

# Comparative risk assessment of carcinogens in alcoholic beverages using the margin of exposure approach

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Alcoholic beverages have been classified as carcinogenic to humans. As alcoholic beverages are multicomponent mixtures containing several carcinogenic compounds, a quantitative approach is necessary to compare the risks. Fifteen known and suspected human carcinogens (acetaldehyde, acrylamide, aflatoxins, arsenic, benzene, cadmium, ethanol, ethyl carbamate, formaldehyde, furan, lead, 4-methylimidazole, *N*-nitrosodimethylamine, ochratoxin A and safrole) occurring in alcoholic beverages were identified based on monograph reviews by the International Agency for Research on Cancer. The margin of exposure (MOE) approach was used for comparative risk assessment. MOE compares a toxicological threshold with the exposure. MOEs above 10,000 are judged as low priority for risk management action. MOEs were calculated for different drinking scenarios (low risk and heavy drinking) and different levels of contamination for four beverage groups (beer, wine, spirits and unrecorded alcohol). The lowest MOEs were found for ethanol (3.1 for low risk and 0.8 for heavy drinking). Inorganic lead and arsenic have average MOEs between 10 and 300, followed by acetaldehyde, cadmium and ethyl carbamate between 1,000 and 10,000. All other compounds had average MOEs above 10,000 independent of beverage type. Ethanol was identified as the most important carcinogen in alcoholic beverages, with clear dose response. Some other compounds (lead, arsenic, ethyl carbamate, acetaldehyde) may pose risks below thresholds normally tolerated for food contaminants, but from a cost-effectiveness point of view, the focus should be on reducing alcohol consumption in general rather than on mitigative measures for some contaminants that contribute only to a limited extent (if at all) to the total health risk.

Since the first observation in France in the beginning of the last century that the consumption of absinthe was related to esophageal cancer,<sup>1</sup> epidemiology has established a causal

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**Abbreviations:** BMD: Benchmark dose; BMDL: Confidence limit of the BMD; CPDB: Carcinogenic potency database; EFSA: European food safety authority; EPA: Environmental protection agency; IARC: International agency for research on cancer; IPCS: International programme on chemical safety; JECFA: Joint FAO/WHO expert committee on food additives; MOE: Margin of exposure; NDMA: *N*-nitrosodimethylamine; NTP: National toxicology program  
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relationship between alcohol consumption in general (*i.e.*, independent of beverage type) and the occurrence of cancer. In 1988, the IARC classified alcoholic beverages into group 1 as carcinogenic to humans.<sup>1</sup> At this time, a causal relationship between alcohol consumption and the occurrence of malignant tumors of the oral cavity, pharynx, larynx, esophagus and liver was established. In the following IARC evaluations, colorectal cancer and female breast cancer were added to the list of cancer sites with causal relationship, while only limited evidence points to stomach and pancreas as further sites.<sup>2,3</sup>

While the epidemiological evidence on the carcinogenicity of alcoholic beverages had been sufficiently established for several decades, the principal mechanism underlying this relationship has been a matter of debate. For a long time, it was assumed that ethanol itself was not a direct carcinogen. The 1988 IARC monograph, for example, stated that there is inadequate evidence for the carcinogenicity of ethanol in experimental animals.<sup>1</sup> However, this statement was based on lack of well-controlled and well-designed experimental studies rather than on a clear absence of effect. Since then, two adequately designed long-term animal studies have clearly demonstrated that ethanol causes a dose-related increase in cancer in mice and rats at sites similar to those observed in humans (liver and oral cavity).<sup>4,5</sup> As a result of this new evidence, the 2007 IARC evaluation concluded that there is sufficient evidence in experimental animals for the

**Table 1.** Summary of WHO International Agency for Research on Cancer (IARC) evaluation of carcinogenicity of substances that may be present in alcoholic beverages (updated from IARC<sup>2</sup>)

Agent	IARC Monographs evaluation of Carcinogenicity			IARC Monographs (Volume Number)
	In animals	In humans	IARC group <sup>1</sup>	
Acetaldehyde associated with consumption of alcoholic beverages	Sufficient	Sufficient	1	36, Sup 7, 71, 100E
Acrylamide	Sufficient	Inadequate	2A	60
Aflatoxins	Sufficient	Sufficient	1	56, 82, 100F
Arsenic	Sufficient	Sufficient	1	23, Sup 7, 100C
Benzene	Sufficient	Sufficient	1	29, Sup 7, 100F
Cadmium	Sufficient	Sufficient	1	58, 100C
Ethanol in alcoholic beverages	Sufficient	Sufficient	1	44, 96, 100E
Ethyl carbamate (urethane)	Sufficient	Inadequate	2A	7, Sup 7, 96
Formaldehyde	Sufficient	Sufficient	1	88, 100F
Furan	Sufficient	Inadequate	2B	63
Lead compounds, inorganic	Sufficient	Limited	2A	87
4-Methylimidazole	Sufficient	Inadequate	2B	101
N-Nitrosodimethylamine	Sufficient	Inadequate	2A	17, Sup 7
Ochratoxin A	Sufficient	Inadequate	2B	56
Safrole	Sufficient	Inadequate	2B	10, Sup 7

<sup>1</sup>Group 1: Carcinogenic to humans; Group 2A: Probably carcinogenic to humans; Group 2B: Possibly carcinogenic to humans (for definitions of groups, see [monographs.iarc.fr](http://monographs.iarc.fr)).

carcinogenicity of ethanol.<sup>2</sup> Furthermore, substantial mechanistic evidence has become available in humans who are deficient in aldehyde dehydrogenase that acetaldehyde, which is the first metabolite of ethanol, may accumulate and contribute to the causation of malignant esophageal tumors. Acetaldehyde reacts with DNA to form various DNA adducts, and elevated levels of acetaldehyde-derived DNA adducts have been detected in white blood cells of individuals who are heavy alcoholic beverage drinkers. Some of the DNA adducts that are increased after alcoholic beverage consumption are mutagenic in human cells. In addition, these adducts can undergo rearrangements in double-stranded DNA, which can result in the formation of DNA-protein crosslinks and DNA interstrand crosslinks, which are mechanistically consistent with the generation of chromosomal aberrations. Elevated levels of chromosomal aberrations have been observed in human cells in culture after exposure to acetaldehyde as well as *in vivo* in human alcoholics.<sup>2</sup> This mechanistic evidence combined with the results in experimental animals and the epidemiological observation that all alcoholic beverages cause cancer demonstrate that ethanol is an important carcinogenic compound in alcoholic beverages. In their most recent evaluation, IARC has therefore classified both “ethanol in alcoholic beverages” as well as “acetaldehyde associated with alcohol consumption” into Group 1 as “human carcinogens.”<sup>3</sup>

