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Is contaminated unrecorded alcohol a health problem in the European Union? A review of existing and methodological outline for future studies

Dirk W. Lachenmeier[1], Kerstin Schoeberl[1], Fotis Kanteres[2,3], Thomas Kuballa[1], Eva-Maria Sohnius[1] & Jürgen Rehm[2,4,5]

Chemisches und Veterinäruntersuchungsamt (CVUA) Karlsruhe, Karlsruhe, Germany,[1] Centre for Addiction and Mental Health (CAMH), Toronto, Canada,[2] Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, the Netherlands,[3] Dalla Lana School of Public Health, University of Toronto, Toronto, Canada[4] and Institute for Clinical Psychology and Psychotherapy, TU Dresden, Dresden, Germany[5]

ABSTRACT

Aims Some European countries with high levels of unrecorded alcohol consumption have anomalously high rates of death attributable to liver cirrhosis. Hepatotoxic compounds in illegally produced spirits may be partly responsible. Based on a review of the evidence on the chemical composition and potential harm from unrecorded alcohol, the Alcohol Measures for Public Health Research Alliance (AMPHORA) project's methodology for identifying, analysing and toxicologically evaluating such alcohols is provided. **Methods** A computer-assisted literature review concentrated on unrecorded alcohol. Additionally, we refer to our work in the capacity of governmental alcohol control authority and a number of pilot studies. **Results** The risk-oriented identification of substances resulted in the following compounds probably posing a public health risk in unrecorded alcohol: ethanol, methanol, acetaldehyde, higher alcohols, heavy metals, ethyl carbamate, biologically active flavourings (e.g. coumarin) and diethyl phthalate. Suggestions on a sampling strategy for identifying unrecorded alcohol that may be most prone to contamination include using probable distribution points such as local farmers and flea markets for selling surrogate alcohol (including denatured alcohol) to focusing on lower socio-economic status or alcohol-dependent individuals, and selecting home-produced fruit spirits prone to ethyl carbamate contamination. **Conclusions** Standardized guidelines for the chemical and toxicological evaluation of unrecorded alcohol that will be used in a European-wide sampling and are applicable globally are provided. These toxicological guidelines may also be used by alcohol control laboratories for recorded alcohol products, and form a scientific foundation for establishing legislative limits.

Keywords Acetaldehyde, alcoholic beverages, ethyl carbamate, methanol, unrecorded alcohol.

Correspondence to: Dirk W. Lachenmeier, Chemisches und Veterinäruntersuchungsamt (CVUA) Karlsruhe, Weissenburger Strasse 3, D-76187 Karlsruhe, Germany. E-mail: lachenmeier@web.de
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INTRODUCTION

Some European countries, such as Hungary, Slovenia and Romania, have anomalously high rates of death attributable to liver cirrhosis, which rank among the highest in the world [1]. Although these high rates have yet to be explained fully, some research suggests that hepatotoxic compounds in illegally produced spirits may be partly responsible [2], as these countries have been identified as having very high levels of unrecorded consumption [3]. This raises the question of the extent to which unrecorded alcohol (e.g. surrogate, home and illegally produced alcohol; for detailed definition see [4,5]) contains potentially health-threatening properties, including contaminants and high alcoholic strengths [4]. The Alcohol Measures for Public Health Research Alliance (AMPHORA) project addresses this issue by examining chemically samples of unrecorded alcohol from all European Union (EU) countries. The aim of this project is not only to determine more clearly the public health threat from unrecorded alcohol, but to produce practical recommendations for reducing the harm. This is the first time such a study has been undertaken. The subgroups of unrecorded alcohol we mainly expect to find are artisanal

home-made alcohol (e.g. fruit distillates, home-brewed beer, home-made wine) throughout all of Europe, as well as surrogate alcohol (e.g. cosmetics or medicinal alcohol), predominantly in the eastern regions of Europe. While cross-border shopping may be more common in some parts of Europe (e.g. Nordic countries), this form of alcohol is recorded and quality controlled in its originating jurisdiction and should thus not have any additional health consequences over and above those of ethanol [6].

