Regulatory Toxicology and Pharmacology 86 (2017) 128–136

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

ALDH2-deficiency as genetic epidemiologic and biochemical model for the carcinogenicity of acetaldehyde

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ARTICLE INFO

Article history:
Received 8 November 2016
Received in revised form
24 February 2017
Accepted 27 February 2017
Available online 1 March 2017

Chemical compounds studied in this article:
Acetaldehyde (PubChem CID: 177)
Ethanol (PubChem CID: 702)

Keywords:
Acetaldehyde
Genetic epidemiology
ALDH2
Cancer
Alcohol
Ethanol

ABSTRACT

Humans are cumulatively exposed to acetaldehyde from various sources including alcoholic beverages, tobacco smoke, foods and beverages. The genetic-epidemiologic and biochemical evidence in ALDH2-deficient humans provides strong evidence for the causal relationship between acetaldehyde-exposure due to alcohol consumption and cancer of the upper digestive tract. The risk assessment has so far relied on thresholds based on animal toxicology with lower one-sided confidence limit of the benchmark dose values (BMDL) typically ranging between 11 and 63 mg/kg bodyweight (bw)/day dependent on species and endpoint. The animal data is problematic for regulatory toxicology for various reasons (lack in study quality, problems in animal models and appropriateness of endpoints - especially cancer - for transfer to humans). In this study, data from genetic epidemiologic and biochemical studies are reviewed. The increase in the daily exposure dose to acetaldehyde in alcohol-consuming ALDH2-deficients vs. ALDH2-actives was about twofold. The acetaldehyde increase due to ALDH2 inactivity was calculated to be 6.7 μg/kg bw/day for heavy drinkers, which is associated with odds ratios of up to 7 for head and neck as well as oesophageal cancer. Previous animal toxicology based risk assessments may have underestimated the risk of acetaldehyde. Risk assessments of acetaldehyde need to be revised using this updated evidence.

1. Introduction

Acetaldehyde (ethanal) is a compound to which humans are regularly exposed from multiple sources such as foods, beverages, cigarettes and the environment (Cavalcante et al., 2005; Feron et al., 1991; Homann et al., 1997; Lachenmeier et al., 2009b; Lachenmeier and Sohnius, 2008; Nazaroff and Singer, 2004; Salaspuro, 2009a, 2009b; Uebelacker and Lachenmeier, 2011). Highest exposure results from consumption of alcoholic beverages and is localized to mucosal surfaces of the upper digestive tract. This is due to the fact that after alcohol intake some ethanol is metabolized locally by oral microbes and mucosal cells to acetaldehyde. Because of the inefficient ability of mucosa and microbes to eliminate acetaldehyde, the compound accumulates in the saliva and gastric juice (Homann et al., 1997, 2001; Kurkivuori et al., 2007; Lachenmeier and Monakhova, 2011; Linderborg et al., 2011; Salaspuro, 2003; Salaspuro and Salaspuro, 2004; Väkeväinen et al., 2000, 2001a, 2001b, 2002). Furthermore, acetaldehyde is found in high concentrations in some spirits, but it is also regularly present in wine and beer (Boffetta et al., 2011; Lachenmeier and Sohnius, 2008; Linderborg et al., 2008; Paiano et al., 2014).

A point mutation in *ALDH2*-gene resulting in deficient activity of the main acetaldehyde metabolizing mitochondrial enzyme (ALDH2) provides conclusive evidence for the causal relationship between local acetaldehyde exposure and upper digestive tract cancer. When drinking alcohol, the upper digestive tract mucosa of ALDH2-deficients is exposed via saliva to about 2 times and via gastric juice to 5–6 times higher acetaldehyde concentrations than in persons with active ALDH2-enzyme (Maejima et al., 2015; Väkeväinen et al., 2000, 2001b; Yokoyama et al., 2016; Yokoyama et al., 2008). Parallel to increased local acetaldehyde exposure, the risk of ALDH2-deficient alcohol drinkers for oral, pharyngeal, oesophageal and gastric cancer is many fold compared to alcohol drinking ALDH2-actives (Boccia et al., 2009; Matsuo et al., 2013; Tsai et al., 2014; Yang et al., 2010). Moreover, the difference in cancer risk between ALDH2-deficients and ALDH2-actives increases with increasing alcohol consumption. Thus, ALDH2-

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http://dx.doi.org/10.1016/j.yrtph.2017.02.024

deficiency provides a unique human cancer model for local acetaldehyde exposure in the upper digestive tract. Based on new gene-epidemiological and gene–biochemical evidence, the International Agency for Research on Cancer (IARC) has reclassified acetaldehyde associated with the consumption of alcoholic beverages as a Group 1 human carcinogen (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2012; Secretan et al., 2009).

For the risk assessment of acetaldehyde, no human data has been available so far, so that toxicological thresholds based on animal experiments have been suggested (Lachenmeier et al., 2009b). Some risk assessment bodies such as the German Federal Institute for Risk Assessment (BfR, 2010) or the MAK Commission (2013) have questioned the use of the available animal data for oral exposure, while other bodies such as the SCCS (2012) used them to provide quantitative risk estimates and suggest risk management actions such as implementation of limits in consumer products such as cosmetics.

ALDH2-deficiency provides an entirely new human model for the quantitative estimation of increased acetaldehyde exposure via saliva in the upper digestive tract of alcohol drinking ALDH2-deficients compared to ALDH2-actives. Point mutation in *ALDH2* gene has “randomized” millions of alcohol drinkers to abnormally high acetaldehyde exposure via saliva for decades thus providing a natural model. Therefore, the intention of this article was to review the human data that has become available from genetic epidemiological and biochemical research during the last decade and discuss its relevance for risk assessment.

2. Methods

Data on genetic epidemiological and genetic biochemical studies regarding the connection between aldehyde dehydrogenase 2 (*ALDH2*)-polymorphism, alcohol consumption, upper digestive tract cancer and salivary acetaldehyde concentrations in the presence of ethanol were obtained by a computer-assisted literature search. Searches were carried out in the PubMed database (U.S. National Library of Medicine, Bethesda, MD). We specifically aimed to identify studies that specified several dose groups of alcohol intake and compared ALDH2 active with ALDH2 deficient individuals, and hence might provide evidence of a clear dose-response effect.

3. Results

Three studies were identified that reported about the frequency of the *ALDH2* polymorphism among oesophageal cancer and head and neck cancer cases and controls according to two variant *ALDH2* genotypes [*1*2(deficient) and *1*1(active)] with similar levels of alcohol intake thus providing dose-response data (Table 1): one meta-analysis and one study on head and neck cancer (Boccia et al., 2009; Tsai et al., 2014) and one meta-analysis on oesophageal cancer (Yang et al., 2010). The data were dichotomized according to the drinking status of the original studies.

For the estimation of local acetaldehyde exposure via saliva in different levels of alcohol intake five studies reporting *in vivo* salivary acetaldehyde levels in ALDH2 deficients vs. ALDH2 actives after alcohol intake were identified (Maejima et al., 2015; Väkeväinen et al., 2000, 2001b; Yokoyama et al., 2016; Yokoyama et al., 2008). The main characteristics of these studies are presented in Table 2.

Mean blood and salivary acetaldehyde levels of ALDH2 actives and deficients after alcohol intake were averaged from the data presented in each study. In Table 3, salivary acetaldehyde levels represent sampling time points when ethanol had been evenly distributed to the whole-body water including saliva after alcohol intake. In three studies the areas under the curve (AUCs) of acetaldehyde exposure via saliva during the follow up ranging from 2 to 4 h was either reported or could be calculated. Thereafter differences in salivary acetaldehyde exposure of ALDH2 actives and deficients were calculated. The average difference was estimated to be 2.0fold at the sampling time point (5 studies) and according to AUCs 2.2fold (3 studies) for the deficients vs the actives (Table 3). The average of 2.1fold was selected for further calculations in Tables 4 and 5.

