Risiken erkennen – Gesundheit schützen

# 5. Sitzung der BfR-Kommission für Lebensmittelzusatzstoffe, Aromastoffe und Verarbeitungshilfsstoffe (LAV-Kommission)

Protokoll der Sitzung vom 28. Oktober 2010

Die BfR-Kommission für Lebensmittelzusatzstoffe, Aromastoffe und Verarbeitungshilfsstoffe wurde 2008 gegründet. Sie besteht aus externen unabhängigen Sachverständigen und berät das Bundesinstitut für Risikobewertung zu Fragen, die in den Bereich der Risikobewertung von Lebensmittelzusatzstoffen, Aromastoffen und Verarbeitungshilfsstoffen fallen.

## 1 Begrüßung

Der Vorsitzende begrüßte die Teilnehmer.

## 2 Tagesordnung

Die Tagesordnung wurde angenommen.

## 3 Deklaration von Interessen

Nach mündlicher Auskunft der Teilnehmer lagen keine Interessenkonflikte in Bezug auf die in der Sitzung behandelten Themen vor.

## 4 Annahme des Protokolls der letzten Sitzung

Das Protokoll der Sitzung vom 18. Februar 2010 wurde angenommen.

## 5 Verwendung von Acetaldehyd als Aromastoff

Acetaldehyd ist ein in alkoholischen Getränken vorkommender aromawirksamer Stoff, der als Nebenprodukt der alkoholischen Gärung entsteht. Acetaldehyd kommt auch natürlicherweise in vielen Lebensmitteln vor. Im menschlichen Organismus entsteht Acetaldehyd u.a. als Zwischenprodukt beim Ethanolabbau durch das Enzym Alkoholdehydrogenase.

Acetaldehyd kann als Aromastoff verwendet werden. Maßgeblich ist hierfür die Entscheidung der Kommission Nr. 1999/217/EG vom 23. Februar 1999 über ein Verzeichnis der in oder auf Lebensmitteln verwendeten Aromastoffe, das gemäß Verordnung (EG) Nr. 2232/96 des Europäischen Parlaments und des Rates vom 28. Oktober 1996 erstellt wurde, zuletzt geändert durch Entscheidung der Kommission Nr. 2009/163/EG. Diese Entscheidung gilt gemäß Verordnung (EG) Nr. 1334/2008 bis zur Erstellung einer Gemeinschaftsliste. Die meisten der in diesem Verzeichnis aufgeführten Aromastoffe wurden im Rahmen eines Bewertungsprogramms von der European Food Safety Authority (EFSA) gesundheitlich bewertet. Acetaldehyd gehört zu den Aromastoffen, die vom Joint FAO/WHO Expert Committee on Food Additives (JECFA) bereits vor dem Jahr 2000 bewertet wurden und deshalb gemäß Artikel 2 der Verordnung (EG) Nr. 1565/2000 im Rahmen des in dieser Verordnung angesprochenen Be-

wertungsprogramms von der EFSA nicht erneut bewertet werden müssen, es sei denn, dass neue Erkenntnisse zu anderen Bewertungsergebnissen führen würden. Im Lichte der neuen Daten aus dem CVUA Karlsruhe zum Vorkommen von Acetaldehyd in Lebensmitteln sowie neuer Daten zur Toxizität erhob sich die Frage, ob eine Verwendung von Acetaldehyd als Aromastoff weiterhin akzeptabel ist, ob die neuen Daten zu einem anderen (von der JECFA-Bewertung abweichenden) Bewertungsergebnis führen könnten und ob insofern auch die EFSA damit zu befassen sei.

Diese Fragen wurden von der LAV-Kommission in mehreren Sitzungen intensiv diskutiert. Abschließend wurde hierzu folgende Stellungnahme verfasst (zur Begründung siehe Anhang):

