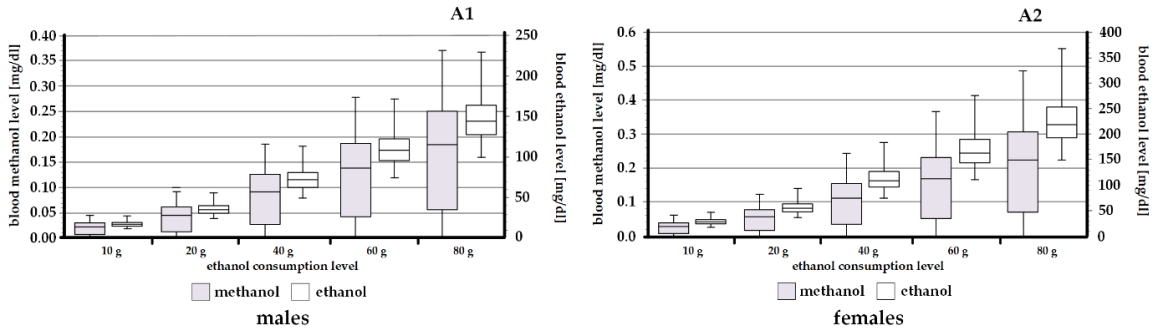
⁺ The values in brackets show the volume of unrecorded fruit spirit containing 40.0 V/V % ethanol.

4.3.3. Estimated blood methanol and ethanol levels associated with the consumption of unrecorded fruit spirits

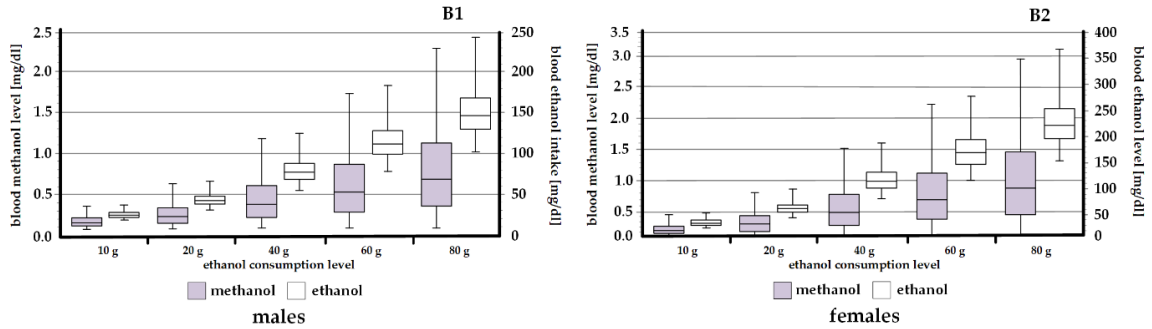
Methanol levels found in wine-derived spirit, schnapps, mouro, koumaro, pálinka, țuică, brandy, and a HFS were used to estimate the distributions of the BMLs separately for men and women. The results of this assessment are presented in **Figures 15, 16 and 17**. As demonstrated in Panels I1-I2 of **Figure 17**, the distribution of BMLs for males and females reached above the MTBML when drinking țuică containing methanol and at least 80 g and 60 g of ethanol, respectively. The distributions of BMLs related with drinking of unrecorded fruit spirits included in our research differed significantly from those associated with consumption of a HFS at each drinking level both for men and women ($p < 0.001$).

Table 14 demonstrates the methanol levels in unrecorded fruit spirits required to reach the MTBML at different consumption levels. As shown, consumption of unrecorded fruit spirits containing methanol at a level of 17 476.1 mg/l of p.a. or 17 384.2 mg/l of p.a., as well as 80 g (equivalent to 253.4 ml of fruit spirit with an ethanol content of 40 % V/V) or 60 g ethanol (equivalent to 190.1 ml of fruit spirit with an ethanol content of 40 % V/V) can lead to methanol intakes that can reach the MTBML in men and women, respectively.

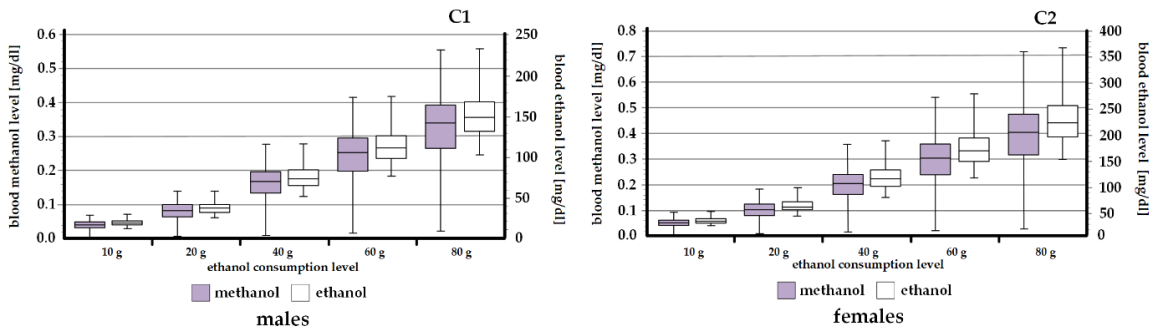
wine-derived spirits analysed by Hanousek-Čiča et al., 2019



schnapps analysed by Huckenbeck et al., 2003



mouro analysed by Soufleros et al., 2004



koumaro analysed by Soufleros et al., 2005

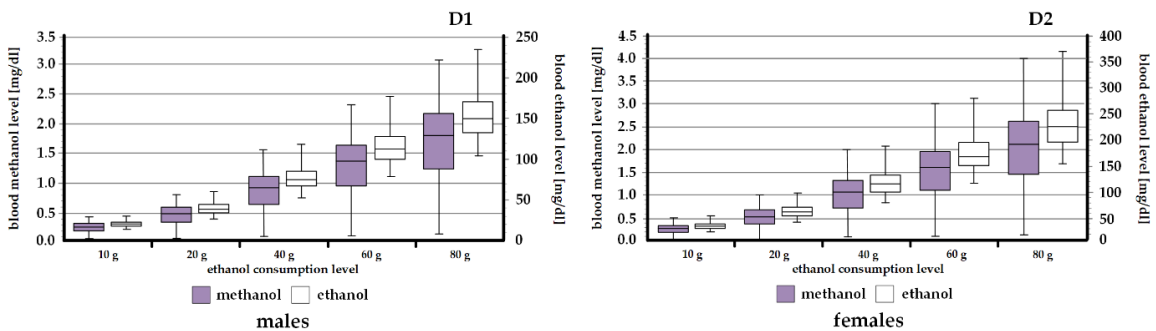


Figure 15. Estimated blood methanol and ethanol concentrations in males and females when consuming unrecorded fruit spirits containing methanol