This paper presents results from the first stage of the AMPHORA project, which consists of a review of the evidence on the chemical composition and potential harm from unrecorded alcohol. The AMPHORA project methodology of identifying, sampling and analysing unrecorded alcohols across all European countries is described, including the selection criteria for the analysis and toxicological evaluation of samples. The strengths and weaknesses of the methodology and the probable contribution of the results to alcohol policy are also discussed.

MATERIALS AND METHODS

The methodology of the chemical analysis and toxicological evaluation of alcoholic beverages is based primarily on our previous work and knowledge in the capacity of governmental alcohol control authority in the German Federal State of Baden-Württemberg, as well as on knowledge gathered during pilot studies in several countries world-wide.

In addition, a computer-assisted literature search was conducted using a combination of the keywords: alcohol, unrecorded, home-made, illegal, illicit, clandestine and surrogate. The criteria for inclusion were that the literature had to contain information on the chemical composition or toxicological evaluation of unrecorded alcohol with focus on the European Union. As this paper aims to examine only health-relevant aspects (e.g. high alcoholic strength, contaminants), we excluded information on other quality parameters (e.g. sugar content or authenticity control due to labelling).

The searches were carried out using the following databases: PubMed, Toxnet and ChemIDplus (US National Library of Medicine, Bethesda, MD, USA), Web of Science (Thomson Reuters, Philadelphia, PA, USA), IPCS/INCHEM [International Programme on Chemical Safety/Chemical Safety Information from Intergovernmental Organizations, World Health Organization (WHO), Geneva, Switzerland], Food Science and Technology Abstracts (International Food Information Service, Shinfield, UK) and Scopus (Elsevier B.V., Amsterdam, the Netherlands). This was accompanied by a hand search of the reference lists of all papers for any relevant studies not included in the databases. The references, including abstracts, were imported into Reference Manager version 12 (Thomson Reuters, Carlsbad, CA, USA) and the relevant papers were identified manually and purchased in full text. In addition to the results from the literature review, all sections incorporate knowledge from our work in the capacity of governmental alcohol control authority, as well as findings from a number of pilot studies conducted in European and global settings [5,7–15]. From 690 references in our database, 67 were found to be relevant to be used, structured into four sections: current knowledge on toxic contaminants in alcoholic beverages in Europe, methodology for chemical analysis of alcohol for health-relevant compounds, methodology for the toxicological evaluation of compounds and contaminants in alcohol and sampling of unrecorded alcohol.

RESULTS

Current knowledge on toxic contaminants in European alcohol—a risk-oriented identification of substances for study by AMPHORA

The literature search for contaminants in European alcohol was essentially conducted in order to identify substances that are most likely to pose a public health risk and would thus be most relevant for analysis and toxicological evaluation. This corresponds to the 'risk oriented approach' for selecting samples and parameters that is applied regularly in food and alcohol control [16]. This approach is necessary, as systematic 'complete analyses' for every thinkable compound are unrealistic due to technical and economical limitations, and are in many cases unnecessary. This is especially relevant for alcohol, which although potentially containing more than 1000 different components [17], most do not pose a health threat.

As such, the first step in selecting compounds is to refer to the expert knowledge of alcohol control authorities based on recorded alcohol evaluations. In the European Union, the safety and quality of alcoholic beverages is regulated in the context of food laws. Most importantly, every food must be deemed 'safe' [18]. A more specific regulation than this demand of general safety is that of contaminants in foods, which is also applied to alcoholic beverages [19]. This requires that contaminant levels remain as low as reasonably possible, and that contamination be reduced by applying appropriate technology in food production, handling, storage, processing and packaging. In our experience, the only health-relevant contaminant that occurs regularly in recorded alcoholic beverages is ethyl carbamate (e.g. see the annual reports of food control in Baden-Württemberg, Germany [20]). A second possible health-relevant compound in recorded alcohol is acetaldehyde [21,22].