Ausgehend von der Neueinstufung von Acetaldehyd durch IARC (*Secretan B et al. on behalf of the WHO IARC Monograph Working Group, 2009. A review of human carcinogens – Part E: tobacco, areca nut, alcohol, coal smoke, and salted fish. The Lancet Oncology 10, 1033-1034, November 2009*) und den von Lachenmeier et al. publizierten Daten (*Lachenmeier DW, Sohnius EM 2008. The role of acetaldehyde outside ethanol metabolism in the carcinogenicity of alcoholic beverages: evidence from a large chemical survey. Food Chem Toxicol. 46(8), 2903-2911. Lachenmeier et al., 2009. Carcinogenicity of acetaldehyde in alcoholic beverages: risk assessment outside ethanol metabolism. Addiction 104, 533-550. Lachenmeier et al., 2009. Salivary acetaldehyde increase due to alcohol-containing mouthwash use: a risk factor for oral cancer. Int J Cancer. 125(3):730-735*) hat sich die LAV-Kommission eingehend mit der Datenlage zur Risikobewertung von Acetaldehyd befasst und dabei unter anderem neue Daten zur Genotoxizität und Informationen zur Toxikokinetik berücksichtigt. Die Kommission kam zu dem Ergebnis, dass für eine abschließende Bewertung von Acetaldehyd als Aromastoff zusätzliche Studien erforderlich sind. Es gibt ausreichende Hinweise auf Genotoxizität von Acetaldehyd *in vitro*. Unter *in vivo*-Bedingungen ist die Genotoxizität oral aufgenommenen Acetaldehyds jedoch nicht ausreichend untersucht worden. Es kann davon ausgegangen werden, dass Acetaldehyd nach oraler Aufnahme in Darm und Leber effizient detoxifiziert wird, und es ist unwahrscheinlich, dass Acetaldehyd systemisch verfügbar wird. Deshalb ist anzunehmen, dass das genotoxische Potential *in vivo* auf die direkt exponierten Gewebe wie z.B. auf den oberen Aerodigestivtrakt (Mund- und Rachenraum und Ösophagus) begrenzt bleibt, in Analogie zu Formaldehyd (BfR 2006).

Die LAV-Kommission hält es für möglich, einen sicheren Höchstwert (“safe limit”) für die Aufnahme von Acetaldehyd über Lebensmittel abzuleiten. Die derzeit verfügbaren Daten reichen für einen derartigen Ansatz jedoch nicht aus. Folglich sollten weitere Studien auf die offenen Fragen hinsichtlich mechanistisch relevanter Endpunkte eingehen (z.B. Konzentration von Acetaldehyd in den Zielgeweben und deren metabolische Kapazität, Induktion von Zytotoxizität und Langzeittoxizität).

Gegenwärtig kann die Sicherheit von Acetaldehyd als Aromastoff nicht abschließend bewertet werden.

## 6 Verwendung von Ammoniumchlorid zur Herstellung von Lakritzwaren gemäß § 3 der Aromenverordnung

6.1 Mögliche Bildung von 4-Methylimidazol (4-MEI) und 2-Acetyl-tetrahydroxy-butylimidazol (THI) bei der Herstellung von Lakritzerzeugnissen

Nach § 3 in Verbindung mit Anlage 5 der Aromenverordnung ist Ammoniumchlorid zur Herstellung von Lakritzwaren in einer Höchstmenge von 20.000 mg/kg zugelassen. Dies entspricht einem Gehalt von 2 %. Für die Verwendung von Ammoniumchlorid in Mengen bis zu 7,99 % wurden Ausnahmegenehmigungen erteilt mit der Auflage, die Erzeugnisse besonders zu kennzeichnen.

Im BfR fiel jetzt auf, dass die Ausgangsverbindungen (Ammoniumverbindungen und reduzierende Zucker) zur Herstellung des Farbstoffs Ammoniak-Zuckerkulör (E 150c) auch bei der Herstellung von Salzlakritz eingesetzt werden und dass die Reaktionsbedingungen (Erhitzen auf über 100°C) ebenfalls ähnlich sind.

Bei der Herstellung von Ammoniak-Zuckerkulör entstehen die unerwünschten Imidazolderivate 4-Methylimidazol (4-MEI) und 2-Acetyl-tetrahydroxy-butylimidazol (THI). Mit 4-MEI wurden in Mäusen kanzerogene Wirkungen beobachtet. THI hat in tierexperimentellen Untersuchungen immunmodulatorische Wirkungen gezeigt. Für 4-MEI und THI wurden Höchstmengen von 250 mg/kg bzw. 10 mg/kg Zuckerkulör festgelegt (Richtlinie 2008/128/EG der Kommission vom 22. Dezember 2008 zur Festlegung spezifischer Reinheitskriterien für Lebensmittelfarbstoffe, Amtsblatt der Europäischen Union vom 10.01.2009, L 6/20).

