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Acetaldehyde as a Food Flavoring Substance: Aspects of Risk Assessment

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The Senate Commission on Food Safety (SKLM) of the German Research Foundation (DFG) has reviewed the currently available data in order to assess the health risks associated with the use of acetaldehyde as a flavoring substance in foods. Acetaldehyde is genotoxic in vitro. Following oral intake of ethanol or inhalation exposure to acetaldehyde, systemic genotoxic effects of acetaldehyde in vivo cannot be ruled out (induction of DNA adducts and micronuclei). At present, the key question of whether acetaldehyde is genotoxic and mutagenic in vivo after oral exposure cannot be answered conclusively. There is also insufficient data on human exposure. Consequently, it is currently not possible to reliably assess the health risk associated with the use of acetaldehyde as a flavoring substance. However, considering the genotoxic potential of acetaldehyde as well as numerous data gaps that need to be filled to allow a comprehensive risk assessment, the SKLM considers that the use of acetaldehyde as a flavoring may pose a safety concern. For reasons of precautionary consumer protection, the SKLM recommends that the scientific base for approval of the intentional addition of acetaldehyde to foods as a flavoring substance should be reassessed.

1. Introduction

Acetaldehyde (ethanal; Chemical Abstract Service [CAS] No. 75-07-0; European Commission [EC] No. 200-836-8) occurs naturally in many foods, including alcoholic beverages as a by-product of alcoholic fermentation. Due to its fruity aroma, acetaldehyde is also used as a flavoring substance. Acetaldehyde is listed in the European register of flavoring substances (Regulation [European Union (EU)] No 872/2012 and [EC] No 1334/2008)[1,2] and has "Generally Recognized as Safe" (GRAS) status in the US.[3] As a flavoring substance, acetaldehyde was evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1997. The Committee at that time concluded that there were no health concerns concerning the use of acetaldehyde as a flavoring

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substance.[4] In contrast, the International Agency for Research on Cancer (IARC) classified acetaldehyde as "possibly carcinogenic to humans (Group 2B)" and, in combination with its oral intake via alcoholic beverages, as "carcinogenic to humans (Group 1)".[5] According to the criteria set out in Regulation (EC) No 1272/2008 (Classification, Labeling and Packaging [CLP] regulation),[6] acetaldehyde is globally harmonized classified as Carc. 1B (Carcinogenicity category 1B: may cause cancer) and Muta. 2 (Germ cell mutagenicity category 2: suspected of causing genetic defects) and thus meets the criteria of a CMR substance (substances that are classified as carcinogenic, mutagenic, and/or toxic for reproduction). In light of the classification of acetaldehyde by IARC, the Committee for Food Additives, Flavorings and Processing Aids of the German Federal Institute for Risk Assessment (BfR-LAV Committee) previously discussed whether the use of acetaldehyde as a flavoring substance is still justifiable.[7] However, based on the data available in 2010, it was not possible to arrive at a conclusive assessment on the safety of acetaldehyde as a flavoring substance.[7] Considering the key role of local acetaldehyde formation in the pathogenesis of alcohol-related oropharyngeal cancer, which is comprehensively summarized in ref. [8], the Senate Commission on Food Safety (SKLM) of the German Research Foundation (DFG) discussed the current data base on acetaldehyde and examined whether new findings have become available that would allow an assessment of the health risk associated with the use of acetaldehyde as a flavoring substance. Thus, with the aim to re-assess the safety of acetaldehyde as a flavoring substance and to identify knowledge gaps that need to be filled to allow a science based risk assessment, the SKLM updated the current state of knowledge on the formation, occurrence, exposure, biotransformation, genotoxicity, and carcinogenicity of acetaldehyde, with a focus on new data available since the BfR-LAV Committee assessment. In particular, the SKLM considered systemic and local effects after oral intake of acetaldehyde, including the possible impact of polymorphisms of xenobiotic metabolizing enzymes, and the relative contribution of acetaldehyde as a flavoring substance to the overall human exposure to acetaldehyde.

2. Previous Scientific Opinions and Legal Regulations

As a flavoring in food, acetaldehyde has GRAS status. This was established by the US Food and Drug Administration (FDA)[9] and by JECFA[4] or, following the concept of the GRAS Notification Program of the FDA, by the Expert Panel of the Flavor and Extract Manufacturers Association (FEMA).[10] Acetaldehyde is also included in the European list of flavorings.[6]

Considering production figures and estimated intake levels in Europe and the US, JECFA identified acetaldehyde as one of the toxicologically important aliphatic substances of a group of 38 flavorings.[4] Based on a tiered safety assessment of flavorings, JECFA assigned acetaldehyde to the class I of chemicals according to Cramer et al.[11] This class includes substances with a simple chemical structure that are rapidly metabolized to harmless, non-toxic substances, indicating low oral toxicity. The acceptable intake of class I substances of 1800 μg per person per day (corresponding to 30 μg kg^{-1} body weight [bw] per day for a person with a bw of 60 kg) was defined by JECFA as the upper limit to be able to assign a flavoring to the class I of chemicals and to be able to designate it as harmless to health.[4] JECFA estimated the intake of acetaldehyde at 9700–11 000 μg per person per day which exceeds the defined upper limit of class I flavorings. However, since acetaldehyde is completely metabolized to endogenous products, and the endogenous level of these metabolites would not give rise to perturbations beyond the physiological range, JECFA considered acetaldehyde to be free of any safety concern as a flavoring agent.[4] Nowadays, acetaldehyde is still assigned to Cramer class I. However, when applying the threshold of toxicological concern (TTC) decision tree, 0.15 μg day^{-1} (or 0.0025 μg kg^{-1} bw per day) would result as TTC level since there is a genotoxicity concern/structural alert (second question in the TTC decision tree as given by the European Food Safety Authority [EFSA][12,13]). Intake values estimated for acetaldehyde exceed the TTC value of 0.15 μg day^{-1} which would lead to the conclusion "Risk assessment requires a non TTC approach/compound-specific toxicity data".

Based on inadequate evidence on the carcinogenicity in humans, but sufficient evidence from animal studies, IARC assigned acetaldehyde to group 2B, i.e., "possibly carcinogenic to humans".[14] Subsequent studies showed mechanistic evidence for a possible involvement of acetaldehyde from ethanol metabolism in tumor development in individuals with aldehyde dehydrogenase (ALDH, EC 1.2.1.3) deficiencies. In such individuals a substantially increased risk of developing alcohol-related cancers, in particular of the esophagus and other regions of the upper aerodigestive tract, was observed. Based on this evidence, acetaldehyde associated with the consumption of alcoholic beverages was classified as "carcinogenic to humans" (Group 1),[15] while acetaldehyde itself is still classified as "possibly carcinogenic to humans" in the IARC group 2B. The advisory group of

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IARC selected acetaldehyde in 2019 with a high priority for re-evaluation.[16]

The Food Safety Commission of Japan (FSCJ) concluded in 2005 that acetaldehyde, inhaled at high concentrations (750–3000 mL m^{-3}), is carcinogenic and exhibits characteristics of genotoxicity. However, data on genotoxicity in cancer-target organs was not available.[17] It was noted that the estimated exposure via the use of acetaldehyde as a flavoring of 9.6–19.2 mg per person per day (0.192–0.384 mg kg^{-1} bw per day; calculated with the Japanese average bw of 50 kg) exceeds the value of 1800 µg day^{-1}, the upper limit for Cramer class I substances.[4] FSCJ further noted that higher amounts of acetaldehyde are ingested through consumption of foods containing acetaldehyde as a natural constituent, such as fruit and alcoholic beverages, than those due to its addition as a flavoring. Assuming a low oral bioavailability of acetaldehyde due to ALDH mediated metabolism in the gastrointestinal mucosa, rapid protein binding, and effective first pass metabolism in the liver, FSCJ concluded that only minute amounts are expected to reach the systemic circulation. It was also pointed out that acetaldehyde was a biogenic substance that had been used as a flavoring in the US and Europe many years without any health hazards being reported. Although intake levels exceeded the acceptable daily intake for class I substances, FSCJ concluded that there were no safety concerns regarding the use of acetaldehyde as a flavoring agent because of its rapid metabolism to innocuous biogenic substances and the notion that presumed intake-related acetaldehyde levels in the blood would not exceed the physiological range.[17]

In 2008, the Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (MAK Commission) of the DFG assigned acetaldehyde to carcinogenicity category 5 [Substances that cause cancer in humans or animals or that are considered to be carcinogenic for humans and for which an MAK (maximum workplace concentration) value can be derived. A genotoxic mode of action is of prime importance but is considered to contribute only very slightly to human cancer risk, provided the MAK and BAT values are observed. The classification and the MAK and BAT (biological tolerance value for occupational exposure) values are supported by information on the mode of action, dose-dependence and toxicokinetic data. (Extracted from MAK and BAT list, available online: https://series.publisso.de/sites/default/files/documents/series/mak/lmbv/Vol2021/Iss2/Doc002/mbwl_2021_eng.pdf)].[18,19] If the MAK value of 50 mL m^{-3} in the air at the workplace (≙91 mg m^{-3}) is taken into account and assuming that the retained acetaldehyde (i.e., the proportion not exhaled again) is completely systemically available, this results in an additional lifetime exposure of 1.0 µmol L^{-1} blood. This compares to an endogenous lifetime exposure to acetaldehyde, for which a blood level of 2.2 ± 1.1 µmol L^{-1} has been assumed[18,20] (cf. Section 3.2). According to MAK, the contribution of occupational acetaldehyde exposure is, even in the worst case, within the range of the standard deviation of the endogenous exposure, so that no significant contribution to systemic cancer risk in humans is to be expected. This assumption is supported by the absence of systemic tumors in animal experiments at acetaldehyde concentrations that cause local tumors.[18,19] At that time, however, it was unclear whether local (geno)toxic effects were to be expected at a concentration of 50 mL m^{-3}, as no studies on DNA cross-linking or DNA adducts in the nasal mucosa were available at this concentration.[18,19]

In a proposal put forward by a working group of the two DFG Senate Commissions, MAK and SKLM, on the risk assessment of genotoxic carcinogens based on their mechanisms of action, acetaldehyde was discussed as an example.[21] In this context, a dose-dependent increase in DNA adduct levels (determined by reduction of the primary Schiff base formed upon reaction of acetaldehyde with amino groups of DNA bases) was observed in various in vitro and in vivo studies (cf. Section 6.3.). The working group pointed out that the biological significance of the identified DNA adducts for the mutagenicity and carcinogenicity of ethanol and acetaldehyde has not yet been fully elucidated.[21] It was noted that the assessment must consider that ethanol and acetaldehyde are also formed endogenously, e.g., during amino acid metabolism.[21] Studies indicated that the background of endogenously formed DNA adducts ranges from 13 to 150 adducts per 10^8 nucleotides in humans, with considerable intra- and interindividual differences.[21]

The use of acetaldehyde as an ingredient in cosmetic products was evaluated by the Scientific Committee on Cosmetic Products and Non-Food Products intended for consumers (SCCNFP) in 2004[22] and by the Scientific Committee on Consumer Safety (SCCS) in 2012.[23] According to SCCNFP, acetaldehyde could be safely used as a fragrance/flavor ingredient at a maximum concentration of 0.0025% in the fragrance compound.[22] In 2004, no further restrictions were recommended. According to estimates by SCCNFP, such exposure does not pose an increased lifetime cancer risk (cf. Section 7.2). This assessment was applied to acetaldehyde present in the fragrance fraction of cosmetic products; other sources of ethanol or acetaldehyde in cosmetic products were not considered.[22,23] In a reassessment, SCCS concluded that acetaldehyde should not be used as an intentionally added ingredient in cosmetic products, except for the use as a fragrance/flavor ingredient at a maximum concentration of 0.0025% in the fragrance compound (corresponding to about 5 ppm in the final product). Furthermore, acetaldehyde should not be intentionally used in mouth washing products.[23]

The BfR-LAV Committee noted in 2010 that the in vivo genotoxicity of acetaldehyde after oral ingestion had not been adequately investigated, but a genotoxic effect could be assumed as there was sufficient evidence for the genotoxicity of acetaldehyde in vitro.[7] After oral intake, acetaldehyde is efficiently metabolized in the intestine and liver, and it appears unlikely that acetaldehyde will become systemically available.[7] It was assumed that the genotoxic potential in vivo is limited to tissues directly exposed such as the upper aerodigestive tract (including mouth and esophagus), in analogy to formaldehyde. The Committee concluded that the safety of acetaldehyde when used as flavoring substance could not be finally evaluated and that additional studies were needed for a final evaluation of acetaldehyde as a flavoring.[7]

3. Occurrence of Acetaldehyde in Foods

3.1. Literature Review

Acetaldehyde occurs ubiquitously in the environment from both natural and anthropogenic sources.[24] Acetaldehyde is listed on

Table 1. Summary of literature data on the occurrence of acetaldehyde in foods[24] (also see full presentation of Volatile Compounds in Food (VCF) database entries in the Supporting Information).

Food	Acetaldehyde content [mg kg^{-1}]
Yoghurt	0.7–76
Cheese	0.1–7.5
Apples	0.2–2.2
Pears	7
Blackberries, raspberries	26
Citrus fruits	1.2–230
Strawberries	2–5
Peas[a)]	0.56–2.4[a)]
Cucumbers	0.2–2
Carrots	0.45–16.9
Maize	0.7–3.5
Tomatoes	0.015–9
Bread	4.2–9.96
Apple juice	0.2–11.8
Passion fruit and peach juice	3.21–4
Orange juice	0.7–192
Tomato juice	2.31–2.44
Beer	0.6–63
Wine and sparkling wine	2.5–493
Spirits	0.5–800
Honey	0.1–1.7
Vinegar	20–1060

[a)] The VCF database reports a maximum value of 400 mg kg^{-1} for peas. However, this appears to be a transmission error, as the original study reported this value for ethanol and not for acetaldehyde (see[27]). Consequently, the correct value of 2.4 mg kg^{-1} acetaldehyde is shown in Table 1.

the positive list of flavorings that may be used in food production in the EU. This list was published by the EC with the Implementing Regulation (EU) No 872/2012[1] adopting the list of flavoring substances provided for by Regulation (EC) No 2232/96, introducing it in Annex I to Regulation (EC) No 1334/2008.[2] The main sources of exposure to acetaldehyde include tobacco smoke, alcoholic and soft drinks, and foods such as coffee, bread, fruit, and yoghurt.[25-27] Acetaldehyde levels in food are summarized in **Table 1** and in the Supporting Information. These tables show very wide ranges and a high variability of the acetaldehyde content of foods. In addition to the typical natural variances, these may occur as a consequence of oxidative and microbial processes during food storage. Acetaldehyde is produced as a by-product of natural fermentation processes (e.g., in dairy products such as yoghurt) and also during microbially induced alcohol formation, e.g., during storage of sugar containing products such as fruits and fruit juices. The levels reported in foods therefore always present a "snapshot". For this reason, exposure considerations available to date have generally been specified for minimum and maximum levels to cover the possible range in the form of best-case and worst-case considerations.