Finally, European laws provide some specific regulations regarding alcoholic beverages, including maximum limits for certain contaminants and compounds. An example is the European spirits regulation that provides maximum limits for methanol or acetaldehyde in certain product categories [23]. It must, however, be mentioned that the European alcohol regulations are often guided by quality considerations, so limits may not be based on toxicological thresholds but on 'best practices' or thresholds desirable from an organoleptic standpoint. For example, the methanol limit for vodka of 10 g/hl of pure alcohol (g/hl pa) [23] is lower than the maximum tolerable concentration of 5000 g/hl pa [24] by a factor of 500. Therefore, European laws often provide even greater safety, and exceeding a limit in European regulations cannot be interpreted directly as a public health problem. In our experience the limits (e.g. methanol) in the legislation are very seldom exceeded. For this reason, we also screened the Rapid Alert System for Food and Feed (RASFF) of the European Commission for information on alcohol [25]. The RASFF collects notifications from all European member states on foods or feeds presenting a serious risk. In terms of alcoholic beverages, the RASFF contains very few alerts and no consistent trends; this does not allow the selection of health-relevant compounds for our purposes.

It can be concluded that recorded alcohol in the market in the European Union is generally seen as safe. Other than the two compounds mentioned (acetaldehyde, ethyl carbamate), recorded alcohol studies do not indicate the need for the selection of further compounds for the AMPHORA project. We therefore focused our literature research on what is known specifically about unrecorded alcohol, as well as conducting pilot studies in different countries.

Currently, the largest study ($n = 99$) on illegal spirits was conducted by Huckenbeck *et al.* [26] in several central and eastern European countries. While the spectrum of analyses was restricted to methanol and the most common higher alcohols, some products were found with relatively high concentrations of methanol and 1-propanol compared to recorded spirits. The same range of compounds was analysed in studies conducted in Estonia [27] and Hungary [2]. Findings included the same observation of higher concentrations of higher alcohols in some of the products, as well as a general trend to higher alcoholic strengths of surrogate alcohols.

In preparation for the AMPHORA project, we conducted pilot studies in Lithuania, Hungary and Poland [5,15], in which we searched for a wider range of components than in the previous studies. These not only included alcoholic strength and volatiles (methanol, acetaldehyde and higher alcohols), but also ethyl carbamate, all relevant inorganic elements and, when required, flavourings and food additives including preservatives, colours and sweeteners. Additionally, a multitarget screening analysis for toxicologically relevant substances was conducted. While most samples were of no toxicological concern, we confirmed the presence of very high alcoholic strengths in unrecorded alcohols from Lithuania and Poland. Furthermore, we found the substances coumarin and diethyl phthalate at concentrations of toxicological concern in surrogates from Lithuania [14]. In some of the samples, the suspected human carcinogens, acetaldehyde and ethyl carbamate, were found at levels relevant to public health concerns.

In summary, we selected the compounds listed in Table 1 as the most probable candidates for causing problems in unrecorded alcohol. It should be noted that as full analyses could not be conducted, we chose to specifically exclude certain compounds that appear to have no health relevance for both recorded and unrecorded alcohol; these include nitrosamines [28], some halogenated solvents [29] and mycotoxins and pesticides that are unlikely to occur in any alcohol (see [30] for a detailed review on compounds in alcoholic beverages).

Methodology for chemical analysis of alcohol for health-relevant compounds

To ensure the highest validity of results, the AMPHORA project decided to use the same requirements for method validation and analytical quality assurance laws that are demanded for governmental food and alcohol control authorities [31]; specifically, the principles outlined in the International Organization for Standardization (ISO) 17025 [32]. Moreover, all laboratories involved in the project are to be externally accredited and participate regularly in international inter-laboratory trials. The following paragraphs describe the suitable chemical methodology based on own experience rather than systematic literature review. However, detailed reviews on chemical methodology are provided in the cited references.

As our pilot phase [5] demonstrated, the chemical–analytical methods established for recorded alcohol can be applied directly to unrecorded alcohol. European Union reference methods also exist for some parameters [33]. The reference procedure for determining alcoholic strength by volume consists of a distillation followed by a density measurement using pycnometry, electronic densimetry or densimetry using hydrostatic balance. This requires relatively large liquid volumes of up to 200 ml, which are often not easily available in samplings of unrecorded alcohol, and also depletes samples volume unnecessarily. Therefore, the alcoholic strength is to be determined by the more efficient Fourier transform infrared (FTIR) spectroscopy, which is faster and uses a lower sample amount, with necessary precision and accuracy

Table 1 Results of risk-oriented selection of parameters for analysis in unrecorded alcohol.