The distribution of blood methanol and ethanol levels are demonstrated on the left and right y-axes (mg/dl), respectively. Panels: A1-A2 wine-derived spirits, B1-B2 schnapps, C1-C2 mouro, D1-D2 koumaro. Median values, their interquartile ranges, 1st and 99th percentiles are shown.

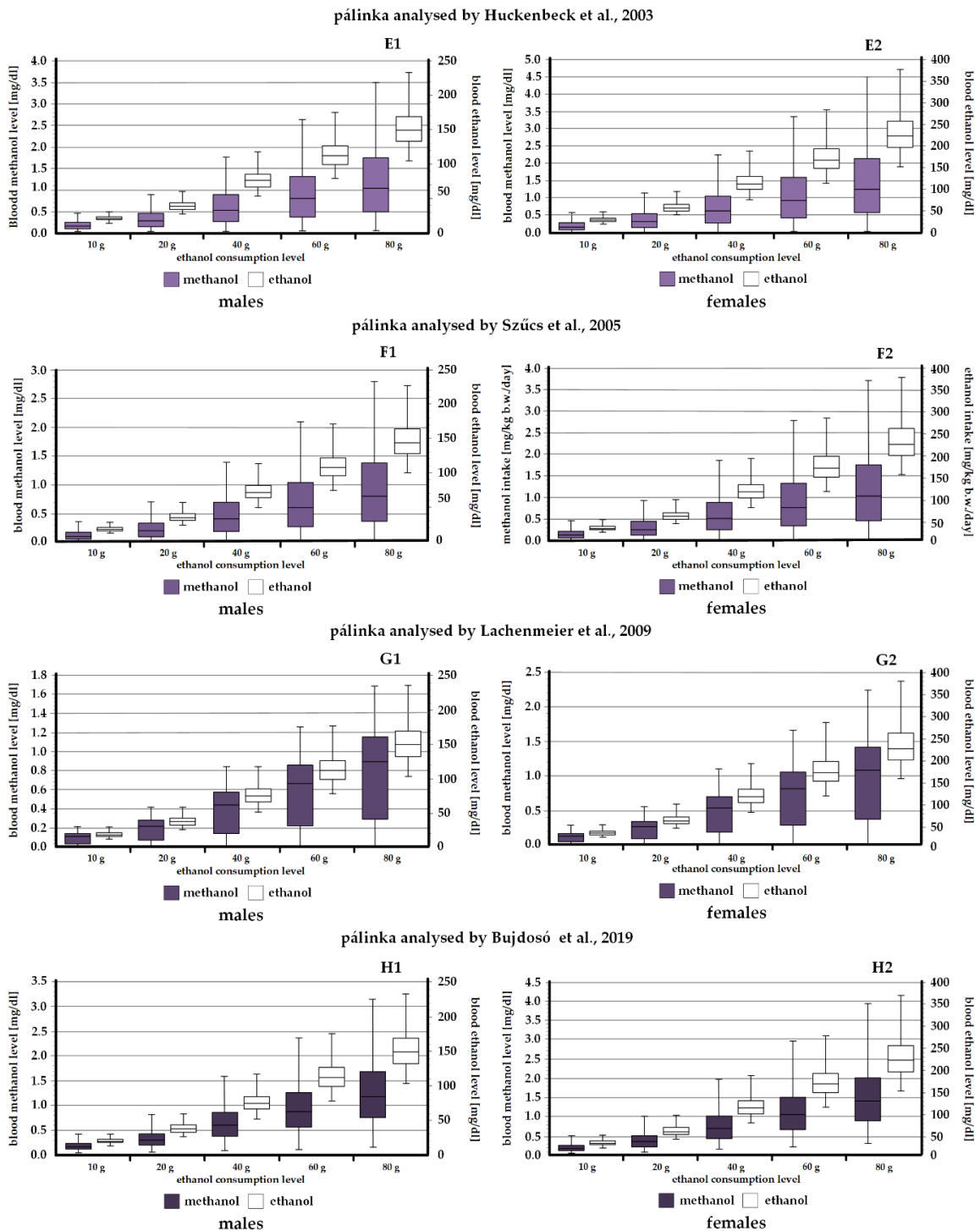


Figure 16. Estimated blood methanol and ethanol concentrations in males and females when consuming unrecorded fruit spirits containing methanol

The distribution of blood methanol and ethanol levels are demonstrated on the left and right y-axes (mg/dl), respectively. Panels: E1-E2 pálinka, F1-F2 pálinka, G1-G2 pálinka, H1-H2 pálinka. Median values, their interquartile ranges, 1st and 99th percentiles are shown.