For the estimation of exposure of the upper digestive tract mucosa to acetaldehyde in μg/kg bodyweight (bw)/day, median unstimulated saliva flow rate was assumed to be 0.5 ml/min (Fenoll-Palomares et al., 2004). Mean alcohol consumption of moderate drinkers was assumed to be 3 drinks/day corresponding to about 4.5 h exposure via saliva (141 ml saliva) to acetaldehyde (Table 4). The mean alcohol consumption of heavy drinkers and corresponding exposure to salivary acetaldehyde was assumed to be 7 drinks (330 ml saliva) per day (Table 4). The additional acetaldehyde exposure for ALDH2 deficient compared to ALDH2 active persons was determined by multiplying the exposure of ALDH2 actives by 2.1 as indicated in Table 3.

Table 5 compares the acetaldehyde daily dose increase with the odds ratios for the cancer types. It can be deduced that for heavy drinkers odds ratios ranging from 4 to 7 are associated with acetaldehyde dose increases of 6.7 μg/kg bw/day.

Finally, Table 6 summarizes toxicological thresholds for acetaldehyde from various literature sources. It can be seen that thresholds based on animal experiments are generally above 10 mg/kg bw/day. Thresholds based on human epidemiological data are not yet available and the study data shown in Tables 5 and 6 did not allow for a dose-response-modelling as none of the study provided absolute or extra risk data. Nevertheless, the very low acetaldehyde doses (6.7 μg/kg bw/day) associated with significantly increased odds ratios for cancer, provide plausibility that the human threshold could lie considerably lower than 0.1 mg/kg bw/day.

4. Discussion

ALDH2-deficiency resulting from a single point mutation in *ALDH2*-gene is a health risk that passes in frequency familiar hypercholesterolemia (FH). The incidence of FH is 1:500 but that of ALDH2-deficiency 1:13. *ALDH2*-gene mutation took place in South China over 2000 years ago, and today its carrier frequency is close to 600 million people of East-Asian descent (Brooks et al., 2009; Li et al., 2009; Luo et al., 2009). Deficient activity of ALDH2-enzyme results in decreased ability to detoxify acetaldehyde locally formed from ethanol and thus provides a unique human model for increased exposure of upper digestive tract mucosa to acetaldehyde via saliva after drinking of alcohol (Helminen et al., 2013; Maejima et al., 2015; Väkeväinen et al., 2000, 2001b; Yokoyama et al., 2016; Yokoyama et al., 2008). With increased exposure to salivary acetaldehyde, oral, pharyngeal and oesophageal cancer risks of alcohol drinking ALDH2 deficients are many folds compared to ALDH2 active drinkers, and the higher their alcohol consumption has been (Boccia et al., 2009; Tsai et al., 2014; Yang et al., 2010).

Acetaldehyde is a cytotoxic, genotoxic and mutagenic compound and carcinogenic in experimental animals (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2012; Seitz and Stickel, 2010). In conjunction with the consumption of alcoholic beverages, acetaldehyde has been reclassified as carcinogenic to humans (Group 1) (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2012; Secretan et al., 2009). The new classification concerns both the acetaldehyde formed from ethanol by local microbial and mucosal oxidation as well as when present in alcoholic beverages.

Table 1
Studies reporting the frequency of the ALDH2 polymorphism among oesophageal cancer (Yang et al., 2010) and head and neck cancer (Boccia et al., 2009; Tsai et al., 2014) cases and controls according to two variant genotypes [*1*2(deficient) and *1*1(active)] with similar levels of alcohol intake.

Study	Cases/controls	Never/rare drinkers Mean alcohol consumption (g/day)	Moderate drinkers Mean alcohol consumption (g/day)	Heavy drinkers Mean alcohol consumption (g/day)
Meta-analysis by Yang et al. (2010) [a]				
- Boonyaphiphat et al. (2002)	202/261	Non-drinkers = 0	≤60 g/day	≥60 g/day
- Yang et al. (2007)	191/198	Non + ex-drinkers = 0	1–50 g/day; <5 d/wk	>50 g/day; ≥5 d/wk
- Ding et al. (2009)	221/191	Non-drinkers = 0	>40 g/week	–
- Lee et al. (2008)	406/656	Non-drinkers = 0	1-40 g/day	>40 g/day
- Yang et al. (2005)	165/494	Non-drinkers = 0	1–50 g/day; <5 d/wk	>50 g/day; ≥5 days/wk.
- Yokoyama et al. (2002)	234/634	Never/rare = 0/1	3-56 g/day	>57 g/day
- Yokoyama et al. (2006a)	52/412	Never/rare = 0/1	3-56 g/day	>57 g/day
- Chao et al. (2000)	88/327	Rare = 0/1	–	>60 g/day
- Yokoyama et al. (2001)	112/526	Rare = 0/1	–	>60 g/day (alcoholics)
- Yokoyama et al. (2006b)	42/273	Rare = 0/1	–	>60 g/day (alcoholics)
- Matsuo et al. (2001)	102/242	Others?	Others?	3 gou/day; ≥5 days/wk[b]
SELECTED AVERAGE[c]		**<1 g/day = 0 g/day**	**3 drinks (a 11 g) = 33 g/day**	**7 drinks (a 11 g) = 77 g/day**
Meta-analysis by Boccia et al. (2009) [a]				
- Katoh et al. (1999)	101/147	Never drinkers = 0	1–59 g/day	>59 g/day
- Nomura et al. (2000)	191/120	Never drinkers = 0	1–59 g/day	>59 g/day
- Asakage et al. (2007)	96/642	Never drinkers = 0	1–59 g/day	>59 g/day
- Hiraki et al. (2007)	329/969	Never drinkers = 0	1–59 g/day	>59 g/day
- Yokoyama and Omori (2001)	36/847	–	1–59 g/day	>59 g/day
SELECTED AVERAGE[c]		**<1 g/day = 0 g/day**	**3 drinks (a 11g) = 33 g/day**	**7 drinks (a 11 g) = 77 g/day**
Tsai et al. (2014)	436/514	Never drinkers = 0	0.1–50 g/day	>50 g/day
SELECTED AVERAGE[c]		**<1 g/day = 0 g/day**	**3 drinks (a 11g) = 33 g/day**	**7 drinks (a 11 g) = 77 g/day**

[a] It is reported that the authors of the original papers have been contacted for missing data.
[b] 1 Gou = 23 g alcohol.
[c] Selected average represents the best guess for the median alcohol consumption of never/rare, moderate and heavy drinkers.

Due to its aldehyde group, acetaldehyde is very reactive and easily binds to various tissue components, including DNA, and forms carcinogenic adducts both *in vitro* and *in vivo*. Increasing concentrations of acetaldehyde ranging from 25 to 500 μM in the presence of DNA and polyamines, produce an exponential increase of mutagenic 1,N_2-propanodeoxyguanosine adducts (Theruvathu et al., 2005). Polyamine synthesis is tightly related to cellular proliferation, with the highest levels being found in rapidly dividing cells. This is characteristic for the regenerating upper digestive tract mucosa (Tabor and Tabor, 1984). In human volunteers, low doses of alcohol have been shown to produce a dose-dependent increase in acetaldehyde-DNA adducts (N^2-ethylidene-dGuo) in the oral cavity (Balbo et al., 2012). Corresponding doses of alcohol have been shown to result in 18.7–143.4 μM acetaldehyde levels in saliva (Homann et al., 1997). In contrast to Balbo et al. (2012) N^2-ethylidene-dGuo adducts were not observed in peripheral blood white cells of human volunteers 48 h after exposure to 150 ml of vodka (Singh et al., 2012). This discrepancy, however, is not unexpected, since only very low acetaldehyde concentrations are found in hepatic venous blood of intoxicated non-alcoholic male Caucasians (Nuutinen et al., 1984) and acetaldehyde levels are undetectable (<2 μM) in the peripheral blood (Table 3).

After alcohol intake, several times higher concentrations of acetaldehyde are found in the saliva than in the blood (Table 3) (Väkeväinen et al., 2000, 2001b; Yokoyama et al., 2008). This is due to the fact that parotid glands, oral microbes and upper digestive tract mucosal cells are able to oxidize ethanol to acetaldehyde, but are not sufficiently capable for its detoxification unlike the liver. The high local exposure to acetaldehyde formed from ingested ethanol is in line with epidemiological findings. In alcohol drinking ALDH2-deficient individuals compared to ALDH2-actives, the incidence of those cancers exposed to acetaldehyde derived locally from cellular and microbial oxidation of ethanol is increased, especially of the upper digestive tract including stomach (Boccia et al., 2009; Roerecke et al., 2015; Yang et al., 2010; Hidaka et al., 2014; Wang et al., 2014; Matsuo et al., 2013). On the contrary, the incidence of cancers of organs not covered with microbes appears not to be increased in alcohol drinking ALDH2-deficients compared to ALDH2-actives e.g. cancers of breast, kidney, liver or the evidence is contradictory as in the case of pancreas (Kanda et al., 2009; Kawase et al., 2009; Miyasaka et al., 2005; Yokoyama et al., 1998; Zhou et al., 2012).