Lifestyle factors such as cigarette smoking and alcohol consumption are considered as major sources of acetaldehyde exposure. Alcoholic beverages can contribute to acetaldehyde exposure in two ways. First, acetaldehyde can be present in alcoholic beverages as a by-product of alcoholic fermentation in substantial amounts[28]; second, acetaldehyde is produced in the human body during ethanol metabolism[25] (cf. Section 5).

Table 2. Acetaldehyde concentrations measured in different food groups in Germany (2000–2020).

Food group	Number of samples	Acetaldehyde concentration [mg L^{-1}]
Non-alcoholic beverages, beverage mixes, beverage powder, also calorie-reduced products[a)]	75; 7 > LOD	0.08–9867
Drinking water, mineral water, table water, spring water, water for domestic use[a)]	857; 252 > LOD	0.00013–966
Seasonings[a)]	43; 27 > LOD	2–148
Flavorings[a)]	8; 5 > LOD	7–2039
Fruit juices, fruit nectars, fruit syrups, dried fruit juices[a)]	14; 0 > LOD	–
Fruit juices[b)]	641; 506 > LOD	1–13

LOD, limit of detection. [a)] Data provided by the German Federal Office of Consumer Protection and Food Safety (BVL) [b)] Data provided by the official laboratories for food and animal health control in the state of Baden-Württemberg, Germany.

3.2. Original Data from Food Control in Germany 2000–2020

Except for alcoholic beverages, standardized methods for determining acetaldehyde levels in food are not available.[27] Therefore, in-house methods based on headspace gas chromatography (HS-GC) or NMR are typically used by the testing facilities. The main analytical challenge is to prevent loss of the volatile analyte during extraction of samples. Extraction of acetaldehyde by steam distillation is generally considered problematic, as considerable losses (of up to 30%) may occur. Additionally, this procedure dilutes the analyte, which may require an additional extraction step. Excessive heating during sample preparation should be avoided, as this not only leads to volatilization but also increases the reactivity of acetaldehyde. A strong heating (e.g., in the headspace oven) can also lead to artefactual formation of acetaldehyde from ethanol, which may explain the very high concentrations of acetaldehyde reported in older studies (see also the comments in Section 4.2 on identical problems in the analysis of biological matrices). For this reason, a simple static head space injection is typically suited. The dynamic variant ("purge and trap") is not possible as acetaldehyde adsorbs insufficiently to the used chromatographic materials (e.g., Tenax[27]). NMR, which does not require heating and calibration, has also proven to be advantageous for analysis of acetaldehyde in liquid samples such as fruit juices and lemonades, with a detection limit of 0.7 mg L^{-1}.[29]

Upon request by the SKLM Working group on Food Constituents, the German Federal Office of Consumer Protection and Food Safety (BVL) provided data on acetaldehyde levels of the most important food groups (**Table 2**). According to BVL, nationally coordinated control programmes did not yet explicitly include analysis of acetaldehyde in food. The available analytical data do not allow to distinguish between acetaldehyde content due to its use as a flavoring or due to its natural occurrence. A

total of 16 052 official food control data sets from 2000 to 2020 were available. Most data related to alcoholic beverages. For exposure assessment of acetaldehyde as a flavoring substance, acetaldehyde levels in other food groups, e.g., non-alcoholic beverages, mineral water, fruit juices, were of particular interest. However, in most cases only small data sets were available, presumably due to the lack of standardized analytical methods in these matrices. Analytical data of 75 samples were available in the food group "Non-alcoholic beverages, beverage mixes, beverage powder, also calorie-reduced products"; in seven samples (9%) the levels of acetaldehyde were above the detection limit (2x flavored mineral water, 3x lemonade, 2x mix of soft drinks). The reported levels covered a wide concentration range from 0.08 to 9867 mg L^{-1} (90th percentile 7451 mg L^{-1}). The two highest levels were reported in flavored mineral water (measured values from 2006): 9867.05 mg L^{-1} (non-packaged) and 5840 mg L^{-1} (bottle made of polyethylene terephthalate [PET]). These two levels are significantly higher than the other values quantified in this food group and also higher than the values quantified for other food groups (except alcoholic beverages), which may indicate analytical errors or errors in the reporting.

High levels of acetaldehyde were also found in the food group "Drinking water, mineral water, table water, spring water, water for domestic use". A total of 857 samples were analyzed; 252 samples were above the detection limit (29%). In 20 samples (2.3%), the concentration of acetaldehyde was above 6 mg L^{-1}, in 17 samples (2%) above 100 mg L^{-1} (95th percentile 222 mg L^{-1}). All 20 samples with high acetaldehyde content were PET bottled waters. The specific migration limit for acetaldehyde from PET bottles is 6 mg L^{-1}. Up to this limit value, adverse health effects were ruled out by the BfR.[30] With few exceptions, the reported levels were generally below the migration limit. To further investigate this issue, the SKLM Working Group on Food Constituents also requested data on acetaldehyde levels from the food industry. The data sets obtained ($n = 285$) on acetaldehyde levels in water from PET bottles indicate that the values are more likely to be in the one to two-digit µg L^{-1} range and in many samples even below the detection limit (<10 µg L^{-1}). Currently, a conclusive assessment of the range of content levels in the food group "Drinking water, mineral water, table water, spring water, water for domestic use" cannot be made. Data from the food industry on other food groups are not yet available.

In the food group "Seasonings", 43 samples were examined, predominantly different types of vinegar. Twenty-seven samples were above the detection limit in a range of 2–148 mg L^{-1}. In the food group "Flavorings", eight samples were analyzed and acetaldehyde content levels of 7–2039 mg L^{-1} were detected (six samples; two samples < LOD). In the food group "Fruit juices, fruit nectars, fruit syrups, dried fruit juices", all 14 samples were below the detection and quantification limit. In a larger-scale investigation of this food group (mainly fruit juices) by the official laboratories for food and animal health control in the state of Baden-Württemberg, Germany, using NMR ($n = 641$, fruit juices), an average acetaldehyde content of 1.66 mg L^{-1} was determined (95th percentile: 4 mg L^{-1}). Acetaldehyde was not detected in 135 samples (21%), and levels of 1–13 mg L^{-1} were measured in 506 samples (79%) (data from the official laboratories for food monitoring and animal health in Baden-Württemberg from 2010 to 2020). The two highest values of 12 and 13 mg L^{-1} were measured in pure apple juice. Fats and oils were also examined by the official laboratories for food and animal health control in Baden-Württemberg. In these samples, only traces of acetaldehyde were detected, with a maximum concentration of 137 µg kg^{-1}.

A statistical evaluation of the BVL data on alcoholic beverages from the years 2000 to 2020 showed that there is no evidence of a trend over time for wine. Beer and brandy showed slight trends, at the border of statistical significance, which are more likely to be accounted for by data quality. In the case of beer, in particular, there are inconsistencies (very high levels in individual years, which may be due to incorrectly transmitted values from the testing facilities to the BVL). In summary, it can be concluded that the content levels in wine, beer and brandy are largely stable or have only changed to a minimal extent over time.

Overall, there is still a lack of representative data on acetaldehyde content levels in the most important food groups, such as non-alcoholic beverages, mineral and table water, fruit juices and nectars, flavorings, seasonings, dairy products (e.g., yoghurt, kefir) and bakery products (e.g., yeast dough pastries).

4. Exposure

4.1. Exposure Estimations Found in the Literature

There are no detailed and up-to-date exposure assessments on acetaldehyde, e.g., based on systematic chemical analyses and consumption studies. It is also not possible to obtain quantitative estimates based on the declaration of foodstuffs, since acetaldehyde is hidden in the ingredient "flavoring" as a group designation and does not require separate labeling. There is thus a large knowledge gap as to how much acetaldehyde is added to foods, making it necessary to systematically analyze the acetaldehyde content of the most important food groups.

Overall, the database is insufficient to perform a reliable assessment of human exposure to acetaldehyde. Based on limited data available in the literature, at best only rough estimates of human exposure to acetaldehyde can be made (**Table 3**). Currently, there are also no biomarkers that would allow differentiation between ethanol exposure and acetaldehyde exposure (cf. Section 4.3).

In 1998, JECFA estimated exposure to acetaldehyde as a flavoring in Europe based on the total acetaldehyde production for flavorings of 300 t in Europe in 1995.[4] Using this approach, an average daily intake of 9.7–11 mg per person per day was estimated (approx. 0.14–0.16 mg kg^{-1} bw per day for a person weighing 70 kg) (Table 3). Fenaroli's Handbook of Flavor Ingredients[31] used industry data to estimate a similar exposure of 0.16 mg kg^{-1} bw per day. The Food Safety Commission of Japan estimated the daily intake of acetaldehyde resulting from its use as a food additive to be 9.6–19.2 mg per person (corresponding to 0.14–0.27 mg kg^{-1} bw for a person weighing 70 kg) and stated that this was probably around 20% of the total intake of acetaldehyde from food.[17] Based on these values, an overall daily acetaldehyde exposure via food (100%) of 0.7–1.35 mg kg^{-1} bw may be estimated. This calculation matches very well with another exposure estimate for food, which was carried out based on an extensive literature analysis (more than 600 data sets) in the Volatile Compounds in Food (VCF) database and test results from food monitoring in Baden-Württemberg.[24] Assuming

Table 3. Overview of estimates of direct daily intake of acetaldehyde via food.

Reference	Food category	Daily intake of acetaldehyde via food	
		[mg absolute]	[mg kg^{-1} body weight][a)]
[203]		40–80 (Worst case 200)	0.57–1.14 (Worst case 2.9)
[4]	Flavored foods	9.7–11	0.14–0.16
[31]	Flavored foods	11.2	0.16
[17]	Flavored foods	9.6 (Europe), 19.2 (US)	0.14 (Europe), 0.27 (US)
[7]	Flavored foods	12.9–15.4	0.18–0.22
[24,27] Based on data from refs. [28, 32, 33]	Food	2–108	0.03–1.54
[204]	Fruit juice, wine	0.6 (Fruit juice)–9 (wine)	0.009 (Fruit juice)–0.13 (wine)

[a)] Calculated for a body weight of 70 kg.

typical consumption levels, a daily acetaldehyde exposure via food of 0.03–1.54 mg kg^{-1} bw was estimated[27,28,32,33] (see Table 3). For children with a bw of 12–15 kg, an intake of 0.27–0.33 mg kg^{-1} bw may be estimated based on consumption of 1 L of apple juice containing 4 mg L^{-1} acetaldehyde (95th percentile, cf. Section 3.2).

In 2010, the BfR-LAV Committee estimated human exposure to acetaldehyde resulting from its use as a flavoring. For this purpose, the "Maximized Survey-Derived Daily Intake" (MSDI) approach was used, which is based on the annual production volume reported by producers and population numbers.[7] Taking into account the population of the 15 EU countries in 2004, the BfR-LAV Committee obtained an MSDI value of 15.4 mg per person per day. Based on the population of 25 EU countries in 2005 (10 countries joined the EU on May 1, 2004), an MSDI value of 12.9 mg per person per day was calculated.

An alternative exposure assessment to the MSDI approach based on the "Theoretical Added Maximum Daily Intake" (TAMDI) or the modified TAMDI (mTAMDI) approach was not possible at that time because the necessary data were not available.[7] The TAMDI approach involves calculation of exposure based on the assumption that the consumer will consume a fixed amount (standard portions) of flavored food and beverages day-to-day and that these items will always contain the specific flavoring at its specified upper use level. The mTAMDI approach involves taking the normal use levels instead of the upper use levels.

Overall, there is a lack of data on acetaldehyde in food, both from natural sources as well as deliberate addition as a flavoring substance, and thus reliable assessment of human dietary exposure to acetaldehyde is currently not possible.

4.2. Endogenous Background

Acetaldehyde is formed endogenously in mammalian intermediary metabolism, e.g., during oxidative decarboxylation of pyruvate or in the course of amino acid metabolism, e.g., threonine aldolase-mediated degradation of threonine.[19] Oxidative decarboxylation of pyruvate has been reported to be catalyzed by the enzyme pyruvate dehydrogenase and results in the production of acetyl-CoA.[34] In the course of the reaction free acetaldehyde is released from the decarboxylating component of this multienzyme complex.[34] Furthermore, acetaldehyde is formed by microorganisms, including those that colonize the human and animal oral cavity and intestine.[18] It was suggested that non-oxidative decarboxylation of pyruvate catalyzed by pyruvate decarboxylase is involved in glucose-derived acetaldehyde formation and that alcohol dehydrogenase (ADH, EC 1.1.1.1) and NADH oxidase are involved in ethanol-derived acetaldehyde formation.[19,34,35] Microbial acetaldehyde production is affected by environmental conditions (pH, oxygen level). Anaerobic commensal microorganisms form acetaldehyde and ethanol from glucose.[35] Under aerobic or microaerobic conditions, for example, near the mucosal surfaces, facultative anaerobic, and aerobic microorganisms form acetaldehyde from endogenous or exogenous ethanol or acetaldehyde and ethanol from glucose (details of proposed metabolic pathways see e.g.[35]). The ability to eliminate locally formed acetaldehyde by microorganisms and the mucosa is limited, leading to accumulation of acetaldehyde in saliva and gastric juice of humans and in colonic contents of experimental animals.[36-38]

In humans, a relatively broad endogenous concentration range of <0.5–3.6 µmol acetaldehyde L^{-1} blood is reported in the literature (3.6 ± 1.0 µmol L^{-1}[39]; >2.5 µmol L^{-1}[40]; 1.25 µmol L^{-1}[41]; < 1 µmol L^{-1}[42]; <0.5 µmol L^{-1}[43]). According to Fukunaga et al., acetaldehyde from exogenous or endogenous sources can be present in the blood in "free, loosely bound and more strongly bound" forms.[20] Depending on sample preparation and analytical method, these forms are detected in different ways, which might partly explain the reported wide concentration range. However, the terms "free," "loosely bound," and "more strongly bound" are not well defined, but examples for forms of bound acetaldehyde will be given below (Section 4.3.3). In particular, earlier data on the endogenous formation of acetaldehyde must be critically questioned against the background of artefact formation of acetaldehyde mainly by oxidation of blood alcohol during sample preparation or in the gas chromatographic system.[43,44] A lifetime endogenous exposure to acetaldehyde of 2.2 ± 1.1 µmol L^{-1} blood was assumed for the assessment by the MAK Commission[18] and the BfR-LAV Committee,[7] resulting from the lowest mean endogenous acetaldehyde concentration in whole blood.[20]

This value derives from the original study by Fukunaga et al. for samples analyzed after perchloric acid (PCA) treatment without separation of the precipitates.[20] In this study, the

endogenous acetaldehyde concentrations in the blood of healthy individuals without alcohol use disorders were investigated by means of headspace GC using different sample preparation methods (no processing, hemolysis, treatment with PCA). The formation of acetaldehyde artefacts during sample preparation or the analytical process was observed in all applied methods, protein precipitation with PCA being the least problematic in this respect. However, according to the authors small artefactual portions due to formation from ethanol during sample preparation cannot be ruled out. In whole blood with PCA precipitates, which also contained the bound acetaldehyde, the acetaldehyde concentration was higher (2.2 ± 1.1 μM), while lower levels were detected in whole blood after centrifugation of the precipitates (0.7 ± 0.5 μM). Low acetaldehyde levels of 0–1 μM were detected in plasma.