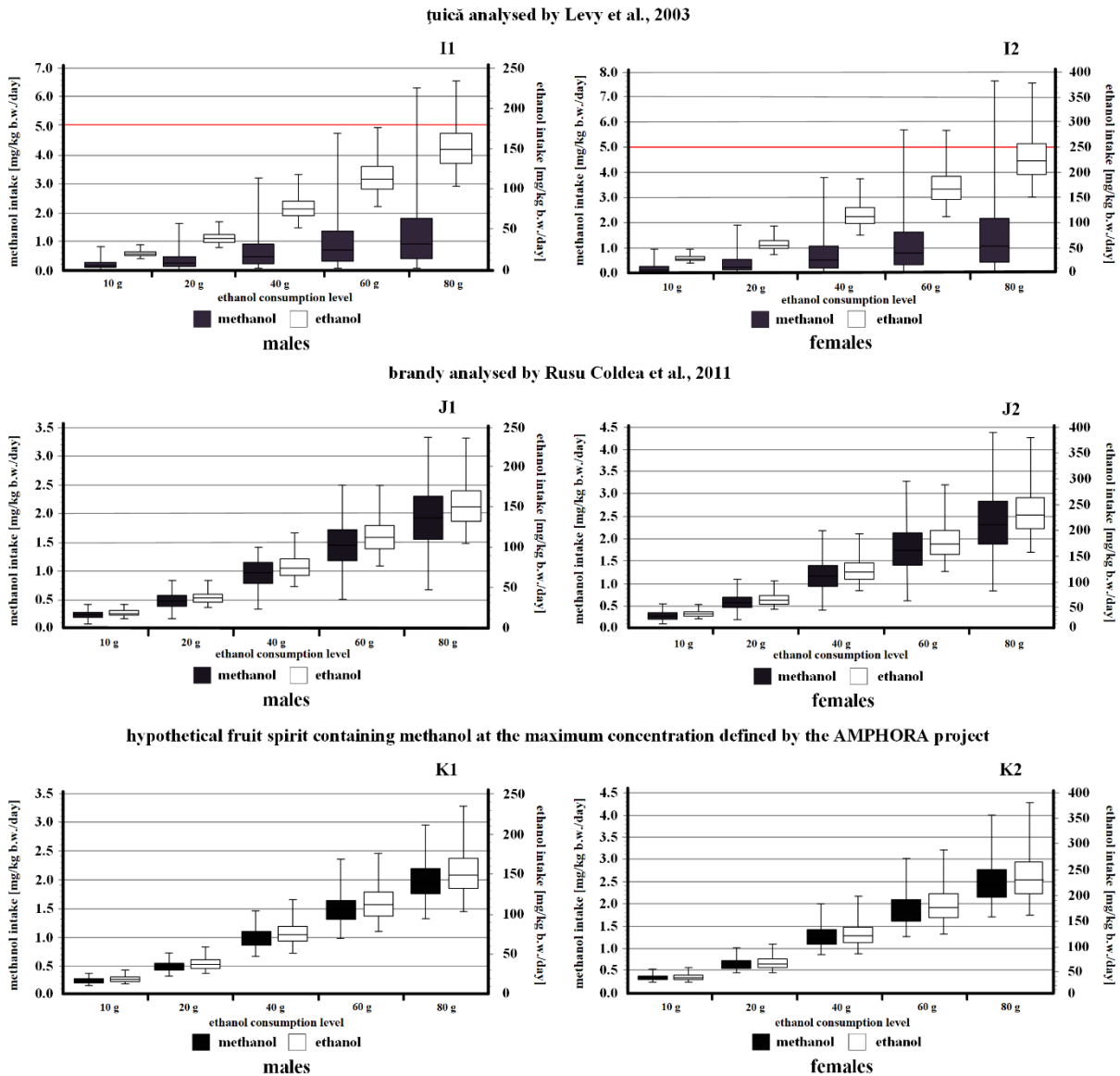


Figure 17. Estimated blood methanol and ethanol concentrations in males and females when consuming unrecorded fruit spirits containing methanol

The distribution of blood methanol and ethanol levels are demonstrated on the left and right y-axes (mg/dl), respectively. Panels: I1-I2 țuică, J1-J2 brandy, K1-K2 hypothetical fruit spirit. Median values, their interquartile ranges, 1st and 99th percentiles are illustrated. The maximum tolerable blood methanol level is shown by the red line.

Table 14. Levels of methanol in unrecorded fruit spirits required to reach the maximum tolerable blood methanol level at different intake levels

percentiles	body weight distribution of males [kg]	methanol concentration * in unrecorded fruit spirits required to reach the reference dose of 2.0 mg/kg body weight/day at different ethanol consumption levels					body weight distribution of females [kg]	methanol concentration * in unrecorded fruit spirits required to reach the reference dose of 2.0 mg/kg body weight/day at different ethanol consumption levels				
		10 g ethanol (31.7 ml ⁺)	20 g ethanol (63.4 ml ⁺)	40 g ethanol (126.7 ml ⁺)	60 g ethanol (190.1 ml ⁺)	80 g ethanol (253.5 ml ⁺)		10 g ethanol (31.7 ml ⁺)	20 g ethanol (63.4 ml ⁺)	40 g ethanol (126.7 ml ⁺)	60 g ethanol (190.1 ml ⁺)	80 g ethanol (253.5 ml ⁺)
1%	55.0	-	69904.4	34952.2	23301.5	17476.1	40.8	-	52152.5	26076.3	17384.2	13038.1
25%	73.4	-	-	46758.6	31172.4	23379.3	58.8	-	74987.2	37493.6	24995.7	18047.9
50%	82.0	-	-	52412.7	34941.8	26206.3	67.2	-	-	42958.3	28638.9	21479.1
75%	90.6	-	-	58240.1	38826.7	29120.0	75.6	-	-	48619.2	32412.8	24309.6
99%	108.9	-	-	71474.0	47649.3	35737.0	93.6	-	-	61002.9	40668.6	30501.5

* Only methanol concentrations possible at the given consumption levels are shown in mg/l of pure alcohol

+ The values in brackets show the volume of unrecorded fruit spirit containing 40.0% V/V ethanol