Although only acetaldehyde associated with alcohol consumption has been shown to be carcinogenic to humans, any genotoxic, mutagenic and carcinogenic compound irrespective of its origin should concern equal regulatory rules and restrictions (EFSA, 2005). This is particularly true with acetaldehyde that is easily water and lipid soluble and thus passes readily through cell membranes. Acetaldehyde probably is one of the most prevalent human carcinogens. It is present, sometimes in high concentrations, in several alcoholic beverages. It is accumulating in the digestive tract including saliva, gastric juice and colonic contents due to its local formation from ethanol by mucosal cells and microbes (Salaspuro, 2003, 2009a). Tobacco smoking may lead to considerable exposure, due to the presence of acetaldehyde in the smoke (Baumung et al., 2016; Cunningham et al., 2011; Salaspuro and Salaspuro, 2004; Xie et al., 2012), which dissolves into the saliva and is by that means distributed to the mucosal surfaces of the whole upper digestive tract. Moreover, it has also been detected in smoking cessation products such as electronic cigarettes (Hahn et al., 2014). Fermented foods such as yoghurt may naturally contain acetaldehyde, and the substance may also be added to certain foods as a flavouring compound, especially for its capability to improve orange flavour (Lachenmeier et al., 2010; Uebelacker and Lachenmeier, 2011). Acetaldehyde has also been detected in certain cosmetics (Lachenmeier et al., 2009a; SCCNFP, 2004), as well as in household and urban air (Bakeas et al., 2003; Nazaroff and Singer, 2004).

Despite its ubiquitous presence and the expected daily lifetime intake of humans in industrial societies, the toxicological assessment of acetaldehyde has not reached much attention and there are no regulatory limits for any of the mentioned products (the only regulation in the EU for acetaldehyde restricts its migration from packaging materials such as PET into foods to a certain level

Table 2
Summary of studies about the effect of *ALDH2* genotype on salivary acetaldehyde concentrations in the presence of ethanol. ALDH2+ = active aldehyde dehydrogenase enzyme; ALDH2- = deficient aldehyde dehydrogenase enzyme.

Study	Model of alcohol administration	Salivary acetaldehyde	Blood ethanol and acetaldehyde	Other remarks
1. Väkeväinen et al. (2000) - ALDH2+; n = 13 - ALDH2 -; n = 7	Oral ingestion of ethanol (0.5 g/kg bw), 10% vol in orange juice within 20 min	- At 20 min intervals from 0 to 240 min - Parotid gland saliva at 60–80 min (three ALDH2+ and ALDH2-) - AUCs[a] could be calculated from figure 1	At 60 min after alcohol intake	
2. Väkeväinen et al. (2001b) - ALDH2+; n = 6 - ALDH2-; n = 5	Oral ingestion of ethanol (0.4 g/kg bw),10% vol in orange juice within 20 min	- At 20 min intervals from 0 to 240 min - AUCs not available	At 60 min after alcohol intake	The study was repeated after one week with 4-methylpyrazole (10–15 mg/kg) 2 h before alcohol[b]
3. Yokoyama et al. (2008) - ALDH2+; n = 12 - ALDH2 -; n = 7	Oral ingestion of alcohol (0.6 g/kg), 13% vol Calvados, *shochu*, red wine or beer at 3 weeks' intervals	- At 30 min intervals from 0 to 180 min - AUCs reported in the study	At 30 min intervals from 0 to 180 min	
4. Maejima et al. (2015) - ALDH2+; n = 10 - ALDH2 -; n = 10	Intragastric infusion of ethanol (0.5 g/kg), 15% w/v via nasogastric tube	- At 30 min intervals from 0 to 120 min - AUCs reported in the study	Not measured	Also gastric juice ethanol and acetaldehyde levels were analysed and the effects of PPI-treatment and slow-release L-cysteine were studied[c]
5. Yokoyama et al. (2016) - ALDH2+; n = 81 - ALDH2 -; n = 18	Alcoholics in withdrawal treatment that had stopped drinking >4 h (mean 10 h) before saliva sampling	Twice at 1 h intervals - AUCs could not be calculated (only 2 time points)	Not measured	
6. Helminen et al. (2013)[d] - ALDH2+; n = 11 - ALDH2-; n = 6	Rinsing of mouth with 5 ml of 40% alcohol for 5 s, after which the oral contents were discharged	At 0.5, 2.5, 5, 10, 15 and 20 min after rinsing	Not measured	No differences in salivary acetaldehyde levels between ALDH2-actives and −deficients. Presence of ethanol in systemic blood circulation is required for the elevation of salivary acetaldehyde among ALDH2-deficients

[a] AUC = area under the salivary acetaldehyde curve of ALDH2-actives and -deficients.
[b] 4-Methylpyrazole (4 MP) is an effective inhibitor of alcohol dehydrogenase (ADH) enzyme of somatic cells. Its inhibitory effect on microbial ADH enzymes, however, is poor.
[c] PPIs are powerful gastric acid secretion inhibitors resulting in microbial colonization of the gastric contents. PPI-inhibitor rabeprazole 10 mg b.i.d. was administered for 7 days before the experiments with or without slow-release L-cysteine. L-cysteine binds effectively to acetaldehyde and inactivates its reactive aldehyde group.
[d] Excluded, since only local acetaldehyde formation in the mouth was studied. No ethanol was delivered to saliva via systemic blood circulation.

(Choodum et al., 2007; Mutsuga et al., 2006)). The lack of regulation can be probably explained historically because acetaldehyde received the 'generally recognized as safe' (GRAS) status by the Flavor and Extract Manufacturers Association (FEMA) in 1965 (Hall and Oser, 1965). This status was corroborated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1998 (JECFA, 1998). However, in the case of acetaldehyde, the application of JECFA's decision tree for flavouring substances includes a crucial misconception with regard to acetaldehyde's metabolism (WHO, 2000). Because of the lack of low K_m aldehyde dehydrogenase enzymes in the oral mucosa, acetaldehyde accumulates in the presence of ethanol in the saliva and is not metabolized into innocuous products in the oral cavity (Dong et al., 1996). Via saliva, carcinogenic acetaldehyde is distributed to the mucosal surfaces of the mouth, oesophagus and stomach.

As a matter of fact, a large body of evidence has been acquired over the last decade that may question these early risk assessments. Specifically, the evidence for carcinogenic and genotoxic effects of acetaldehyde has been improved leading the International Agency for Research on Cancer to assign acetaldehyde into group 2B as being 'possibly carcinogenic to humans' in 1987 and 1999 (IARC, 1987; IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 1999), while it was upgraded in association with alcohol consumption into group 1 (i.e., the highest level of evidence) in 2009 (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2012; Secretan et al., 2009).

The differences in the order of magnitude of toxicological threshold values for acetaldehyde between animal experiments and human epidemiology are not unexpected. In this study, human data was restricted to upper digestive tract cancers i.e. on the local carcinogenic potential of acetaldehyde. With regard to acetaldehyde, the use of human data has several advantages as compared to animal data. In animal model studies with rodents, acetaldehyde has been administered without the presence of ethanol, but only acetaldehyde associated with alcoholic beverages has been classified as human group 1 carcinogen. Gastrointestinal tract mucosa of rodents is known to be able to metabolize both ethanol and acetaldehyde (Koivisto and Salaspuro, 1996; Pronko et al., 2002). Without the competing presence of ethanol, some of the acetaldehyde given to rodents in drinking water is metabolized by mucosal cells and the rest of it completely by the liver to acetate (Matysiak-Budnik et al., 1996). Thus, the first-pass metabolism of acetaldehyde mediated mostly by the liver totally eliminates possible systemic effects of acetaldehyde via blood circulation to other organs.