Nevertheless, misinformation is still spread that ethanol is not a carcinogen at all or that alcohol-related cancer is exclusively caused by something else. For example, promotional material on an ethanol-containing mouthwash states that

“ethanol is not a carcinogen; however, alcoholic beverages contain numerous carcinogenic compounds such as urethane, nitrosamines, polycyclic hydrocarbons and aflatoxins.”<sup>6</sup> While there is certainly ample evidence pointing to the fact that ethanol is the major carcinogenic compound in alcoholic beverages, the assumption about other carcinogens cannot be directly negated. Alcoholic beverages are multi compound mixtures and (similar to tobacco) may regularly contain various carcinogens such as those in the promotional material mentioned above. The IARC also remarked that identification of ethanol as a known carcinogenic agent in alcoholic beverages does not rule out the possibility that other components may also contribute to their carcinogenicity.<sup>2</sup> A summary of known and suspected human carcinogens typically occurring in alcoholic beverages is provided in Table 1. In fact some of these substances in alcoholic beverages, and specifically ethyl carbamate (urethane), are seen by international bodies such as the JECFA or the EFSA as public health risk independent of ethanol.<sup>7,8</sup> For this reason, the European Commission has advised the member states to monitor the ethyl carbamate contamination in certain alcoholic beverages.<sup>9</sup> Another example is NDMA, which was first found in German beers in 1978, when concentrations of up to 68 µg/L caused worldwide concern.<sup>10</sup> A change in the target organ specificity of NDMA by coadministration of ethanol was observed: when NDMA was given in combination with ethanol, rats and mice developed tumors in the nasal cavity, which is not a target site for this nitrosamine. This suggests that ethanol may influence the initiation of carcinogenesis in some manner,

but it is also possible that the process is enhanced due to some mechanistic events: the facilitation of entry into the target cell by ethanol, a change in intracellular metabolism or suppression of DNA repair. The hypothesis of competitive inhibition of hepatic metabolism of the carcinogen, which allows it to reach the target organs, has also been proposed.<sup>2</sup> The questions about the risk posed by substances other than ethanol is especially important for unrecorded (*i.e.*, illicitly or home-produced) alcohol, which is assumed to potentially contain higher concentrations of contaminants, especially ethyl carbamate and acetaldehyde.<sup>11</sup>

The literature currently offers no quantitative information if and how much other carcinogenic constituents or contaminants of alcoholic beverages are comparable with and contribute to the risk generated by ethanol. Such information is necessary especially to inform risk management to prioritize cancer prevention measures.

Several approaches were suggested in the past for quantitative risk assessment of carcinogens. From these, the so-called MOE approach is currently preferred by international bodies such as WHO<sup>12</sup> or EFSA.<sup>13</sup> Our study will, therefore, apply the MOE approach to provide a comparative risk assessment of carcinogens occurring in alcoholic beverages. The results will be used to point out options for alcohol policy.

## Methods

The selection of substances and their occurrence in alcoholic beverages was based on the most recent detailed IARC review,<sup>2</sup> for exceptions see remarks in results section. The assessment of toxicological endpoints and BMD for the selected known and suspected human carcinogens was generally based on literature data, as own dose–response modeling would have gone beyond the scope of our study. Suitable risk assessment studies including endpoints and dose–response modeling results were typically identified in monographs of national and international risk assessments bodies such as WHO IPCS, JECFA, US EPA and EFSA. For substances without available monographs or with missing data on dose–response modeling results, the scientific literature in general was searched for such data. Searches were carried out in September 2011 in the following databases: PubMed (US National Library of Medicine, Bethesda, MD), Web of Science (Thomson Reuters, Philadelphia, PA), Scopus (Elsevier B.V., Amsterdam, The Netherlands) and Google Scholar (Google, Mountain View, CA).

The BMD/MOE approach was used for risk assessment.<sup>13,14</sup> In short, the BMD is the dose of a substance that produces a pre-determined change in response rate (benchmark response) of an adverse effect compared to background based on dose–response modeling.<sup>14</sup> The benchmark response is generally set near the lower limit of responses that can be measured (typically in the range of 1–10%). The result of BMD-response modeling can then be used in combination with exposure data to calculate a MOE for quantitative risk assessment. The MOE is defined as the ratio between the lower one-sided confidence limit of the BMD

(BMDL) and estimated human intake of the same compound. It can be used to compare the health risk of different compounds and in turn prioritize risk management actions. By definition, the lower the MOE, the larger the risk for humans; generally, a value under 10,000 used to define public health risks.<sup>15</sup>

If BMDL values were unavailable in the literature, no observed effect level (NOEL) or no observed adverse effect level (NOAEL) values were identified as surrogate thresholds instead. The MOEs were then calculated by dividing the NO(A)EL by the estimated human intake.

For each beverage group (*i.e.*, beer, wine, spirits and unrecorded alcohol), the human intakes were calculated for two different drinking scenarios (low risk drinking and heavy drinking) based on the drinking guidelines for Canada, which consider that 13.6 g pure alcohol constitute a standard drink.<sup>16</sup> For both drinking scenarios, MOEs for average contamination as well as maximum contamination with the different compounds were additionally calculated to estimate a range for average and worst case contamination scenarios.

## Results

Alcoholic beverages may contain more than 1,000 different components,<sup>1</sup> from which several are potentially carcinogenic. The compounds can either be naturally occurring (from raw materials or fermentation), being present as residue or contamination or even intentionally added.<sup>2</sup>

In the first step of the comparative risk assessment, a selection of compounds for further evaluation has to occur. The IARC Monographs Working Group Vol. 96<sup>2</sup> compared the complete IARC list of known and suspected human carcinogens with the list of compounds regularly occurring in alcoholic beverages (Appendix 1 in the IARC 1988 monograph<sup>1</sup>) and provided a summary of carcinogens that may be present in alcoholic beverages (see Table 1.14, p. 113 in the IARC 2010 monograph<sup>2</sup>). From this summary, we have chosen the compounds set into IARC Group 1 (carcinogenic to humans), IARC Group 2A (probably carcinogenic to humans) and IARC Group 2B (possibly carcinogenic to humans) to be included in our evaluation. Compounds set into IARC Group 3 (not classifiable as to its carcinogenicity to humans) such as deoxynivalenol, nivalenol, organolead compounds and patulin were excluded from our evaluation. The remaining compounds in Groups 1, 2A and 2B were acetaldehyde, acrylamide, aflatoxins, arsenic, benzene, cadmium, ethanol, ethyl carbamate, furan, inorganic lead compounds, NDMA and ochratoxin A (Table 1).