A study by Helander et al., in which the distribution of free and bound acetaldehyde in the blood was investigated by HPLC, arrived to a similar conclusion.[41] In plasma, acetaldehyde levels were generally below the detection limit (0.2 μM), whereas significantly higher levels were detected in whole blood (>2.5 μM).[40] The value for whole blood was determined after PCA precipitation without centrifugation of the total sample. About 70% of the total content was identified as bound (i.e., PCA-insoluble) acetaldehyde. The authors concluded that most of the endogenously circulating acetaldehyde in the blood is more or less reversibly associated with erythrocytes/hemoglobin (Hb). This bound fraction also remained at the same level after addition of ethanol to the blood sample before PCA treatment or in blood samples from subjects after ethanol intoxication. Artefactual formation of acetaldehyde was found in both cases only in the soluble fraction of the supernatant. This means that – as already stated by the MAK Commission – the formation of acetaldehyde artefacts interfered only with the determination of the "free" or "total" acetaldehyde, not with the determination of the acetaldehyde that was "bound" in the blood.[18] The physiological relevance and availability of the bound acetaldehyde fraction in peripheral tissues remain unclear.[40] The potential artefactual formation of acetaldehyde during the analyses therefore remains a problem and should be rechecked, e.g., by testing control blood spiked with different ethanol concentrations and treated in the same way as the blood samples.[40,43] Furthermore, some studies have shown that degradation of acetaldehyde, e.g., to acetate, could also occur during sample preparation and analysis.[43]

Some authors assume low to non-detectable concentrations of "free" or "loosely bound" acetaldehyde (<0.5 μmol L^{-1}) in the blood provided that there is no genetic deficiency in ALDH2 activity or chronic heavy alcohol consumption.[43] Based on the extrapolation of respiratory acetaldehyde concentrations, which are easier to determine than acetaldehyde concentrations in blood, Eriksson estimated a very low endogenous blood acetaldehyde concentration in the range of 0.00–0.05 μmol L^{-1}.[43] In fasting subjects without alcohol use disorders, acetaldehyde concentrations between 0.7 and 11 ng L^{-1} (i.e., between 0.016 and 0.25 nmol L^{-1}) were determined in the breath,[45] which correspond to an acetaldehyde concentration in the blood of 0.004–0.05 μM after application of a conversion factor.[43] The only recent study published after the assessment by the MAK Commission reported low endogenous blood acetaldehyde concentrations (<1 μmol L^{-1}[42]). However, no information on the data basis is provided; thus, the study appears inadequate for assessment. There are no other studies available on this topic.

In the opinion of the SKLM, the data available on endogenous acetaldehyde formation is very limited, and no reliable estimate of the endogenous exposure to acetaldehyde can be made at the present time.

Studies on endogenous background levels of DNA adducts specific for acetaldehyde (especially N^2-ethylidene-dG, cf. Section 6.3) support the assumption that endogenous acetaldehyde formation occurs in the organism, which should be taken into account in the assessment. As summarized by MAK and SKLM, the endogenous background of acetaldehyde-derived DNA adducts in humans ranges from 13 to 150 adducts per 10^8 nucleotides, indicating considerable differences among individuals.[21]

4.3. Biomarkers of Exposure

In principle, acetaldehyde in blood or saliva as well as acetaldehyde-related DNA or protein adducts in blood or tissue samples may be used to monitor endogenous or exogenous exposure to acetaldehyde.

4.3.1. Free Acetaldehyde in Blood and Saliva

As mentioned above, it is at present difficult to reliably estimate the endogenous acetaldehyde concentration in blood. Reported levels range between 0.5 and 3.6 μM (≈0.1 mg L^{-1}).[18] In healthy adults, the concentration of acetaldehyde in blood was shown to correlate with ethanol intake and ALDH genotype.[46,47] In ALDH2-deficient subjects (*ALDH2**1/*2, cf. Section 5), maximum acetaldehyde concentrations of 50–60 μM were measured after consumption of 0.25–0.75 g ethanol kg^{-1} bw [Based on a body weight of 70 kg, 0.25–0.75 g ethanol kg^{-1} bw corresponding to an intake of 17.5–52.5 g ethanol per person may be achieved by consumption of 440–1320 mL beer (5% vol), 180–540 mL wine (12% vol), or 55–165 mL spirit (40% vol)]. In subjects with an active genotype (*ALDH2**1/*1), significantly lower maximum blood acetaldehyde concentrations of <10 μM were reached with the same ethanol dose. In a study in premature infants, the concentration of acetaldehyde in blood was determined after administration of two medicinal preparations, iron and furosemide, containing ethanol (0.3%–7.5%). The concentration of acetaldehyde in blood was increased in both treatment groups (iron: median: 0.16 mg L^{-1}, range 0–8.89 mg L^{-1}; furosemide: median: 0.21 mg L^{-1}; range: 0–2.43 mg L^{-1}) as compared to the control group (median 0.01 mg L^{-1}; range: 0–0.14 mg L^{-1}).[48]

Since acetaldehyde is rapidly and efficiently metabolized after oral intake (cf. Section 5: "Metabolism and toxicokinetics"), it seems unlikely that acetaldehyde present in food is systemically bioavailable in the organism for a sufficient period of time. Consequently, determination of acetaldehyde in blood does not seem to be suitable as a biomarker of exposure to acetaldehyde from food.

Determination of acetaldehyde in saliva is used as a marker for local exposure of the oral cavity (and the upper digestive tract) to acetaldehyde. Increased concentrations of acetaldehyde in saliva

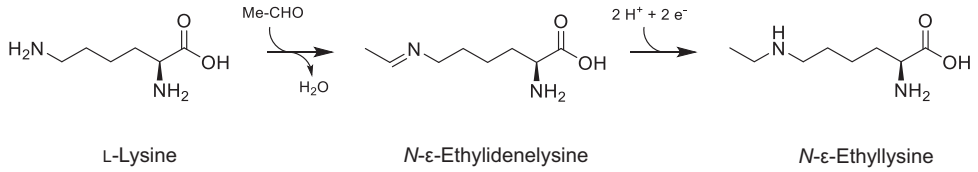

Figure 1. Formation of the Schiff base between the ϵ-amino group of lysine and acetaldehyde and its reduction to *N*-ϵ-ethyllysine.

are observed after ingestion of alcoholic beverages, use of alcohol-containing mouthwashes, and smoking.[49] L-Cysteine can inactivate acetaldehyde and, thus, significantly reduce its concentration in saliva.[50-53]

Whether consumption of foods containing acetaldehyde also leads to an increase in the concentration of acetaldehyde in saliva has been investigated to a very limited extent so far. In human volunteers, a transient increase in salivary acetaldehyde (from <20 µM before administration to approximately 0.06–1 mM after ingestion) was recorded within 30 s of sipping alcoholic beverages with a high acetaldehyde content (470 µM to >15.5 mM).[54] Within a few minutes, the concentration of acetaldehyde in saliva dropped again significantly.[54] Since local formation of acetaldehyde from ethanol is slightly delayed, the authors attributed the rapid increase in salivary acetaldehyde to acetaldehyde present in the beverages.[54] A similar conclusion was reached in a study by Linderborg et al., in which the concentration of acetaldehyde in saliva was determined after sipping calvados (40 vol%) with a high acetaldehyde content (2400 µM) as compared to ethanol (40 vol%).[55] The concentration of acetaldehyde in saliva (up to 258 µM) was significantly higher 30 s after consumption of calvados as compared to acetaldehyde-free 40 vol% ethanol (89 µM), while no significant differences were observed after 2, 5, and 10 min. The area under the curve (AUC) for the concentration of acetaldehyde in saliva within 10 min was not significantly different in the pure ethanol group as compared to the calvados group. The rapid increase and decline in salivary acetaldehyde concentrations observed after sipping alcoholic beverages containing acetaldehyde suggest a transiently increased local acetaldehyde exposure of the oral cavity and the upper digestive tract when consuming foods with a high acetaldehyde content.

Studies investigating the role of the oral cavity microbiome in acetaldehyde production revealed an association between acetaldehyde levels in the oral cavity air and bacterial counts, bacterial diversity, and relative abundance of *Gemella sanguinis*, *Veillonella parvula*, and *Neisseria flavescens*.[56] In esophageal cancer patients, dental hygiene was reported to reduce bacterial counts and acetaldehyde levels in the oral cavity air.[57] Alcohol consumption has also been demonstrated to influence the oral cavity microbiome,[58] and there appears to be an association between alcohol consumption, poor oral cavity health status, and increased acetaldehyde levels.[59]

4.3.2. DNA Adducts

Acetaldehyde-related DNA adducts may serve as biomarkers of exposure. After ingestion of a moderate dose of alcohol, a significant increase in the N^2-ethylidene-dG levels (quantified as its reduced form N^2-ethyl-dG; cf. Section 6.3) of up to 100 times the baseline value was observed in cells of the oral cavity.[60] Studies on the formation of N^2-ethylidene-dG in peripheral leucocytes provided contradictory results. While alcohol ingestion at doses adjusted for each individual to reach a blood alcohol level of 0.03, 0.05% and 0.07% led to a significant increase in the N^2-ethylidene-dG levels in peripheral granulocytes and lymphocytes,[61] no significant effects on the formation and persistence of N^2-ethylidene-dG in leucocyte DNA were observed in volunteers after intake of 150 mL vodka (42% pure ethanol).[62] However, Balbo et al. pointed out the high intraindividual variability,[61] which suggests that there may be further significant sources for this DNA adduct besides alcohol.

In rhesus monkeys that consumed an average of 2.3 ± 0.8 g ethanol per kg bw for 1 year, the content of N^2-ethylidene-dG, detected as N^2-ethyl-dG, was determined in the DNA of the oral mucosa, the esophagus, and the mammary gland.[63] Background adduct levels were comparable in all three tissues. In the alcohol-exposed group, there was a significant increase in N^2-ethylidene-dG only in the oral mucosa. However, a significant correlation was found between N^2-ethylidene-dG levels in oral mucosa and esophageal mucosa. Alcohol exposure had no effect on the levels of N^2-ethylidene-dG in the mammary gland.

In ethanol-treated mice, significantly increased N^2-ethylidene dG levels, detected as N^2-ethyl-dG, were observed in liver DNA, which correlated with the ALDH genotype.[64]

4.3.3. Protein Adducts

Acetaldehyde also reacts with the amino groups of free amino acids, peptides, and proteins. Mainly the *N*-termini and the side chains of lysine, histidine, and cysteine are targets of the reaction. In particular, free cysteine rapidly reacts with acetaldehyde, giving rise to 2-methylthiazolidine-4-carboxylic acid. Although occurrence of 2-methylthiazolidine-4-carboxylic acid in blood can be attributed to alcohol consumption, its value as a marker for alcohol intake was questioned due to its low stability.[65]

Acetaldehyde first binds to the ϵ-amino group of lysine to form a Schiff base (*N*-ϵ-ethylidenelysine), which can be reduced to *N*-ϵ-ethyllysine (NEL) in vivo (**Figure 1**).[66,67] At the *N*-termini of peptides resembling the *N*-terminal region of the Hb chains, the formation of a cyclic imidazolidinone was suggested[68] but later attributed to artificial formation during tryptic digestion.[69]

Both the formation of the Schiff base and NEL have been assessed in human proteins. Alcohol consumption leads to an increase in acetaldehyde-Hb adducts, which decreases during the first 5 days after exposure, making those adducts potential biomarkers for short-term ethanol consumption.[69] Similar to Hb_{A1C} as a marker for the mean blood glucose concentration in the recent months, acetaldehyde-Hb adducts were proposed as

markers for alcohol consumption in the preceding months.[70] In isolated plasma proteins of subjects suffering from alcohol use disorder (daily consumption of alcohol, 152 ± 49 g), the concentration of NEL was significantly higher than in completely abstinent control volunteers (1.14 ± 0.38 NEL modifications per 1000 lysine vs. 0.26 ± 0.07 NEL modifications per 1000 lysine residues, each $n = 10$). None of the abstinent persons showed a higher lysine-to-NEL conversion than any of the persons with alcohol use disorder.[67]

Having been hampered by the application of unspecific and insensitive methods,[71] research regarding the individual structures formed from acetaldehyde at reactive sites on proteins and their role as biomarkers of exposure to acetaldehyde and alcohol is largely lacking.