Parameter	*Rationale for inclusion of parameter*
Alcoholic strength (ethanol content)	Limited studies indicate that the alcoholic strength of unrecorded alcohol may be higher than that of commercially produced products [4]. Outcomes: acute intoxication and poisoning, as well some cancer types such as oesophageal cancer [56,60]
Volatile substances (including methanol, acetaldehyde, higher alcohols, ethyl acetate)	Some alcoholic beverages contain volatile components; other than ethanol, they include the toxins methanol and acetaldehyde. Methanol has been described to be the most common cause for surrogate toxicity [4,24], while acetaldehyde may contribute to the carcinogenicity of alcoholic beverages [22]. Higher alcohols have also been speculated as a cause for unrecorded alcohol toxicity in eastern Europe [52]
Heavy metals	Toxic and/or carcinogenic metals (e.g. lead, arsenic, antimony, cadmium, copper, zinc) may be found in unrecorded alcohol due to deficiencies in production technology. Lead intoxications were commonly described in consumers of American moonshine. Lead is an IARC group 2B carcinogen, while cadmium and arsenic are IARC group 1 carcinogens [61]
Ethyl carbamate (in addition to volatile substances)	Typical carcinogenic contaminant of alcoholic beverages (IARC group 2A). Previously found in unrecorded alcohol from Hungary and Poland [5,15]
Biologically active flavourings (e.g. coumarin)	Potential to occur in non-beverage alcohols (e.g. aftershaves), used as alcoholic beverages. Found in unrecorded alcohol from Lithuania [5]. Different effects (e.g. hepatotoxicity of coumarin [62])
Nitrate, water quality	Contamination of water source. Nitrate, ingested under conditions that result in endogenous nitrosation, is an IARC group 2A carcinogen [61]
Diethyl phthalate	Potential to occur in denatured alcohols. Found in unrecorded alcohol from Lithuania [14]

IARC: International Agency for Research on Cancer.

[34,35]. If FTIR is not possible (e.g. for highly viscous samples), an optimized reference procedure using steam distillation requiring only 25 ml of sample can be applied [36–38].

For volatile components analysis, the AMPHORA project uses the European Community Reference Method for the Analysis of Spirits: gas chromatography (GC) with a flame-ionization detector (FID) [33]. The reference method quantifies the following parameters: methanol, acetaldehyde, higher alcohols (including 1-propanol, 1-butanol, 2-butanol, iso-butanol, 2-methyl-1-butanol and 3-methyl-1-butanol) and ethyl acetate. We have added some parameters to this spectrum that can be determined simultaneously along with those detailed in the reference procedure. We quantify 1-hexanol, benzyl alcohol, 2-phenyl ethanol, methyl acetate, benzyl acetate, ethyl lactate, ethyl caprylate, ethyl benzoate and benzaldehyde. Furthermore, we qualitatively determine 29 further compounds.

To gain additional analytical safety, the volatiles are determined on two different GC columns of different polarity. Additional details on the GC-FID procedure are published elsewhere [8]. For the establishment of the GC-FID reference method, a large European inter-laboratory trial was conducted [39]; validation data are available. Since this trial, we have participated successfully in three to four of such trials per year, and will continue to do so. This ensures the highest possible validity of results gathered during the AMPHORA project as well as the comparability of results between different studies or laboratories.

The methodologies for the chemical analysis of the other compounds can be described only in an itemized manner; however, all methods were validated and evaluated using inter-laboratory proficiency tests. Ethyl carbamate (urethane) is determined using GC with tandem mass spectrometry (GC-MS/MS) [40]. Anionic composition is analysed using ion chromatography [41]. Inorganic elements are analysed using semi-quantitative inductively coupled plasma mass spectrometry (ICP-MS) after evaporation of the sample and reconstitution in ultrapure water. Analyses for flavourings can be conducted using GC-MS or high performance liquid chromatography (HPLC)-MS [5,42]. Moreover, we have developed an analytical method for phthalates (i.e. denaturing agents) using GC/MS with deuterated internal standards [14].