5. Discussion

Several studies have shown that, in addition to ethanol, other aliphatic alcohols including methanol, as well as metalloids and metals such as Cu, Cd, Pb, Zn, and Ni can be detected in alcoholic beverages (56, 85, 106, 110, 115, 120-123, 190-198). Some of them have been described to be toxic or even carcinogenic to humans (64, 83, 92, 125-127, 153, 173, 199-201). Although these substances are often present in recorded alcoholic beverages, their concentrations have been shown to be significantly higher in their unrecorded counterparts, especially in spirits (10). Previous research suggests that almost all unrecorded alcohol is consumed entirely in the form of unrecorded spirits in the countries of the WB and CEE including Albania and Hungary (10, 82, 202, 203). Consequently, consumers of unrecorded spirits, particularly heavy drinkers, may be exposed to higher levels of these harmful substances compared to those who consume only recorded spirits (10, 56, 69, 85, 130, 202, 204). This raises the question of whether these beverages pose any health risk beyond those related to ethanol alone. However, there have been no studies in the scientific literature that determined the levels of metals in both recorded and unrecorded spirits and compared the health risks related to their consumption. In addition, the composition of unrecorded Albanian spirit samples has not been studied before. Furthermore, it has not been investigated in countries of CEE whether exposure to methanol consumed in unrecorded spirits is expected to cause any acute or chronic health effects. To fill the first and the second gap, we have used data obtained by the analysis of recorded and unrecorded spirit samples collected in Hungary and unrecorded rakia from Albania. To address the third question, we have estimated EDMI and BML using information extracted from previous studies reporting concentrations of methanol in unrecorded fruit spirits consumed in countries of CEE.

While toxicological limits have been determined for several metals, a comparison with their levels in spirit samples can only determine whether their concentrations are below or above the threshold (114). Nevertheless, this method fails to capture the entirety of exposure and is unsuitable for a quantitative risk assessment since exceeding toxicological limits does not always indicate an elevated health risk. Therefore, a more complex approach incorporating the THQ analysis is required to carry out detailed health risk estimation (191, 192, 205). Furthermore, factors, including sex and drinking patterns, influencing the health risk associated with the consumption of recorded and unrecorded spirits should be also included in the assessment. Although, a previous investigation was carried out using this methodology, it analysed data only on the concentration of heavy metals in unrecorded spirits (191, 192, 205). In addition, the health risk associated with drinking recorded and unrecorded spirits has not been compared. Therefore, our work is the first that provides a comprehensive health risk estimation using a probabilistic risk assessment approach, including THQ analysis with different exposure scenarios for the Hungarian population. First, as described previously, levels of heavy metals in recorded and unrecorded spirits collected in Hungary were measured. These concentration values were compared with those reported by previous investigations. A Serbian study showed that unrecorded spirits distilled from plums contained on average 0.3, 0.4, 1.4, and 3.9 mg/l of Zn, Mn, Fe, and Cu, respectively (190). The same study found that the mean concentrations of these metals in recorded plum spirits were 1.1 (Zn), 0.4 (Mn), 0.4 (Fe), and 3.3 (Cu) mg/l (190). A critical review on the levels of Zn, Fe, and Cu in recorded brandy, whiskey, and vodka reported concentrations ranging from 0.0 to 20, from 0.0 to 2.3, and from 0.1 to 14.6 mg/l, respectively (121). Our results are in agreement with those reported in previous papers (see **Table 8** and **Table 9**). Due to lack of data, we were not able to compare the levels of Sn in recorded and unrecorded spirits collected in Hungary with results in other studies. Furthermore, only a small number of research published data on the level of Ni in spirits (82,

191). Of the studies available, one found a mean Ni concentration of 0.23 mg/l in unrecorded spirits, whereas another reported that the average concentration of this heavy metal was 0.13 mg/l in their recorded counterparts (82, 192). Although, the level of Ni in unrecorded spirit samples collected in Hungary (0.21 mg/l) was comparable to that of in previous investigations, this was not so for the recorded samples (4.29 mg/l) (118). There could be a number of reasons for this difference. Among them, the type of spirits investigated and their ethanol content can have the greatest impact (191). In our study, the type of recorded spirits and their alcohol concentration (varying between 31% and 80% V/V) was different from those included in previous studies (ranging from 5% to 60% V/V) (192). Furthermore, we have expressed the concentrations of metals in mg/l of p.a. to ensure comparability of samples with varying ethanol content. This may not have been done so in other investigations, complicating comparisons. Another possible explanation for this discrepancy is that our dataset incorporated outlier values for Ni levels, which could contribute to the higher mean concentrations reported in our study. Three groups of metals have been shown in recorded spirits by factor analysis incorporating Mn, Zn, Co (factor 1), Co, Cr, Ni (factor 2) and Sn, Fe (factor 3). Distillation equipment and storage containers are typically composed of materials containing metals in factor 1 and factor 2, respectively (121, 206). Consequently, we can assume that metals in factor 1 and 2 leach into recorded spirits from these sources. Since metals in factor 3 are negatively correlated, we hypothesise that they originate from different sources. Although a significant share of our recorded and unrecorded samples contained levels of heavy metals above the AMPHORA limit, our comprehensive risk estimation indicates that the health risk is low for average, regular and chronic heavy drinkers (version A). In contrast, a small proportion of chronic heavy drinkers (version B) may be at increased risk when they consume recorded spirits containing heavy metals. However, when compared to the adverse effects of ethanol, this may only have a small impact on the overall health of population in Hungary.