Experimentelle Daten zu dem möglichen Vorkommen von 4-MEI und THI in Lakritzerzeugnissen sind nicht bekannt. Aus Sicht des BfR ist es jedoch vorstellbar, dass die beiden unerwünschten Stoffe 4-MEI und THI, die bei der Herstellung von Ammoniak-Zuckerkulör entstehen, auch bei der Herstellung von Stark-Lakritz (mit bis zu 7,99 % Ammoniumchlorid) und bei der Verwendung von Ammoniumchlorid als Aromavorstufe entstehen könnten. Es erhob sich die Frage, ob diese Einschätzung von der Kommission geteilt wird.

Die Kommission hat den Sachverhalt intensiv diskutiert. Sie hält es für möglich, dass Imidazolderivate ebenso wie bei der Herstellung von Ammoniak-Zuckerkulör, auch bei Verwendung von Ammoniumchlorid zur Herstellung von Lakritzerzeugnissen sowie bei der Verwendung von Ammoniumchlorid als Aromavorstufe entstehen könnten.

6.2 Analytik von 4-Methylimidazol (4-MEI) und 2-Acetyl-tetrahydroxy-butylimidazol (THI)

Für die Bestimmung von 4-MEI und THI existiert derzeit keine validierte Referenzmethode. Aus der Sicht der LAV-Kommission sollte eine gemeinsame Analytik beider Verbindungen mit HPLC-MS-MS möglich sein.

## 7 Verarbeitungshilfsstoffe

Verarbeitungshilfsstoffe werden auch als technische Hilfsstoffe bezeichnet, ihre Verwendung regelt die Technische Hilfsstoff-Verordnung (THV). Diese Verordnung gilt für Extraktionslösungsmittel und bestimmte Bleichmittel. Da die Bewertungen dieser Stoffe durch internationale Expertengremien zum Teil schon mehrere Jahre zurückliegen, wurde die LAV-

Kommission vom BfR gebeten zu prüfen, inwieweit Neubewertungen notwendig sind und ob die damals angewandten Bewertungsprinzipien weiterhin angemessen sind.

### 7.1 Wasserstoffperoxid

Wasserstoffperoxid darf entsprechend Anlage 5 zur Technischen Hilfsstoff-Verordnung (THV) als Bleichmittel für Stärke, Gelatine und Fischmarinaden „quantum satis“ eingesetzt werden. Die Kommission diskutierte den Einsatz und die Wirkungsweise von Wasserstoffperoxid. Wie schon in den vorhergehenden Sitzungen beschlossen, sollte eine abschließende Bewertung einzelner Verarbeitungshilfsstoffe jedoch erst später erfolgen, wenn auch über die Bewertungsprinzipien abschließend beraten wird.

### 7.2 Bewertungsprinzipien

Die Kommission diskutierte die mögliche Anwendbarkeit der für den Bereich der Lebensmittelverpackungen (Stoffe aus Materialien in direktem Kontakt mit Lebensmitteln) etablierten spezifischen Migrationsgrenzwerte auf die Verarbeitungshilfsstoffe. Die Kommission kam überein, dass die bei Lebensmittel-Verpackungsmaterialien angewandten Verfahren nicht direkt auf die Verarbeitungshilfsstoffe übertragen werden können. Da die Datenlage zu Verarbeitungshilfsstoffen recht unterschiedlich ist, wurde diskutiert, ob Mindestanforderungen an die Datenlage zweckmäßig sind. Die Kommission kam überein, diese Frage in der nächsten Berufungsperiode weiter zu diskutieren.

## 8 Termine der nächsten Sitzungen

Als Termin der nächsten Sitzung wurde Donnerstag, der 17. Februar 2011, festgelegt.

## 9 Sonstiges

Das Mandat der laufenden Berufungsperiode dieser Kommission endet am 31. Dezember 2010. Die neue Berufungsperiode beginnt am 01. Januar 2011. Der Vorsitzende würdigte die Organisation und inhaltliche Vorbereitung der Sitzungen durch die Geschäftsführung. Der Geschäftsführer bedankte sich bei den Kommissionsmitgliedern für die konstruktive Zusammenarbeit und die in dieser Berufungsperiode geleistete wissenschaftliche Beratung.

