REVIEW

Toxicology and risk assessment of 5-Hydroxymethylfurfural in food

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5-Hydroxymethylfurfural (5-HMF) as a product of the Maillard reaction is found in many foods. Estimated intakes range between 4 and 30 mg per person and day, while an intake of up to 350 mg can result from, e.g., beverages made from dried plums. In vitro genotoxicity was positive when the metabolic preconditions for the formation of the reactive metabolite 5-sulphoxymethylfurfural were met. However, so far in vivo genotoxicity was negative. Results obtained in short-term model studies for 5-HMF on the induction of neoplastic changes in the intestinal tract were negative or cannot be reliably interpreted as "carcinogenic". In the only long-term carcinogenicity study in rats and mice no tumours or their precursory stages were induced by 5-HMF aside from liver adenomas in female mice, the relevance of which must be viewed as doubtful. Hence, no relevance for humans concerning carcinogenic and genotoxic effects can be derived. The remaining toxic potential is rather low. Various animal experiments reveal that no adverse effect levels are in the range of 80–100 mg/kg body weight and day. Safety margins are generally sufficient. However, 5-HMF exposure resulting from caramel colours used as food additives should be further evaluated.

Received: November 12, 2010
Revised: February 2, 2011
Accepted: February 9, 2011

Keywords:
5-Hydroxymethylfurfural / Carcinogenicity / Exposure / Genotoxicity / Toxicokinetics

1 Introduction

5-Hydroxymethylfurfural (5-hydroxymethyl-2-furfuraldehyde, 5-HMF, CAS No. 67-47-0) is formed during the thermal treatment of carbohydrate-containing foods. Reactants are reducing hexoses in the presence of amino acids or proteins (Maillard reaction). It is generated by acid-catalysed thermal dehydration from fructose, saccharose and to a lesser degree from glucose. 5-HMF was likewise detected in heat-sterilised glucose/fructose solutions for parenteral nutrition. It is used as a flavouring substance in food and is also present in wood smoke and liquid smoke [1].

Since the 1950s there have been reports of 5-HMF in food. It has been identified in a wide variety of heat-processed foods. Depending on production technology and storage, levels in food vary considerably. While 5-HMF is practically not present in fresh food, high levels in the g/kg range can be found in dried fruits, coffee and caramel products. In honey and some other food, concentrations of 5-HMF can be used as an indicator of heating and storage changes. For example, the Codex Alimentarius standard sets a maximum limit for 5-HMF in honey of 40 mg/kg (80 mg/kg in tropical honey) as a way of assuring that the product has not undergone heating during processing [2].

The detection of 5-HMF in several food items prompted an assessment of possible health risks with consideration of

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Abbreviations: 5-HMF, 5-Hydroxymethylfurfural; **ACF,** aberrant crypt foci; **bw,** body weight; **CAFAM,** 5-carboxylic-2-furoyl-amino-methane; **CAFG,** 5-carboxylic-2-furoyl-glycine; **EFSA,** European Food Safety Authority; **FDCA,** 2,5-furan dicarboxylic acid; **FGE.13,** Flavouring Group Evaluation 13; **GSH,** glutathione; **HMFA,** 5-hydroxymethyl-2-furoic acid; **HMFG,** 5-hydroxymethyl-2-furoyl glycine; **m-TAMDI,** modified-Theoretical Added Maximum Daily Intake; **NTP,** National Toxicology Program; **SMF,** 5-sulphoxymethylfurfural; **SULT,** sulphotransferase

dietary intake. Therefore, in this review, we summarize the available toxicological data, including recent research and findings on the carcinogenic potential of 5-HMF and the possible relevance of sulphotransferase (SULT) activity in humans compared to rodents. Furthermore, an estimation of 5-HMF exposure in Germany is performed based on occurrence and consumption data; these results are compared with estimates from other countries. Finally, it is evaluated whether the dietary intake of 5-HMF in food might be related to possible health risks.

2 Toxicity of 5-HMF

2.1 Toxicokinetics and metabolism

So far, several metabolic pathways could be detected for 5-HMF (Fig. 1). The primary step involves oxidation of the aldehyde function into 5-hydroxymethyl-2-furoic acid (HMFA). This is followed by conjugation with glycine into 5-hydroxymethyl-2-furoyl glycine (HMFG). Both metabolites, HMFA and HMFG, are rapidly eliminated in urine. The ratio of the concentrations of HMFA to HMFG is elevated at higher 5-HMF doses as the availability of free glycine is limited. This results in increased excretion of free HMFA and/or 2,5-furan dicarboxylic acid (FDCA) [3].

^{14}C-labelled 5-HMF was administered orally to mice and rats as well as intravenously to rats. Following rapid absorption, radioactivity was quickly and almost completely eliminated within 24–48 h via urine. Whole-body autoradiography found some radioactivity in the liver shortly after administration; however, there was a greater amount in the kidneys and bladder. The only major difference between oral and intravenous administration was a high level of radioactivity in the brain of intravenously treated animals [3].