The levels of metals in unrecorded spirit samples collected in countries of CEE have been reported by a number of prior studies (85, 180, 190, 195). However, only limited information is available on their concentration in traditional home-made fruit spirits, including rakia, frequently consumed in the WB (47, 82, 118, 195, 202). In addition, no previous research has investigated the concentrations of metals and ethanol content in unrecorded rakia obtained from Albania (10, 198). We have, therefore, complemented the existing research by analysing alcohol strength and metal content in unrecorded Albanian rakia and then compared the concentration obtained with AMPHORA limits. A significant proportion of our rakia samples (63.3%, n=19) contained more than 40% V/V ethanol, which is the most common alcohol level in recorded spirits (105, 113). Our findings are consistent with that of in previous studies demonstrating that, compared to recorded spirits, the ethanol content of unrecorded spirits is frequently higher (75, 82, 195). In addition, compared to the alcohol strength obtained by GC/MS measurements, individuals selling unrecorded spirits significantly underestimated the ethanol content of their products. Lachenmeier et al. 2011, also identified a discrepancy between the reported (20% V/V) and measured (44% V/V) ethanol content of a rakia sample collected in Albania (82). This difference may be attributed to the use of hydrometers by the sellers of the unrecorded rakia to determine the alcohol content of the spirits. Despite the fact that this device is commonly applied to measure the ethanol level of alcoholic beverages based on their density, it provides only semi-quantitative results (194, 207). Improper use of hydrometers or not adjusting ethanol levels for temperature can result in inaccuracies in the alcohol content determined in unrecorded spirits (207). As a consequence, the underestimation of alcohol strength in unrecorded rakia can result in heavy drinking and related adverse effects in consumers of those beverages. Our results are in agreement with those of in previous studies indicating that the level of various metals in unrecorded spirits can exceed the permissible limits (85, 190, 194, 202, 208). Of these, Cu and Pb are most concerning (85, 118, 202, 209, 210).

The mean level of Cu in our rakia samples was 13.1 ± 9.09 mg/l of p.a. This is comparable to the findings of a Hungarian (5.51 ± 8.06 mg/l of p.a.) and a Serbian research group (8.18 ± 8.37 mg/l of p.a.) in unrecorded spirits (118, 202). Furthermore, a Slovakian and a Serbian study found that the level of Pb in unrecorded spirits ranged from 0.0 to 0.581 mg/l of p.a. and from 0.0 to 1.87 mg/l of p.a., respectively (85, 202). These findings are in line with our results showing that Pb concentrations in rakia varied from 0.0 to 1.337 mg/l of p.a. Levels of Pb and Cu were above the AMPHORA limits in 33% and 90% of our samples, respectively. To evaluate the significance of these results, it was important to estimate the daily intake of these heavy metals related to drinking rakia and compare them with their RfDs. Therefore, we considered an average body weight of 73.9 kg, an exposure duration of 365 days, and the mean annual per capita unrecorded alcohol consumption of 2.5 litres for both sexes in Albania (183). The average concentrations of Cu (13.1 mg/l of p.a.) and Pb (0.2 mg/l of p.a.) in our rakia samples were also taken into account (10). The daily intake of these metals was estimated by multiplying the mean level of Cu and Pb by the average per capita alcohol consumption in Albania, and then dividing by the average body weight and duration of exposure (74). The results of this calculation show that an average Albanian alcohol consumer can ingest 0.001 mg/kg bw/day and 0.02 μ g/kg bw/day of Cu and Pb in unrecorded alcohols, assuming that all is consumed exclusively in the form of home-made rakia, respectively. Therefore, the daily intake of Cu (0.001 mg/kg body weight/day) is considerably lower than its RfD of 0.04 mg/kg bw/day. Consequently, it is unlikely that exposure to Cu from unrecorded rakia can pose any health risk to average alcohol consumers in Albania (211). There is no accepted RfD for Pb. However, the lower one-sided confidence limit of the benchmark dose (BMDL) of this heavy metal has been found to be 1.5 μ g/kg bw/day (212). Therefore, the consumption of unrecorded rakia is unlikely to pose a risk to the average drinkers, as the estimated daily intake of Pb (0.02 μ g/kg body weight/day) is below the BMDL. However, financial constraints may result in the

consumption of unrecorded spirits above the level of average drinkers in a significant share of the Albanian population since these alcoholic beverages can be considerably cheaper than their recorded counterparts. This may be especially true for chronic heavy drinkers (72, 213-215). Therefore, the intake of Pb in unrecorded rakia may pose an additional health risk in chronic heavy drinkers in Albania (216).

Since a large proportion of alcohol drinkers in Croatia, Germany, Greece, Hungary, and Romania could potentially be exposed to methanol when consuming unrecorded fruit spirits, it was important to estimate the consumption level at which the daily methanol intake from this type of spirits might be higher than the toxicological limits (186). Comparison of the level of methanol in unrecorded fruit spirits, obtained from the selected studies, with its AMPHORA limit demonstrated that 2-35% of samples in 6 of the included investigations contained methanol above the threshold value (see **Table 12**). In addition, our estimations showed that consumption of brandy, țuică, pálinka, and koumaro with a methanol level of no less than 8598.1 mg/l of p.a and 10 g ethanol can lead to a methanol intake identical to the RfD in males. Furthermore, our findings indicate that methanol intake in females can reach the RfD when consuming brandy, țuică, pálinka, koumaro and schnapps containing methanol at a minimum level of 6382.1 mg/l of p.a. and 10 g ethanol. These findings suggest that individuals who consume unrecorded fruit spirits on a regular basis may have a realistic risk of being exposed to methanol doses exceeding the RfD over an extended period. However, only a few studies, based on animal experiments, are available on the adverse effects of chronic low-dose methanol intake (217, 218). They suggest that there might be an association between the long-term exposure to methanol and the onset of a neurological disorder showing similarities with the Alzheimer's disease (217, 218). Therefore, the possible adverse health effects of long-term intake of methanol from unrecorded fruit spirits exceeding the RfD of 2.0 mg/kg body weight/day should also be investigated in humans. In addition, the AMPHORA limit of

methanol (10 g/l) for unrecorded spirits was derived using a prior RfD of 20.0 mg/kg bw/day (104, 114). Nevertheless, we have taken into account the RfD of 2.0 mg/kg bw/day published by the United States Environmental Agency in 2013. If this RfD is applied, the current limit for methanol levels in unrecorded spirits should be reconsidered. After demonstrating that the levels and the daily intake of methanol in unrecorded fruit spirits can exceed the AMPHORA threshold and the RfD, respectively, it was reasonable to estimate whether the BML in drinkers of these type of beverages can equal or be above the MTBML of methanol (5 mg/dl) (174). Our findings indicate that drinking unrecorded fruit spirits with methanol levels of 17476.1 and 17384.2 mg/l and minimum 80 g and 60 g ethanol by males and females, respectively, can lead to BMLs exceeding the MTBML. Acute methanol toxicity is expected to occur at BMLs exceeding 50.0 mg/dl (111). However, levels so high are only found in patients with acute methanol intoxication (93-95, 97-99, 109, 111). Therefore, our findings indicate that drinking unrecorded fruit spirits is unlikely to result in acute methanol poisoning (203).