# Statement of the BfR Committee on Food Additives, Flavourings and Processing Aids (LAV) on the use of acetaldehyde as flavouring substance

**Summary**

Based on the new classification by IARC (*Secretan B et al. on behalf of the WHO IARC Monograph Working Group, 2009. A review of human carcinogens – Part E: tobacco, areca nut, alcohol, coal smoke, and salted fish. The Lancet Oncology 10, 1033-1034, November 2009*) and the data published by Lachenmeier et al. (*Lachenmeier DW, Sohnius EM 2008. The role of acetaldehyde outside ethanol metabolism in the carcinogenicity of alcoholic beverages: evidence from a large chemical survey. Food Chem Toxicol. 46(8), 2903-2911. Lachenmeier et al., 2009. Carcinogenicity of acetaldehyde in alcoholic beverages: risk assessment outside ethanol metabolism. Addiction 104, 533-550. Lachenmeier et al., 2009. Salivary acetaldehyde increase due to alcohol-containing mouthwash use: a risk factor for oral cancer. Int J Cancer. 125(3):730-735*) the LAV Committee thoroughly reviewed the existing data relevant for the risk assessment of acetaldehyde. In particular, new data regarding genotoxicity and information on toxicokinetics have been considered.

The LAV Committee concluded that additional studies are required for a final evaluation of acetaldehyde as flavouring substance. There is sufficient evidence for genotoxicity of acetaldehyde *in vitro*. However, under *in vivo* conditions acetaldehyde genotoxicity upon oral uptake has not been adequately studied. Upon oral uptake acetaldehyde is anticipated to be efficiently detoxified in intestine and liver and it appears unlikely that acetaldehyde will become available systemically. Therefore, any *in vivo* genotoxic potency is considered to be limited to directly exposed tissues such as the upper aerodigestive tract (including mouth and esophagus), in analogy to formaldehyde (BfR, 2006).

The LAV Committee is of the opinion that it should be possible to establish a safe limit for dietary exposure of acetaldehyde, but the presently available data are not sufficient to support such an approach. Consequently, further studies should address the open questions regarding mechanistically relevant endpoints (e.g. concentration of acetaldehyde in target tissues and their metabolic capacity, induction of cytotoxicity, long-term toxicity).

At present, the safety of acetaldehyde when used as flavouring substance cannot be finally evaluated.

**Zusammenfassung**

Ausgehend von der Neueinstufung von Acetaldehyd durch IARC (*Secretan B et al. on behalf of the WHO IARC Monograph Working Group, 2009. A review of human carcinogens – Part E: tobacco, areca nut, alcohol, coal smoke, and salted fish. The Lancet Oncology 10, 1033-1034, November 2009*) und den von Lachenmeier et al. (*Lachenmeier DW, Sohnius EM 2008. The role of acetaldehyde outside ethanol metabolism in the carcinogenicity of alcoholic beverages: evidence from a large chemical survey. Food Chem Toxicol. 46(8), 2903-2911. Lachenmeier et al., 2009. Carcinogenicity of acetaldehyde in alcoholic beverages: risk assessment outside ethanol metabolism. Addiction 104, 533-550. Lachenmeier et al., 2009. Salivary acetaldehyde increase due to alcohol-containing mouthwash use: a risk factor for oral cancer. Int J Cancer. 125(3):730-735*) publizierten Daten hat sich die LAV-Kommission eingehend mit der Datenlage zur Risikobewertung von Acetaldehyd befasst und dabei unter anderem neue Daten zur Genotoxizität und Informationen zur Toxikokinetik berücksichtigt. Die Kommission kam zu dem Ergebnis, dass für eine abschließende Bewertung von Acetaldehyd als Aromastoff zusätzliche Studien erforderlich sind. Es gibt ausreichende Hinweise auf Genotoxizität von Acetaldehyd *in vitro*. Unter *in vivo*-Bedingungen ist die Genotoxizität oral aufgenommenen Acetaldehyds jedoch nicht ausreichend untersucht worden. Es kann davon ausgegangen werden, dass Acetaldehyd nach oraler Aufnahme in Darm und Leber effizient detoxifiziert wird, und es ist unwahrscheinlich, dass Acetaldehyd systemisch verfügbar wird. Deshalb ist anzunehmen, dass das genotoxische Potential *in vivo* auf die direkt exponierten Gewebe wie z.B. auf den oberen Aerodigestivtrakt (Mund- und Rachenraum und Ösophagus) begrenzt bleibt, in Analogie zu Formaldehyd (BfR 2006).