If the mentioned routine and screening analyses suggest further constituents or contaminants (e.g. due to large, unidentified peaks in the GC-FID chromatograms), the samples may be screened for unknown substances using a non-targeted HPLC with a diode-array detector

Table 2 Toxicological evaluation of metals and contaminants in alcohol, for which drinking water regulations or specific regulations for wine exist.

Constituent	*WHO guidelines for drinking water quality* [45] *[mg/l]*	*EU drinking water quality standards* [46] *[mg/l]*	*OIV international limits for wine* [49] *[mg/l]*	*German national limits for wine* [47] *[mg/l]*	*Maximum limits chosen by the AMPHORA project for evaluation of unrecorded alcohol*[a] *[mg/l]*
Aluminium, Al	(No limit)	0.200	(No limit)	8.00	2.0
Arsenic, As	0.01	0.010	0.2	0.10	0.1
Lead, Pb	0.01	0.010	0.15	0.25	0.2[b]
Boron, B	0.5	1.0	80 (as boric acid)	80 (as boric acid)	5
Cadmium, Cd	0.003	0.005	0.01	0.01	0.01
Copper, Cu	2	2.0	1	2.00	2.0
Zinc, Zn	(No limit)	(No limit)	5	5.00	5.0
Tin, Sn	(No limit)	(No limit)	(No limit)	1.00	1.0
Iron, Fe	(No limit)	0.200	(No limit)	(No limit)	2.0
Nickel, Ni	0.07	0.020	(No limit)	(No limit)	0.2
Antimony, Sb	0.02	0.005	(No limit)	(No limit)	0.05
Mercury, Hg	0.006	0.001	(No limit)	(No limit)	0.01
Fluoride, F	1.5	1.5	1–3	1–3	1.0
Chromium, Cr	0.05	0.050	(No limit)	(No limit)	0.5
Manganese, Mn	0.4	0.050	(No limit)	(No limit)	0.5
Selenium, Se	0.01	0.010	(No limit)	(No limit)	0.1
Benzene	0.01	0.001	(No limit)	(No limit)	0.01
Nitrite	0.2	0.50	(No limit)	(No limit)	2.0
Nitrate	50	50	(No limit)	(No limit)	500
Chloride	(No limit)	250	(No limit)	(No limit)	2500
Sulphate	(No limit)	250	1000–2500	1000–2500	1000

[a]The Alcohol Measures for Public Health Research Alliance (AMPHORA) evaluation generally assumes a daily consumption of water 10 times higher than of alcohol. The AMPHORA limit was therefore calculated from the lowest available drinking water limit [either European Union (EU) or World Health Organization (WHO)] with a factor of 10. The Organisation Internationale de la Vigne et du Vin/International Organization of Vine and Wine (OIV) or German wine limits were used when no drinking water limit was available, or if they were lower than the drinking water limits. [b]This corresponds to the Codex Alimentarius maximum level for lead in wine. [48]

(HPLC-DAD) or GC-MS, each followed by spectral library search for ultraviolet (UV) or MS spectra, similar to our study conducted in Nigeria [9]. In special cases, high-resolution nuclear magnetic resonance (NMR) spectroscopy can also be commissioned, with the aim of identifying substances that are not assignable by other spectroscopic and chromatographic methods, or for non-targeted screening analysis [43,44].

Methodology for the toxicological evaluation of compounds and contaminants in alcohol

The toxicological evaluation of many compounds in alcoholic beverages is problematic, as even for the most common compounds such as higher alcohols no European or international maximum limits have been established, as our review of international regulatory documents shows (e.g. on IPCS/INCHEM, see Materials and methods). For certain compounds (metals, ions) that occur in drinking water, specific international [45] or European [46] water regulations exist that can be extrapolated to alcohol. For example, there are German national limits for certain metals in wine [47], with the Codex Alimentarius similarly providing a limit for lead in wine [48]. Some limits for wine are also provided by the Organisation Internationale de la Vigne et du Vin (OIV) [49]. The final AMPHORA project evaluations for these compounds are detailed in Table 2. We decided against directly using the water limits to evaluate alcohol, as the average daily consumption of water is considerably higher than that of alcohol, which might lead to an exaggeration of the inherent risk of alcohol.