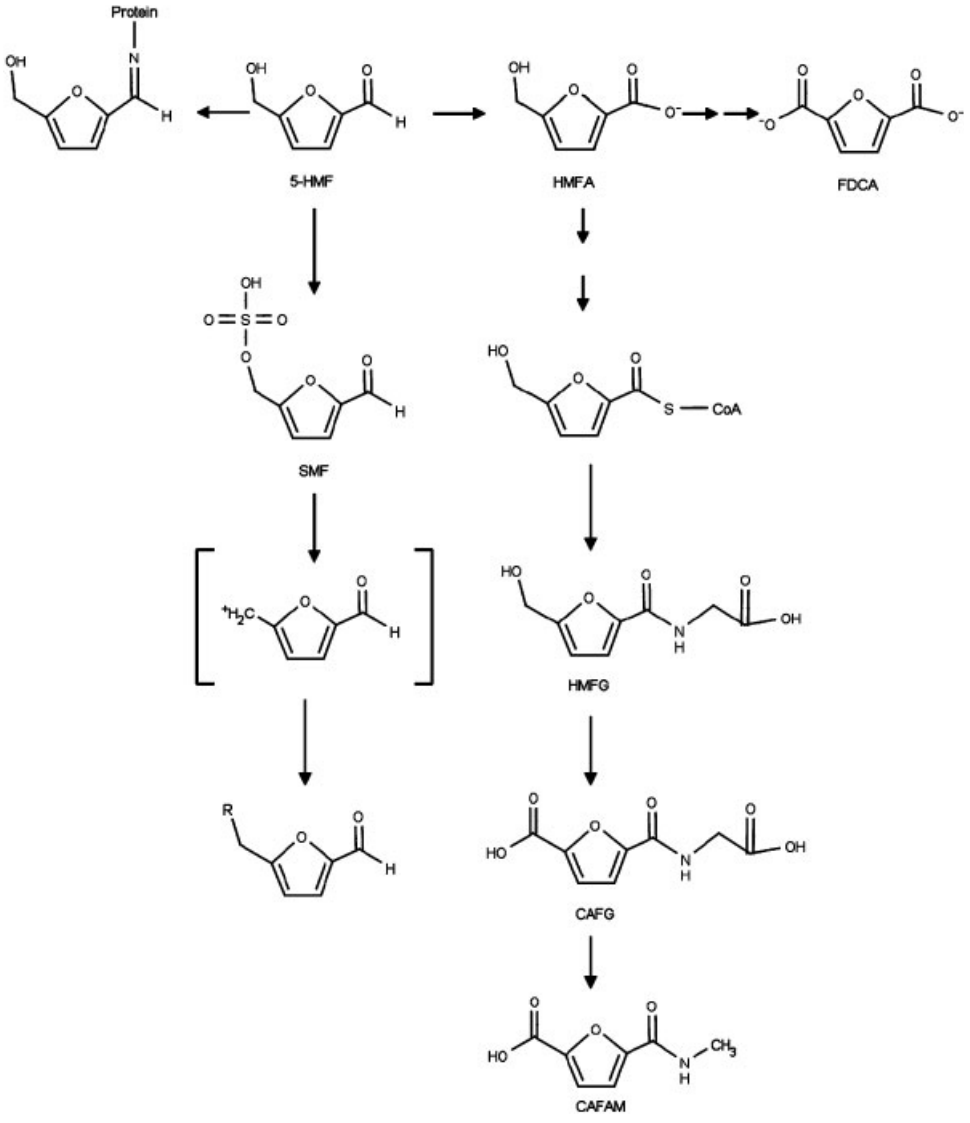

Figure 1. Biotransformation of 5-HMF. Metabolites have been detected in laboratory animals and/or humans and/or in vitro. R might represent protein, glutathion, DNA or RNA.

A relatively low level of non-extractable radioactivity was found in the liver, kidneys and small intestine, which might be an indication of covalent binding [4]. The authors identified and quantified HMFA, HMFG and FDCA in the urine of rats and mice in amounts of 78–85, 5–8 and 2–6% of the dose administered. The elimination rate was not dependent on the dose level. As 5-HMF and its metabolites were almost completely eliminated, there was no evidence for accumulation of the compounds [4].

So far, HMFA, HMFG and FDCA have been detected in human urine – e.g. after administration of fructose-containing infusion solutions for parenteral nutrition [5, 6]. Pryor et al. [7] were able to detect HMFA, HMFG, 5-carboxylic-2-furoyl-glycine (CAFG) and 5-carboxylic-2-furoylamino-methane (CAFAM) in the urine of humans who had eaten dried plums or drunk the juice of dried plums. In the individuals who consumed 3944 μmol (497 mg) 5-HMF in juice, the recovery rates 6 h after consumption were 36.9% for HMFA, 3.4% for HMFG, 4.2% for CAFG and 1.9% for CAFAM with regard to the administered 5-HMF dose. The ratio of HMFA to HMFG in urine was 10.7. These findings in humans are comparable with the results from animal experiments performed by Germond et al. [3] and Godfrey et al. [4]. However, the metabolites CAFG and CAFAM were not observed in the rat. By contrast, FDCA was not detected in this investigation in humans.

The metabolic pathway for possible bioactivation, which has become more important in recent times – sulphonation of the allylic hydroxyl function of 5-HMF catalyzed by SULTs – was initially identified in vitro. The resulting sulphate ester 5-sulphoxymethylfurfural (SMF) can induce genotoxic and mutagenic effects through a highly electrophilic allyl carbocation. The formation of SMF could also be demonstrated recently in vivo in the FVB/N mouse. Following intravenous application of 793 μmol 5-HMF/kg body weight (bw), biphasic elimination kinetics were observed in plasma with half-lives of 1.7 and 28 min for the initial and terminal elimination phase. The maximum SMF plasma level was reached 2.5 min after 5-HMF administration. It was estimated that between 452 and 551 ppm (approx. 0.5 pro mille) of the 5-HMF dose was converted into SMF and reached the bloodstream [8]. It is likely that some of the SMF reacts with cellular structures at the site of formation. Hence, the total amount of SMF formed cannot be quantified. So far, there is no evidence that SMF is also formed from 5-HMF in humans. SMF has not been detected in human urine and this is plausible given its instability [9].

5-HMF induced a concentration-dependent decrease in glutathione (GSH) in various cells in culture [10]. The underlying mechanism has not been elucidated. However, the effect was similar in V 79 cells, which are SULT-deficient, and in primary hepatocytes, which probably were SULT-proficient.