6. Conclusions

Our aim was to investigate the health risks associated with the consumption of recorded and unrecorded spirits in Albania and Hungary. We also aimed to determine the risk of acute and chronic methanol toxicity when consuming unrecorded fruit spirits containing methanol in several CEE countries.

1. We demonstrated that levels of Cu, Zn, and Sn in unrecorded spirits collected in Hungary were significantly higher than those in their recorded counterparts. In addition, the concentrations of Fe, Mn, and Ni were significantly higher in recorded spirits. Our results demonstrated that intake of heavy metals in recorded spirits can pose a potential risk to chronic heavy drinkers in Hungary.
2. Our findings showed that there was a significant difference between the measured and the reported ethanol levels in unrecorded Albanian rakia. Of the metals detected in unrecorded rakia, Cu and Pb were found to be the greatest concern. Although the estimated daily intake of these heavy metals was below their toxicological limit, the concentrations of Cu and Pb were above their limit in 90% and 33% of our rakia samples, respectively. Therefore, the possibility of adverse health effects cannot be excluded completely.
3. We found that the consumption of unrecorded fruit spirits containing methanol at levels above 8598.1 or 6382.1 mg/l of p.a. and 10 g ethanol, can result in a methanol intake exceeding the RfD in men and women, respectively. These findings suggest that individuals who consume unrecorded fruit spirits containing methanol on a regular basis may have a realistic risk of being exposed to methanol doses exceeding the RfD over an extended period. We also showed that consumption of unrecorded fruit spirits containing methanol at levels published in the literature is unlikely to result in BMLs above the MTBML in alcohol drinkers living in CEE countries.

7. Recommendations for future research

To decrease the uncertainty of health risk assessments on unrecorded spirits, it is recommended for future studies to incorporate a greater number of samples from a wide range of CEE countries. Given that drinking patterns and with them health risks are subject to change over time, it is advisable to conduct studies that take into account these temporal variations. Recognizing the limitations of the (THQ_c) approach, future research should explore alternative risk assessment methods that take into account target organ toxicity as well as potential interactions between heavy metals, and the combined effects of heavy metals and ethanol. These studies should aim to provide a more precise understanding of the synergistic or antagonistic effects and their implications for health risk assessments. Therefore, they can contribute to a more accurate estimation of health risks associated with unrecorded alcohol consumption.

Our study on unrecorded Albanian rakia indicates that there is a clear need for food safety authorities in Albania to include these beverages within their existing consumer safety measures. Our findings highlight the quality concerns that should be addressed as part of a comprehensive strategy to decrease the unrecorded alcohol-related disease burden in Albania. To obtain a more accurate estimation of the health risks associated with consuming unrecorded Albanian rakia, further studies using a larger sample size are required.

Although EU policymakers and producers of alcoholic drinks have taken multiple measures to decrease the methanol level in recorded fruit spirits below the limit of 10 g/l, less consideration has been given to the management and reduction of methanol levels in unrecorded fruit spirits (115, 203). Given that chronic exposure to methanol from unrecorded fruit spirits can pose a risk to the health of consumers, EU policymakers, particularly in the countries included in our investigation, should also contemplate the implementation of interventions to prevent or to decrease the intake of methanol from these types of beverages. Prior studies have suggested

several measures to mitigate the adverse effects of drinking unrecorded alcohol including fruit spirits (114, 203). They comprise more stringent control by fiscal authorities, monitoring of quality by food safety laboratories, and providing incentives to small-scale manufacturers to register and to subject their alcoholic beverages to quality control (80, 203). Furthermore, it is important to raise public awareness concerning the potential health consequences of methanol intake in unrecorded alcoholic beverages.

8. Limitations

Our research has several limitations. First, all spirit samples were collected in Hungary and Albania, which restricts the generalizability of our results. In addition, the number of spirit samples obtained in Albania was relatively small and they were exclusively rakia. Third, drinking patterns can change over time but the data required to consider how they change were not available for us. Fourth, our risk assessments were based on the estimation of combined THQs. However, this method does not consider the target organ toxicity and the health effects that could arise from the interaction among heavy metals and between them and ethanol when consumed simultaneously. Furthermore, THQc values do not completely consider the differences in toxicity of various heavy metals and their interactions. This could result in an under- or overestimation of THQc in our study. Therefore, further toxicological studies are needed to provide more precise health risk assessments. Another limitation is that although we reviewed over 300 publications, only a few reported data on methanol concentrations in unrecorded fruit spirits and most reported only average levels. As we included only studies with 10 or more unrecorded fruit spirit samples, some smaller ones were not considered. Individual data on methanol concentrations required for our Monte Carlo Simulations were available only from 5 EU countries, which limits the generalizability of our results to other countries. Therefore, further studies are required that determine and report individual data on the methanol levels in large number of unrecorded fruit samples collected in each EU member state allowing more precise probabilistic toxicological assessments.