4.3.4. Conclusion on Biomarkers of Exposure

In summary, information on potential biomarkers of acetaldehyde exposure are almost exclusively based on the formation of acetaldehyde from ethanol. There is a lack of controlled studies on biomarkers of exposure to acetaldehyde from food to be able to distinguish the contribution of acetaldehyde intake via food from endogenous formation and exposure to acetaldehyde from alcohol. Moeller et al. exposed thymidine kinase (TK) 6 cells to isotopically labeled [$^{13}C_2$] acetaldehyde and then investigated the concentration–response relationship for N^2-ethylidene-dG as a biomarker of exposure and for cytotoxicity and the formation of micronuclei as effect biomarkers over a wide dose range (50 nM–2 mM).[72] While the endogenous adduct levels remained almost constant, a clear increase in the N^2-ethylidene-dG levels was observed at a [$^{13}C_2$] acetaldehyde concentration of ≥1 μM upwards. The exogenous adduct levels reached the endogenous levels of N^2-ethylidene-dG only at concentrations of acetaldehyde ≥50 μM. Statistically significant effects on micronucleus formation were found at acetaldehyde concentrations ≥1000 μM. The above mentioned studies indicate a non-linear concentration–response for DNA adduct formation and challenge linear extrapolation to low exogenous acetaldehyde exposures for risk assessment. In vivo studies confirming these observations are not available up to now.

5. Metabolism and Toxicokinetics

Generally, acetaldehyde can be converted to ethanol via the ADH enzyme system and to acetic acid through aldehyde dehydrogenase 2 (ALDH2) (**Figure 2**). The reaction catalyzed by ADH is reversible. The cosubstrate for the conversion of acetaldehyde to ethanol is the reduced co-enzyme nicotinamide adenine dinucleotide (NADH/H$^+$), while the cosubstrate for the reverse reaction is the oxidized form of nicotinamide adenine dinucleotide (NAD$^+$).[73] The ADH-mediated ethanol oxidation rate is regulated by the enzyme activity but also by the mitochondrial reoxidation of NADH/H$^+$ to NAD$^+$.[74,75] Accordingly, ADH-mediated conversion of acetaldehyde to ethanol can only proceed when NADH/H$^+$ is available, which may be relevant under certain conditions (e.g., during prolonged fasting). Fasting conditions are considered to slow down the rate of ethanol oxidation[76] (as cited by[75]), most likely due to limitations in the mitochondrial reoxidation of NADH/H$^+$[77] (as cited by[75]).

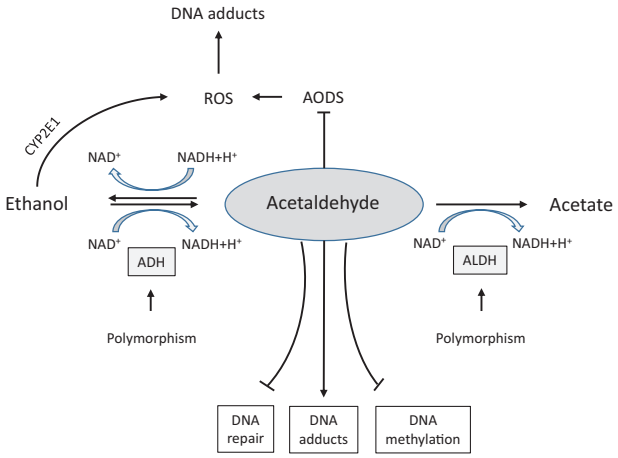

Figure 2. Metabolism of acetaldehyde (modified from refs. [73, 78]). ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; AODS, antioxidative defense system; CYP2E1, cytochrome P450 2E1; NAD$^+$, oxidized form of the co-enzyme nicotinamide adenine nucleotide; NADH/H$^+$, reduced form of the co-enzyme nicotinamide adenine dinucleotide; ROS, reactive oxygen species.

Seven isoenzymes of human ADH have been identified and ADH activity can be detected in almost all tissues.[78] The isoenzymes ADH1B and ADH1C are polymorphically expressed and the genes encode enzymes that can produce different amounts of acetaldehyde.[78] There are two alleles for ADH1B, *ADH1B*1* (less active ADH1B) and *ADH1B*2* (active ADH1B) and correspondingly three genotypes: *ADH1B*1/*1*, less active slow metabolizing ADH1B, *ADH1B*1/*2* and *ADH1B*2/*2*, active ADH1B[1,79] (cf. Section 7.3.2).

The enzyme mainly responsible for the degradation of acetaldehyde is ALDH2. ALDH2 metabolizes acetaldehyde to acetate, which can be transported out of the cell through a carrier or converted into acetyl coenzyme A (acetyl-CoA).[73] The co-substrate for the reaction is NAD$^+$. The reaction is irreversible, because ALDH is located intramitochondrially and acetyl-CoA enters different physiological pathways, including energy metabolism pathways such as the tricarboxylic acid (TCA) cycle or the oxidative phosphorylation (OXPHOS) pathway (summarized by[73]). ALDH2 is encoded by the *ALDH2* gene, for which two different alleles, *ALDH2*1* and *ALDH2*2*, are known.[78] The *ALDH2*2* allele differs from the normal *ALDH2*1* allele by a nucleotide substitution (G > A) in the *ALDH2* gene, which results in the ALDH enzyme being largely inactive.[78] The phenotypic loss of ALDH2 activity is found in both heterozygous (*ALDH2*1/*2*) and homozygous (*ALDH2*2/*2*) genotypes.[79] Three ALDH2 genotypes are classified: *ALDH2*1/*1*, active (100% activity) ALDH2; *ALDH2*1/*2*, inactive (<10% activity) ALDH2; and *ALDH2*2/*2*, inactive (0% activity) ALDH2.[79]

Toxicokinetic data show that acetaldehyde is systemically available after inhalation and oral uptake and is usually rapidly and efficiently metabolized.[79,80] A detailed discussion of the available data is presented in refs. [19, 80].

In rats ($n = 3$) exposed to acetaldehyde by inhalation (1–20 mM, about 44–882 mg L^{-1} = 24 000–480 000 mL m^{-3}) for 1 h, the concentration of acetaldehyde in blood immediately after the end of exposure was 1200 µM and rapidly declined with a half-life of 3.1 min.[19,81] Compared to blood, the initial concentrations observed in the liver were significantly lower (55 µmol kg^{-1}), presumably due to the rapid metabolism of acetaldehyde, as suggested by the European Chemicals Agency (ECHA).[19,80,81]

After intragastric administration of acetaldehyde to rats, a similar half-life of about 3 min was estimated in portal vein blood.[82] In this study, rats ($n = 4$) were administered a single intragastric dose of 9 mg kg^{-1} bw or an intracolonic dose of 5 mg kg^{-1} bw.[19,82] Highest acetaldehyde levels were detected in the portal blood 5 min after intragastric (235 µM) and intracolonic administration (344 µM). The acetaldehyde concentration in the portal blood was about 17 times higher than that in the femoral vein after liver passage,[82] indicating extensive hepatic first pass metabolism.[19]

A rapid first pass effect was also observed in dogs.[19] In male dogs ($n = 6$) given a single acetaldehyde dose of 600 mg kg^{-1} bw by gavage, acetaldehyde plasma levels peaked at 15 min and rapidly declined thereafter. Oral administration of acetaldehyde at this dose level caused vomiting, resulting in substantial interindividual differences in acetaldehyde plasma levels (<50 µM to 13.6 mM). While this limits the validity of the study to link acetaldehyde exposure to plasma levels, it appears that even high oral doses of acetaldehyde may only produce a transient increase in plasma levels due to rapid first pass metabolism.[19,83]

Limited experimental data suggest that acetaldehyde administered by intraperitoneal (i.p.) injection may be partially transferred from maternal to fetal blood.[80] In pregnant CD-1 mice (number of animals not specified) given a single i.p. dose of acetaldehyde of 200 mg kg^{-1} bw on day 10 of gestation, the maximum concentrations of acetaldehyde (77.3 ± 10.3 µg g^{-1}; mean value ± SD) were detected in embryo tissue 5 min after injection. The corresponding concentrations in the maternal blood were around 185 ± 13.6 µg mL^{-1} (4.2 mM). The concentrations decreased rapidly and were below the detection limit 2 h after the treatment.[19,84]

In humans, a significant uptake (45%–70%) of inhaled acetaldehyde (exposure to 0.4–0.6 µg mL^{-1} [220–330 mL m^{-3} inhaled air]) via the respiratory tract was observed after a very short exposure duration of 45–75 s.[19,85] There are no data available for the half-life of acetaldehyde in humans.[19]

For local (saliva) and systemic (blood) acetaldehyde concentrations after intake of acetaldehyde-rich alcoholic beverages and/or ethanol see Section 4.3.1 ("Free acetaldehyde in blood and saliva").

6. Genotoxicity and Mutagenicity

6.1. Genotoxicity In Vitro

Results from genotoxicity tests have been comprehensively described and referenced in the reports by the DFG MAK Commission, CERI and by ECHA, and are briefly summarized here[19,80,86] (see also **Table 4**). In the Ames test with various different *Salmonella typhimurium* strains, acetaldehyde did not show mutagenic effects with or without metabolic activation. In further bacterial mutagenicity tests with *Escherichia coli*, inconsistent results were obtained. In mammalian cells, sister-chromatid exchanges in human lymphocytes and Chinese hamster ovary (CHO) cells (starting at acetaldehyde concentrations of 40 and 30 µM, respectively) and DNA strand breaks in human lymphocytes as well as human gastric mucosa and colon cells (comet assay, starting at acetaldehyde concentrations of 1.56 and 3 mM, respectively) were detected without metabolic activation. DNA strand breaks were not detected by alkaline elution, but DNA–DNA cross-links were observed starting at 1.5 mM acetaldehyde. In numerous chromosome aberration tests, a clastogenic effect of acetaldehyde was confirmed (in rat fibroblasts with 0.1 mM, in human lymphocytes and human fibroblasts starting at ≈0.4 mM, and in CHO cells starting at 1.1 mM acetaldehyde), whereby it was not possible to rule out entirely aneugenic effects. In the hypoxanthine-guanine phosphoribosyltransferase (HPRT) assay with human lymphocytes and human fibroblasts, as well as in the TK assay with L5178Y mouse lymphoma cells, mutations induced by acetaldehyde were detected without metabolic activation (starting at 1.2 and 4 mM, respectively). Furthermore, acetaldehyde induced micronuclei in human lymphocytes and rat fibroblasts (starting at 0.6 and 0.5 mM, respectively). Beginning at a low millimolar concentration range, acetaldehyde induced DNA–protein and DNA–DNA cross-links (in human and CHO cells, among others), though less potently than formaldehyde at similar concentration ranges. Finally, acetaldehyde induced the formation of several DNA adducts (especially N^2-ethylidene-dG and methyl-γ-hydroxy-N^2-propano-deoxyguanosine [CrPdG], see **Figure 3** and Section 6.3).

6.2. Genotoxicity In Vivo

After a single i.p. administration, acetaldehyde was found to be genotoxic as evidenced by a significant increase in sister-chromatid exchanges in the bone marrow of mice and hamsters (at ≥0.5 mg acetaldehyde kg^{-1} bw), and positive findings in the mouse[147,148] and rat micronucleus test (bone marrow and peripheral blood; at ≥190 mg acetaldehyde kg^{-1} bw, 24 h) (**Table 5**). Further information on toxicity/mortality was not provided in some of these studies and the purity of the test substance was sometimes not reported or low (89.4%).[147-149] Acetaldehyde was negative in the micronucleus test with mouse spermatids (125–500 mg kg^{-1} bw i.p.).[150]

Inhalative (125 and 500 ppm) and oral acetaldehyde administration (100 mg kg^{-1} bw per day) for 2 weeks significantly induced micronuclei in reticulocytes and gene mutations in *ALDH2* knock-out mice, but not in wild-type mice.[151]

Weak but reproducible somatic mutations and recombinations were observed in *Drosophila melanogaster* exposed to acetaldehyde (0.18 mM in the diet).[152] Acetaldehyde-induced sex-linked recessive lethal (SLRL) mutations were also observed in *D. melanogaster* after single injection, but not after administration via feed (Table 5), which may be due to the reaction of acetaldehyde with feed components or due to rapid detoxification following oral exposure.[153]

After inhalation, acetaldehyde caused DNA–protein cross-links in the respiratory epithelium of rats already after 6 h (starting at 1000 mL m^{-3}), in the olfactory epithelium after 5 days (1000

Table 4. Genotoxicity studies of acetaldehyde in vitro (adapted from refs. [19, 80, 86] and updated).