Sickle cell disease is caused by abnormal haemoglobin that polymerises under hypoxic conditions. Some chemicals that form Schiff's base with the terminal valine residue of the α-chain can stabilise haemoglobin. Abdulmalik et al. [11] have shown that 5-HMF can exert this anti-sickling effect in a transgenic mouse expressing human haemoglobin. Almost 100% of human haemoglobin α-chain as well as the endogenous murine haemoglobin α-chain were modified 1 h after oral administration of 100 mg 5-HMF per kg bw. This level persisted for 3 h, followed by a gradual decrease to undetectable levels by 6 h. Thus, the protein adducts disappeared shortly after the elimination of 5-HMF from blood, suggesting reversibility of this Schiff's base.

2.2 Genotoxicity and mutagenicity

5-HMF itself mainly tested negative in in vitro genotoxicity tests; however it is mutagenic in the presence of the cytosolic fraction of the rat liver with 3′-phosphoadenosine-5′-phosphosulphate as the cofactor for SULTs and in genetically modified bacteria and mammalian cells that express the human SULT 1A1 [10, 12–21].

SULTs from rats and human SULTs bioconvert 5-HMF into the reactive allyl ester SMF. This substance is mutagenic in bacteria and mammalian cells in vitro in the absence of an activating system [16]; the mechanism probably involves reactions of its highly electrophilic allyl carbocation. SMF is inactivated by GSH in the presence of GSH *S*-transferases. A genotoxic effect in *Salmonella typhimurium* TA104 in the absence of a metabolic system could be reduced in the presence of GSH and GSH *S*-transferases [17].

5-HMF is negative in the in vitro micronucleus test with HepG2 cells that express SULTs [22]. In the in vitro Comet assay, 5-HMF is weakly positive in various cell lines (with different SULT activity), however, only at high concentrations (25–100 mM) that are also cytotoxic [23]. In HepG2 cells, 5-HMF also induced DNA damage at somewhat lower concentrations (8–25 mM) in the Comet assay [22]. These concentrations were also comparatively high since these tests are generally conducted up to a concentration of 10 mM.

Discrepancies between positive and negative results of in vitro tests may be due to different conditions under which these assays were performed including expression of SULT in the cell lines used and presence or absence of 3′-phosphoadenosine-5′-phosphosulphate in the metabolising system.

In vivo, 5-HMF did not induce any micronuclei in the micronucleus test with the peripheral blood cells of male and female B6C3F1 mice, which were given 5-HMF over 90 days by gavage at doses of 0, 47, 94, 188, 375 and 750 mg/kg bw and day [24]. The ratio of polychromatic erythrocytes to total erythrocytes was not changed and thus it is not clear whether the substance reached the bone marrow. The study design deviated from the standard protocol in terms of duration of administration but is nonetheless acceptable.

SMF was positive in the micronucleus test in mice [25]. However, these two studies are not directly comparable because they were conducted with different mouse strains and treatment protocols.

2.3 Animal experiments on acute and subchronic toxicity

The acute toxicity of 5-HMF is very low. In a study in rats, an acute oral LD50 of 3100 mg/kg bw was observed. For mice there was an LD50 of 1910 mg/kg bw and in another study an LD50 of >2000 mg/kg bw [26]. Rats were administered 250 mg 5-HMF/kg bw per day over a 40-wk period. There were no significant differences in terms of weight increase, feed consumption or final weight between these rats and the control animals. The histological examination of various organs like for instance heart, liver and kidneys did not detect any differences between the two groups either [27].

In a longer-term study, rats were given 0, 40, 80 or 160 mg 5-HMF/kg bw 6 days a week over a period of 11 months. Protein and lipid metabolism, ascorbic acid levels in the adrenal glands, the activity of the hepatic succinate dehydrogenase, organ morphology and bw were unchanged in comparison to the controls. At 160 mg/kg bw there were, however, minor changes in the clinical chemical parameters (a temporary increase in the γ globulin levels in the serum and a tendency to increased activity of the hepatic tributyrase) and an increase in the relative spleen weight; 40 and 80 mg/kg bw did not have any effect [26, 28].

In a study with mice over a 3-month period within the framework of the US National Toxicology Program (NTP) studies, there was a significantly lower weight increase at the highest dose of 750 mg/kg bw (5 days per week) compared with the controls. Effects like minor-to-mild cytoplasmic changes in the kidneys were significant in the male animals at the doses of 188 mg/kg bw (5 days per week) and above. At 94 mg/kg bw (5 days per week) and below, no adverse effects were observed [24].

Hence, the maximum dose observed with no adverse effects (NOAEL) regarding acute and subacute toxicity in animal experiments is in the range of 80–100 mg/kg bw per day.

2.4 Animal experiments on carcinogenicity

2.4.1 Heat-treated saccharose promotes azoxymethane-initiated microadenomas in the intestines of mice and rats

A potential carcinogenic effect plays a major role in the assessment of 5-HMF and its reactive allyl ester SMF. Discussions focus above all on the possible formation of intestinal tumours. In 1990 Corpet et al. [29] examined a tumour-inducing effect after initiation with azoxymethane in CF1 mice and Fisher344 rats who had been given different dietary components heat-treated at 180°C. After 100 days a significantly higher number of large microadenomas of the intestines were observed in animals that had been given heat-treated (caramelised) sugar or heat-treated casein and fat (compared with control animals given the same but unheated feed). Five additional heat-treated food components had no significant effects. The authors thought that compounds formed during the heating process are promoters of colon cancer. However, this study could not draw any conclusions about which compounds were responsible.