9. Summary

Alcohol attributable disease burden is the highest in the European Region of the World Health Organization. However, significant differences exist within this region. Previous investigations have suggested that the intake of low-quality unrecorded alcohol can contribute to the elevated alcohol-attributable disease burden in Central and Eastern Europe and Western Balkan countries including Hungary and Albania. Therefore, our aims were to determine the chemical composition of recorded and unrecorded spirits collected in Hungary and unrecorded Albanian rakia and to estimate the health risks associated with their intake. In addition, the daily methanol intake and related blood methanol levels (BMLs) attributable to drinking unrecorded fruit spirits in the European Union (EU) was estimated. Inductively coupled plasma optical emission spectrometric analysis was used to determine the concentration of 24 elements and in 97 recorded and 100 unrecorded Hungarian spirit samples, as well as in 30 unrecorded Albanian rakia. Ethanol concentration was determined using gas chromatography/mass spectrometry. Concentration data on the metals detected in spirit samples were used to carry out a statistical analysis and a probabilistic risk assessment, characterized by the combined target hazard quotient (THQc) method with different scenarios considering average, regular and chronic heavy drinkers. THQc values higher than 1 were considered as public health concerns. In addition, data on methanol concentrations in unrecorded fruit spirits across EU member states were collected from the literature and the health risk associated with their consumption was estimated. We demonstrated that levels of Cu, Zn, and Sn in unrecorded spirits collected in Hungary were significantly higher than those in their recorded counterparts. In addition, the concentrations of Fe, Mn, and Ni were significantly higher in recorded spirits. The distributions of THQc values associated with the consumption of recorded spirits, brandy, pálinka, whiskey, and vodka containing heavy metals were less than 1.0 for both sexes, and separately for men and women. Average and regular consumers, as well as chronic heavy drinkers consuming

recorded pálinka ($p < 0.001$), whiskey ($p < 0.001$), and vodka ($p < 0.001$) collected in Hungary had significantly lower THQc values than those who drink recorded brandy. THQc values were also less than 1.0 at each drinking level, when consuming unrecorded spirits. The distributions of THQc values for men consuming recorded spirits, brandy and pálinka reached above 1.0 and were significantly lower for chronic heavy drinker males consuming recorded vodka ($p < 0.001$). Distributions of THQc values reached above 1.0 for women consuming recorded spirits, brandy and pálinka and were significantly lower for chronic heavy drinker females consuming recorded whiskey ($p < 0.001$) and vodka ($p < 0.001$) collected in Hungary. In addition, compared to chronic heavy drinker males and females consuming recorded spirits and recorded pálinka, THQc values were significantly lower for those who drink unrecorded spirits ($p < 0.001$).

Our findings showed that there was a significant difference between the measured and the reported ethanol levels in unrecorded Albanian rakia. Of the metals detected in unrecorded rakia, Cu and Pb were found to be the greatest concern. Although the estimated daily intake of these heavy metals was below their toxicological limit, the concentrations of Cu and Pb were above their limit in 90% and 33% of our rakia samples, respectively.

We also found that the consumption of unrecorded fruit spirits containing methanol at levels above 8598.1 or 6382.1 mg/l of pure alcohol and 10 g ethanol, can result in a methanol intake exceeding the oral reference dose of methanol in men and women, respectively.

Our results indicate that the intake of heavy metals in recorded spirits can poses a potential risk for Hungarian chronic heavy drinkers. Additionally, we found that the occurrence of adverse health effects associated with the consumption of unrecorded Albanian rakia cannot be entirely excluded. Furthermore, our study suggests that individuals regularly consuming unrecorded fruit spirits with methanol may face a realistic risk of exceeding the RfD over an extended period. However, it was also demonstrated that the levels of methanol published in the literature for unrecorded fruit spirits with methanol are unlikely to result in BMLs above the maximum

tolerable blood methanol level in alcohol drinkers residing in CEE countries. In light of our findings, there is a compelling need for the establishment of a comprehensive public health surveillance system to monitor both the safety and quality of recorded and unrecorded spirits consumed in Albania and Hungary.

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Key words

Albania, Aliphatic alcohols, Blood methanol level, Ethanol, Fruit spirit, Health risk estimation, Heavy metals, Hungary, Methanol, Unrecorded alcohol

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Nunu, besove vërtet se unë mundesha. <https://www.youtube.com/watch?v=8kzj3kNcy3E> . A do të kish kuptim ndryshe? JO (e madhe). Të kam...

Ma, faleminderit që vazhdon të kujdesesh për mua edhe pse tani jam e rritur.

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Candidate: Teuta Muhollari
Doctoral School: Doctoral School of Health Sciences

List of publications related to the dissertation

1. Muhollari, T., Szűcs, S., Sajtos, Z., McKee, M., Baranyai, E., Ádány, R., Pál, L.: Heavy metals in unrecorded Albanian rakia: A pilot study on a potential public health risk.
Heliyon. 9 (3), 1-24, 2023.
DOI: <http://dx.doi.org/10.1016/j.heliyon.2023.e13717>
IF: 4 (2022)
2. Muhollari, T., Szűcs, S., Ádány, R., Sándor, J., McKee, M., Pál, L.: Methanol in unrecorded fruit spirits. Does it pose a health risk to consumers in the European Union? A probabilistic toxicological approach.
Toxicol. Lett. 357, 43-56, 2022.
DOI: <http://dx.doi.org/10.1016/j.toxlet.2021.12.019>
IF: 3.5
3. Pál, L., Muhollari, T., Bujdosó, O., Baranyai, E., Nagy, A. C., Árnnyas, E., Ádány, R., Sándor, J., McKee, M., Szűcs, S.: Heavy metal contamination in recorded and unrecorded spirits. Should we worry?
Regul. Toxicol. Pharmacol. 116, 1-10, 2020.
DOI: <http://dx.doi.org/10.1016/j.yrtph.2020.104723>
IF: 3.271





List of other publications

4. Pál, L., Jenei, T., McKee, M., Kovács, N., Vargha, M., Bufa-Dórr, Z., Muhollari, T., Bujdosó, O., Sándor, J., Szűcs, S.: Health and economic gain attributable to the introduction of the World Health Organization's drinking water standard on arsenic level in Hungary: a nationwide retrospective study on cancer occurrence and ischemic heart disease mortality. *Sci. Total Environ.* 851, 1-11, 2022.
DOI: <http://dx.doi.org/10.1016/j.scitotenv.2022.158305>
IF: 9.8