Since the writing of the exposure section in the IARC Monograph Vol. 96 in 2007 (two of the authors of this article, D.W.L. and J.R., were members of this working group and contributed to the initial evaluation), additional evidence for some compounds has become available. For example, the regular occurrence of formaldehyde, an IARC Group 1 carcinogen, in alcoholic beverages was detected.<sup>17</sup> Furthermore, 4-methylimidazole a contaminant of caramel colors with known use in certain alcoholic beverages,<sup>18,19</sup> was newly

**Table 2.** Occurrence of WHO International Agency for Research on Cancer (IARC) known and suspected human carcinogens in alcoholic beverages

Agent	Amount in alcoholic beverages (Average/Maximum) <sup>a</sup>	Amount in unrecorded alcohol (Average/Maximum) <sup>b</sup>
Acetaldehyde associated with consumption of alcoholic beverages	9/63 mg/L (beer); 34/211 mg/L (wine); 66/1,159 mg/L (spirits) <sup>50</sup>	90/822 mg/L
Acrylamide	0–72 µg/kg (beer) <sup>c</sup>	(no data)
Aflatoxins	0.002/0.230 µg/L (beer) <sup>23</sup>	(not detectable in all samples)
Arsenic	0/102.4 µg/L (beer); 4/14.6 µg/L (wine); 13/27 µg/L (spirits)	(not detectable in all samples)
Benzene	10/20 µg/L in beer produced with contaminated CO <sub>2</sub>	(no data)
Cadmium	0.9/14.3 µg/L (beer); 1.0/30 µg/L (wine); 6/40 µg/L (spirits)	0/0.04 mg/L
Ethanol in alcoholic beverages	(2–80% vol)	(10–89% vol)
Ethyl carbamate (urethane)	0/33 µg/kg (beer); 5/180 µg/kg (wine); 93/6,730 µg/kg (spirits); 744/22,000 µg/kg (fruit spirits) <sup>7</sup>	0.5/5.4 mg/L
Formaldehyde	0 mg/L (beer); 0.13/1.15 mg/L (wine); 0.50/14.37 mg/L (spirits) <sup>17</sup>	0.22/6.71 mg/L <sup>17</sup>
Furan	3.3/28 µg/kg (beer) <sup>24</sup>	(no data)
Lead compounds, inorganic	2/15 µg/L (beer) <sup>25</sup> ; 57/326 µg/L (wine) <sup>26</sup> ; 31/600 µg/L (spirits)	0.03/1.4 mg/L
4-Methylimidazole	Caramel colored products: 9/28 µg/L in dark beer <sup>18</sup> ; 0/0.14 mg/L in whisky <sup>19</sup>	(No data)
<i>N</i> -Nitrosodimethylamine	0.1/1.3 µg/kg (beer)	(No data)
Ochratoxin A	0.05/1.5 µg/L (beer); 0.23/7.0 µg/L (wine)	(No data)
Safrole	0/6.6 mg/L (bitters/liqueurs/aperitifs) <sup>21</sup>	(No data)

<sup>a</sup>If no other source is stated, the data are taken from the IARC literature review<sup>2</sup> by calculating the average over all studies. Historical data (*i.e.* before implementation of mitigation measures) was not included. <sup>b</sup>If no other source is stated, the data are taken from an European sample of unrecorded alcohol.<sup>11</sup> <sup>c</sup>Few surveys on acrylamide in alcoholic beverages are available. The majority of analyzed samples contained levels below the detection limit. The level of 72 µg/kg was reported in a single sample of wheat beer.<sup>27</sup>

evaluated by IARC in 2011 and set into group 2B.<sup>20</sup> Safrole, another group 2B substance, may also potentially occur in alcoholic beverages.<sup>21</sup> Safrole is a flavor compound with a comparably high ranking in the Berkeley carcinogenic potency project due to its occurrence in spices.<sup>22</sup> Therefore, formaldehyde, 4-methylimidazole and safrole were added to our list (Table 1).

The data on occurrence of the chosen compounds in alcoholic beverages are summarized in Table 2. Data on recorded alcohol (*i.e.*, commercial wine, beer and spirits) were predominantly based on the summaries in the IARC 2010 monograph.<sup>2</sup> In some instance, actualized data from international surveys (*e.g.*, from EFSA) were available (see details in Table 2<sup>23–27</sup>). Less data on unrecorded alcohol is generally available.<sup>28</sup> The data were, therefore, taken from an own survey recently conducted in the European Union.<sup>11</sup>

Generally, the contamination of alcoholic beverages with the selected compounds is subject to a wide variation depending on product category, raw material or diligence during manufacturing. The substances typically occur at ppb levels or below, *e.g.*, for aflatoxins, cadmium or ochratoxin A. The exception are ethyl carbamate and formaldehyde, which may reach ppm levels but only in certain products, while acetaldehyde typically occurs in ppm levels in all product categories (besides vodka and neutral alcohol-based products), and may even exceed 1 g/L in certain highly contaminated

products. No clear difference between commercial and unrecorded alcoholic beverages was detected with the exception of inorganic lead that may exceed 1 mg/L in highly contaminated unrecorded alcohol.

The toxicological endpoints used for dose–response modeling and the chosen points of departure for MOE assessment are shown in Table 3.<sup>8,15,29–41</sup> According to international guidelines for risk assessment using the MOE approach,<sup>12–14,42</sup> the most sensitive toxicological endpoint was chosen, when several endpoints were available. For some agents such as formaldehyde, benzene or lead, noncancer endpoints were more sensitive than cancer endpoints or cancer endpoints were unavailable. To provide a conservative assessment, we decided to use these noncancer endpoints in these cases. For a third of the compounds, human epidemiological data were available suitable for dose–response modeling. For the rest of the compounds, the assessments have to be based on animal data.