2.4.2 5-HMF induces and promotes preneoplastic lesions in the colon of rats

The same working group carried out an HPLC analysis of slightly caramelised sugar in which a level of 1% 5-HMF was identified as the most important reaction product in terms of quantity [30]. In subsequent animal experiments, Fisher344 rats were again pre-treated with azoxymethane and divided into four groups. The control group was given untreated saccharose, other groups were given heat-treated sugar, heat-treated sugar without 5-HMF (butanol extraction) or feed with 1% added 5-HMF. The results of the first study (heat-treated saccharose, [29]) were confirmed, whereas heat-treated sugar, from which 5-HMF had been extracted, did not show any effect. In the group given feed with 1% 5-HMF, there were significantly larger aberrant crypt foci (ACF, precursors of microadenomas) compared to the control group as well as a higher number of large ACF. In additional studies which were then carried out with 5-HMF dissolved in water (given twice at an interval of 1 wk, doses of 100 up to 300 mg/kg bw, no pre-treatment with azoxymethane, evaluation after 30 days), dose-related increases were observed in the numbers of ACF per colon and in the fraction of animals with ACF. Hence, it was reported that 5-HMF could have not only a promoting but also an initiating potential for triggering cancer of the colon. However, regarding the evidence, it should be noted that the study design had shortcomings (three separate studies with different numbers of animals; in two cases far higher ACF numbers in the control groups than in the third study; choice of dose groups not convincing), making a more sophisticated overall assessment necessary [31].

2.4.3 Studies with transgenic mice (min/+)

Similar results were observed with mice. Svendsen et al. [32] treated C57BL/6J mice (wt/wt) shortly after birth with a single subcutaneous injection of 5-HMF (500 mg/kg bw). Examination of the animals aged 12 wk identified ''flat'' ACF in four out of 18 animals (22%), which had not been observed in any of the control animals (no treatment). The

authors reported this result as not significant but did not provide any information about the general incidence of ''flat'' ACF in untreated animals; however, if this tissue change would never occur in a spontaneous fashion, then the results could be interpreted as relevant eventually.

A genetic variant of the animals (C57BL/6J Min/+) was also examined, which is particularly predisposed to the occurrence of multiple intestinal neoplastic changes using the same study design. Treated animals were found to have a higher number of adenomas in the small intestine than untreated animals (mean 119 versus 102). The difference in the number was significant only if the evaluation was undertaken for the middle and distal sections of the small intestine (15–26 cm from stomach; $p = 0.033$). This form of specifying a specific section of the intestines only after histological evaluation is deemed to be problematic from the methodological–statistical angle and is primarily suited for generating hypotheses, e.g. that activation of 5-HMF to carcinogenic metabolites might take place only in a specific section of the intestine. In the large intestine of treated animals a higher number of ''flat'' ACF than in the control group was found (mean 32 versus 21), which was not, however, significant. Nor were there any significant differences in the rate of adenomas in the large intestine.

2.4.4 NTP studies

The relevance of the short-term models described above (Sections 2.4.1–2.4.3) is unclear with regard to the assessment of the potential hazard of 5-HMF causing intestinal cancer. Greater elucidation of this potential could be expected from the NTP study conducted a few years ago [24]. In the 2-year studies in F344/N rats and B6C3F1 mice, groups of 50 female and 50 male animals were given 0, 188, 375 or 750 mg 5-HMF/kg bw 5 days a week by gavage.

In the rats, the survival rate of the 188 and 750 mg/kg bw group was higher than that of the control group. No noticeable differences in bw gains were observed in treated and untreated animals. Degenerative effects were observed in the olfactory epithelium of rats (incidences significantly increased in males at 750 mg/kg bw and in females at 188 and 375 mg/kg bw). The incidences of olfactory epithelium respiratory metaplasia and respiratory epithelium squamous metaplasia were increased in both genders in the highest dose group. The only other treatment-related, non-neoplastic effect was a significantly higher incidence of clear cell foci of the liver in the male rats in the highest dose group.

In the mice of the highest dose group of both genders, there was a significantly lower survival rate than in the control group. Furthermore, a lower weight increase was observed. Lesions of the olfactory and respiratory epithelium of the nose similar to those observed in the rats were found in mice in the dose groups 375 and 750 mg/kg bw. Starting with month 8, neurological symptoms were observed in the highest dose group in both genders within the first minutes of administration (for instance decreased exploratory behaviour, piloerection, salivation, catatonia, excitation, dyspnea, clonic-tonic seizures and unconsciousness). Because of these clinical symptoms and the reduced survival rate in the highest dose group, these animals were excluded from the evaluation of the carcinogenic potential.

The overall result was classified as some evidence of carcinogenic activity of 5-HMF in female B6C3F1 mice, based on elevated incidences of hepatocellular adenomas of 53% in the dose group 188 mg/kg bw and of 52% in the group 375 mg/kg bw. In the control group, the incidence was 28%, which is in accordance with historical control data for hepatocellular adenomas in female B6C3F1 mice (incidence on average around 22%). No evidence of a tumourigenic effect was found in rats or male mice.

The indications observed in short-term studies of a possible potential of 5-HMF to induce intestinal cancer in rodents could therefore not be confirmed in the NTP long-term study.

2.4.5 Studies with SMF

Regarding the possible carcinogenic potential of 5-HMF, the discussions focus in particular on the reactive metabolite, SMF. When applied topically to the skin of mice, SMF initiated a higher incidence of papillomas than 5-HMF itself [15].

In the study by Svendsen et al. [32] SMF was applied subcutaneously. The dose was a single administration of 25 mg/kg bw ($1/20^{th}$ of the 5-HMF dose). Results were similar like those obtained for 5-HMF: an elevated incidence of ''flat'' ACF (two out of 15 mice, 13%) in the wild-type animals, which was not significant compared with the controls. With the particularly sensitive genetic variant, the C57BL/6J Min/+ mouse, an elevated number of small intestinal adenomas and ''flat'' ACF in the colon were observed, but with no or only borderline significance.