The BfR (2010) remarked in their criticism of the dose-response relationship in the oral lifetime rat feeding study of Soffritti et al. (2002) that the statistical power has not been sufficient for the relevant endpoints to evaluate the acetaldehyde carcinogenicity such as tumours of the oral cavity and oesophagus. Therefore, they expected the toxicological threshold to be below the first dose (i.e. 5 mg/kg bw/day) or lower. In light of the sensitivity of the animal tests alone, the lower results in humans appear plausible.

Using a unique genetic human model for increased local acetaldehyde exposure of the upper digestive tract has major advantages over animal data. Nature has randomized millions of alcohol drinking persons to different quantities of acetaldehyde exposure. This type of human model based on a single point mutation is not available for any other of the 119 IARC group 1 carcinogens. The model makes it possible to minimize the effect of important confounding factors such as smoking, diet, oral hygiene, HPV, different beverages, drinking habits, BMI and under reporting that are

Table 3
Mean blood and salivary acetaldehyde levels of ALDH2-actives (ALDH2+) compared to ALDH2-deficients (ALDH2-) after oral intake of alcohol. Difference in salivary acetaldehyde exposure was calculated in two ways: 1. Salivary acetaldehyde concentration at sampling time point of ALDH2-deficients was divided by that of ALDH2-positives. 2. Area under the curve (AUC) of the salivary acetaldehyde curves during the follow-up period (2–4 h) of ALDH2-deficients was divided by that of ALDH2-positives.

Study	Blood acetaldehyde		Salivary acetaldehyde		Salivary ethanol level at sampling time point mM/‰	Difference in salivary acetaldehyde exposure ALDH2-/ALDH2+	
	ALDH2+ (μM / mg/l) (means)	ALDH2- (μM / mg/l) (means)	ALDH2+ (μM / mg/l) (means)	ALDH2- (μM / mg/l) (means)		At sampling time point	According to AUC
1. Väkeväinen et al. (2000) [a]	Not detectable	6.6/0.29 (at 60 min)	26/1.1 (at 60 min)	58/2.6 (at 60 min)	≈12/≈0.55 (at 60 min)	2.3fold (at 60 min)	2.3fold
2. Väkeväinen et al. (2001b) [b]	Not detectable	6.4/0.28 (at 60 min)	12, 17, 20/0.53, 0.75, 0.89 (at ethanol levels of 4,6,8 mM)	19, 27, 33/1.45, 1.19, 1.45 (at ethanol levels of 4,6,8 mM)	4, 6, 8/0.19, 0.28, 0.37	1.5fold (mean)	
3. Yokoyama et al. (2008)	≈2/0.09 (at 90 min)	≈10/0.44 (at 90 min)	30/1.32 (at 90 min)	52/2.29 (at 90 min)	≈12/≈0.55 (at 90 min)	1.7fold (at 90 min)	1.6fold
4. Maejima et al. (2015) [c]	Not measured	Not measured	6/0.26 (at 60 min)	16/1.06 at (60 min)	≈15/≈0.63 (at 60 min)	2.7fold (at 60 min)	2.7fold
5. Yokoyama et al. (2016) [d]	Not measured	Not measured	41/1.80	73/3.20	≥21/≥ 1	1.8fold	
MEAN			**25/1.1** [e]	**46/2.1** [e]		**2.0fold**	**2.2fold**
Remarks	- In ALDH2-actives in general under the limit of detection - In ALDH2-deficients low levels ranging from 6.4 to ≈12 μM (0.28–0.53 mg/l)		- Levels in saliva are several times higher than in blood - In ALDH2-deficients levels are 1.5–2.3fold higher than in ALDH2-actives		Salivary ethanol levels range from very low 2–4 mM to very high 61 mM	The difference in salivary acetaldehyde exposure between ALDH2-actives and –deficients appears to be equal when averaged either from single sampling time points or from AUCs. Mean **2.1** was selected for further calculations in Tables 4 and 5	

[a] Acetaldehyde levels of sterile parotid gland saliva were undetectable in three ALDH2-actives and 75.0, 21.8 and 3.9 μM in three ALDH2-deficients. Suggests that additional salivary acetaldehyde in ALDH2-deficients is derived from salivary glands.

[b] 4-Methylpyrazole (ADH inhibitor) treatment inhibited totally the increase in salivary acetaldehyde among ALDH2-deficients but had no effect on salivary acetaldehyde in ALDH2-actives. Suggests that additional salivary acetaldehyde in ALDH2-deficients is derived from salivary glands.

[c] Alcohol was infused intragastrically that may result in lower salivary acetaldehyde levels. Nevertheless, differences in salivary acetaldehyde levels between ALDH2-actives and -deficients were comparable to those found in other studies.

[d] In line with earlier findings, higher salivary ethanol levels result in higher salivary acetaldehyde levels. This is caused by the kinetics of different microbial alcohol dehydrogenase enzymes.

[e] The significance of differences in salivary acetaldehyde levels between ALDH2-actives and –deficients. Study 1: $p < 0.001$; Study 2: $p < 0.001$; Study 3: $p < 0.0001$; Study 4: $p = 0.009$ for AUC; Study 5: $p = 0.0008$. In study 2, the acetaldehyde level at 8 mM ethanol was used for the calculation of the mean.

Table 4
Calculation of the exposure of upper digestive tract mucosa to acetaldehyde via saliva in ALDH2-actives and -deficients.

Acetaldehyde exposure via saliva	Never/rare drinkers (see Table 1)	Moderate drinkers (see Table 1) (3 drinks = 33 g/day)	Heavy drinkers (see Table 1) (7 drinks = 77 g/day)
Exposure time	0	4.7 h/day[a]	11 h/day[a]
Amount of saliva secreted		141 ml/day[b]	330 ml/day[b]
ALDH2-actives			
- in mg/l	0	1.1[c]	1.1
- in µg/day	0	155	363
- in µg/kg bw/day[d]	0	2.6	6.1
ALDH2-deficients[e]			
- in mg/l	0	2.3	2.3
- in µg/day	0	326	762
- in µg/kg bw/day[d]	0	5.5	12.8
Acetaldehyde daily dose increase due to ALDH2-deficiency[f]			
- in µg/kg bw/day[d]	0	**2.9**[f]	**6.7**[f]

[a] Acetaldehyde exposure time is 1.5 h/drink (1 drink = 11 g ethanol) based on the normal elimination rate of ethanol (7 g/h) (Cederbaum, 2012).
[b] Unstimulated saliva flow rate is assumed to be 0.5 ml/min (Fenoll-Palomares et al., 2004).
[c] 1.1 mg/l is calculated as indicated in Table 3.
[d] Mean weight = 60 kg.
[e] x 2.1 (Table 3).
[f] ALDH2 def. − ALDH2 act.

Table 5
Summary of data from genetic epidemiological studies regarding the cancer risk of ALDH2-deficient people in connection with alcohol consumption.

Drinking status	Acetaldehyde daily dose increase due to ALDH2 inactivity (µg/kg bw/day)[a]	Odds Ratio (LCI/UCI) (ALDH2-deficient compared to active)
1. Yang et al. (2010) **(oesophageal cancer in Japan and China)**		
Never drinkers	0	1.28 (0.91/1.80)
Moderate Drinkers	2.9	3.12 (1.95/5.01)
Heavy Drinkers	6.7	7.12 (4.67/10.86)
2. Boccia et al. (2009) **(head and neck cancer in Japan)**		
Never drinkers	0	0.97 (0.65/1.46)
Moderate Drinkers	2.9	1.68 (1.27/2.22)
Heavy Drinkers	6.7	3.57 (1.41/9.05)
3a. Tsai et al. (2014) **(head and neck cancer in Taiwan)**		
(fast *ADH1B*(*2*2); slow *ALDH2*(*1*2 + *2*2)		
0 g	0	1.00
0.1–50 g	2.9	2.61 (1.19/5.75)[b]
>50 g	6.7	7.28 (2.00/26.49)[b]
3b. Tsai et al. (2014) **(head and neck cancer in Taiwan)**		
(slow *ADH1B*(*1*1 + *1/*2); slow *ALDH2*(*1*2 + *2*2)		
0 g	0	1.00
0.1–50 g	2.9	1.99 (0.92/4.34)[b]
>50 g	6.7	7.09 (2.88/17.42)[b]

[a] Authors estimation based on data shown in Tables 2–4.
[b] The results indicate that fast ADH enzyme does not correlate with increased head and neck cancer risk among ALDH2-deficients. This is in accordance with no effect of fast ADH on salivary acetaldehyde levels in the presence of ethanol in ALDH2-deficient alcoholics (Yokoyama et al., 2016).

generally hampering most epidemiological studies focusing on alcohol and cancer. Among ALDH2-deficient and ALDH2-active individuals these important confounding factors can be assumed to be more or less evenly distributed.