In general, endpoints for the oral route of exposure were identified when available, as the inhalation exposure (*e.g.*, by evaporation during drinking) appears to be negligible. Benzene is the only agent for which an oral study was unavailable. However, the US EPA has provided an oral BMDL for benzene based on route-to-route extrapolation from an inhalation exposure study,<sup>33</sup> which we have decided to use. The second exception is lead, for which the toxicological assessment was based on a total exposure study based on blood lead as

**Table 3.** Dose response modelling results of WHO International Agency for Research on Cancer (IARC) known and suspected human carcinogens occurring in alcoholic beverages

Agent	Toxicological endpoint for modelling (route of exposure) <sup>1</sup>	Reference for dose–response modeling study <sup>2</sup>	BMDL <sub>10</sub> <sup>3</sup> [mg/kg bw/day]
Acetaldehyde	Tumour-bearing animals in male rats (oral)	29	56
Acrylamide	Harderian gland tumours in mice (oral)	30	0.18
Aflatoxin B <sub>1</sub>	Liver cancer in humans (food)	31	0.00087
Arsenic	Lung cancer in humans (water)	32	BMDL <sub>0.5</sub> : 0.003
Benzene	Lymphocyte count in humans (inhalation extrapolated to oral)	33	1.2 <sup>4</sup>
Cadmium	Human studies involving chronic exposures (food)	34	NOAEL: 0.01 <sup>5</sup>
Ethanol	Hepatocellular adenoma or carcinoma in rats (oral)	15	700
Ethyl carbamate (urethane)	Alveolar and bronchiolar neoplasms in mice (oral)	8	0.3
Formaldehyde	Histological changes in the aerodigestive tract, including oral and gastrointestinal mucosa of rats (oral)	35	NOEL: 15 <sup>5</sup>
Furan	Hepatocellular adenomas and carcinomas in female mice (oral)	36	0.96
Lead	Cardiovascular effects in humans (dietary exposure based on blood lead levels)	37	BMDL <sub>01</sub> : 0.0015 <sup>6</sup>
4-Methylimidazole	Cancer of the lung in mice (oral)	38	NOAEL: 80 <sup>5</sup>
N-Nitrosodimethylamine	Total liver tumors (oral)	39	0.029
Ochratoxin A	Kidney adenoma and carcinoma in male rats (oral)	40	0.025
Safrole	Hepatic tumors in mice (oral)	41	3 <sup>7</sup>

<sup>1</sup>Human data were preferred over animal data, if available. Non-cancer endpoints were chosen if dose–response modeling for cancer effects was unavailable (such as in the case of lead). The most sensitive endpoint was chosen if dose–response data for several organ sites were available.

<sup>2</sup>The references for the original data used for dose–response modeling are provided as additional supporting information for online publication.

<sup>3</sup>BMDL<sub>x</sub>: lower one-sided confidence limit of the benchmark dose (BMD) for a x% incidence of health effect. <sup>4</sup>The original endpoint was based on inhalation exposure. BMDL for oral exposure was derived by route-to-route extrapolation.<sup>33</sup> <sup>5</sup>No usable BMD-modeling for oral exposure was identified in the literature. The no effect level (NOEL) or no observed adverse effect level (NOAEL) are used in these cases instead. <sup>6</sup>The values are based on total exposure determined by blood lead levels. The used BMDL was calculated for dietary exposure.<sup>37</sup> <sup>7</sup>A range of “approximately 3–29 mg/kg bw/day” was provided as BMDL<sub>10</sub> for safrole.<sup>41</sup> As no further rationale was provided in the study, we chose the minimum of this range to provide a conservative assessment.

biomarker. However, the EFSA has adjusted the BMDL for dietary exposure.<sup>37</sup> In summary, all chosen toxicological thresholds were intended to evaluate the oral route of exposure.

The effective doses of the compounds as expressed by BMDL vary over a very wide range, from 0.00087 mg/kg bw/day for aflatoxin B<sub>1</sub> to 700 mg/kg bw/day for ethanol.

Table 4 shows the corresponding MOEs for several scenarios and alcoholic beverage groups. An average over all groups is provided in Figure 1. The lowest MOEs were calculated for ethanol, with 3.1 for low risk drinking and 0.8 for heavy drinking. Inorganic lead and arsenic have average MOEs between 10 and 300, followed by acetaldehyde, cadmium and ethyl carbamate between 1,000 and 10,000. Safrole, ochratoxin A, NDMA, 4-methylimidazole, furan, formaldehyde, aflatoxin B<sub>1</sub> and acrylamide have average MOEs above 10,000, even in the heavy drinking scenario.

## Discussion

Our study provides the first comprehensive comparison of the risk related to compounds in alcoholic beverages. It is interesting to note that from all evaluated agents, ethanol

exhibits the lowest potency in terms of BMDL in mg/kg bw/day required to produce an effect. Nevertheless, due to its very high exposure as a major constituent of alcoholic beverages, this situation is completely reversed in terms of MOE, where now ethanol has the highest potency, as all other substances occur at considerably lower concentrations to produce the same effect. The observation that the MOE of ethanol is already in an effective dose range for the low risk drinking guideline for females is absolutely in line with epidemiological observations. For breast cancer, as an example, the largest pooled study on breast cancer shows significant effects at levels lower than one drink daily.<sup>43</sup>

Interestingly, a similar comparative risk assessment that was recently conducted for tobacco carcinogens<sup>44</sup> did not detect a single compound responsible for the carcinogenic effect as it was in our case for ethanol in alcoholic beverages. In tobacco, acrolein, formaldehyde and cadmium all had MOEs down to below 10 and several other compounds had MOEs below 1,000.<sup>44</sup>

Our result for ethanol (MOE of 3.1 for one drink per day) is in excellent agreement with the result from the Berkeley CPDB project,<sup>22</sup> which reported a MOE of 3 for moderate

**Table 4.** Margin of exposure (MOE) of WHO International Agency for Research on Cancer (IARC) known and suspected human carcinogens occurring in alcoholic beverages calculated for different drinking and contamination scenarios (MOE = BMDL or NO(A)EL/Exposure)

Agent	Type of alcohol	Scenario 1: one standard drink per day (low risk drinking guideline for females) <sup>a</sup>		Scenario 2: heavy drinker (four standard drinks per day, own categorization) <sup>a</sup>	
		MOE for average contamination	MOE for maximum contamination (Worst case)	MOE for average contamination	MOE for maximum contamination (Worst case)
Acetaldehyde <sup>b</sup>	Beer	1,095	156	274	39
	Wine	696	112	174	28
	Spirits	1,184	67	296	17
	Unrecorded	868	95	217	24
Acrylamide	Beer	∞ <sup>c</sup>	440	∞	110
Aflatoxin B <sub>1</sub>	Beer	76,540	666	19,135	166
Arsenic	Beer	∞	5	∞	1
	Wine	317	87	79	22
	Spirits	322	155	81	39
Benzene	Beer	21,114	10,557	5,279	2,639
Cadmium	Beer	1,955	123	489	31
	Wine	4,225	141	1,056	35
	Spirits	2,326	349	581	87
	Unrecorded	∞	349	∞	87
Ethanol	All	3.1	–	0.8	–
Ethyl carbamate (urethane)	Beer	∞	1,600	∞	400
	Wine	25,352	704	6,338	176
	Spirits	4,501	62	1,125	16
	Fruit spirits	563	19	141	5
	Unrecorded	837	78	209	19
Formaldehyde	Beer	∞	∞	∞	∞
	Wine	48,754	5,511	12,189	1,378
	Spirits	41,860	1,457	10,465	364
	Unrecorded	95,137	3,119	23,784	780
Furan	Beer	51,186	6,033	12,797	1,508
Lead	Beer	132	17.6	33	4.4
	Wine	11	1.9	2.8	0.5
	Spirits	68	3.5	17	0.9
	Unrecorded	70	1.5	17	0.4
4-Methylimidazole	Caramel-coloured Beer	1,564,027	502,723	391,007	125,681
	Caramel-coloured Whisky	∞	797,342	∞	199,336
<i>N</i> -Nitrosodimethylamine	Beer	51,026	3,925	12,757	981
Ochratoxin A	Beer	87,977	2,933	21,994	733
	Wine	45,928	1,509	11,482	377
Safrole	Bitters/Liqueurs/Aperitifs	∞	634	∞	159