Die LAV-Kommission hält es für möglich, einen sicheren Höchstwert (“safe limit”) für die Aufnahme von Acetaldehyd über Lebensmittel abzuleiten. Die derzeit verfügbaren Daten reichen für einen derartigen Ansatz jedoch nicht aus. Folglich sollten weitere Studien auf die offenen Fragen hinsichtlich mechanistisch relevanter Endpunkte eingehen (z.B. Konzentration von Acetaldehyd in den Zielgeweben und deren metabolische Kapazität, Induktion von Zytotoxizität und Langzeittoxizität).

Gegenwärtig kann die Sicherheit von Acetaldehyd als Aromastoff nicht abschließend bewertet werden.

## 1. Background and terms of reference

Acetaldehyde (AA) was evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1999. According to Article 2 of Commission Regulation (EC) 1565/2000 AA does not need to be re-evaluated within the present EU evaluation programme unless new information becomes available, which might lead to a change in the evaluation.

In response to a report on the occurrence of acetaldehyde in alcoholic beverages (Lachenmeier and Sohnius 2008), the BfR performed a risk assessment concerning the occurrence of acetaldehyde in wine and alcoholic beverages in December 2008 (updated version published in May 2010 (BfR 2010)). In this context, the following questions arose:

1. Is the use of AA as flavouring substance, i.e. its intentional addition to beverages and food, acceptable anymore?
2. Should the use of AA as flavouring substance be re-evaluated by EFSA?

## 2. Existing evaluations and current regulatory status

The use of acetaldehyde as a flavouring substance was evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) at its 49th meeting in 1997. The Committee concluded that acetaldehyde would not present a safety concern at the current levels of intake (JECFA 1999)[1].

According to Commission Decision 1999/217/EC, last amended by Commission Decision 2009/163/EC, acetaldehyde [FL-no 05.001] may be used as a flavouring substance.

According to Commission Directive 2002/72/EC acetaldehyde may be used as monomer or starting substance in the manufacture of plastic materials and articles intended to come into contact with foodstuffs. Its specific migration limit in food or in food simulant expressed as total of moiety or substance(s) indicated (SML(T)) is 6 mg/kg (Annex II of Directive 2002/72/EC). SML(T) in this specific case means that the restriction shall not be exceeded by the sum of the migration of the following substances mentioned as Ref. Nos: 10060 [acetaldehyde] and 23920 [Propionic acid, vinyl ester].

The former Scientific Committee on Food (SCF) evaluated the use of acetaldehyde in the manufacture of plastic materials and articles intended to come into contact with foodstuffs and derived for acetaldehyde and vinyl propionate a Group TDI of 0.1 mg/kg bw (SCF 1998).

According to Directive 67/548/EEC, acetaldehyde was classified into the carcinogenicity category 3 and, accordingly, the risk phrase R40 (limited evidence of a carcinogenic effect) was allocated.

Acetaldehyde has been evaluated by the International Agency for Research on Cancer (IARC 1985, 1999, Baan et al. 2007, Secretan et al. 2009). In the course of its last evaluation in 2009, IARC updated the cancer assessments of several personal habits and household exposures that cause cancer, including consumption of alcoholic beverages and the resulting exposure to acetaldehyde. It was concluded that acetaldehyde associated with alcohol consumption is carcinogenic to humans (Group 1) (Secretan et al. 2009). It should be emphasized that the classification of acetaldehyde into group 1 specifically applies to its exposure resulting from ethanol consumption, AA as a chemical still remains allocated to group 2B[2].

[1] JECFA 1999: "*Conclusion based on current levels of intake: No safety concern*". Cleared at step A4, i.e. the answer to the question "Is AA endogenous or metabolized to endogenous substances? is YES. Comment in JECFA evaluation: AA is oxidized to acetic acid which is metabolized via the citric acid cycle; AA can be also reduced to ethanol.