Endpoint	Test system	Concentration/dose	Results		Comment	Reference
			With metabolic activation	Without metabolic activation		
Bacteria/Fungi						
Reverse mutation	*S. typhimurium* TA98, TA 100, TA 1535, TA 1537	5000 µg mL^{-1}	Negative	Negative	Diluted in 95% ethanol. Also 10 000 µg per plate were tested (negative) but cytotoxicity was observed at this concentration	[87]
	S. typhimurium TA 100, TA 1535, TA 1537	0.5% (in air)	Negative	Negative		[88]
	S. typhimurium TA 98	1% (in air)	Negative	Negative		
	E. coli WP2 *uvr*A	0.5% (in air)	Negative	Negative		
	S. typhimurium TA 102, TA 104 + metabolic activation	up to 1 mg/plate (2515 µg mL^{-1})	Negative	n.a.	Highest non-toxic dose at >114 µmol (5 mg)	[89]
	S. typhimurium TA 1535, TA 1538	10 µL/ plate (7830 µg mL^{-1})	Negative	n.a.		[90]
	S. typhimurium TA 100, TA 102, TA 104	0.1–1.0 µg/plate	Negative	Negative		[91]
	S. typhimurium TA 1535	10 µg/plate	Negative	Negative	Cytotoxicity not specified	[92]
	S. typhimurium TA 100	Not specified	Negative	n.a.	Cytotoxicity not specified	[92]
	S. typhimurium TA 1535	0.01–2.5 µmol/plate (0.44–110 µg/plate)	Negative	Negative	Cytotoxicity not specified	[93]
	S. typhimurium TA 100	not specified	n.d.	Negative	Cytotoxicity not specified	[94]
	S. typhimurium TA 102	0–3 µg/plate	n.d.	Negative	Cytotoxicity> 5000 µg/plate; metabolic activation not explicitly specified	[95]
	E. coli WP2 *uvr*A	880 µM (39 µg mL^{-1})	Positive	n.a.	Lethal for 31% of the culture after 30 min	[96]
	E. coli WP2 *uvr*A	20–10 000 µM (0.88–441 µg mL^{-1})	Negative	n.a.	Cytotoxicity not specified	[97]
	E. coli WP2 *uvr*A trp^{-}	0.1% (780 µg mL^{-1})	Positive	n.a.	Cytotoxicity not specified	[98]
Chromosome aberration (nondisjunction)	*Aspergillus nidulans*	200 µg mL^{-1}	Positive (chromosomal malsegregation)	n.a.	Percentage of survivors decreased from 100 µg mL^{-1} onwards	[99]
Differential killing	*E. coli* K-12 343/636 and 343/591	Up to 370 mM (16.3 mg mL^{-1})	Negative	n.a.	No surviving colony at 123 mM (5.4 mg mL^{-1})	[100]
	E. coli pol A^{+}/pol A^{-}	10 µg/plate (7830)	Positive (weak)	n.a.	Cytotoxicity not specified	[90]
Forward mutation	Yeast	23 400 µg mL^{-1}	Positive (weak)	n.a.		[101]
Mutation signature	Yeast (derivatives of CG379)	0.2%, 24 h	Positive (G-to-T transversions at ssDNA)	n.a.	Evaporation was avoided	[102]
	Yeast (ySR127 strain)	25, 50, 75, and 100 mM, 3 h	Positive (predominantly C/G-to-A/T, C/G-to-T/A, and T/A-to-C/G substitutions)	n.a.	Evaporation was avoided; an excess of deletion events longer than 4 bases was observed	[103]

(*Continued*)

Table 4. (Continued)

Endpoint	Test system	Concentration/dose	Results		Comment	Reference
			With metabolic activation	Without metabolic activation		
Mammalian cells						
Gene mutation	Mouse lymphoma L5178Y cells *tk* gene locus	4–8 mM (176–352 μg mL^{-1})	Positive	n.a.	Dose-dependent, 4 h treatment; toxic at highest concentration, colony size not specified	[104]
	Human TK6 cells; mutants determined at the *hprt* and *tk* gene locus	0.001, 0.005, 0.01, 0.05, 0.25, 0.5, 1.0, 2, and 4 mM for 24 h	Negative at *hprt* locus; Positive at *tk* locus (dose-dependent increase at ≥ 0.05 mM)	n.a.		[105]
	Human lymphocytes *hprt* gene locus	0.6–2.4 mM (27–106 μg mL^{-1}, 24 h) 0.2–0.6 mM (8.8–27 μg mL^{-1}, 48 h)	Positive at ≥ 1.2 mM (24 h) and at ≥ 0.2 mM (48 h)	n.a.	Relative survivors: 0.6% at 3.6 mM (158 μg mL^{-1}) (24 h)	[106]
	Human lymphocytes *hprt* gene locus	1–12 mM (44–528 μg mL^{-1})	Positive at ≥ 5 mM	n.a.	50% survivors at 5 mM (220 μg mL^{-1})	[107]
	Human fibroblast cell line with shuttle vector plasmid containing *supF* suppressor tRNA gene	0, 0.25, 0.5, 1.0, and 2.0 M	Positive (after replication)	n.a.	Mutations were specified as tandem based substitutions (GG-to-TT); single-strand and double strand DNA mutations increased with increasing dose	[108]
	NER-proficient and NER-deficient xeroderma pigmentosum group A (XPA) cells	0.25, 0.5, and 1 M, 1 h	Positive (GG-to-TT transversions)	n.a.	Transversions (partly) repaired by NER	[109]
Gene mutation spectrum	Human lymphocytes, hprt gene locus	2.4 mM for 22 h	Positive (mutation spectrum of acetaldehyde induced mutations was different from control)	n.a.	Cloning efficiency was 50% at 1.2 mM compared to control	[110]
Chromosomal aberration	SD rat primary skin fibroblasts	0.01–1 mM (0.44–44.4 μg mL^{-1})	Positive at ≥ 0.1 mM	n.a.	Cytotoxicity not specified	[111]
	Human lymphocytes	20–40 μg mL^{-1}	Positive	n.a.	Dose-dependent	[112]
	Human lymphocytes	7.8 μg mL^{-1}	Positive (weak)	n.a.		[113]
	Human lymphocytes	90–1080 μM (4–48 μg mL^{-1})	Positive at ≥ 0.72 mM	n.a.	Cytotoxicity at > 1080 μM (48 μg mL^{-1})	[114]
	Human (Fanconi's anemia) lymphocytes, 1 person	0.18–0.36 mM (0.001; 0.002 % v/v; 7.8–15 μg mL^{-1})	Positive	n.a.	Cytotoxicity not specified	[115]
	Human lymphocytes, 3 persons	0.18–0.36 mM (0.001; 0.002 % v/v; 7.8–15 μg mL^{-1})	Negative	n.a.	Cytotoxicity not specified	[115]
	Human lymphocytes	0.02 mM (0.8 μg mL^{-1}), 2x/day, 4 days	Positive	n.a.	Cytotoxicity not specified	[116]

(*Continued*)

Table 4. (Continued)

Endpoint	Test system	Concentration/dose	Results		Comment	Reference
			With metabolic activation	Without metabolic activation		
	Human lymphocytes	0.1–20 mM (4–880 μg mL^{-1}), 2x/day, 5 days	Positive (metabolic activation not specified)	n.a.	Cytotoxicity not specified	[116]
	Human lymphocytes, human fibroblasts	40–800 μM (1.8–35 μg mL^{-1})	Positive at ≥ 0.4 mM	n.a.	Cytotoxicity at ≥ 800 μM (35 μg mL^{-1})	[117]
	Embryo cells, Chinese hamster	0.35–1.1 mM (16–48 μg mL^{-1}; 0.002%–0.006%)	Positive at 1.1 mM, aneuploidy (usually hypodiploidy, but also hyperdiploidy)	n.a.	Highest concentration: mitosis index decreased	[118]
	CHO cells	2–114 mM (88–5000 μg mL^{-1})	Positive at ≥2 mM	n.a.	Cytotoxicity not specified	[116]
	different DNA-repair deficient CHO cells	0.3, 0.6, 1.0, 1.8, 2.5 and 3.6 mM for 2 h; 100 metaphases scored/group	Positive	n.a.	Concentration-related increase; no positive control	[119]
Chromosome aberration (Aneuploid)	Chinese hamster embryonic diploid fibroblasts	15.6 μg mL^{-1}	Positive	n.a.		[118]
Micronucleus test	SD rat primary skin fibroblasts	0.1–10 mM (4.4–440 μg mL^{-1})	Positive at ≥0.5 mM	n.a.	12 h treatment	[111]
	Human lymphocytes	0.2–2 mM (8.8–88 μg mL^{-1})	Positive at ≥ 0.8 mM	n.a.	Cytotoxicity at > 1 mM	[120]
	Human lymphocytes	0.6–1 mM (26–44 μg mL^{-1})	Positive at 0.6 mM and above	n.a.	No cytotoxicity; additional test (FISH) showed aneugenic effects possible	[121]
	Human lymphoblastoid TK6 cells	0.005, 0.01, 0.05, 0.25, 0.5, 1.0, and 2 mM; plates sealed due to volatility substances	Positive at ≥0.25 mM	n.a.	Dose-related increase	[105]
	Human lymphoblastoid TK6 cells	0.005–4 mM; 8 different concentrations	Positive (0.25, 0.5, and 1.0 mM)	n.a.	Negative and positive controls included; only data analyzed when cytotoxicity was below 55%	[80] (study report 2010)
	HepG2 and Hep3B cells	0.9 and 9 mM for 24 h	Positive	n.a.	Per experimental point 1500 cells evaluated; concentration-related increase, no data on cytotoxicity	[122]
	MCL-5 human lymphoblastoid cell line	0–2% v/v; six different concentrations for 22 h	Positive at ≥ 0.4%, dose-dependent increase; Negative: aneuploidy	n.a.	>4000 cells per dose examined	[123]
	V79 Chinese hamster cells	0.5–10 mM	Positive	n.a.	Dose-dependent increase; no positive control	[124]
Sister chromatid exchange	Human lymphocytes	16 and 78 μg mL^{-1} (0.002 and 0.01% v/v)	Positive	n.a.	Cytotoxicity not specified	[125]

(*Continued*)

Table 4. (Continued)

Endpoint	Test system	Concentration/dose	Results		Comment	Reference
			With metabolic activation	Without metabolic activation		
	Human lymphocytes	4–8 μg mL^{-1} (0.0005–0.001% v/v)	Positive at ≥ 4 μg mL^{-1}	n.a.	> 0.001% (8 μg mL^{-1}) cytotoxicity	[126]
	Human lymphocytes	0.063–2 mM (2.8–88 μg mL^{-1})	Positive at ≥0.25 mM	n.a.	Proliferation index decreased at 0.5 mM and above	[127]
	Human lymphocytes	0.0005%–0.002% (4–16 μg mL^{-1})	Positive at ≥0.001% (8 μg mL^{-1}; approx. 0.182 mM)	n.a.	Cytotoxicity not specified	[113, 128]
	Human lymphocytes	90–1080 μM (4–48 μg mL^{-1}), 90 h	Positive at ≥720 μM (29 μg mL^{-1})	n.a.	Cytotoxicity at > 1080 μM (48 μg mL^{-1})	[114]
	Human lymphocytes	1–100 μM (0.04–4.4 μg mL^{-1}), 70 h	Positive at 100 μM (4.4 μg mL^{-1})	n.a.	No cytotoxicity in the tested concentration range	[129]
	Human lymphocytes	100–400 μM (4.4–18 μg mL^{-1})	Positive at ≥0.1 mM (4.4 μg mL^{-1})	n.a.	No cytotoxicity in the tested concentration range	[130]
	Human lymphocytes	250–500 μM (11–22 μg mL^{-1})	Positive at ≥0.25 mM (11 μg mL^{-1})	n.a.	No cytotoxicity in the tested concentration range	[131]
	Human lymphocytes	20, 40 μg mL^{-1} (0.45, 0.91 mM)	Positive at 20 μg mL^{-1}	n.a.		[112]
	Human lymphocytes	0.1–0.3 mM (4.4–13 μg mL^{-1}), 70 h; 0.6–2.4 mM (26–106 μg mL^{-1}), 1 h	Positive at ≥0.1 mM (4.4 μg mL^{-1}) at 70 h; Positive at ≥0.6 mM (26 μg mL^{-1}) at 1 h	n.a.	No drastic decrease in proliferation indices in tested concentration range	[132]
	Human lymphocytes	0.1–2.4 mM (4.4–106 μg mL^{-1})	Positive at ≥0.1 mM (4.4 μg mL^{-1})	n.a.	Cytotoxicity not specified	[133]
	Human lymphocytes	20–400 μM (0.9–18 μg mL^{-1})	Positive at ≥40 μ (1.8 μg mL^{-1})	n.a.	400 μM (18 μg mL^{-1}) cytotoxic	[96]
	CHO cells	20–400 μM (0.9–18 μg mL^{-1})	Positive at ≥40 μM (1.8 μg mL^{-1})	n.a.	880 μM (39 μg mL^{-1}) 100% of cells dead	[96]
	CHO cells	30–300 μM (1.3–13 μg mL^{-1})	Positive at ≥30 μM (1.3 μg mL^{-1})	n.a.	Concentration-dependent increase; cytotoxicity not specified	[134]
	CHO cells	8–80 μg mL^{-1} (0.18–1.8 mM) without metabolic activation; 0.8–40 μg mL^{-1} (0.02–0.9 mM) with metabolic activation	Positive at ≥40 μg mL^{-1} (0.9 mM)	Positive at ≥ 40 μg mL^{-1} (0.9 mM)	No cytotoxicity in the tested concentration range	[135]
	CHO cells	0.00025%–0.0015% (2–12 μg mL^{-1})	Positive at ≥0.00025% (2 μg mL^{-1})	n.a.	Cytotoxicity not specified	[136]
	CHO cells	0.0005%–0.004% (3.9–31 μg mL^{-1})	Positive at ≥0.0005% (3.9 μg mL^{-1})	n.a.	Cytotoxicity at ≥ 0.003% (23 μg mL^{-1})	[137]
	CHO cells	0.2–5 mM	Positive	n.a.	Concentration-dependent increase; no positive control	[124]
	Different DNA-repair deficient CHO cells	0.3, 0.6, 1.0, 1.8, 2.5 and 3.6 mM for 2 h	Positive	n.a.	250 metaphases scored/group; no positive control	[119]

(*Continued*)

Table 4. (Continued)

Endpoint	Test system	Concentration/dose	Results		Comment	Reference
			With metabolic activation	Without metabolic activation		
Comet assay	Human lymphocytes	1.56–100 mM (69–4400 μg mL^{-1})	Positive: single strand breaks at 1.56 mM; double strand breaks at 100 mM	n.a.	100 mM (4400 μg mL^{-1}) (1 h): <20% surviving cells	[138]
	Human lymphocytes	3–200 mM (132–8800 μg mL^{-1})	Positive at ≥ 3 mM (132 μg mL^{-1})	n.a.	>70% survivors at 200 mM (8800 μg mL^{-1})	[139]
	Mucosa cells, stomach, human	3–200 mM (132–8800 μg mL^{-1})	Positive at ≥3 mM (132 μg mL^{-1})	n.a.	>70% survivors at 200 mM (8800 μg mL^{-1})	[139]
	Mucosa cells, colon, human	3–200 mM (132–8800 μg mL^{-1})	Positive at ≥3 mM (132 μg mL^{-1})	n.a.	>70% survivors at 200 mM (8800 μg mL^{-1})	[139]
Alkaline elution	Human lymphocytes	10, 20 mM (440, 880 μg mL^{-1})	Negative: strand breaks; Positive: DNA crosslinks at ≥10 mM (440 μg mL^{-1})	n.a.	No other details	[140]
	Bronchial epithelial cells, human	1 mM (44 μg mL^{-1}), 6 h	Negative	n.a.	ID_{50} (concentration which decreases the growth rate by 50%): 30 mM (1320 μg mL^{-1})	[141]
	Bronchial epithelial cells, human	1–100 mM (44–4400 μg mL^{-1})	Negative: single strand breaks; Positive: DNA crosslinks at ≥ 3 mM (132 μg mL^{-1})	n.a.	ID_{50} (concentrations which lead to 50% inhibition): 25 mM (1100 μg mL^{-1}), colony-forming efficiency; 125 mM (5500 μg mL^{-1}), viability	[107]
	CHO cells	0.5–4.5 mM (22–198 μg mL^{-1})	Negative: single strand breaks; Positive: DNA crosslinks at ≥1.5 mM (66 μg mL^{-1})	n.a.	Viability (% of controls) over the entire concentration range 100%	[142]
	Rat hepatocytes	0.03–3 mM (1.3–132 μg mL^{-1})	Negative	n.a.	Viability (% of controls) > 58%	[143]
Alkaline unwinding	Mouse lymphoma cells	1.5–44 mM (66–1900 μg mL^{-1})	Negative	n.a.	84% not viable at 44 mM (1900 μg mL^{-1})	[144]
Cell transformation	C3H/10T1/2 cells, mouse	10–100 μg mL^{-1}	Negative; Positive at ≥ 10 μg mL^{-1} in the presence of 0.25 μg mL^{-1} TPA	n.a.	LC50: 25 μg mL^{-1}	[145]
	Cell line, HRRT, kidney, rat	up to 3 mM (132 μg mL^{-1})	Negative; Positive at 3 mM with 0.1 μg mL^{-1} TPA	n.a.	No cytotoxicity up to 3 mM (132 μg mL^{-1})	[146]

bw, body weight; CHO cells, Chinese hamster ovary cells; n.a., not assessed; NER, nucleotide excision repair; TPA, 12-*O*-tetradecanoyl phorbol-13-acetate.