The high toxicity of SMF was demonstrated by Bauer-Marinovic et al. (personal communication; Prof. Dr. H. Glatt, Department of Nutritional Toxicology, German Institute of Human Nutrition, Potsdam-Rehbrücke, Nuthetal, Germany). SMF was administered intraperitoneally to FVB/N mice in a single, high dose of 250 mg/kg bw. Most of the animals died within 5–11 days as a consequence of massive damage to the proximal tubules of the kidneys. Here, the uptake of SMF into the proximal tubule cells mediated by the transporters OAT1 and OAT3 may play a role [33]. In subsequent studies with various lower doses and observation periods of up to 40 wk, changes in the kidney tubules were also observed as a main effect. Furthermore, hepatotoxicity and serositis were observed in organs with direct contact to the peritoneum. No ACF and/or adenocarcinomas were observed in the animals treated with SMF. In a positive control (20 animals treated with azoxymethane), a total of 1064 ACF and five adenocarcino-

mas could, however, be induced (personal communication Prof. Dr. H. Glatt).

If SMF should prove to be responsible for a possible carcinogenicity of 5-HMF, then attention would focus on the question of the activity of the respective SULT in various species. For humans it is known that the most important SULT – SULT1A1 – has far higher enzymatic activity in conjunction with 5-HMF than the SULT of rodents, which have been examined [34]. Hence, humans could be more sensitive to the toxic effects of 5-HMF and laboratory rodents would not then be the ideal species to elucidate this issue. To clarify this question, transgenic mice were examined, which carry multiple copies of the human SULT1A1-SULT1A2 gene cluster in the middle region of chromosome 9 (FVB/N-hSULT1A1/2 mice). In different tissues (including colonic mucosa), a three up to 12-fold higher SULT activity could be achieved (personal communication Prof. Dr. H. Glatt).

In this study, the FVB/N-hSULT1A1/2 mice and the wild-type mice (FVB/N) were given 5-HMF in drinking water at the dose of 0 (control), 134 and 536 mg/kg bw for 12 wk. The experiment consisted of 48 animals treated with 5-HMF and 24 untreated control animals. In the evaluation, only minor histological tissue changes were observed in the kidneys, which surprisingly did not differ between the mice with high SULT activity and the wild-type mice. In addition, no ACF or tumours were observed either in the control animals or in the animals treated with high or low SULT activity (personal communication Prof. Dr. H. Glatt).

2.5 Assessment of the possible carcinogenicity 5-HMF

The incidence of colorectal carcinomas varies around the world up to 25-fold between the different countries. With growing industrialisation and urbanisation the rates increase. This implies that lifestyle could play an important role in the onset of intestinal cancer. Results from so-called migrant studies also indicate this [35]. There are, however, a small number of chemicals that induce colon cancer in laboratory rodents like, for instance, heterocyclic aromatic amines (PhIP). It was, therefore, surprising that heated saccharose promoted azoxymethane-initiated preneoplasias in the colon of rat and mice [29]. 5-HMF was identified as the possible active component of heated saccharose [31]. Furthermore, it was shown that 5-HMF itself – without prior treatment with azoxymethane – is also capable of inducing an elevated number of preneoplastic ACF [31]. This initiation implied the induction of gene mutations in the colon mucosa by 5-HMF. For instance, Femia et al. [36] detected K-ras mutations up to 100% (14/14) in ACF, which were induced in the rat colon by 1,2-dimethyl hydrazine.

5-HMF itself is not mutagenic. However, its metabolite SMF has mutagenic potential. The decisive question now is whether this metabolite is formed in vivo and is responsible for the induction of preneoplastic ACF in rats treated with 5-HMF.

This question was therefore examined in several experiments in which however mice were used instead of rats. SMF has been detected in vivo in the plasma of mice that had been administered 5-HMF intravenously. Furthermore, transgenic mice were used, which express a high level of human SULT1A1 and 1A2 in many tissues. This genetic modification leads to a clear increase of 5-HMF sulphation to SMF, detected in sub-cellular preparations of colon mucosa and other tissue (personal communication Prof. Dr. H. Glatt).

After administration in drinking water, 5-HMF probably reaches the intestinal mucosa of the luminal side and may be directly activated in the target tissue. If absorption takes place, 5-HMF would reach the intestinal mucosa along systemic pathways. In this short-term experiment, 5-HMF did not, however, induce any preneoplastic ACF either in transgenic or in wild-type mice (personal communication Prof. Dr. H. Glatt).

These negative results for 5-HMF were surprising as the overall exposure in this short-term study was far higher than the exposure that showed the positive results in the original rat study by Zhang et al. [31]. It is not clear whether major species differences between mice and rats could play a role. There are indeed no indications of this. It should, however, be pointed out that the spontaneous rates of ACF in the Zhang study (1993) varied between the experiments and that the number of ACF in the animals treated with 5-HMF was only slightly higher than the spontaneous rate. Moreover, the publication does not clearly describe how the statistical analyses were conducted.

In the only long-term study within the framework of the NTP in rats and mice, 5-HMF did not demonstrate any neoplastic or other non-neoplastic effects in the intestinal tract of either species. Nonetheless, an elevated incidence of hepatocellular adenomas was observed in female mice. The incidence was not, however, dose-dependent and the B6C3F1 strain is known for its genetically driven high susceptibility to the formation of liver tumours. No carcinogenic evidence was seen in male mice and in rats of both genders.

In the study with neonate min/+mice, 5-HMF elevated the number of adenoma in the small intestine whereas SMF increased the number of so-called ''flat ACF'' in the colon [32]. Both effects were indicated as statistically significant in relation to the vehicle control. However, in terms of methodology, this is difficult to interpret because an evaluation was only undertaken with regard to certain sections of the intestines. These positive effects were possibly also caused by the high sensitivity in this specific sensitive model. The min/+mouse is heterozygous for a mutation in the tumour-suppressor gene Apc, which spontaneously leads to the development of numerous adenomas, mainly in the small intestine but also in the colon. Furthermore, the test substances were administered

subcutaneously to the neonate animals and this constitutes artificial intake.