5. Conclusions

Our data based on human epidemiology corroborate the findings of previous studies based on animal toxicology (see overview in Table 6) in the fashion that these studies at least have not overestimated the risk of acetaldehyde. The unique human gene–epidemiological and gene–biochemical evidence, even if limited by some uncertainty, points in the direction that the risk of acetaldehyde has been underestimated rather than overestimated.

In light of this evidence, it is commendable that the European Commission has recently stated that they will raise the issue of acetaldehyde with the competent authorities of the member states and, if appropriate, the European Food Safety Authority (EFSA) will be requested to assess the risk for human health related to the presence of acetaldehyde in food as process contaminant or as natural flavour (Borg, 2014). We basically agree with this assessment of the commission and clearly believe in a considerable appropriateness of an EFSA assessment, however, we think that the addition of acetaldehyde as artificial flavour to foods, which still is a common and legal practice, might specifically require scrutiny. It must be noted that the German Federal Institute for Risk Assessment remarked in 2010 that the safety of acetaldehyde when used as flavouring substance cannot be finally evaluated (BfR, 2010). This raises the question why potentially unsafe flavour additives may be used in foods prior to the proof of their safety by adequate studies conducted by the industry. There is currently no evidence that acetaldehyde present in 'non-alcoholic' foods and beverages is more safe than acetaldehyde associated with the use of alcoholic beverages.

Table 6
Summary of toxicological thresholds (benchmark doses) for acetaldehyde.

Study containing the original experimental data	Study with dose-response modelling to establish thresholds	Species/Route/Study type	Endpoint	BMD [a] (mg/kg bw/day)	BMDL [b] (mg/kg bw/day)
Yang et al. (2010)	This study	Humans/oral/ epidemiology	Oesophageal cancer	≈<0.1 [c]	≈<0.1 [c]
Boccia et al. (2009)	This study	Humans/oral/ epidemiology	Head and neck cancer	≈<0.1 [c]	≈<0.1 [c]
Tsai et al. (2014)	This study	Humans/oral/ epidemiology	Head and neck cancer	≈<0.1 [c]	≈<0.1 [c]
Soffritti et al. (2002)	Lachenmeier et al. (2009b)	Rats/oral/lifetime	Tumour-bearing animals, M	114	56
Soffritti et al. (2002)	Lachenmeier et al. (2009b)	Rats/oral/lifetime	Total malignant tumours, M	58	41
Soffritti et al. (2002)	Lachenmeier et al. (2009b)	Rats/oral/lifetime	Total malignant tumours, F	152 [d]	63 [d]
Woutersen et al. (1986)	Xie et al. (2012)	Rats/inhalation/27 months	Nasal squamous cell carcinomas, M	15 [e]	11 [e]
Sanner et al. (2001)	Sanner et al. (2001)	(no data provided)	(no data provided)	(no data provided)	15.5 [f]
Unclear; several studies (Woutersen et al. (1986); Woutersen and Feron (1987); including pers. comm.)	Gold et al. (2007)	Unclear (Rats/inhalation /26 months)	Unclear (Nose and larynx cancer)	(no data provided)	17.4
Unclear; Feron et al. (1982) and pers. comm.	Gold et al. (2007)	Unclear (Hamsters/ inhalation/52 weeks)	Unclear (Larynx cancer)	(no data provided)	41.9

[a] BMD: benchmark dose. The benchmark response was 10% in the animal studies.
[b] BMDL: lower one-sided confidence limit of the BMD.
[c] The available data do not allow for BMD modelling. However, the values are estimated as considerably being below 0.1 mg/kg bw/day (compare exposure data in Table 5).
[d] Non-significant model.
[e] The BMC and BMCL in mg/m^3 were re-calculated to BMD/BMDL using the following conversion of (SCCNFP, 2004): BMD (mg/kg bw/day) = BMC (mg/m^3)/9. This formula considers an inhalation rate of 20.5 l/h and an exposure time of 6 h/d, 5 d/week for 27 months.
[f] Calculated from LED_1 value (1.55) by multiplication with 10.

Conflicts of interest statement

DWL declares no conflicts of interest; MS is board member of Biohit Oyj. No funding was specific to the production of this manuscript.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.yrtph.2017.02.024.

References

Asakage, T., Yokoyama, A., Haneda, T., Yamazaki, M., Muto, M., Yokoyama, T., Kato, H., Igaki, H., Tsujinaka, T., Kumagai, Y., Yokoyama, M., Omori, T., Watanabe, H., 2007. Genetic polymorphisms of alcohol and aldehyde dehydrogenases, and drinking, smoking and diet in Japanese men with oral and pharyngeal squamous cell carcinoma. Carcinogenesis 28, 865–874.

Bakeas, E.B., Argyris, D.I., Siskos, P.A., 2003. Carbonyl compounds in the urban environment of Athens, Greece. Chemosphere 52, 805–813.

Balbo, S., Meng, L., Bliss, R.L., Jensen, J.A., Hatsukami, D.K., Hecht, S.S., 2012. Kinetics of DNA adduct formation in the oral cavity after drinking alcohol. Cancer Epidemiol. Biomarkers Prev. 21, 601–608.

Baumung, C., Rehm, J., Franke, H., Lachenmeier, D.W., 2016. Comparative risk assessment of tobacco smoke constituents using the margin of exposure approach: the neglected contribution of nicotine. Sci. Rep. 6, 35577.

BfR, 2010. Statement of the BfR Committee on Food Additives, Flavourings and Processing Aids (LAV) on the Use of Acetaldehyde as Flavouring Substance. Bundesinstitut für Risikobewertung (BfR), Berlin, Germany.

Boccia, S., Hashibe, M., Galli, P., De, F.E., Asakage, T., Hashimoto, T., Hiraki, A., Katoh, T., Nomura, T., Yokoyama, A., van Duijn, C.M., Ricciardi, G., Boffetta, P., 2009. Aldehyde dehydrogenase 2 and head and neck cancer: a meta-analysis implementing a Mendelian randomization approach. Cancer Epidemiol. Biomarkers Prev. 18, 248–254.

Boffetta, P., Kaihovaara, P., Rudnai, P., Znaor, A., Lissowska, J., Swiatkowska, B., Mates, D., Pandics, T., Salaspuro, M., 2011. Acetaldehyde level in spirits from central European countries. Eur. J. Cancer Prev. 20, 526–529.

Boonyaphiphat, P., Thongsuksai, P., Sriplung, H., Puttawibul, P., 2002. Lifestyle habits and genetic susceptibility and the risk of esophageal cancer in the Thai population. Cancer Lett. 186, 193–199.

Borg, T., 2014. Parliamentary questions. E-004538/2014. Quantities of acetaldehyde in food products. Answer given by Mr Borg on behalf of the commission. Off. J. Eur. Union C399, 94–95.

Brooks, P.J., Enoch, M.A., Goldman, D., Li, T.K., Yokoyama, A., 2009. The alcohol flushing response: an unrecognized risk factor for esophageal cancer from alcohol consumption. PLoS Med. 6, e50. http://dx.doi.org/10.1371/journal.pmed.1000050.

Cavalcante, R.M., Seyffert, B.H., D'Oca, M.G.M., Nascimento, R.F., Campelo, C.S., Pinto, I.S., Anjos, F.B., Costa, A.H.R., 2005. Exposure assessment for formaldehyde and acetaldehyde in the workplace. Indoor Built Environ. 14, 165–172.

Cederbaum, A.I., 2012. Alcohol metabolism. Clin. Liver Dis. 16, 667–685.

Chao, Y.C., Wang, L.S., Hsieh, T.Y., Chu, C.W., Chang, F.Y., Chu, H.C., 2000. Chinese alcoholic patients with esophageal cancer are genetically different from alcoholics with acute pancreatitis and liver cirrhosis. Am. J. Gastroenterol. 95, 2958–2964.