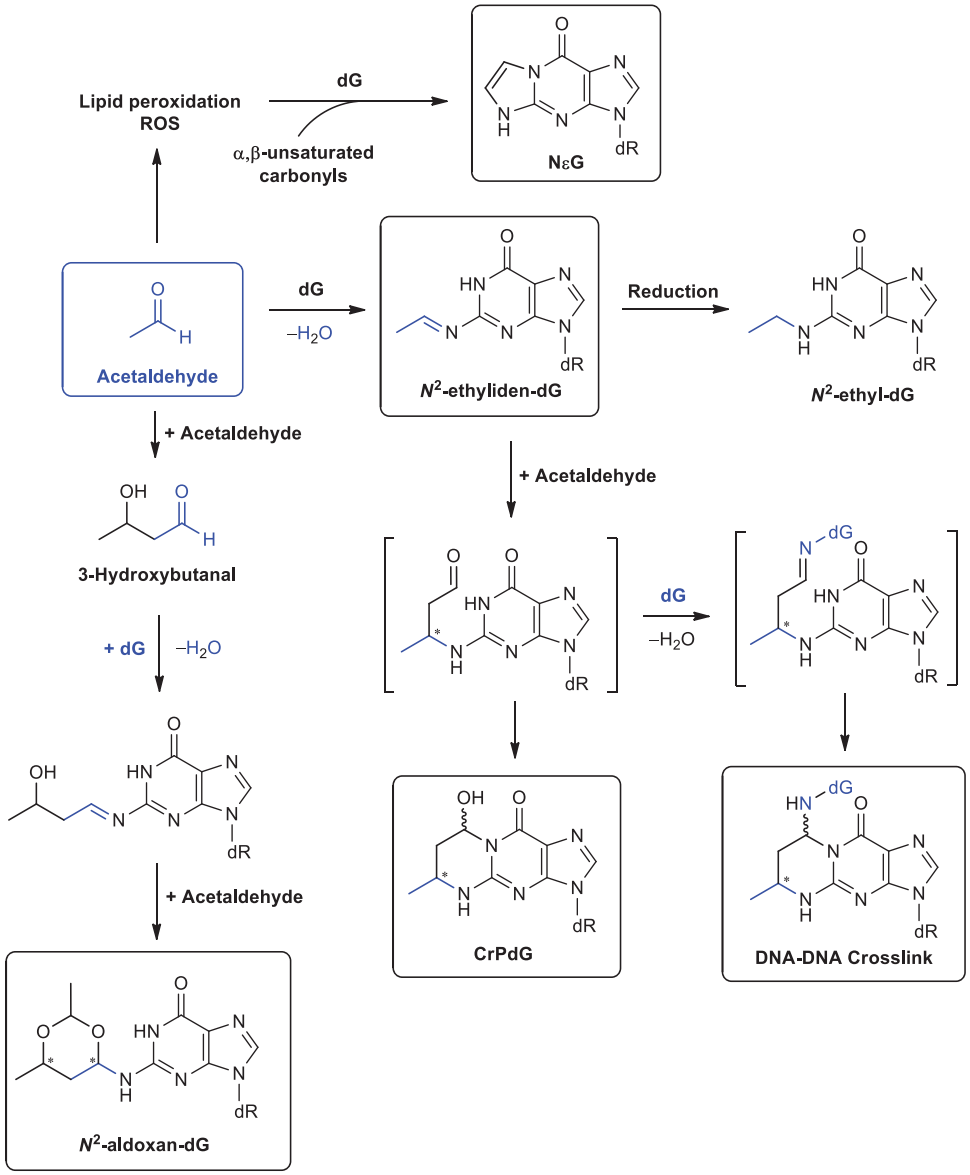

Figure 3. Acetaldehyde mediated DNA adduct formation in vitro (modified from refs. [79, 162]). CrPdG, methyl-γ-hydroxy-N^2-propano-dG; dG, 2′-deoxyguanosine; NεG, 1,N^2-etheno-dG.

mL m^{-3}, 6 h per day)[154] (Table 5). However, the finding in the respiratory epithelium was not confirmed in a similar study (rat, 1500 mL m^{-3}, 6 h), although it should be noted that a different sample processing/determination method was used in the latter case.[155]

6.3. DNA Adducts

Acetaldehyde forms DNA adducts with 2′-deoxyguanosine (dG) in vitro. The main adduct is N^2-ethylidene-dG (Figure 3): in this case acetaldehyde forms a Schiff base with the exocyclic amino group in the N^2-position of dG after elimination of water (condensation). This DNA adduct is unstable as a nucleoside, but relatively stable in DNA with a half-life of around 24 h,[161] which complicates its analysis.[79,162] Through chemical reduction (e.g., with $NaBH_3CN$), N^2-ethylidene-dG can be converted into the stable and thus analytically more easily detectable N^2-ethyl-dG, a reaction that probably also occurs to a limited extent in vivo, e.g., with glutathione or vitamin C.[163] To the best of our knowledge, the biological activity of the primary Schiff base adducts formed, their kinetics, stability under physiological conditions and persistence have not been thoroughly investigated. However, since the basal endogenous levels of N^2-ethyl-dG are low,[164] this nucleoside can be used as a biomarker for acetaldehyde-related DNA damage. Alcohol and tobacco consumption locally increased the level of this DNA adduct in vivo (in the oral mucosa, stomach, and esophagus) and systemically (in the liver) depending on the ALDH genotype in humans as well as in animal experiments.[60,61,63,64,161,165-167] Systemically

Table 5. Genotoxicity studies of acetaldehyde in vivo (adapted from refs. [19, 80, 86] and updated).

Endpoint	Species/organism/strain	Concentration/dose	Result	Comment	Reference
Soma cells					
SMART	*Drosophila melanogaster*, mwh-flr3 CROSS, 160 wings evaluated	0.18 mM in diet, 48 h	Positive (weak, but reproducible)		[152]
SCE, bone marrow	Mouse, CBA, 1 male	0.5,1 mL 10^{-4}% v/v, = ≈0.125 and 0.25 mg kg^{-1} bw; single i.p. treatment	Positive after 28 h	Non-standardized test; toxicity not specified	[115]
	Hamster, Chinese, 6–7 animals	0.01, 0.1, 0.5 mg kg^{-1} bw, single i.p. treatment	Positive at 0.5 mg kg^{-1} bw	Non-standardized test; mortality at 0.6 mg kg^{-1} bw and above	[156]
	Male mouse (NIH)	0.4, 4.0, 40, and 400 mg kg^{-1} bw, single i.p. injection	Positive at 40 and 400 mg kg^{-1} bw	Mitotic index and average generation time did not differ from control; number of mice per group not given; no positive control	[157]
Gene mutation and micronuclei formation	Wildtype and knock-out mice with inactive *ALDH2* gene; micronuclei determined in reticulocytes; mutations were determined by T-cell receptor (TCR) gene mutation assay	125 and 500 ppm (inhalation), continuously for 2 weeks; negative control was inhalation of clean air	Micronuclei: Positive in knock-out mice Negative in wild-type mice Mutation (TCR mutant frequency): Positive in knock-out mice; Negative in wild-type mice		[151]
	Wildtype and knock-out mice with inactive ALDH2 gene; micronuclei determined in reticulocytes; mutations were determined by T-cell receptor (TCR) gene mutation assay	100 mg kg^{-1} bw (oral), once a day for 2 weeks; 5–10 animals/group	Micronuclei: Positive in knock-out mice; Negative in wild-type mice Mutation (TCR mutant frequency): Positive in knock-out mice; Negative in wild-type mice		[151]
Micronucleus test, bone marrow, peripheral blood	Mouse, CD-1, 6 males/dose	0, 95, 190, 380 mg kg^{-1} bw, 24 h, single i.p. treatment (in sodium chloride solution, purity: 89.4%)	Positive at ≥ 190 mg kg^{-1} bw	LD_{50} 470 mg kg^{-1} bw	[147]
	Mouse, CD-1, 5 males	0, 100, 200, 300, 400 mg kg^{-1} bw, 24 h single i.p. treatment (in sodium chloride solution, purity: > 99.5%)	Positive at ≥ 200 mg kg^{-1} bw	LD_{50} 338 mg kg^{-1} bw, dose-related increase	[147]
	SD Rat, at least 4 animals/group	250 mg kg^{-1} bw (i.p.), two treatments with 24 h intervals, sampling 24 h after last dosage, vehicle: saline 0.9%	Positive	Purity of test item not specified	[149]

(*Continued*)

Table 5. (Continued)

Endpoint	Species/organism/strain	Concentration/dose	Result	Comment	Reference
Micronucleus test, peripheral blood	Mouse, C57BL/6J, 4 males, control animals: 2 males	6, 12 mg kg^{-1} bw, 5 days, i.p. (in sodium chloride solution), purity: not specified	Positive	Toxicity not specified	[148]
	Male Han rats, 5 animals/group	125 or 250 mg kg^{-1} bw, single i.p. injection, blood samples collected after 0, 24, 48, and 72 h	Positive at 24 and 48 h, no data at 72 h due to toxicity	Dose-related increase	[158]
Chromosomal aberration	Rat embryo cells	7800 mg kg^{-1} bw, single administration through the amnion (on gestation day 13)	Positive	Administration through the amnion (on gestation day 13)	[159]
DNA-protein cross-links	Fischer 344 rat nasal mucosa	100, 300, 1000, and 3000 mL m^{-3}, Inhalation exposure, 6 h per day, 5 days	Positive at ≥ 1000 mL m^{-3} (respiratory epithelium, after 6 h, and after 5 days with 6 h exposure per day) Negative (olfactory epithelium, 6 h, up to highest concentration) Positive at 1000 mL m^{-3} (olfactory epithelium, 5 days, 6 h per day)		[154]
	Fischer 344 rat nasal mucosa	1500 mL m^{-3}, nose-only inhalation	Negative (olfactory epithelium)	Increase in DNA-protein cross-links after incubation with 500 mM acetaldehyde. Different extraction method used than in the study of Lam et al. (KCl-SDS precipitation instead of chloroform-isoamyl alcohol-phenol extraction)	[155]
Germ cells					
SLRL (Basctechnique)	*Drosophila melanogaster*, > 20 males/brood (total of 3), 1 male mated with 3 females	25 µL mL^{-1} in 10% ethanol, 3 days, in diet	Negative	Mortality 3%	[153]
SLRL (Basctechnique)	*Drosophila melanogaster*, >20 males/brood (total of 3), 1 male mated with 3 females	22.5 µL mL^{-1} in 10% ethanol, single injection	Positive	Mortality 29%	[153]
Micronucleus test, early spermatids	Mouse, hybrid (C57BL/6J×C3H/He), 4 males, control animals: 7 males	0, 125, 250, 500 mg kg^{-1} bw, single i.p. treatment (in sodium chloride solution), 13 days	Negative	Only one specific stage investigated, not the entire spermatogenesis	[150]
Sister chromatid exchange	Mouse spermatogonial cells; 4–5 animals/dose	0.4, 4.0, 40, and 400 mg kg^{-1} bw; single i.p. injection; cells were isolated 53 h after injection	Positive (all doses applied, $p < 0.05$); no clear exposure-response relationship	Authors did test for intoxication; concentrations used were considered non-toxic/-lethal	[160]

bw, body weight; SLRL, sex-linked recessive lethal mutations; SMART, somatic mutagenicity and recombination test.