Nevertheless, the most relevant and potentially harmful compounds in alcohol—ethyl carbamate and acetaldehyde—lack regulations. Ethyl carbamate was identified recently by the European Food Safety Authority (EFSA) as a potential health concern in certain alcoholic beverages [50]; however, legislative limits are non-existent in the European Union. For acetaldehyde, we have some limits derived from the quality and organoleptic aspects; however, maximum limits that consider its identified carcinogenic potential are also non-existent [21].

For these reasons, the AMPHORA project conducted a literature review with the aim of identifying toxicological evaluations for the compounds identified for analysis that lack regulatory limits. With these evaluations, we thus developed AMPHORA maximum limits, which present the best available toxicological knowledge and are to be used in the course of the project for the evaluation of the analysed unrecorded alcohol samples.

Compounds with threshold-based toxic mechanisms were evaluated using acceptable daily intakes (ADI) for foods with the assumption of a life-time daily exposure. ADI values were taken from international evaluations, with preference to those of the Joint Food and Agriculture Organization (FAO)/WHO Expert Committee on Food Additives [51], and from an own evaluation when otherwise unavailable [52]. The ADI values are usually based on 'no observed effect levels' in animal experiments with the application of safety factors for extrapolation from animals to humans. The evaluations for threshold-based compounds are summarized in Table 3.

For non-threshold-based compounds, analyses were conducted according to the EFSA harmonized approach for the risk assessment of substances which are genotoxic and carcinogenic [53]. The EFSA has developed and recommends an approach known as the margin of exposure (MOE). This approach uses the value of doses of substances that have been observed to cause low but measurable harmful responses in animals as a reference point, and compares this with relevant substance-specific dietary intake estimates in humans, taking into account differences in consumption amounts and patterns. To obtain the MOE, a benchmark dose lower confidence limit (BMDL) of 10% was used for ethyl carbamate [50], as well as for acetaldehyde [21]. In general, a MOE of 10 000 or higher, if based on a BMDL from an animal study, would be considered of low public health concern, and subsequently low priority for risk management actions [53]. We use this threshold value of 10 000 to provide an estimation for preliminary maximum limits to be used in the AMPHORA project evaluation. This estimation is based on a life-time daily consumption of 0.1 litre of the product in question (Table 4). This is, of course, a worst-case scenario, but the daily consumption of unrecorded alcohol in this amount cannot be excluded completely, especially in alcohol-dependent people of lower socio-economic status, a group associated with high hazardous drinking patterns [54].

Finally, it must be mentioned that the AMPHORA project evaluations of toxicity should not be viewed as definite, or interpreted in a way that if a single sample exceeds the limits, a public health problem can be established. However, surpassing AMPHORA limits clearly justifies the implementation of larger studies and sampling on a national level, in countries with such instances.

Table 3 Toxicological evaluation of some threshold-based constituents and contaminants using acceptable daily intakes (ADI).

Constituent	*ADI/TDI/RfD*[a] *(mg/kg bw/day) [Reference]*	*Concentration in g/hl pa required to reach ADI/TDI/RfD (calculated for a 60- kg person drinking 100 ml of alcohol at 40% vol per day)*	*European Union maximum limits for distilled spirits* [23] *(g/hl pa)*	*Maximum limit chosen by the AMPHORA project for evaluation of unrecorded alcohol (g/hl pa)*
Methanol	20 [52]	3000	10 (vodka), 30 (neutral alcohol), 200 (wine spirit/brandy), 1000 (grape marc, fruit spirits, cider), 1200 (certain fruit spirits), 1350 (certain fruit spirits), 1500 (fruit marc). No limits for all other groups.	1000 (similar to the general EU limit for fruit spirits)
Higher alcohols (Sum)	1–20 [52]	150–3000	0.5 (neutral alcohol). No limits for all other groups. Minimum contents for some groups: 225 (rum), 125 (wine spirit/brandy), 140 (grape marc), 200 (fruit marc, fruit spirits, cider)	1000 (as suggested in [52])
Ethyl acetate	25 [51]	3750	1.3 (total esters in neutral alcohol). No limits for all other groups	1000
Benzaldehyde	5 [51]	750	(No limit)	500
Diethyl phthalate	0.8 [63]–5 [64]	120–750	(No limit)	120[b]
Coumarin	0.1 [65]	15	10 mg/kg (approx. 39 g/hl pa) (Codex Alimentarius limit [66])	15[b]