By way of summary, these results lead to doubts about whether 5-HMF has any carcinogenic potential at all. Importantly, there is so far no reliable evidence that 5-HMF induces tumours in the colon or small intestine. The genotoxic relevance of the low levels of the metabolite SMF detected in vivo has still to be clarified. A verification of its detection in vivo seems to make sense. Based on the available results, the possible risks of carcinogenic effects, if present at all, are not currently identifiable or only to be estimated as extremely low.

3 Levels of 5-HMF in food and exposure estimates

Depending on processing, the levels of 5-HMF may vary considerably between the individual food groups and even in the same food. In order to be able to record the variability as representatively as possible, sufficient measurements for certain foods should be available. Detailed information on the processing and production processes, giving hints for the formation of 5-HMF in food, is not systematically recorded in the data on contamination levels. This leads to un-quantifiable uncertainties with regard to intake estimates. A number of studies [9, 37–42] have been identified in which 5-HMF levels in different kinds of food have been measured. Two studies from Spain [42, 43] additionally provided consumption figures; a third study from Norway [38] estimated the intake of 5-HMF by means of dietary recall. Furthermore, intake estimates have been calculated on the basis of primary food consumption data from the German national nutrition survey II provided by the Max-Rubner-Institute [44] and on data from the German food surveillance programme [45]. Respective data are presented in Table 1. For exposure estimation, the following 13 particular food groups may be relevant: dried fruits and beverages prepared from dried fruits; fresh fruits; ketchup and mustard; cereals and bread; sweets; nuts; coffee; jams, marmalade and honey; fruit juices; alcoholic beverages; milk; vinegar; miscellaneous.

In the first group (dried fruits, plums, grapes and dates), dried fruit exhibit the highest concentrations of 5-HMF. On the basis of the German data (Table 1) and four studies [9, 37–39], the average concentrations range from 5.5 to 1350 mg/kg. Maximum levels of 3500 mg/kg have been reported in dried apples and pears [37]. Lower values can be found in dates and raisins. Plums may serve as a special source of 5-HMF as even high concentrations were found in fresh fruits (up to 2200 mg/kg). Most of the information of 5-HMF in fresh fruits is based on very small numbers of measurements [9]. Therefore, the contribution of fresh fruit to 5-HMF exposure currently can hardly be assessed.

A high intake of 5-HMF from consumption of pastes and dried tomatoes in Spain has been estimated by Rufian-Henares et al. [42]. They reported an average intake of 5.4 μg/kg bw and day and a high intake value of 37.3 μg/kg bw and day (assuming a bw of 60 kg). However, the latter results from a high consumption figure of 0.2 g/kg bw per day.

Cereals and cereal products, in particular bread, may serve as a major source of 5-HMF exposure. The majority of

Table 1. Mean HMF levels in various foods in mg/kg (data from Germany 2005–2010)[a)]

Food	*n*	<LOQ (%)	Mean levels (mg/kg)	Uncertainties/description
Honey	726	10	9.1	Values from blossom honey and mixtures (consumed frequently)
Apple juice	234	12	7.4	
Orange juice	10	0	0.4	Few measurements
Multivitamin nectar	16	19	40.9	Few measurements
Pineapple juice	29	24	2.6	
Grape juice	80	9	6.3	Red and white
Berry juice	11	9	5.7	Few measurements, types of berry not known
Plum juice beverage	11	0	707.7	Few measurements
Plum butter/jam	174	0	410.9	
Apricot jam	15	0	36.3	Few measurements
Cereal bar	117	9	43.2	
Chocolates/praline	13	0	273.8	Few measurements, non-specific (large variability)
Dried plums	153	0	350.8	
Beverage powder with coffee	23	0	286.1	Non-specific (large variability)
Rye-wheat bread	20	10	44.5	Large variability in production and composition
Almonds, roasted and coated	28	0	155.5	
Mulled wine	192	27	13.7	
Cocoa-containing beverage powder	14	0	503.8	Few measurements, non-specific (large variability)
Beverage from dried plums	71	0	1022.1	

a) Surveillance programs/food monitoring; levels <LOQ/LOD = 0.5*LOQ/LOD.

average concentrations ranged from 14 to 53 mg/kg. With regard to cereals having low consumption figures (e.g. bisquits, pastries), the average intake for 5-HMF is estimated to be 2 μg/kg bw per day. For cereals consumed in higher amounts such as white bread [42] or rye-wheat bread, the estimated intake is about 30–50 μg/kg bw per day. Based on data from Norway [38] and Germany (Table 1), nuts obviously are not a relevant source of 5-HMF.

Coffee is an important source of 5-HMF. Values of up to 4000 mg/kg have been detected in coffee powder, but most of the levels were much lower (about 12–300 mg/kg). 5-HMF exposure related to drinking coffee has been described in the study of Arribas-Lorenzo et al. [43], evaluating different sorts of coffee in Spain. Levels of 1119 mg/kg (median) and 3824 mg/kg (maximum) in ground and soluble coffees were found. A huge range of dietary intake of 5-HMF from drinking coffee was observed. The estimated daily 5-HMF exposure of 5.26 mg (median) and 8.57 mg (high consumption) corresponds to 87 and 143 μg/kg bw per day, respectively. Hence, coffee accounted for about 50% of the estimated total 5-HMF exposure in Spain [42] and to about 63% in Norway [38].

5-HMF has been determined in a number of sweet bread spreads. Although spreads made from plums have the highest concentrations, the low consumption rate is the cause for the low intake. In addition, other jams and marmalades as well as honey will not contribute to a high extent of 5-HMF exposure. Average values of 0.2–0.8 μg/kg bw per day can be derived for intake estimates from bread spreads based on data from Germany and Rufian-Henares et al. [42], while high consumption may account for 3.9 μg/kg bw per day as a maximum. It should be noted that storage may lead to an increase of 5-HMF concentrations in jams and fruit-based infant foods [46].