(in lymphocytes and granulocytes), increased levels of N^2-ethyl-dG (presumably formed from N^2-ethylidene-dG after reduction with $NaBH_4$) were observed in 24 examined patients with alcohol use disorders (^{32}P post-labeling; structural verification by co-elution with an N^2-ethyl-dG standard).[168] On average, 3.4 ± 3.8 adducts/10^7 nucleotides were detected in granulocytes and 2.1 ± 0.8 adducts/10^7 nucleotides in lymphocytes. In 12 volunteers with no or only moderate alcohol consumption, the adduct levels were below the limit of detection of 0.5 adducts/10^7 nucleotides. It was possible to rule out smoking, a source of acetaldehyde exposure, as a cause for the increased adduct levels found in the patient group.[168] In a study of male non-smokers, no statistically significant exposure-dependent changes in N^2-ethyl-dG adduct levels were detected in the DNA of peripheral white blood cells (basal level: 34.6 ± 21.9 adducts/10^8 nucleosides) after alcohol consumption,[62] while in another study, the levels after ethanol exposure were increased systemically (in lymphocytes and granulocytes) and locally 5- and 100-fold, respectively, when compared to the unexposed control group.[60,61] N^2-ethyl-dG strongly blocks polymerase α but not polymerase η.[169] Upon inhalation, acetaldehyde resulted in a marked increase in N^2-ethyl-dG adduct levels locally in the nasal pharynx, lung, and skin of ALDH-deficient mice if compared to wild-type animals, whereas lower adduct levels were found in the livers of ALDH-deficient animals when compared to the wild type.[170] Although DNA repair does not seem to be a major factor in vivo, the mutagenic potential of N^2-ethylidene-dG in vivo is still unclear.[163] Because of the instability of N^2-ethylidene-dG, N^2-ethyl-dG has been used as a model adduct and thus there is no direct information as to the mutagenic potential of N^2-ethylidene-dG, or its susceptibility and/or stability to DNA repair.[163]

By the reaction of two molecules of acetaldehyde with dG or one molecule of acetaldehyde with N^2-ethylidene-dG, the cyclic or open-chained α-*S*-and α-*R*-configured CrPdG (Figure 3) can be formed in vitro. Intramolecular and intermolecular DNA–DNA or DNA–protein cross-links may form after further formation of a Schiff base with another molecule of dG or by reaction with proteins. CrPdG is responsible for the genotoxic, mutagenic, and carcinogenic effect of crotonaldehyde, from which this DNA adduct is also directly formed by reaction with dG.[171] For this reason, some authors consider CrPdG – in contrast to N^2-ethylidene-dG – to be a toxicologically relevant (genotoxic/mutagenic) DNA adduct and biomarker of acetaldehyde. However, since two molecules of acetaldehyde are required for the reaction, the formation and, therefore, the toxicological significance of this DNA adduct has been questioned in view of the low acetaldehyde levels detected in humans compared to the high doses of acetaldehyde in animal studies.[19,163] Human studies in smokers were not able to determine whether this adduct was formed from acetaldehyde or crotonaldehyde, as exposure to both substances occurs through tobacco smoke. Elevated levels of CrPdG and N^2-ethyl-dG were measured in ALDH-2-deficient smokers with alcohol use disorder.[172] In contrast, no accumulation of CrPdG adducts was observed in ethanol-exposed ALDH2 knock-out mice – while the N^2-ethylidene-dG/N^2-ethyl-dG levels strongly increased in the liver and stomach depending on the genotype.[64,167] These data either suggest that CrPdG was not formed in the mouse after ethanol exposure or that it was efficiently repaired, e.g., by nucleotide excision repair.[163] Moreover, CrPdG (in contrast to N^2-ethyl-dG) was not detected in rhesus monkeys exposed to ethanol.[63] In a recent study, CrPdG was detected in lung and brain tissues of rats after inhalation of $^{13}C_2$-isotope-labeled acetaldehyde (10 ppbv [Parts per billion by volume of acetaldehyde]; 50 days). This suggests systemic availability and systemic DNA reactivity of acetaldehyde even at low inhalation doses such as those found in ambient air of large cities.[173,174] Mutagenicity studies in vitro showed that both N^2-ethyl-dG and CrPdG have mutagenic potential ([21] and references therein[175,176]). However, the potential role of both adducts in the induction of mutagenicity in vivo or even carcinogenicity remains unclear at the present time.

Furthermore, N^2-aldoxane-DNA adducts (see Figure 3) which are built up from three molecules of acetaldehyde, were detected in vitro. In this case, 3-hydroxybutanal, the product of the aldol addition of two molecules acetaldehyde, reacts with the exocyclic amine function of dG, thereby giving rise to an imine, which then cyclizes with another molecule of acetaldehyde to form N^2-aldoxane-dG. Furthermore, acetaldehyde (155 µM) led to increased levels of another DNA adduct, 1,N^2-etheno-dG (NϵG) in human cells.[177] This DNA adduct probably does not originate from a direct reaction of acetaldehyde with dG, but is an indirect product resulting from acetaldehyde-mediated ROS formation and subsequent induction of lipid peroxidation with further α,β-unsaturated aldehydes (Figure 3).[79,177] The formation of further acetaldehyde-derived DNA adducts after ethanol exposure has been reported.[178]

6.4. Conclusions on Genotoxicity of Acetaldehyde

The currently available data on the genotoxicity and mutagenicity of acetaldehyde indicate that acetaldehyde is genotoxic in vitro. The lowest concentrations at which positive results were obtained in in vitro studies were in the range of 30–45 µM[96,134,136] and, thus, in the same range as maximum acetaldehyde concentrations reached in plasma of ALDH2-deficient subjects (ALDH2*1/*2) after consumption of 0.25–0.75 g ethanol kg^{-1} bw (50–60 µM). In subjects with an active ALDH2 genotype (*ALDH2*1/*1) exposed to the same dose of ethanol, however, maximum blood acetaldehyde concentrations did not exceed 10 µM. While this supports the notion that acetaldehyde associated with alcohol consumption may produce systemic genotoxic effects, particularly in ALDH2 deficient subjects, there are however no data on acetaldehyde plasma concentrations in humans after oral exposure to acetaldehyde via food. Considering its rapid metabolism and short half-life (approximately 3 min in rats), it appears unlikely that acetaldehyde concentrations at which mutagenic/genotoxic effects were observed in vitro may be achieved and sustained in blood following dietary intake.

On the other hand, the few studies investigating acetaldehyde in saliva after sipping alcoholic beverages with high acetaldehyde content suggest high local acetaldehyde concentrations (258 µM[55]; >1 mM[54]) immediately following intake, with a rapid return to baseline within minutes (see also 4.3.1 "Free acetaldehyde in blood and saliva"). It is possible that transiently increased local acetaldehyde exposure of the oral cavity and the upper digestive tract may also occur when consuming other foods with an equally high acetaldehyde content, but evidence for this hypothe-

Table 6. Carcinogenicity studies after oral intake of acetaldehyde.

Species/number of animals	Study design	Treatment duration	Effects	Reference
Sprague-Dawley rat (♀, ♂); $n = 50$/group	0, 50, 250, 500, 1500, 2500 mg L^{-1} in drinking water (ad libitum; dose in mg kg^{-1} bw not given; about 0, 2.5, 12.5, 25, 75, 125 mg kg^{-1} bw per day assuming a daily intake of 20 mL drinking water and a bw of 400 g)	Lifelong: administration from week 6 after birth up to death (last animal died in week 161)	• No difference between control and exposed animals on daily feed consumption, bw, and survival • No treatment-related non-neoplastic pathological changes were detected by gross inspection or histopathological examination • Tumor incidences were not significantly increased in the Zymbal gland, auditory canal, nasal and oral cavities, stomach, intestine, lungs, and mammary gland • Osteosarcomas significantly increased in ♂ in the highest dose group • Number of total malignant tumors per 100 animals was significantly increased at 50 mg L^{-1} (only ♀) and 2500 mg L^{-1} (♂ u. ♀) • Limitations: lack of comprehensive statistical analysis, lack of dose response, limited examination of non-neoplastic end-points, possibly infection with *Mycoplasma pulmonis*	[179]
Wistar rat, 10/group (only ♂)	0, 120 mM in drinking water (ad libitum; corresponding to 324 mg kg^{-1} bw per day)	8 months	• No difference between control and exposed animals on daily fluid and feed consumption, bw, and survival. • Immuno-histochemistry and histopathological examination of the tongue, epiglottis, and forestomach: no tumors • Hyperplasia and increased proliferation indices of the basal layers of the squamous epithelia (tongue, epiglottis, forestomach) • Limitations: small number of animals, only a single dose group, short exposure duration, limited examination of tissues	[182]

sis is not available up to now. Furthermore, higher acetaldehyde concentrations in saliva might be reached in ALDH-2 deficient individuals (see 7.3.2 "Influence of polymorphisms of xenobiotic metabolizing enzymes"). While it is unclear if short-term exposure of directly exposed tissues to such peak concentrations will result in genotoxic and carcinogenic effects, such effects cannot be ruled out at the present time.

Although in vivo studies demonstrate systemic genotoxic effects of acetaldehyde predominantly after i.p. administration, it is not possible to relate the applied doses to oral exposures. The only available in vivo study investigating acetaldehyde genotoxicity after oral exposure in rodents reported gene mutations and micronuclei in ALDH2 knock-out but not in wild-type mice following a 2-week treatment at 100 mg kg^{-1} bw acetaldehyde, i.e., a dose about two orders of magnitude higher than the estimated human acetaldehyde exposure via food (0.03–1.54 mg kg^{-1} bw, cf. Section 4 "Exposure").

There is evidence for increased levels of acetaldehyde-derived DNA adducts following ethanol intake in humans (oral cavity, peripheral blood) and animals (upper gastrointestinal tract, liver) and following inhalation exposure to acetaldehyde in animals (lung, brain). However, the effects strongly depended on the ALDH2 genotype, further highlighting the particular susceptibility of ALDH2-deficient animals/individuals to acetaldehyde genotoxicity and the efficient detoxification of acetaldehyde by ALDH2. However, there are no data on acetaldehyde-derived DNA adducts following oral exposure to acetaldehyde. Based on the available data, it is therefore still not possible to conclude on acetaldehyde genotoxicity and mutagenicity in vivo after oral exposure in situations without concomitant ethanol exposure.

7. Carcinogenicity

7.1. Oral Exposure

Data on the carcinogenicity of acetaldehyde after oral intake are limited (**Table 6**). Up to now, there is only one long-term animal study available in the scientific literature, in which acetaldehyde was applied orally.[179] Acetaldehyde was administered life-long (from week 6 after birth up to death) in the drinking water (0, 50, 250, 500, 1500, or 2500 mg L^{-1}) to Sprague-Dawley rats (50 animals/sex/group). A limited number of tumors in several organs, including the oral cavity, were reported. Only one carcinoma (2%) was detected in a medium dose group (500 mg L^{-1}) in the pharynx and larynx (only female). Moreover, two carcinomas (4%, female and male) occurred in the highest dose group (2500 mg L^{-1}; ≈125 mg acetaldehyde kg^{-1} bw per day) in the oral cavity (lips and tongue), but the effects were not dose-dependent

and showed no statistical significance (carcinomas: control: 1 [female]; 50 mg L^{-1}: 0; 250 mg L^{-1}: 1 [male]; 500 mg L^{-1}: 1 [male]; 1500 mg L^{-1}: 0). Furthermore, this study was not compliant with the OECD guidelines.[7] The BfR-LAV Committee concluded that this study could not be used for assessing cancer risk and does not provide reliable evidence of a carcinogenic effect of acetaldehyde after oral exposure.[7,180] Other organizations such as the MAK Commission, the ECHA and the Health Council of the Netherlands also classified the study as inadequate for the purposes of a quantitative risk assessment.[18,19,80,181] The SKLM agrees with these assessments. Up to now, there are no new long term oral animal studies available.

In a study with a limited number of Wistar rats (10 animals/group), 120 mM acetaldehyde was administered in the drinking water for 8 months.[182] No tumorigenic lesions but increased proliferation indices and hyperplasia of the basal layers of the squamous epithelia (tongue, epiglottis, and forestomach) were observed. However, this study is considered inadequate to assess acetaldehyde carcinogenicity. The number of animals per group was small and the duration of treatment too short for a carcinogenicity study. Moreover, only a single dose group was studied, precluding characterization of the dose–response relationship. Additionally, only a few organs were examined.

Acetaldehyde has repeatedly been evaluated by IARC as a single substance as well as linked to alcohol consumption[14,15,75,183,184] (cf. Section 2). In animal experiments, a link between acetaldehyde exposure due to oral ethanol intake and cancer was demonstrated in various organ systems and species.[75] Epidemiological and mechanistic studies based on ALDH2-deficiency provide further evidence for a causal and dose-dependent role of local acetaldehyde in the carcinogenesis of oral cavity, pharynx, and esophagus linked to ethanol consumption. Overall, IARC assigned acetaldehyde associated with consumption of alcoholic beverages to the carcinogenicity group 1 [Sufficient evidence in humans for the carcinogenicity of alcohol consumption and for the carcinogenicity of acetaldehyde associated with the consumption of alcoholic beverages].[75]

There are no epidemiological studies investigating the association between acetaldehyde alone and cancer risk.[80]

7.2. Inhalation Exposure

Upon inhalation exposure, acetaldehyde induced the formation of adenocarcinomas and squamous cell carcinomas of the olfactory and respiratory epithelium in the rat and nose and larynx in the hamster (**Table 7**).[7,185-189]

The SCCNFP and the SCCS derived a quantitative risk assessment from the inhalation study by Woutersen et al.,[185] with lifetime cancer risks calculated based on a T25 of 121 mg kg^{-1} bw per day.[22,23] A corresponding human dose descriptor (HT25) of 36.7 mg kg^{-1} bw per day was calculated. Assuming a maximum consumer exposure to acetaldehyde in fragranced cosmetic products of 0.1 μg kg^{-1} bw per day, a lifetime cancer risk of 7×10^{-7} was estimated. The SCCNFP concluded that this exposure does not represent any cancer risk.[22]

7.3. Consideration of Local Effects Following Oral Exposure and Polymorphisms

7.3.1. Local Effects Following Oral Exposure

Similar to formaldehyde, chronic local tissue damage due to the cytotoxicity of acetaldehyde is considered to play a key role in tumor development in the nasal mucosa.[18,19] Assuming complete systemic bioavailability of retained acetaldehyde, MAK estimated a lifetime additional internal exposure of 1.0 μmol acetaldehyde L^{-1} blood when the MAK value of 50 mL m^{-3}, which is based on the avoidance of irritant effects of acetaldehyde after inhalation exposures, is observed. According to MAK, the contribution made by occupational exposure to acetaldehyde is, even in the worst case, within the range of the standard deviation of its endogenous levels in blood (2.2 ± 1.1 μmol L^{-1} [18,19]; so that no notable contribution to the systemic cancer risk in humans is to be expected.

However, consideration of systemic availability after oral exposure of acetaldehyde may not be sufficient to rule out any damage to health,[54,190] as this does not consider the possible local effects of acetaldehyde in the gastrointestinal tract. Local formation of acetaldehyde from ethanol occurs in the upper digestive tract, both in the mucosa cells and by the microflora of the upper digestive tract. Enzyme activities of these tissues, characterized, e.g., by a very low level of ALDH2 activity, differ from those of other organ systems, resulting in accumulation of acetaldehyde in saliva and gastric juice.[38,191-195] For example, after administration of a standard dose of ethanol (0.5–0.6 g kg^{-1} bw; observation period 30–180 min), salivary acetaldehyde concentrations (≈24–53 μM) were approx. ten-fold higher than the blood acetaldehyde concentrations in ALDH2-active individuals.[196] However, there are no studies on acetaldehyde alone that specifically addressed local effects in the upper digestive tract.