[a]Nomenclature dependent on reference: acceptable daily intake (ADI), tolerable daily dose (TDI), or oral reference dose (RfD). [b]Diethyl phthalate and coumarin are generally undesirable compounds in any beverage alcohol. Therefore the concentration should be as low as technologically possible. ADI: acceptable daily intake; TDI: tolerable daily dose; RfD: reference dose; AMPHORA: Alcohol Measures for Public Health Research Alliance; bw: body weight; pa: pure alcohol.

Table 4 Toxicological evaluation of some carcinogenic and genotoxic constituents in alcohol products using the margin of exposure (MOE) model.

Constituent	*BMDL value [mg/kg bw/day]*	*Concentration to reach the MOE threshold of 10 000 (calculated for a 60-kg person drinking 100 ml of alcohol at 40% vol per day)*	*European Union maximum limits for distilled spirits*	*Maximum limit chosen by the AMPHORA project for evaluation of unrecorded alcohol*
Acetaldehyde	56 [21]	0.8 g/hl pa	No general limit. Limit for neutral alcohol is 0.5 g/hl pa [23].	50 g/hl pa (most recorded spirits contain less than 50 g/hl pa, stricter limits would also exclude most recorded spirits).
Ethyl carbamate	0.3 [67]	0.018 mg/L	No limit in the EU. Canadian limits are 0.15 mg/L for distilled spirits and 0.4 mg/L for fruit spirits [50].	0.4 mg/L (similar to Canadian limit for fruit spirits).

BMDL: benchmark dose lower confidence limit; bw: body weight; AMPHORA: Alcohol Measures for Public Health Research Alliance.

Therefore the limits are appropriate to the purpose of our project: to identify problematic compounds, problematic subgroups of unrecorded alcohol and countries or regions with problematic alcohol production. For a final risk assessment, a population-based exposure estimate is required.

Sampling of unrecorded alcohol

The AMPHORA project made a public open call for sampling of unrecorded alcohol on its webpage (http://www.amphoraproject.net). All interested parties were invited to send samples from all European Union member states. The methodology is suitable for all types of unrecorded alcohols (i.e. not only spirits but also home-produced wine or beer). The information in Table 5 was provided to guide the samplers in procuring unrecorded alcohol. Additionally, the samplers were asked to provide the information outlined in Table 6 in the form of a detailed sampling protocol. To allow for the diverse range of different analytical methods, we asked for a sample size of 300 ml but at least 100 ml. As alcoholic beverages generally have a shelf-life of at least 1 year at room temperature (even low-alcoholic beers), no specific

Table 5 Alcohol Measures for Public Health Research Alliance (AMPHORA) criteria for selecting samples of unrecorded alcohol.

- Choose only unrecorded alcohol clearly intended for human consumption (i.e. no aftershaves or similar products sold in drug stores clearly not for human consumption)
- 5–10 samples from each country
- Choose locations (e.g. local farmers and flea markets) most likely to be selling unrecorded alcohol to people of lower socio-economic status or alcohol-dependent people
- Focus on collecting products that could be contaminated (e.g. surrogate alcohols: non-beverage alcohol sold for human consumption)
- Focus on products based on stone-fruits (e.g. plums, cherries) for probable contamination with ethyl carbamate
- Focus on home produced/home-distilled spirits, but also home-produced beers and wines may be included

Table 6 Information to be recorded in a sampling protocol.

- Unique sample ID
- Sample location (country, city, site, form of retailer, market)
- Time and date of sampling
- Description (name, category of beverage, original vessel size)
- List of ingredients/raw materials and alcohol content in % vol. (if known or provided by the seller)
- Price per litre (€)
- Price of equal legal product per litre (€)
- Peculiarities and observations about the samples, suspicions on contamination of product

requirement for transport to the laboratory were required. After submission to the laboratory, the samples were treated according to the guidelines of ISO 17025 [32]. For example, unique ID-numbers were given to each sample to avoid mismatch. The samples were stored in the dark at 8°C until analysed.