<sup>a</sup>A standard drink in Canada is considered to have a total of 13.6 g of alcohol.<sup>16</sup> To recalculate the amount of contaminants per L or per kg to standard drink, portions of 341 mL (beer), 142 mL (wine), 43 mL (spirits and unrecorded) were chosen<sup>16</sup>. As no density was given in any of the contamination studies, 1 kg was set to equal 1 L for recalculation to volume if necessary. The exposure was estimated for the different drinking scenarios based on the occurrence data in Table 2 and a body weight of 60 kg. <sup>b</sup>Acetaldehyde directly contained in the beverages excluding metabolically formed acetaldehyde. <sup>c</sup>The lemniscate symbol indicates that the MOE was not calculable as the exposure was zero (*i.e.*, below the detection limit of the applied analytical methodology).

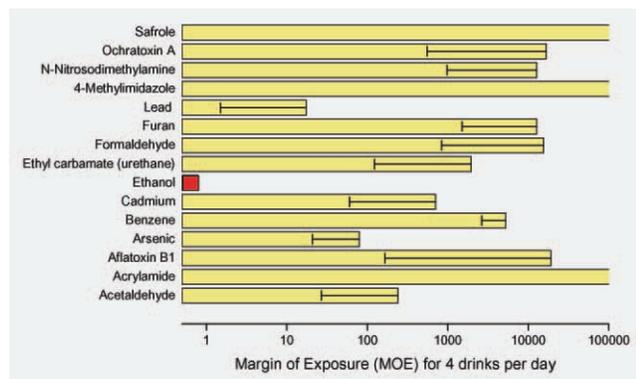


Figure 1. Margin of Exposure (MOE) for carcinogens occurring in alcoholic beverages for heavy drinking scenario (averages based on data from Table 4; error bar indicates worst case contamination).

daily drinking (based on ethanol exposure of 326 mg/kg/day). It is of note that the CPDB project uses different methodology to calculate MOE (based on adjusted  $TD_{50}$  (median toxic dose) values from older animal experiments<sup>45</sup> and not  $BMDL_{10}$  from the most recent NTP study as in our case<sup>15</sup>). As the results are almost the same, this independently validates our approach.

The CPDB project also reported data on NDMA in beer before 1979 (MOE of 1,000) and NDMA in beer 1994–1995 (MOE of 50,000), which is also in agreement with our MOE results and the general observation that NDMA in beer is nowadays of negligible risk due to changes in production technology.<sup>10</sup>

The first limitation of our study is the fact that the MOE estimations for several of the other compounds are not as robust as those for ethanol. For ethanol, not only the  $BMDL_{10}$  from animal experiments is available but also human BMD modeling data for several endpoints including liver cirrhosis<sup>15</sup> as well as liver markers and blood pressure,<sup>46</sup> all of which are in the same order of magnitude confirming the validity and interspecies transferability of the animal data. As no human BMDL for cancer effect of ethanol was available in the literature, we used the animal BMDL for our study. For several of the other compounds, no epidemiological data was available or it was inconclusive (signified by classification into IARC Groups 2A and 2B). For two of the compounds (see results), no data for the oral route of exposure was available and extrapolations had to be used. Three major problems of such assessments remain: extrapolating between species, extrapolating from high doses in animals to low doses in humans, as well as extrapolating between routes of exposure if required. Finally, we have chosen noncancer endpoints for some of the compounds, when they were more sensitive than the cancer endpoints. Our approach would therefore rather overestimate the cancer risks of these agents compared to ethanol, for which all these problems do not arise.

The second limitation of the study would also lead to overestimation of the risks of all compounds besides ethanol: the

limited database on occurrence data of these compounds in alcoholic beverages. For most of the compounds large international surveys are missing, which would be necessary to provide more robust exposure estimations. The exception of this is ethyl carbamate, for which large international and EU-wide surveys have been conducted.<sup>7,8</sup> Such data are especially lacking for aflatoxins, cadmium, lead and ochratoxin A. Several compounds also occur in only one category of beverages (*e.g.*, acrylamide and furan are only expected in beer, while 4-methylimidazole may only occur in caramel-colored products). In these cases, the absence of survey data can be explained by the unlikelihood of occurrence, which explains that some groups of beverages were not studied at all in the context of risk-oriented monitoring programs. We also assume that there is a publication bias favoring positive results. From own experience in our research projects about unrecorded alcohol we know that it is much more problematic to publish survey results indicating no public health relevance rather than alarmist reports of methanol deaths, for example. From the typical lack of studies reporting absence of contamination in alcoholic beverages, along with own experience as alcohol control authority (that routinely tests for chemical contamination), we think that the occurrence data reported in Table 2 are most likely biased towards higher levels. This observation even strengthens our argument that ethanol is the real risk factor in alcoholic beverages, as even with the available (most likely biased) occurrence data, the MOEs of all other compounds are considerably higher than the MOE of ethanol.