Choodum, A., Thavarungkul, P., Kanatharana, P., 2007. Acetaldehyde residue in polyethylene terephthalate (PET) bottles. J. Environ. Sci. Health B 42, 577–583.

Cunningham, F.H., Fiebelkorn, S., Johnson, M., Meredith, C., 2011. A novel application of the Margin of Exposure approach: segregation of tobacco smoke toxicants. Food Chem. Toxicol. 49, 2921–2933.

Ding, J.H., Li, S.P., Cao, H.X., Wu, J.Z., Gao, C.M., Su, P., Liu, Y.T., Zhou, J.N., Chang, J., Yao, G.H., 2009. Polymorphisms of alcohol dehydrogenase-2 and aldehyde dehydrogenase-2 and esophageal cancer risk in Southeast Chinese males. World J. Gastroenterol. 15, 2395–2400.

Dong, Y.J., Peng, T.K., Yin, S.J., 1996. Expression and activities of class IV alcohol dehydrogenase and class III aldehyde dehydrogenase in human mouth. Alcohol 13, 257–262.

EFSA, 2005. 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. 282, 1–31.

Fenoll-Palomares, C., Munoz Montagud, J.V., Sanchiz, V., Herreros, B., Hernandez, V., Minguez, M., Benages, A., 2004. Unstimulated salivary flow rate, pH and buffer capacity of saliva in healthy volunteers. Rev. Esp. Enferm. Dig. 96, 773–783.

Feron, V.J., Kruysse, A., Woutersen, R.A., 1982. Respiratory tract tumours in hamsters exposed to acetaldehyde vapour alone or simultaneously to benzo(a)pyrene or diethylnitrosamine. Eur. J. Cancer Clin. Oncol. 18, 13–31.

Feron, V.J., Til, H.P., de Vrijer, F., Woutersen, R.A., Cassee, F.R., van Bladeren, P.J., 1991. Aldehydes: occurrence, carcinogenic potential, mechanism of action and risk assessment. Mutat. Res. 259, 363–385.

Gold, L.S., Ames, B.N., Slone, T.H., 2007. LTD10 Values for Chemicals Evaluated as Carcinogenic in a Least One Experiment in the CPDB. Carcinogenic Potency Project, University of California, Berkeley, Berkeley, CA. http:/toxnet.nlm.nih.gov/cpdb/xls/LTD10.xls. Accessed 28 January 2015. (Archived by WebCite® at http://www.webcitation.org/6W5l6p5xb).

Hahn, J., Monakhova, Y.B., Hengen, J., Kohl-Himmelseher, M., Schussler, J., Hahn, H., Kuballa, T., Lachenmeier, D.W., 2014. Electronic cigarettes: overview of chemical composition and exposure estimation. Tob. Induc. Dis. 12, 23.

Hall, R.L., Oser, B.L., 1965. Recent progress in the consideration of flavoring ingredients under the food additives amendment. III. GRAS substances. Food Technol. 19, 151–197.

Helminen, A., Väkeväinen, S., Salaspuro, M., 2013. ALDH2 genotype has no effect on salivary acetaldehyde without the presence of ethanol in the systemic circulation. PLoS One 8, e74418.

Hidaka, A., Sasazuki, S., Matsuo, K., Ito, H., Sawada, N., Shimazu, T., Yamaji, T.,

Iwasaki, M., Inoue, M., Tsugane, S., for the JPHC Study Group, 2014. Genetic polymorphisms of ADH1B, ADH1C and ALDH2, alcohol consumption, and the risk of gastric cancer: the Japan Public Health Center-based prospective study. Carcinogenesis 36, 223–231.

Hiraki, A., Matsuo, K., Wakai, K., Suzuki, T., Hasegawa, Y., Tajima, K., 2007. Gene-gene and gene-environment interactions between alcohol drinking habit and polymorphisms in alcohol-metabolizing enzyme genes and the risk of head and neck cancer in Japan. Cancer Sci. 98, 1087–1091.

Homann, N., Jousimies-Somer, H., Jokelainen, K., Heine, R., Salaspuro, M., 1997. High acetaldehyde levels in saliva after ethanol consumption: methodological aspects and pathogenetic implications. Carcinogenesis 18, 1739–1743.

Homann, N., Tillonen, J., Rintamäki, H., Salaspuro, M., Lindqvist, C., Meurman, J.H., 2001. Poor dental status increases acetaldehyde production from ethanol in saliva: a possible link to increased oral cancer risk among heavy drinkers. Oral Oncol. 37, 153–158.

IARC, 1987. Acetaldehyde. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Supplement 7, Overall Evaluations of Carcinogenicity: an Updating of IARC Monographs Volumes 1 to 42. International Agency for Research on Cancer, Lyon, France, pp. 77–78.

IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 1999. Acetaldehyde, vol.71. IARC Monogr. Eval. Carcinog. Risks Hum., pp. 319–335

IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2012. Consumption of Alcoholic Beverages, vol. 100E. IARC Monogr. Eval. Carcinog. Risks Hum., pp. 373–499

JECFA, 1998. Saturated Aliphatic Acyclic Linear Primary Alcohols, Aldehydes, and Acids. WHO Food Additives Series 40. Safety Evaluation of Certain Food Additives and Contaminants. World Health Organization, Geneva, Switzerland, pp. 148–188.

Kanda, J., Matsuo, K., Suzuki, T., Kawase, T., Hiraki, A., Watanabe, M., Mizuno, N., Sawaki, A., Yamao, K., Tajima, K., Tanaka, H., 2009. Impact of alcohol consumption with polymorphisms in alcohol-metabolizing enzymes on pancreatic cancer risk in Japanese. Cancer Sci. 100, 296–302.

Katoh, T., Kaneko, S., Kohshi, K., Munaka, M., Kitagawa, K., Kunugita, N., Ikemura, K., Kawamoto, T., 1999. Genetic polymorphisms of tobacco- and alcohol-related metabolizing enzymes and oral cavity cancer. Int. J. Cancer 83, 606–609.

Kawase, T., Matsuo, K., Hiraki, A., Suzuki, T., Watanabe, M., Iwata, H., Tanaka, H., Tajima, K., 2009. Interaction of the effects of alcohol drinking and polymorphisms in alcohol-metabolizing enzymes on the risk of female breast cancer in Japan. J. Epidemiol. 19, 244–250.

Koivisto, T., Salaspuro, M., 1996. Aldehyde dehydrogenases of the rat colon: comparison with other tissues of the alimentary tract and the liver. Alcohol. Clin. Exp. Res. 20, 551–555.

Kurkivuori, J., Salaspuro, V., Kaihovaara, P., Kari, K., Rautemaa, R., Grönroos, L., Meurman, J.H., Salaspuro, M., 2007. Acetaldehyde production from ethanol by oral streptococci. Oral Oncol. 43, 181–186.

Lachenmeier, D.W., Gumbel-Mako, S., Sohnius, E.M., Keck-Wilhelm, A., Kratz, E., Mildau, G., 2009a. Salivary acetaldehyde increase due to alcohol-containing mouthwash use: a risk factor for oral cancer. Int. J. Cancer 125, 730–735.

Lachenmeier, D.W., Kanteres, F., Rehm, J., 2009b. Carcinogenicity of acetaldehyde in alcoholic beverages: risk assessment outside ethanol metabolism. Addiction 104, 533–550.

Lachenmeier, D.W., Monakhova, Y.B., 2011. Short-term salivary acetaldehyde increase due to direct exposure to alcoholic beverages as an additional cancer risk factor beyond ethanol metabolism. J. Exp. Clin. Cancer Res. 30, 3.

Lachenmeier, D.W., Sohnius, E.M., 2008. The role of acetaldehyde outside ethanol metabolism in the carcinogenicity of alcoholic beverages: evidence from a large chemical survey. Food Chem. Toxicol. 46, 2903–2911.

Lachenmeier, D.W., Uebelacker, M., Hensel, K., Rehm, J., 2010. Acetaldehyde in the human diet: an underestimated risk factor for cancer. Deut. Leb. Rundsch. 106, 30–35.