DISCUSSION

Strength and weaknesses of the approach

The major strength of this approach is that established chemical reference methodologies with accurate results are to be used. The methodologies have been proven in the routine analysis of commercial alcoholic beverages and feasibility studies of unrecorded alcohol.

The weaknesses include the restriction to selected parameters for analysis, for which it is possible that relevant compounds that cannot be detected by the usual GC/MS or HPLC approaches may be overlooked. It is also apparent that our approach only allows for the assignment of compounds that are included in the usual spectral databases [e.g. with mass spectra or UV/visible (VIS) spectra]. As such, unknown or new compounds may be detected, but structural elucidation is outside the scope of this project. We would, however, certainly investigate and publish this information to facilitate future studies into such unknown compounds.

While the problems in toxicological evaluation were discussed above, in summation, due to limited or non-existent data on dose–response effect in humans, it is difficult to derive a final evaluation for many compounds.

The final limitation of the project is that the sample size is restricted to approximately five to 10 samples per country (i.e. we expect to analyse approximately 300 samples from the European Union member states and surrounding countries with high prevalence for unrecorded consumption, such as Russia or the Balkan countries). We will therefore most probably be seeing just the 'tip of the iceberg' and it is, of course, difficult to extrapolate a public health risk from one positive sample. However, the major aims of the project are to gain the first European-wide glimpse into the composition of unrecorded alcohol and to identify compounds that might be problematic on a country level. It is expected that there will be large differences due to the diversity of countries; for example, we expect flavour compounds in surrogate alcohols to be problematic in the Baltic countries, while ethyl carbamate contamination may be prevalent in fruit spirit-producing countries such as Hungary, Poland, Romania, Germany or France.

Probable contribution of the results to alcohol policy

In a scenario where there is no substantial compositional difference between recorded alcoholic beverages and unrecorded alcohols, policy measures can focus on the standard health risk indicators of volume and patterns of drinking. Certain additional actions may still be required, as the illegal market is obviously not impacted directly by policy measures such as tax increases [55]. For instance, the raw materials for alcohol production could be influenced by increases in price, or the tax-reduction status for denatured or industrial alcohols could be changed in circumstances where significant amounts of this alcohol are going towards human consumption. However, the measurement of the amount of unrecorded consumption is outside the scope of the AMPHORA project. These measures are based on two main methods [3,56]: for countries with good health statistics, total consumption can be inferred indirectly by using proxy indicators such as forensic reports of accidental and violent deaths (e.g. see Nemtsov [57] for Russia). Total consumption can also be estimated from surveys (e.g. [58]). Comparative research has shown that both methods can supplement each other [59].

Another scenario would entail a major part of unrecorded alcohol being more toxic than recorded alcohol. In this case, measures may range from legalizing unrecorded alcohol with subsequent quality control to instructing the producers of unrecorded alcohol how to avoid the detected problems. If, for example, we find that the toxic components are those used predominantly to denature alcohol, a policy measure could be to prohibit unsuitable compounds, particularly methanol and diethyl phthalate (both of which cannot be tasted in alcohol).

Finally, the results of the AMPHORA study will also be usable for selecting susceptible countries and types of unrecorded alcohol for a more detailed sampling strategy.

CONCLUSION

The AMPHORA project methodology for the chemical-toxicological examination of unrecorded alcohol provides for the first time standardized guidelines on how to analyze unrecorded alcohol and interpret the results. The established toxicological guidelines for compounds in alcohol may also be used by alcohol control laboratories for recorded alcohols, and lay a scientific foundation for establishing limits in European legislation for some of the compounds.

This methodology will now be used to conduct the first systematic overview of compounds in unrecorded alcohol from all European Union countries. It is our hope that the AMPHORA project will lead to strategies and guidelines for alcohol policy that improve the protection of consumers of unrecorded alcohol in Europe and the world.

Declarations of interest

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