## Conclusions

There are two main conclusions. First, the MOE approach is well-suited to provide comparative risk assessments for lifestyle factors that are mixtures of several toxic compounds such as alcoholic beverages. Second, ethanol was confirmed as by far the most important carcinogen in alcoholic beverages. This confirms deductions by other approaches (such as genetic epidemiology and mechanistic considerations, see Introduction). This observation ultimately leads to the question if mitigation measures for the other known and suspected human carcinogens (*e.g.*, as currently conducted for ethyl carbamate) are an adequate policy or if the money should not rather be spent on reducing alcohol consumption *per se*, for which several cost-effective measures are already available.<sup>47</sup> The focus on alcohol policy would also not only reduce alcohol-related cancer but alcohol-related harm in general. The German Federal Institute for Risk Assessment, for example, holds the view in their assessment of acetaldehyde as contaminant of alcoholic beverages that mitigation measures are not required in this case, as alcoholic beverages are health damaging anyway.<sup>48</sup> On the one hand, we agree of course with this statement as alcoholic beverages *per se* certainly pose inherent health risks. However, it also disregards the obligation of the regulating agency to provide the safest possible environment. In modern societies, we accept the fact that citizens take risks, including risks, which are potentially lethal (*e.g.*, by

drinking alcohol or exercising risky sports). However, within this risk taking the regulating agencies have to make sure that the environment in which individual risk taking occurs is the safest possible (see Ref. <sup>49</sup> for further elaboration of these arguments). We would not argue to tolerate not closing a ski slope with present danger of avalanche based on the reasoning that skiing is dangerous anyway. In other words, reducing directly contained acetaldehyde in alcoholic beverages, which is technically possible,<sup>29,50</sup> should be targeted by regulating agencies, as it would reduce risk of cancer independent of any individual risk decision. Our society cannot on the one hand tolerate the use of alcoholic beverages and regulate them within food laws (as is the case in the European Union) but then allow an exception regarding quality and safety. The individual drinker would also most certainly select uncontaminated alcohol over contaminated alcohol.

In this context, it is noteworthy that for many of the mentioned contaminants, no maximum limits are set by legislation that would allow adequate control and enforcement of quality standards.<sup>28</sup> At least for one of the compounds, ethyl carbamate, mitigative risk management approaches are ongoing but only on a "recommendation" basis.<sup>9</sup> Inorganic compounds such as lead or arsenic could be relevant for future research.

However, the problem of lead is not restricted to alcoholic beverages, which contribute only about 7% to the total lead exposure from foods and beverages.<sup>37</sup> As the MOEs for total lead exposure may reach down to 1,<sup>37</sup> risk management strategies outside of alcohol policy appear to be necessary for this metal.

A final conclusion is the interesting observation that there is basically no substantial difference in risk between unrecorded and recorded alcohol. We also see no scientific basis for advertizing claims that certain alcoholic beverages are more or less carcinogenic than others (e.g., red wine less than spirits).

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### References

- IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Alcohol Drinking. *IARC Monogr Eval Carcinog Risks Hum* 1988;44:1-416.
- IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Alcohol consumption and ethyl carbamate. *IARC Monogr Eval Carcinog Risks Hum* 2010;96:1-1428.
- Secretan B, Straif K, Baan R, Grosse Y, El Ghissassi F, Bouvard V, Benbrahim-Tallaa L, Guha N, Freeman C, Galichet L, Coglian V. A review of human carcinogens—Part E: tobacco, areca nut, alcohol, coal smoke, and salted fish. *Lancet Oncol* 2009;10:1033-4.
- Beland FA, Benson RW, Mellick PW, Kovatch RM, Roberts DW, Fang JL, Doerge DR. Effect of ethanol on the tumorigenicity of urethane (ethyl carbamate) in B6C3F1 mice. *Food Chem Toxicol* 2005;43:1-19.
- Soffritti M, Belpoggi F, Cevolani D, Guarino M, Padovani M, Maltoni C. Results of long-term experimental studies on the carcinogenicity of methyl alcohol and ethyl alcohol in rats. *Ann N Y Acad Sci* 2002;982:46-69.
- Johnson & Johnson. *Listerine—FAQs for Professionals*. Available at: <http://www.listerine.com.sg/faqs-for-professionals.html>. Accessed October 18, 2011. (Archived by WebCite® at <http://www.webcitation.org/62Wfznhtc>): Johnson & Johnson Pte. Ltd., 2011.
- EFSA. Ethyl carbamate and hydrocyanic acid in food and beverages. *EFSA J* 2007;551:1-44.
- Vavasour E, Renwick AG, Engeli B, Barlow S, Castle L, DiNovi M, Slob W, Schlatter J, Bolger M. Ethyl carbamate. WHO Food Additives Series 55. Safety evaluation of certain contaminants in food. Prepared by the sixty-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Geneva, Switzerland: WHO and FAO, 2006:205-316.
- European Commission. Commission Recommendation of 2 March 2010 on the prevention and reduction of ethyl carbamate contamination in stone fruit spirits and stone fruit marc spirits and on the monitoring of ethyl carbamate levels in these beverages. *Off J Eur Union* 2010;L52:53-7.
- Lachenmeier DW, Fügél D. Reduction of Nitrosamines in Beer—review of a success story. *Brew Sci* 2007;60:84-9.
- Lachenmeier DW, Leitz J, Schoeberl K, Kuballa T, Straub I, Rehm J. Quality of illegally and informally produced alcohol in Europe: results from the AMPHORA project. *Adicciones* 2011;23:133-40.
- IPCS. Environmental health criteria 240: principles and methods for the risk assessment of chemicals in food. Geneva: World Health Organization, 2009.
- EFSA. Opinion of the Scientific Committee on a request from EFSA related to a harmonised approach for risk assessment of substances which are both genotoxic and carcinogenic. *EFSA J* 2005;282:1-31.
- U.S.EPA. The use of the benchmark dose approach in health risk assessment. EPA/630/R-94/007. Washington, DC.: Office of Research and Development, US Environmental Protection Agency, 1995.
- Lachenmeier DW, Kanteres F, Rehm J. Epidemiology-based risk assessment using the benchmark dose/margin of exposure approach: the example of ethanol and liver cirrhosis. *Int J Epidemiol* 2011;40:210-8.
- CAMH. Low-risk drinking guidelines [pamphlet]. Toronto, Canada: Centre for Addiction and Mental Health, 2005.
- Jendral JA, Monakhova YB, Lachenmeier DW. Formaldehyde in alcoholic beverages: large chemical survey using purpald screening followed by chromotropic acid spectrophotometry with multivariate curve resolution. *Int J Anal Chem* 2011;Article ID 797604.
- Klejdus B, Moravcová J, Lojtková L, Vacek J, Kubán V. Solid phase extraction of 4(5)-methylimidazole (4MeI) and 2-acetyl-4(5)-(1,2,3,4-tetrahydroxybutyl)-imidazole (THI) from foods and beverages with subsequent liquid chromatographic electrospray mass spectrometric quantification. *J Sep Sci* 2006;29:378-84.
- Yoshikawa S, Fujiwara M. Determination of 4(5)-methylimidazole in food by thin layer chromatography. *J Food Hyg Soc Jap* 1981;22:189-96.
- Grosse Y, Baan R, Secretan-Lauby B, El Ghissassi F, Bouvard V, Benbrahim-Tallaa L, Guha N, Islami F, Galichet L, Straif K. Carcinogenicity of chemicals in industrial and consumer products, food contaminants and flavourings, and water chlorination byproducts. *Lancet Oncol* 2011;12:328-9.
- Curro P, Micali G, Lanuzza F. Determination of beta-asarone, saffrole, isosaffrole and anethole in alcoholic drinks by high-performance liquid chromatography. *J Chromatogr* 1987;404:273-8.
- Gold LS, Ames BN, Slone TH. How many fold lower is human exposure than the dose that gave rodents cancer: margin of exposure, MOE (rodent cancer dose/human exposure). Berkeley, CA. Available at: <http://potency.berkeley.edu/MOETable.html>. Accessed 8 November, 2009 (Archived by WebCite® at <http://www.webcitation.org/5ix20FMx>): Carcinogenic Potency Project, University of California, Berkeley, 2008.