Total IF of journals (all publications): 20,571

Total IF of journals (publications related to the dissertation): 10,771

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

02 November, 2023



Appendix

Supplement 1

Estimation of per capita consumption of ethanol from recorded and unrecorded spirits

1. scenario: average consumption on a population level

Estimates of the volume of total (11.4 litres of absolute ethanol), recorded (9.9 litres of absolute ethanol, 86.8 % of total) and unrecorded (1.5 litres of absolute ethanol, 13.2 % of total) per capita alcohol consumption in Hungary and separately by men (19.1 litres of absolute alcohol) and women (4.5 litres of absolute alcohol) were obtained from the Global Status Report on Alcohol and Health 2018 (10). The share of recorded alcohol intake from spirits was reported to be 32.3 % in Hungary. These figures equate to 3.2 litres of absolute alcohol per capita, with 28.1 % of the total in the form of recorded spirits. The volumes of per capita of recorded spirits for each man and woman were calculated to be 5.35 litres and 1.26 litres, respectively. Data on the share of alcohol consumption from unrecorded spirits are not available. However, previous studies showed that non-commercial alcoholic beverages consumed in Hungary are, almost exclusively, spirits (219). Therefore, the volume of alcohol intake from unrecorded spirits per capita was considered to be equal to that of from unrecorded alcohols (1.5 litres of absolute alcohol) which was 13.2 % of total per capita alcohol consumption. The corresponding figures for men (2.52 litres) and women (0.59 litres) were obtained by applying this proportion (13.2 %). Based on these consumption data, daily ethanol intakes (g/day) were calculated by multiplying the volume of alcohol (in litre) by the density of ethanol (789 g/l) and dividing the result (in grams) by the number of days in a year (365 days). These consumption data are shown in Table 4. The results of this risk assessment are presented in Figure 7.

2. scenario: regular drinkers (drinkers only)

Data on the volume of total, recorded and unrecorded per capita alcohol consumption by regular drinkers (“drinkers only” categorised by WHO; 17.1 litres of absolute alcohol) and separately for men (24.0 litres of absolute alcohol) and women (8.3 litres of absolute alcohol) were also obtained from the Global Status Report on Alcohol and Health 2018 (10). Data on the share of alcohol intake by regular drinkers from recorded and unrecorded spirits are not available. Therefore, the proportion of alcohol consumption from recorded (28.1%) and unrecorded (13.2%) spirits was assumed to be the same as that of in the overall population. In this way, the volumes of recorded and unrecorded spirits drunken by regular drinkers of both sexes were calculated to be 4.80 litres and 2.25 litres of absolute alcohol per capita, respectively. The per capita volumes of alcohol from recorded and unrecorded spirits consumed by men and women were calculated by taking into consideration the same shares (28.1% for recorded and 13.2% for unrecorded spirits) that were used in the first scenario. Thus, the alcohol intake from recorded spirits was considered to be 6.74 litres and 2.33 litres of absolute alcohol per capita for men and women, respectively. Alcohol consumption from unrecorded spirits was calculated to be 3.16 litres and 1.09 litres of absolute alcohol per capita for men and women, respectively. Based on these consumption data, daily ethanol intakes (g/day) were calculated by multiplying the volume of alcohol (litre) with the density of ethanol (789 g/l) and dividing the result (in grams) by the number of days in a year (365 days). These consumption data are shown in Table 4. The results of this risk assessment are presented in Figure 8.

3. scenario: chronic heavy drinkers, version A

The health risk assessment was carried out for chronic heavy drinkers (version A) at a realistic consumption level. The amount of alcohol consumed was defined as 60 g/day (27.75 litres/year/capita) and 40 g/day (18.50 litres/year/capita) for men and women, respectively.

However, the share of recorded (86.8% of total, 24.08 litres/year/capita for men, 16.05 litres/year/capita for women) and unrecorded (13.2% of total, 3.66 litres/year/capita for men, 2.44 litres/year/capita for women) intake was also taken into consideration. The portion of recorded alcohol drinking from spirits was reported to be 32.3% in Hungary. By applying this percentage, the volumes of per capita of recorded spirits for each man and woman were calculated to be 7.77 litres (24.08×0.323) and 5.18 litres (16.05×0.323), respectively. Previous studies showed that non-commercial alcoholic beverages consumed in Hungary are, almost exclusively, fruit spirits (211). Therefore, the volume of alcohol intake from unrecorded spirits per capita was considered to be equal to that of from unrecorded alcohols which was 13.2% of total per capita alcohol consumption. The corresponding volumes for men ($27.75 \times 0.132 = 3.66$ litres) and women ($18.50 \times 0.132 = 2.44$ litres) were obtained by applying this proportion (13.2 %). Based on these consumption data, daily ethanol intakes (g/day) were calculated by multiplying the volume of alcohol (litre) with the density of ethanol (789 g/l) and dividing the result (in grams) by the number of days in a year (365 days). These consumption data are shown in Table 4. The results of this risk assessment are presented in Figure 9.

4. scenario: chronic heavy drinkers, version B

To estimate the health risk at the highest consumption level, the amount of alcohol consumed by chronic heavy drinkers was also considered. This quantity was defined as 60 g/day and 40 g/day for men and women, respectively (177). Supposing that chronic heavy drinkers consume only spirits, their annual alcohol intake was calculated to be 27.75 litres and 18.50 litres of absolute alcohol per capita for men and women, respectively. These alcohol volumes were taken into account when exposure to heavy metals from recorded and unrecorded spirits was estimated. Based on these consumption data, daily ethanol intakes (g/day) were calculated by multiplying the volume of alcohol (litre) with the density of ethanol (789 g/l) and dividing the

result (in grams) by the number of days in a year (365 days). These consumption data are shown in Table 4. The results of this risk assessment are presented in Figure 10.