Lee, C.H., Lee, J.M., Wu, D.C., Goan, Y.G., Chou, S.H., Wu, I.C., Kao, E.L., Chan, T.F., Huang, M.C., Chen, P.S., Lee, C.Y., Huang, C.T., Huang, H.L., Hu, C.Y., Hung, Y.H., Wu, M.T., 2008. Carcinogenetic impact of ADH1B and ALDH2 genes on squamous cell carcinoma risk of the esophagus with regard to the consumption of alcohol, tobacco and betel quid. Int. J. Cancer 122, 1347–1356.

Li, H., Borinskaya, S., Yoshimura, K., Kalina, N., Marusin, A., Stepanov, V.A., Qin, Z., Khaliq, S., Lee, M.Y., Yang, Y., Mohyuddin, A., Gurwitz, D., Mehdi, S.Q., Rogaev, E., Jin, L., Yankovsky, N.K., Kidd, J.R., Kidd, K.K., 2009. Refined geographic distribution of the oriental ALDH2*504Lys (nee 487Lys) variant. Ann. Hum. Genet. 73, 335–345.

Linderborg, K., Joly, J.P., Visapää, J.P., Salaspuro, M., 2008. Potential mechanism for Calvados-related oesophageal cancer. Food Chem. Toxicol. 46, 476–479.

Linderborg, K., Salaspuro, M., Väkeväinen, S., 2011. A single sip of a strong alcoholic beverage causes exposure to carcinogenic concentrations of acetaldehyde in the oral cavity. Food Chem. Toxicol. 49, 2103–2106.

Luo, H.R., Wu, G.S., Pakstis, A.J., Tong, L., Oota, H., Kidd, K.K., Zhang, Y.P., 2009. Origin and dispersal of atypical aldehyde dehydrogenase ALDH2487Lys. Gene 435, 96–103.

Maejima, R., Iijima, K., Kaihovaara, P., Hatta, W., Koike, T., Imatani, A., Shimosegawa, T., Salaspuro, M., 2015. Effects of ALDH2 genotype, PPI treatment and L-cysteine on carcinogenic acetaldehyde in gastric juice and saliva after intragastric alcohol administration. PLoS One 10, e0120397. http://dx.doi.org/10.1371/journal.pone.0120397.

MAK Commission, 2013. Acetaldehyde. The MAK Collection for Occupational Health and Safety. The MAK-collection Part I, MAK Value Documentations 2013. Wiley-VCH Verlag, Weinheim, Germany, pp. 1–59. http://dx.doi.org/10.1002/3527600418.mb7507e4413.

Matsuo, K., Hamajima, N., Shinoda, M., Hatooka, S., Inoue, M., Takezaki, T., Tajima, K., 2001. Gene-environment interaction between an aldehyde dehydrogenase-2 (ALDH2) polymorphism and alcohol consumption for the risk of esophageal cancer. Carcinogenesis 22, 913–916.

Matsuo, K., Oze, I., Hosono, S., Ito, H., Watanabe, M., Ishioka, K., Ito, S., Tajika, M., Yatabe, Y., Niwa, Y., Yamao, K., Nakamura, S., Tajima, K., Tanaka, H., 2013. The aldehyde dehydrogenase 2 (ALDH2) Glu504Lys polymorphism interacts with alcohol drinking in the risk of stomach cancer. Carcinogenesis 34, 1510–1515.

Matysiak-Budnik, T., Jokelainen, K., Kärkkäinen, P., Mäkisalo, H., Ohisalo, J., Salaspuro, M., 1996. Hepatotoxicity and absorption of extrahepatic acetaldehyde in rats. J. Pathol. 178, 469–474.

Miyasaka, K., Kawanami, T., Shimokata, H., Ohta, S., Funakoshi, A., 2005. Inactive aldehyde dehydrogenase-2 increased the risk of pancreatic cancer among smokers in a Japanese male population. Pancreas 30, 95–98.

Mutsuga, M., Kawamura, Y., Sugita-Konishi, Y., Hara-Kudo, Y., Takatori, K., Tanamoto, K., 2006. Migration of formaldehyde and acetaldehyde into mineral water in polyethylene terephthalate (PET) bottles. Food Addit. Contam 23, 212–218.

Nazaroff, W.W., Singer, B.C., 2004. Inhalation of hazardous air pollutants from environmental tobacco smoke in US residences. J. Expo. Anal. Environ. Epidemiol. 14 (Suppl. 1), S71–S77.

Nomura, T., Noma, H., Shibahara, T., Yokoyama, A., Muramatusu, T., Ohmori, T., 2000. Aldehyde dehydrogenase 2 and glutathione S-transferase M 1 polymorphisms in relation to the risk for oral cancer in Japanese drinkers. Oral Oncol. 36, 42–46.

Nuutinen, H.U., Salaspuro, M.P., Valle, M., Lindros, K.O., 1984. Blood acetaldehyde concentration gradient between hepatic and antecubital venous blood in ethanol-intoxicated alcoholics and controls. Eur. J. Clin. Invest. 14, 306–311.

Paiano, V., Bianchi, G., Davoli, E., Negri, E., Fanelli, R., Fattore, E., 2014. Risk assessment for the Italian population of acetaldehyde in alcoholic and non-alcoholic beverages. Food Chem. 154, 26–31.

Pronko, P., Bardina, L., Satanovskaya, V., Kuzmich, A., Zimatkin, S., 2002. Effect of chronic alcohol consumption on the ethanol- and acetaldehyde-metabolizing systems in the rat gastrointestinal tract. Alcohol Alcohol 37, 229–235.

Roerecke, M., Shield, K.D., Higuchi, S., Yoshimura, A., Larsen, E., Rehm, M.X., Rehm, J., 2015. Estimates of alcohol-related oesophageal cancer burden in Japan: systematic review and meta-analyses. Bull. World Health Organ 93, 329–338. C.

Salaspuro, M., 2009a. Acetaldehyde as a common denominator and cumulative carcinogen in digestive tract cancers. Scand. J. Gastroenterol. 44, 912–925.

Salaspuro, M., 2009b. Acetaldehyde: a cumulative carcinogen in humans. Addiction 104, 551–553.

Salaspuro, M.P., 2003. Acetaldehyde, microbes, and cancer of the digestive tract. Crit. Rev. Clin. Lab. Sci. 40, 183–208.

Salaspuro, V., Salaspuro, M., 2004. Synergistic effect of alcohol drinking and smoking on in vivo acetaldehyde concentration in saliva. Int. J. Cancer 111, 480–483.

Sanner, T., Dybing, E., Willems, M.I., Kroese, E.D., 2001. A simple method for quantitative risk assessment of non-threshold carcinogens based on the dose descriptor T25. Pharmacol. Toxicol 88, 331–341.

SCCNFP, 2004. Opinion of the Scientific Committee on Cosmetic Products and Non-food Products Intended for Consumers Concerning Acetaldehyde. http://ec.europa.eu/health/ph_risk/committees/sccp/documents/out275_en.pdf.

SCCS, 2012. Opinion on Acetaldehyde, 18 September 2012-revision 11 December 2012 (SCCS/1468/12). Scientific Committee on Consumer Safety (SCCS), Brussels, Belgium. https://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_104.pdf.

Secretan, B., Straif, K., Baan, R., Grosse, Y., El Ghissassi, F., Bouvard, V., Benbrahim-Tallaa, L., Guha, N., Freeman, C., Galichet, L., Cogliano, V., 2009. A review of human carcinogens - Part E: tobacco, areca nut, alcohol, coal smoke, and salted fish. Lancet Oncol. 10, 1033–1034.

Seitz, H.K., Stickel, F., 2010. Acetaldehyde as an underestimated risk factor for cancer development: role of genetics in ethanol metabolism. Genes Nutr. 5, 121–128.

Singh, R., Gromadzinska, J., Mistry, Y., Cordell, R., Juren, T., Segerbäck, D., Farmer, P.B., 2012. Detection of acetaldehyde derived N(2)-ethyl-2'-deoxyguanosine in human leukocyte DNA following alcohol consumption. Mutat. Res. 737, 8–11.

Soffritti, M., Belpoggi, F., Lambertin, L., Lauriola, M., Padovani, M., Maltoni, C., 2002. Results of long-term experimental studies on the carcinogenicity of formaldehyde and acetaldehyde in rats. Ann. N. Y. Acad. Sci. 982, 87–105.