23. Mably M, Mankotia M, Cavlovic P, Tam J, Wong L, Pantazopoulos P, Calway P, Scott PM. Survey of aflatoxins in beer sold in Canada. *Food Addit Contam* 2005;22:1252–7.
24. EFSA. Update on furan levels in food from monitoring years 2004–2010 and exposure assessment. *EFSA J* 2011;9:2347.
25. Donhauser S, Wagner D, Jacob F. Critical trace-elements in brewing technology. 2. occurrence of arsenic, lead, cadmium, chromium, mercury and selenium in beer. *Monatsschr Brauwissenschaft* 1987;40:328–33.
26. Andrey D, Beuggert H, Ceschi M, Corvi C, de Rossa M, Herrmann A, Klein B, Probst-Hensch N. Monitoring-Programm 'Schwermetalle in Lebensmitteln'. IV. Blei, Cadmium, Kupfer und Zink in Weinen auf dem Schweizer Markt. Teil B: Vorgehen, Resultate und Diskussion. [Monitoring programme for heavy metals in food. IV. Lead, cadmium, copper and zinc in wine on the Swiss market. Part B: methods, results and discussion.]. *Mitt Geb Lebensm Hyg* 1992;83:711–36.
27. Gutsche B, Weisshaar R, Buhlert J. Acrylamide in food—screening results from food control in Baden-Württemberg. *Deut Lebensm Rundsch* 2002;98:437–43.
28. Lachenmeier DW, Schoeberl K, Kanteres F, Kuballa T, Sohnius E-M, Rehm J. Is contaminated alcohol a health problem in the European Union? A review of existing and methodological outline for future studies. *Addiction* 2011;106(Suppl 1):20–30.
29. Lachenmeier DW, Kanteres F, Rehm J. Carcinogenicity of acetaldehyde in alcoholic beverages: risk assessment outside ethanol metabolism. *Addiction* 2009;104:533–50.
30. Mueller U, Agudo A, Carrington C, Doerge D, Hellenäs KE, Leblanc JC, Rao M, Renwick A, Slob W, Wu Y. Acrylamide (Addendum). WHO Food Additives Series 63. Safety evaluation of certain contaminants in food. Prepared by the seventy-second meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Geneva, Switzerland: WHO and FAO, 2011: 1–151.
31. EFSA. Opinion of the scientific panel on contaminants in the food chain on a request from the commission related to the potential increase of consumer health risk by a possible increase of the existing maximum levels for aflatoxins in almonds, hazelnuts and pistachios and derived products. *EFSA J* 2007;446:1–127.
32. Benford DJ, Alexander J, Baines J, Bellinger DC, Carrington C, Devesa i Peréz VA, uxbury J, Fawell J, Hailemariam K, Montoro R, Ng J, Slob W, et al. Arsenic (Addendum). WHO Food Additives Series 63. Safety evaluation of certain contaminants in food. Prepared by the seventy-second meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Geneva, Switzerland: WHO and FAO, 2011:153–316.
33. U.S.EPA. Benzene (CASRN 71–43-2). Integrated Risk Information System. Document 0276. Washington, DC: U.S. Environmental Protection Agency, 2003.
34. U.S.EPA. Cadmium (CASRN 7440-43-9). Integrated Risk Information System. Document 0141. Washington, DC: U.S. Environmental Protection Agency, 1998.
35. IPCS. Formaldehyde. Concise international chemical assessment document 40. Geneva: World Health Organization, 2002.
36. Williams GM, Ariseto AP, Baines J, DiNovi M, Feeley M, Schlatter J, Slob W, Toledo MCF, Vavasour E. Furan. WHO Food Additives Series 63. Safety evaluation of certain contaminants in food. Prepared by the seventy-second meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Geneva, Switzerland: WHO and FAO, 2011:487–603.
37. EFSA. Scientific opinion on lead in food. *EFSA J* 2010;8:1570.
38. EFSA. Scientific opinion on the re-evaluation of caramel colours (E 150 a,b,c,d) as food additives. *EFSA J* 2011;9:2004.
39. Zeilmaker MJ, Bakker MI, Schothorst R, Slob W. Risk assessment of N-nitrosodimethylamine formed endogenously after fish-with-vegetable meals. *Toxicol Sci* 2010;116:323–35.
40. Barlow S, Bolger M, Pitt JI, Verger P. Ochratoxin A (addendum). WHO Food Additives Series 59. Safety evaluation of certain contaminants in food. Prepared by the sixty-eighth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Geneva, Switzerland: WHO and FAO, 2008:357–429.
41. Martati E, Boersma MG, Spengelink A, Khadka DB, Punt A, Vervoort J, van Bladeren PJ, Rietjens IM. Physiologically based biokinetic (PBBK) model for safrrole bioactivation and detoxification in rats. *Chem Res Toxicol* 2011;24: 818–34.
42. Benford D, Bolger PM, Carthew P, Coulet M, DiNovi M, Leblanc JC, Renwick AG, Setzer W, Schlatter J, Smith B, Slob W, Williams G, et al. Application of the margin of exposure (MOE) approach to substances in food that are genotoxic and carcinogenic. *Food Chem Toxicol* 2010;48: S2–S24.
43. Hamajima N, Hirose K, Tajima K, Rohan T, Calle EE, Heath CW, Jr, Coates RJ, Liff JM, Talamini R, Chantarakul N, Koetsawang S, Rachawat D, et al. Alcohol, tobacco and breast cancer—collaborative reanalysis of individual data from 53 epidemiological studies, including 58,515 women with breast cancer and 95,067 women without the disease. *Br J Cancer* 2002;87:1234–45.
44. Cunningham FH, Fiebelkorn S, Johnson M, Meredith C. A novel application of the Margin of Exposure approach: segregation of tobacco smoke toxicants. *Food Chem Toxicol* 2011;49:2921–33.
45. Gold LS, Slone TH, Bernstein L. Summary of carcinogenic potency and positivity for 492 rodent carcinogens in the carcinogenic potency database. *Environ Health Perspect* 1989;79:259–72.
46. Dakeishi M, Murata K, Tamura A, Iwata T. Relation between benchmark dose and no-observed-adverse-effect level in clinical research: effects of daily alcohol intake on blood pressure in Japanese salesmen. *Risk Anal* 2006;26:115–23.
47. Babor T, Caetano R, Casswell S, Edwards G, Giesbrecht N, Graham K, Grube J, Hill L, Holder H, Homel R, Livingstone M, Österberg E, et al. Alcohol: no ordinary commodity. research and public policy, 2nd edn. Oxford, UK: Oxford University Press, 2010.
48. BfR. Gesundheitliche Bewertung von Acetaldehyd in alkoholischen Getränken. Aktualisierte Stellungnahme Nr. 022/2010 des BfR vom 04. Mai 2010. Berlin: Bundesinstitut für Risikobewertung, 2010.
49. Rehm J, Patra J. Different guidelines for different countries? On the scientific basis of low-risk drinking guidelines and their implications. *Drug Alcohol Rev* 2011;31:156–61.
50. Lachenmeier DW, Sohnius E-M. The role of acetaldehyde outside ethanol metabolism in the carcinogenicity of alcoholic beverages: evidence from a large chemical survey. *Food Chem Toxicol* 2008;46:2903–11.