Fruit juices can be a major source of 5-HMF exposure due to high concentrations of 5-HMF (e.g. plum juice) or due to the high consumption of juices with lower levels (e.g. apple juice). For many juices, the average 5-HMF intake was estimated to be below 1 μg/kg bw per day. In case of apple juice, intake estimates are 16.8 and 92.5 μg/kg bw per day for mean and high consumption, respectively, using the mean level of 7.4 mg/kg from 234 analyses in Germany (Table 1). Highest 5-HMF levels were found in beverages prepared from dried plums (mean 1022 mg/kg, 71 analyses from Germany). While these beverages are not ingested by the vast majority of consumers, persons drinking one glass (250 mL) per day may be additionally exposed to 256 mg 5-HMF on average (4.26 mg/kg bw per day).

5-HMF has been studied in a number of jarred and canned baby foods. Although average concentrations in these foods do not seem to contribute considerably to 5-HMF exposure, some foods may contain elevated concentrations. In the studies evaluated, the highest amount reported was 22 mg/kg [37]. Taking into account that a baby weighing 10 kg is fed with an amount of 50 g of such food, the dietary intake of 5-HMF corresponds to 110 μg/kg bw per day.

Delgado-Andrade et al. [47] reported highest values of 5-HMF in paella, kid stew and churros, a typical Spanish dish. No consumption figures are available, but taking a default of 50 g of consumption and an average contamination of 20–50 mg/kg would account for an intake of 16–40 μg/kg bw per day.

Beer and other alcoholic beverages may also contribute to 5-HMF exposure [38]. Concentrations of 5-HMF in milk have been measured in ultra-high temperature milk as well as in sterilized samples, exhibiting median values between 0.14 and 1.6 mg/L [42]. Surprisingly, the consumption figure for ultra-high temperature milk (3.6 g/kg bw per day) presented by Rufian-Henares et al. [42] differs exorbitantly from that of pasteurized milk (0.068 g/kg bw per day). The former value, however, is in accordance with data from different European countries as documented in the European Food Safety Authority (EFSA) database (2–5 g/kg bw per day in adults). Based on these figures, the intake of 5-HMF from milk would range from about 0.7 to 8 μg/kg bw per day, taking the low and high level and the high consumption value.

Interestingly, balsamic vinegar may exhibit high concentrations of 5-HMF. An exposure estimate of about 1.5 μg/kg bw per day each from balsamic vinegar and the less-contaminated but higher consumed wine vinegar can be calculated from maximum levels and consumption data from Spain [42].

Taken all together, in spite of many measurements of 5-HMF performed in a range of food groups, the database is limited to estimate exposures precisely. However, some major conclusions can be drawn. First of all, drying of fruits, in particular of plums, can lead to high concentrations of 5-HMF. Second, cereals and bread may contribute considerably to 5-HMF exposure, due partly to elevated concentrations and partly to high consumption. The third major source of 5-HMF is coffee, due to increased levels of 5-HMF formation during roasting, as well as high consumption rates.

For Germany, estimates of total 5-HMF intake of 67 and 215 μg/kg bw per day have been calculated for mean concentrations in combination with mean and high consumption figures, respectively. An even higher value of 265 μg/kg bw per day was calculated for mean consumption and high levels; however, this may not be relevant if one considers long-term exposure. Due to missing occurrence data from Germany, it was not possible to include coffee as source of 5-HMF exposure. Based on the 5-HMF data in coffee from Spain and Norway described above, total daily 5-HMF exposure in Germany could be distinctly higher; as a rough estimate, high exposure may reach 400 μg/kg bw per day.

In the study by Rufian-Henares et al. [42], the mean total intake estimates for Spain are 10 mg/day (about 170 μg/kg bw per day, scenario median levels) and 23 mg/day (about 380 μg/kg bw per day, scenario maximum levels). Similar estimates of 5-HMF for the Norwegian population made by Husøy et al. [38] revealed a mean

and median estimate of 5-HMF exposure of 5.56 and 3.04 mg per day, respectively (93 and 51 μg/kg bw per day). However, in the same study, the mean and medium urinary excretion of HMFA was 12.4 and 11.8 mg per day, respectively (about 200 μg/kg bw per day), indicating a greater lack of sources of exposure and thus showing clearly an underestimation of the calculated exposure, which may be even higher taken into account that besides the analysed HMFA there are further metabolites of 5-HMF. High exposure to 5-HMF (95th percentile) was estimated to be 27.6 mg per day (460 μg/kg bw per day, with an respective urinary HMFA excretion of 28.6 mg per day). The calculations in this study should be interpreted carefully, because they were based on 24 h dietary recalls from 53 volunteers only.

In summary, the estimates from Germany, Norway und Spain are roughly in the same (relatively broad) range. However, direct comparisons are difficult to draw because of possible major differences in food consumption behaviour. Furthermore, the estimates are not based on consistent data. For example, in the German estimate, coffee is not a major source. On the other hand, fruit juices have not been considered in the Spanish study [42]. Therefore, the estimates of total intake in these two studies may present an underestimate of total exposure, which is due to uncertainties regarding to representativity of the food list and insufficient data on 5-HMF concentrations.