Tabor, C.W., Tabor, H., 1984. Polyamines. Annu. Rev. Biochem. 53, 749–790.

Theruvathu, J.A., Jaruga, P., Nath, R.G., Dizdaroglu, M., Brooks, P.J., 2005. Polyamines stimulate the formation of mutagenic 1,N^2-propanodeoxyguanosine adducts from acetaldehyde. Nucleic Acids Res. 33, 3513–3520.

Tsai, S.T., Wong, T.Y., Ou, C.Y., Fang, S.Y., Chen, K.C., Hsiao, J.R., Huang, C.C., Lee, W.T., Lo, H.I., Huang, J.S., Wu, J.L., Yen, C.J., Hsueh, W.T., Wu, Y.H., Yang, M.W., Lin, F.C., Chang, J.Y., Chang, K.Y., Wu, S.Y., Liao, H.C., Lin, C.L., Wang, Y.H., Weng, Y.L., Yang, H.C., Chang, J.S., 2014. The interplay between alcohol consumption, oral hygiene, ALDH2 and ADH1B in the risk of head and neck cancer. Int. J. Cancer 135, 2424–2436.

Uebelacker, M., Lachenmeier, D.W., 2011. Quantitative determination of acetaldehyde in foods using automated digestion with simulated gastric fluid followed by headspace gas chromatography. J. Autom. Methods Manag. Chem. 2011, 907317. http://dx.doi.org/10.1155/2011/907317.

Väkeväinen, S., Mentula, S., Nuutinen, H., Salmela, K.S., Jousimies-Somer, H., Färkkilä, M., Salaspuro, M., 2002. Ethanol-derived microbial production of carcinogenic acetaldehyde in achlorhydric atrophic gastritis. Scand. J. Gastroenterol. 37, 648–655.

Väkeväinen, S., Tillonen, J., Agarwal, D.P., Srivastava, N., Salaspuro, M., 2000. High salivary acetaldehyde after a moderate dose of alcohol in ALDH2-deficient subjects: strong evidence for the local carcinogenic action of acetaldehyde. Alcohol. Clin. Exp. Res. 24, 873–877.

Väkeväinen, S., Tillonen, J., Blom, M., Jousimies-Somer, H., Salaspuro, M., 2001a. Acetaldehyde production and other ADH-related characteristics of aerobic bacteria isolated from hypochlorhydric human stomach. Alcohol. Clin. Exp. Res. 25, 421–426.

Väkeväinen, S., Tillonen, J., Salaspuro, M., 2001b. 4-Methylpyrazole decreases salivary acetaldehyde levels in ALDH2-deficient subjects but not in subjects with normal ALDH2. Alcohol. Clin. Exp. Res. 25, 829–834.

Wang, H.L., Zhou, P.Y., Liu, P., Zhang, Y., 2014. ALDH2 and ADH1 genetic polymorphisms may contribute to the risk of gastric cancer: a meta-analysis. PloS one 9, e88779.

WHO, 2000. Safety Evaluation of Certain Food Additives and Contaminants. Food Additives series: 44. International programme on chemical safety. World Health Organization, Geneva, Switzerland. http://www.inchem.org/documents/jecfa/jecmono/v44jec08.htm.

Woutersen, R.A., Appelman, L.M., Van Garderen-Hoetmer, A., Feron, V.J., 1986. Inhalation toxicity of acetaldehyde in rats. III. Carcinogenicity study. Toxicology 41, 213–231.

Woutersen, R.A., Feron, V.J., 1987. Inhalation toxicity of acetaldehyde in rats. IV. Progression and regression of nasal lesions after discontinuation of exposure. Toxicology 47, 295–305.

Xie, J., Marano, K.M., Wilson, C.L., Liu, H., Gan, H., Xie, F., Naufal, Z.S., 2012. A probabilistic risk assessment approach used to prioritize chemical constituents in mainstream smoke of cigarettes sold in China. Regul. Toxicol. Pharmacol. 62, 355–362.

Yang, C.X., Matsuo, K., Ito, H., Hirose, K., Wakai, K., Saito, T., Shinoda, M., Hatooka, S., Mizutani, K., Tajima, K., 2005. Esophageal cancer risk by ALDH2 and ADH2 polymorphisms and alcohol consumption: exploration of gene-environment and gene-gene interactions. Asian Pac. J. Cancer Prev. 6, 256–262.

Yang, S.J., Wang, H.Y., Li, X.Q., Du, H.Z., Zheng, C.J., Chen, H.G., Mu, X.Y., Yang, C.X., 2007. Genetic polymorphisms of ADH2 and ALDH2 association with esophageal cancer risk in southwest China. World J. Gastroenterol. 13, 5760–5764.

Yang, S.J., Yokoyama, A., Yokoyama, T., Huang, Y.C., Wu, S.Y., Shao, Y., Niu, J., Wang, J., Liu, Y., Zhou, X.Q., Yang, C.X., 2010. Relationship between genetic polymorphisms of ALDH2 and ADH1B and esophageal cancer risk: a meta-analysis. World J. Gastroenterol. 16, 4210–4220.

Yokoyama, A., Kamada, Y., Imazeki, H., Hayashi, E., Murata, S., Kinoshita, K., Yokoyama, T., Kitagawa, Y., 2016. Effects of ADH1B and ALDH2 genetic polymorphisms on alcohol elimination rates and salivary acetaldehyde levels in intoxicated Japanese alcoholic men. Alcohol. Clin. Exp. Res. 40, 1241–1250.

Yokoyama, A., Kato, H., Yokoyama, T., Igaki, H., Tsujinaka, T., Muto, M., Omori, T., Kumagai, Y., Yokoyama, M., Watanabe, H., 2006a. Esophageal squamous cell carcinoma and aldehyde dehydrogenase-2 genotypes in Japanese females. Alcohol. Clin. Exp. Res. 30, 491–500.

Yokoyama, A., Kato, H., Yokoyama, T., Tsujinaka, T., Muto, M., Omori, T., Haneda, T., Kumagai, Y., Igaki, H., Yokoyama, M., Watanabe, H., Fukuda, H., Yoshimizu, H., 2002. Genetic polymorphisms of alcohol and aldehyde dehydrogenases and glutathione S-transferase M1 and drinking, smoking, and diet in Japanese men with esophageal squamous cell carcinoma. Carcinogenesis 23, 1851–1859.

Yokoyama, A., Mizukami, T., Omori, T., Yokoyama, T., Hirota, T., Matsushita, S., Higuchi, S., Maruyama, K., Ishii, H., Hibi, T., 2006b. Melanosis and squamous cell neoplasms of the upper aerodigestive tract in Japanese alcoholic men. Cancer Sci. 97, 905–911.

Yokoyama, A., Muramatsu, T., Ohmori, T., Yokoyama, T., Okuyama, K., Takahashi, H., Hasegawa, Y., Higuchi, S., Maruyama, K., Shirakura, K., Ishii, H., 1998. Alcohol-related cancers and aldehyde dehydrogenase-2 in Japanese alcoholics. Carcinogenesis 19, 1383–1387.

Yokoyama, A., Muramatsu, T., Omori, T., Yokoyama, T., Matsushita, S., Higuchi, S., Maruyama, K., Ishii, H., 2001. Alcohol and aldehyde dehydrogenase gene polymorphisms and oropharyngolaryngeal, esophageal and stomach cancers in Japanese alcoholics. Carcinogenesis 22, 433–439.

Yokoyama, A., Omori, T., 2001. Alcohol and oropharyngolaryngeal and digestive tract cancer [in Japanese] Nihon Arukoru Yakubutsu Igakkai Zasshi 36, 551–566.

Yokoyama, A., Tsutsumi, E., Imazeki, H., Suwa, Y., Nakamura, C., Mizukami, T., Yokoyama, T., 2008. Salivary acetaldehyde concentration according to alcoholic beverage consumed and aldehyde dehydrogenase-2 genotype. Alcohol. Clin. Exp. Res. 32, 1607–1614.

Zhou, D., Xiao, L., Zhang, Y., Xian, J., Jiang, J., Zong, W., Huang, Z., Yang, Y., 2012. Genetic polymorphisms of ALDH2 and ADH2 are not associated with risk of hepatocellular carcinoma among East Asians. Tumour Biol. 33, 841–846.