7.3.2. Influence of Polymorphisms of Xenobiotic Metabolizing Enzymes

Polymorphisms in the two relevant enzyme systems ADH1B and ALDH2 can lead to a considerable impairment of acetaldehyde metabolism.

Compared to individuals with active ALDH2 there is an approximately two-fold increase in the concentration of acetaldehyde in saliva and an approximately five-fold increase in gastric juice in ALDH2-deficient individuals after ethanol ingestion.[191,196] After administration of a standard dose of ethanol (0.5–0.6 g kg^{-1} bw; observation period 30–180 min), substantially higher acetaldehyde concentrations were measured in the blood (up to 25 μM), saliva (up to 76 μM) and gastric juice (up to 47 μM) of ALDH2-deficient individuals than in the blood, saliva and gastric juice of ALDH2-proficient individuals.[191,196] In vivo studies in ALDH2 knock-out mice also showed an increased occurrence of genotoxic effects (induction of gene mutations and micronuclei) in reticulocytes after oral intake/inhalation of acetaldehyde.[80] These observations indicate that the occurrence of genotoxic effects correlates with the ALDH genotype.

Table 7. Carcinogenicity studies after inhalation exposure to acetaldehyde.

Species/number of animals	Study design	Treatment duration	Effects	Reference
Wistar rat (♀, ♂); $n = 105$/group	0, 750, 1500, or 3000/1000 mL m^{-3}, for 6 h per day, 5 days per week; due to toxicity the highest concentration was gradually decreased from day 142 to 1000 mL m^{-3} on day 360	28 months ($n = 55$/group)	Tumors in the respiratory tract: • Significantly increased incidences of adenocarcinomas in the nose (♂, ♀) at ≥750 mL m^{-3} • Significantly increased incidences of squamous cell carcinomas in the nose (♂: 1500 and 3000/1000, ♀: 3000/1000)	[185]
		Interim investigations: 13/26 weeks ($n = 5$); 52 weeks ($n = 10$)	Nasal lesions in exposed animals	[187]
		52 weeks treatment, followed by a recovery period of up to 12 months ($n = 30$)	Nasal lesions in exposed animals (week 52): • Degeneration of the olfactory epithelium at all concentrations • Hyper- and metaplastic changes of the respiratory epithelium at the top concentrations • Slight to moderate rhinitis in several rats of the top-concentration group After recovery period (26/52 weeks): • Regeneration of the olfactory epithelium at low- and mid-exposure concentration, not at the highest exposure concentration • Olfactory epithelium was frequently replaced by respiratory epithelium • 26 weeks: hyper- and metaplastic changes of the respiratory epithelium frequently progressed to squamous cell carcinomas • 52 weeks: degeneration of the respiratory epithelium was less pronounced; papillomatous hyperplasias were not found, hyper- and metaplastic changes were still present; squamous cell carcinomas were observed in a few animals Comment: only the respiratory tract (nose, larynx, trachea with main bronchi and lungs) was examined for the presence of abnormalities	[186]
Syrian golden hamster (♂), $n = 35$/group	0 or 1500 mL m^{-3}; 7 h per day; 5 days per week	52 weeks (animals killed after 78 weeks)	• No substance-related tumors were found • Epithelial hyperplasia, metaplasia and inflammatory changes in the nasal cavity and trachea were observed in exposed animals	[188]
Syrian golden hamster (♀, ♂); $n = 30$/group (control groups $n = 18$)	0, 2500 mL m^{-3}, 7 h per day, 5 days per week; due to growth inhibition/mortality the concentration was gradually decreased after 9 weeks to 1650 mL m^{-3} by week 45 and thereafter	52 weeks (observation period up to week 81)	Tumors in the respiratory tract: • Pathological changes were observed in the nose, larynx, and trachea: rhinitis, hyperplasia, and metaplasia of the nasal, laryngeal and tracheal epithelium, nasal, and laryngeal carcinomas • Incidences of carcinomas of the larynx were significantly increased • Other increases in the tumor incidences in exposed animals were not statistically significant as compared to controls	[189]

It is estimated that about 40% of the Japanese, Korean, or Chinese population carry the *ALDH2**2 allele in the heterozygous form. The resulting reduced enzyme activity leads to increased acetaldehyde concentrations in the blood, even when comparatively small amounts of alcohol are consumed. A significantly increased risk of upper digestive tract tumors and colorectal tumors has been observed in relation to chronic alcohol consumption in the Asian population, particularly in individuals with decreased ALDH2 activity.[181,197,198]

The *ADH1B*2* allele encodes an enzyme that is approximately 40 times more active than the enzyme encoded by the *ADH1B*1* allele.[78] Thus, acetaldehyde accumulates following alcohol consumption in individuals who carry the highly active *ADH1B*2* allele because ethanol is rapidly converted to acetaldehyde. The *ADH1B*2* allele occurs more frequently in Asian populations but is rarely found in Caucasians.[78] In Caucasians, the *ADH1C**1 genotype encodes an ADH that is approximately 2.5 times more active than the enzyme encoded by the *ADH1C**2 allele.[78] The allele frequency of *ADH1C**1 and the rate of homozygosity are associated with an increased risk of tumors in the mouth/throat as well as the upper digestive tract, liver, colon and female breast in the case of moderate to high alcohol consumption.[78]

Furthermore, it has been reported that also the low activity *ADH1B*1/1** genotype is associated with enhanced exposure to acetaldehyde through saliva. It has been suggested that a lower systemic elimination rate of ethanol from the body can result in prolonged exposure to acetaldehyde produced by oral microbes.[78] Various epidemiological and mechanistic studies have shown that individuals with the less active genotype *ADH1B**1/*1 and those with the inactive genotype *ALDH2**2/*2 are subject to increased acetaldehyde concentrations, e.g., in saliva, and a significantly higher risk of cancer in the upper digestive tract.[8,79,193,196,199–202]

8. Assessment

The currently available data on the genotoxicity and mutagenicity of acetaldehyde indicate a genotoxic effect in vitro. Furthermore, the data that have become available since the previous evaluation by the BfR-LAV Committee in 2010[7] allow the conclusion that systemic genotoxic effects of acetaldehyde in vivo cannot be ruled out after oral ethanol exposure as well as inhalative and i.p. acetaldehyde exposure. Acetaldehyde induced micronuclei in the bone marrow in vivo after inhalation and oral administration in ALDH2 knock-out mice, but not in wild-type mice. DNA adducts (N^2-ethylidene-dG, analyzed as N^2-ethyl-dG, and CrPdG) were identified in different tissues in animal experiments after oral ethanol exposure, inhalation, and after i.p. acetaldehyde exposure, with ALDH2-deficient animals being more sensitive. A dose-dependent increase in DNA adduct levels was observed in various in vitro and in vivo studies. The biological significance of the identified DNA adducts for the mutagenicity and carcinogenicity of ethanol and acetaldehyde is currently not fully elucidated; additional mechanisms could contribute to the carcinogenic effect.[21] It should be noted that N^2-ethyl-dG was used as a surrogate adduct to determine the biological effects of acetaldehyde and no data on the biological properties, e.g., the mutagenic potential of the originally formed adduct N^2-ethylidene-dG, are available. The question of whether acetaldehyde has genotoxic and mutagenic effects after oral exposure in vivo cannot be definitively answered at the present time. Since acetaldehyde is efficiently metabolized in the intestine and liver after oral intake, it seems unlikely that acetaldehyde becomes systemically available to a significant extent. However, genotoxic and carcinogenic effects of acetaldehyde as a flavoring substance in directly exposed tissues (upper airways, oral cavity, gastrointestinal tract, especially esophagus) – in analogy to formaldehyde – cannot be ruled out based on the current state of knowledge. This is especially true for individuals with reduced ALDH enzyme activity. Frequent consumption of alcoholic beverages in larger quantities can result in an increased risk of local tumor development. This locoregionally increased risk may result from direct contact of the epithelia of the oral cavity and esophagus with acetaldehyde present in the drink, but especially from metabolic acetaldehyde formation from ethanol. Polymorphisms of genes, such as those for ADH1B and ALDH2, appear to be of particular importance for this mainly local acetaldehyde formation, as they code for enzymes that control the formation and degradation of acetaldehyde. Local microbial formation of acetaldehyde in the oral cavity, e.g., in ADH1B- and/or ALDH2-deficient individuals, could coincide with a comparatively low detoxification of acetaldehyde through ALDH2. Thus, in order to perform a risk assessment, considering systemic exposure to acetaldehyde alone cannot be considered sufficient. Rather, local events in the oral cavity and esophagus as well as genetic polymorphisms must also be taken into account. Therefore, the available risk assessments of acetaldehyde should be reviewed and complemented, considering new genetic-epidemiological findings and methods, particularly concerning an increased cancer risk of the oral cavity and esophagus.

When assessing the risks of acetaldehyde exposure from exogenous sources, it must be taken into account that ethanol and acetaldehyde are also formed endogenously during amino acid metabolism and energy metabolism. Data on endogenous acetaldehyde formation is limited. At present, there is no conclusive answer to the question of endogenous acetaldehyde levels in the blood. Previous analytical results should be critically questioned and, if necessary, verified using proven artefact-free analytical methods. At this point it is important to note that the endogenous background of acetaldehyde-derived DNA adducts in humans ranges from 13 to 150 adducts per 10^8 nucleotides. The detection of DNA adducts can be considered as a suitable biomarker monitoring after exposure, both for assessing background levels and exogenous exposure to acetaldehyde, as these can be measured reliably and with sufficient sensitivity. Therefore, further research should focus on this issue. The contribution from alcohol intake should also be taken into account in this regard. In a recent publication on the assessment of genotoxic carcinogens, the Senate Commissions MAK and SKLM pointed out that adduct formation by exogenously ingested acetaldehyde, which remains within the deviation range of the endogenous body burden, probably contributes only to a limited extent to cancer risk.[21] This assumption should be verified quantitatively at the DNA adduct level, since local acetaldehyde concentrations and local levels of acetaldehyde-related DNA adducts in the upper respiratory and digestive tracts appear to be a key factor in the development of cancer due to ethanol and acetaldehyde.[21] Therefore, a quantitative comparison with the endogenous background level of DNA

adducts requires consideration of all relevant tissues and body compartments, especially those that first come into contact with acetaldehyde, i.e., the respiratory tract in the case of inhalation and the upper gastrointestinal tract in the case of oral intake.[21] Besides the induction of DNA damage, acetaldehyde is a very reactive substance that can lead to tissue irritation and, therefore, may also exert tumor-promoting activity.[21]

There are no detailed and up-to-date exposure assessments for acetaldehyde. Systematic analyses of the most important food groups are necessary. There is a lack of controlled studies on biomarkers of exposure to acetaldehyde from food that are capable of distinguishing the contribution of ingestion of acetaldehyde via food, especially resulting from its use as a flavoring, from exposure to acetaldehyde via alcohol and from endogenously formed acetaldehyde. In addition, endogenous background exposure to acetaldehyde should be determined in an appropriate manner, e.g., based on biomarker-based controlled and randomized intervention studies.

In the opinion of SKLM, even considering the more recent data, it is still not possible to make a scientifically reliable estimate of the contribution of acetaldehyde used as a flavoring substance, acetaldehyde naturally present in food and endogenously formed acetaldehyde to the overall exposure to acetaldehyde. Furthermore, it is currently not possible to finally assess if acetaldehyde is genotoxic and mutagenic in vivo after oral exposure. Because of the above-mentioned missing data, no full scientific assessment of the health risk of using acetaldehyde as a flavoring is currently possible.

9. Conclusions

In contrast to exposure to acetaldehyde via alcohol consumption, there is insufficient toxicokinetic, toxicity and exposure data related to oral intake of acetaldehyde as a flavoring substance via food to derive strong conclusions on health risks associated with the use of acetaldehyde as a flavoring substance. Based on all available data, however, concerns regarding the genotoxic and/or carcinogenic hazard potential of acetaldehyde after oral intake via food containing acetaldehyde as a flavoring substance cannot be ruled out. In view of the remaining data gaps that need to be filled for a comprehensive risk assessment, and the resulting uncertainties, the SKLM concluded that there are concerns as to the safe use of acetaldehyde (a Globally Harmonized System of Classification and Labeling of Chemicals [GHS] classified CMR substance) as a flavoring substance. Considering all available evidence and for reasons of precautionary consumer protection, the SKLM recommends that the scientific basis for approval of the intentional addition of acetaldehyde to food as a flavoring substance should be re-evaluated.

10. Research Needs

Further data are needed in order to be able to perform a full risk assessment:

- Standardization of analytical methods for the determination of acetaldehyde in foodstuffs.
- Systematic chemical analyses of acetaldehyde in the most important food groups, including flavored foods.
- Toxicokinetic studies combined with improved analytical methods for determination of acetaldehyde and its biomarkers in experimental animals. To this end, transgenic mice carrying the human genes coding for alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) and their various genotypes may prove valuable for assessing endogenous vs. exogenous exposure from food or other sources.
- Establishment of biomarkers of exposure to acetaldehyde from food, particularly in the upper gastrointestinal tract, to determine local effects and to be able to distinguish the contribution of acetaldehyde present in food from exposure to acetaldehyde from alcohol and from endogenously formed acetaldehyde.
- Development of science to assess the biological activity of the primary Schiff base adducts formed, their kinetics, stability under physiological conditions, and persistence toward DNA damage repair.
- Biomarker-based controlled and randomized intervention studies to assess endogenous background exposure to acetaldehyde,
- Studies in vivo on genotoxicity/mutagenicity/carcinogenicity after oral intake of acetaldehyde in experimental animals, including transgenic mice carrying the human genes coding for different genotypes of ADH and ALDH. These mice may also be useful to establish in vitro models as a basis for mechanistic studies.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

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Angela Mally is Professor of Toxicology at the University of Würzburg, Germany. Her main research interests relate to mechanisms of toxicity and carcinogenicity of food contaminants as a basis for risk assessment. She is member of the DFG-Senate Commission on Food Safety (DFG-SKLM), chair of the DFG-SKLM Working Group on Food Constituents, and member of the BfR Committee for Contaminants in the Food Chain. Angela Mally serves as Editor for Toxicology Letters.