3.1 Use of 5-HMF as a flavouring substance

Pursuant to Regulation (EC) No 2232/96 5-HMF may be used as a flavouring substance in food. It is listed under FL-No. 13.139 in the EU Register of Flavouring Substances (Decision of the European Commission 1999/217/EC). 5-HMF was assessed by the EFSA AFC Panel in April 2005 within the framework of the Flavouring Group Evaluation 13 (FGE.13). The panel came to the conclusion that there are no available data on 5-HMF, which could be used to clarify its genotoxic potential in vivo. Based on the production figures provided by the flavourings industry, EFSA estimated 5-HMF intake according to the Maximised Survey-derived Daily Intake (MSDI) approach and indicated this as 0.012 μg/person and day. In addition, the intake was estimated on the basis of the use levels indicated by the flavouring industry according to the modified-Theoretical Added Maximum Daily Intake (m-TAMDI) approach. The European Flavour and Fragrance Association (EFFA) informed EFSA that 5-HMF is used in 15 out of the 18 food categories mentioned in Annex III to Regulation (EC) No. 1565/2000. The normal levels of use envisaged in these food categories are in the range of 1 up to 5 mg/kg whereas maximum use levels are indicated with values of 5 up to 25 mg/kg. The intake estimated on this basis using the m-TAMDI approach in April 2005 is 1600 μg/person and day [48, 49]. This value is higher than the threshold of concern of 540 μg/person and day for the corresponding substance class II, which means that more exact exposure data (''more reliable intake data'') would be needed for a renewed assessment by EFSA. FGE.13 was updated in November 2009 by the CEF Panel now responsible for flavourings with regard to the assessment of additional flavourings (FGE.13Rev1) [49]. 5-HMF was only concerned by this update to the extent that the levels used were now also indicated for the food category 14.2 (''Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts'') [48, 49].

3.2 5-HMF in smoke flavourings and in caramel colours as food additives

5-HMF has been detected in woodsmoke and liquid smoke [1]. Smoke flavourings based on liquid smoke (primary smoke condensates produced by controlled thermal degradation of wood) are added to various foods to replace the traditional smoking process or to impart smoke flavour to foods that are not traditionally smoked [50]. Quantitative data on the 5-HMF levels are available on six of the eleven primary smoke condensates evaluated by EFSA [50–55]. The levels are in the range of 0.8 up to 2.6 mg/kg primary smoke condensate. Between 42 and 78 μg 5-HMF/kg bw and day (equal to 2.5 and 4.7 mg/day, respectively) may be ingested from the consumption of food that contains the primary smoke condensate Scansmoke PB 1110 with the 5-HMF level of 2.6 mg/kg taking into account the intakes of Scansmoke PB 1110 as estimated by EFSA (ranging from 16.2 to 28.3 mg/kg bw and day at normal use levels and 21.8–30.0 mg/kg bw and day at upper use levels [50]). Foods containing liquid smoke thus also contribute to the overall intake of 5-HMF.

5-HMF is also a constituent of caramel colours (class I, II, III and IV), which are widely used as food colours. For example, the 5-HMF contents were estimated to be in the ranges of 0.001–0.39% in caramel colour III (ammonia caramel) [56] and 0.50–2.56% in caramel colour IV (sulphite ammonia caramel) [57]. The four classes differ from each other with respect to manufacturing conditions, chemical and physical properties as well as use in recipes [56]. In an opinion on caramel colours (classes I-IV) published in March 2011, EFSA reported levels of 5-HMF in caramel colours which were in a broad range (0.001–3.37%) [58]. Exposure assessments for these caramel colours also vary extensively. EFSA did not perform any exposure assessment for 5-HMF. Assuming worst case scenarios based on highest reported levels of 5-HMF and the reported 97.5th percentile for exposure of caramel colours would result in very low margins of safety. However, analytical data on the concentrations of 5-HMF in foods containing caramel colours are currently not available and would be decisive for a reliable exposure assessment. The same holds true for foods

containing caramelized sugar. Currently, very few analytical data are available [37].

4 Risk characterisation and discussion

As discussed above, there are contradictory findings on the possible carcinogenicity of 5-HMF. Overall, however, the evidence of a carcinogenic potential is very limited. With regard to other toxic effects, no effects were observed at a daily dose in the range of 80–100 mg/kg bw in animal experiments. However, the number of studies conducted and the species used are limited. In addition, the critical effect is not clearly characterised. Based on these data, no conclusion on mechanisms of toxicity or their relevance for human beings can be drawn. There are no data on reproductive and developmental toxicity. Because of these uncertainties, it is not possible at the present time to establish a tolerable daily intake (TDI).

On the other hand, there is no evidence either that 5-HMF is a substance with high toxic potential. As described above, the maximum 5-HMF exposure resulting from sources other than caramel colours and beverages made from dried plums is expected to be lower than 500 μg/kg bw per day. Hence, there is a margin of safety of more than 100 and the margin of safety would even be higher if calculated based on average exposure. Thus, based on the data presently available, the consumption of 5-HMF-containing food is generally unlikely to be of safety concern. However, the available data on the occurrence of 5-HMF in caramel colours in conjunction with reported use levels of caramel colours in food indicate that the margins of safety may not be sufficient [58]. Therefore, analytical data on the concentrations of 5-HMF in foods containing caramel colours would be required for a reliable risk assessment.

Beverages made from dried plums are a special case and must be assessed separately. A worst case exposure of up to 4260 μg/kg bw per day was estimated (one glass per day with mean levels detected in German products), which is eight times higher than the maximum exposures estimated for other foods. However, even if consumers were drinking one glass of this beverage made from dried plums every day over a longer period, there would, nonetheless, still be a margin of safety of around 20 and so far there is no indication of any concrete health risk for such consumers.

The data on exposure are still incomplete; especially data on levels in coffee (beverage) and dairy products are sparse. However, there is no indication that there are other foods that have particularly high levels. It should be pointed out that on the basis of the data available, there is a need for analysis of the 5-HMF content in foods containing caramel colours (E 150a–E 150d).

Furthermore, an investigation of tissue-specific DNA adducts in order to clarify the relevance of contradictory findings on the carcinogenic potential of 5-HMF would be useful.

Human SULTs were reported to be more efficient with regard to the activation of 5-HMF than SULT in mice and rats [34]. Therefore, in vivo studies on genotoxicity in which the relevance of sulphation of 5-HMF is examined, for instance, studies on DNA adduct formation and induction of mutations in transgenic mice that express human SULT1A1 in comparison to wild-type mice would be considered useful.

The authors have declared no conflict of interest.

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