## Additional supporting information for online publication

### Appendix to Table 3

Agent	Reference for Original Data used for Dose-Response Modelling
Acetaldehyde	1
Acrylamide	2
Aflatoxin B <sub>1</sub>	3
Arsenic	4
Benzene	5
Cadmium	6
Ethanol	7,8
Ethyl carbamate (urethane)	8
Formaldehyde	9
Furan	10
Lead	11
4-Methylimidazole	12
N-Nitrosodimethylamine	13,14
Ochratoxin A	15
Safrole	16,17

### References

1. Soffritti M, Belpoggi F, Lambertin L, Lauriola M, Padovani M, Maltoni C. Results of long-term experimental studies on the carcinogenicity of formaldehyde and acetaldehyde in rats. *Ann N Y Acad Sci* 2002;**982**:87-105.
2. NTP. NTP technical report on the toxicology and carcinogenesis. Studies of acrylamide (CAS No. 79-06-1) in F344/N rats and B6C3F1 mice (drinking water study). *Natl Toxicol Program Tech Rep Ser* 2011;**in press**.
3. Yeh FS, Yu MC, Mo CC, Luo S, Tong MJ, Henderson BE. Hepatitis B virus, aflatoxins, and hepatocellular carcinoma in southern Guangxi, China. *Cancer Res* 1989;**49**:2506-9.
4. Chen CL, Chiou HY, Hsu LI, Hsueh YM, Wu MM, Chen CJ. Ingested arsenic, characteristics of well water consumption and risk of different histological types of lung cancer in northeastern Taiwan. *Environ Res* 2010;**110**:455-62.
5. Rothman N, Li GL, Dosemeci M, Bechtold WE, Marti GE, Wang YZ, Linet M, Xi LQ, Lu W, Smith MT, Titenko-Holland N, Zhang LP, et al. Hematotoxicity among Chinese workers heavily exposed to benzene. *Am J Ind Med* 1996;**29**:236-46.
6. U.S.EPA. *Cadmium (CASRN 7440-43-9). Integrated Risk Information System. Document 0141*. Washington, DC: U.S. Environmental Protection Agency, 1998.
7. Beland FA, Benson RW, Mellick PW, Kovatch RM, Roberts DW, Fang JL, Doerge DR. Effect of ethanol on the tumorigenicity of urethane (ethyl carbamate) in B6C3F1 mice. *Food Chem Toxicol* 2005;**43**:1-19.
8. NTP. NTP technical report on the toxicology and carcinogenesis. Studies of urethane, ethanol, and urethane/ethanol (urethane, CAS No. 51-79-6; ethanol, CAS No. 64-17-5)

- in B6C3F1 mice (drinking water studies). *Natl Toxicol Program Tech Rep Ser* 2004;**510**:1-346.
9. Til HP, Woutersen RA, Feron VJ, Hollanders VH, Falke HE, Clary JJ. Two-year drinking-water study of formaldehyde in rats. *Food Chem Toxicol* 1989;**27**:77-87.
  10. Moser GJ, Foley J, Burnett M, Goldsworthy TL, Maronpot R. Furan-induced dose-response relationships for liver cytotoxicity, cell proliferation, and tumorigenicity (furan-induced liver tumorigenicity). *Exp Toxicol Pathol* 2009;**61**:101-11.
  11. Navas-Acien A, Tellez-Plaza M, Guallar E, Muntner P, Silbergeld E, Jaar B, Weaver V. Blood cadmium and lead and chronic kidney disease in US adults: a joint analysis. *Am J Epidemiol* 2009;**170**:1156-64.
  12. NTP. Toxicology and carcinogenesis studies of 4-methylimidazole (Cas No. 822-36-6) in F344/N rats and B6C3F1 mice (feed studies). *Natl Toxicol Program Tech Rep Ser* 2007;**535**:1-274.
  13. Peto R, Gray R, Brantom P, Grasso P. Dose and time relationships for tumor induction in the liver and esophagus of 4080 inbred rats by chronic ingestion of N-nitrosodiethylamine or N-nitrosodimethylamine. *Cancer Res* 1991;**51**:6452-69.
  14. Peto R, Gray R, Brantom P, Grasso P. Effects on 4080 rats of chronic ingestion of N-nitrosodiethylamine or N-nitrosodimethylamine: a detailed dose-response study. *Cancer Res* 1991;**51**:6415-51.
  15. NTP. Toxicology and carcinogenesis studies of ochratoxin A (CAS No. 303-47-9) in F344/N rats (gavage studies). *Natl Toxicol Program Tech Rep Ser* 1989;**358**:1-142.
  16. Boberg EW, Miller EC, Miller JA, Poland A, Liem A. Strong evidence from studies with brachymorphic mice and pentachlorophenol that 1'-sulfoxysafrole is the major ultimate electrophilic and carcinogenic metabolite of 1'-hydroxysafrole in mouse liver. *Cancer Res* 1983;**43**:5163-73.
  17. Miller EC, Swanson AB, Phillips DH, Fletcher TL, Liem A, Miller JA. Structure-activity studies of the carcinogenicities in the mouse and rat of some naturally occurring and synthetic alkenylbenzene derivatives related to safrole and estragole. *Cancer Res* 1983;**43**:1124-34.