[2] The evaluation performed in October 2009 pertains specifically to "acetaldehyde associated with alcoholic consumption" (Secretan et al. 2009). The reason behind this is that the Working Group reviewed only the literature on alcohol and cancer, and did not review studies of occupational

## 3. Occurrence of acetaldehyde in foods and alcoholic beverages and intake resulting from the use as flavouring substance

### 3.1. Natural occurrence

The natural occurrence of AA was recently reviewed by the ANS-Panel of EFSA in the course of the evaluation of residual AA in sucrose esters of fatty acids (EFSA 2010): *"Acetaldehyde is reported to occur naturally in a variety of fruits and vegetables and other categories of food. According to the former Committee of Experts on Flavouring Substances of the Council of Europe, acetaldehyde occurs naturally in grapefruit juice (0.3 – 50 mg/kg), other fruits (up to 10 mg/kg), peas (1.2 - 400 mg/kg), other vegetables (up to 22 mg/kg), bread (4.2 – 9.96 mg/kg), cereals (up to 3.5 mg/kg), yoghurt (0.7 - 76 mg/kg), other milk products (up to 8 mg/kg), wine (white) (7.3 - 142 mg/kg) (CoE 2000). Acetaldehyde levels of up to 132 and 190 ppm were reported for orange juice and grapefruit juice, respectively (Lund et al 1981). The database "Volatile Compounds in Foods" (TNO, The Netherlands) contains data on the natural occurrence of acetaldehyde in many foods, e.g. carrot (0.45 – 16.9 mg/kg), tomato (0.015 - 9 mg/kg), fig (<1 - 40 mg/kg), grapefruit juice (40 - 230 mg/kg), wheaten bread (7 mg/kg), cheddar cheese (0.1 – 7.5 mg/kg), cheese (various types) (0.4 – 1.4 mg/kg), vinegar (20 - 1060 mg/kg), alcoholic beverages e.g. sherry (100 – 500 mg/kg) (TNO 2009). For many other foods only qualitative data were given in this database."*

### 3.2. Data on acetaldehyde concentrations in alcoholic beverages

Lachenmeier and Sohnius (2008) reported AA-concentrations in dessert wines (on average 118 mg/L, ranging from 12 to 800 mg/L) to be higher than in other alcoholic beverages.

### 3.3. Acetaldehyde intake resulting from its use as flavouring substance

In the course of the evaluation of flavouring substances to be included in the Union list, the dietary exposure considered by EFSA to assess their safety has been a *per capita* estimate, the "Maximised Survey-Derived Daily Intake" (MSDI), based on the annual volume of production reported by the applicant. Considering that the MSDI model may underestimate the intake of flavouring substances by certain groups of consumers, the SCF recommended also taking into account the results of other intake assessments (SCF, 1999).

<u>Estimated Daily per Capita Intake (MSDI) Approach</u>

The Maximised Survey-derived Daily Intake (MSDI (SCF, 1999)) data are derived from surveys on annual production volumes in Europe. These surveys were conducted in 1995 by the International Organization of the Flavour Industry, in which flavour manufacturers reported the total amount of each flavouring substance incorporated into food sold in the EU during the previous year (IOFI 1995). The intake approach does not consider the possible natural occurrence in food. Average *per capita* intake (MSDI) is estimated based on the assumption that the amount added to food is consumed by 10 % of the population[3] (Eurostat, 1998). This is derived for a flavouring substance from estimates of annual volume of production provided by industry and incorporates a correction factor of 0.6 to allow for incomplete reporting (60

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exposure of other possibles routes and modes of exposure. Acetaldehyde as a chemical is still in Group 2B.

[3] EU population: 375 millions (Eurostat, 1998). This population figure relates to EU population at the time production data were available, and is consistent (comparable) with evaluations conducted prior to the enlargement of the EU. No production data are available for the enlarged EU.

%) in the industry surveys (SCF, 1999). The MSDI approach takes into consideration the dietary exposure of a 60 kg adult.

The MSDI for AA was estimated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) to be 9.7 (USA) and 11 (Europe) mg per person per day (JECFA 1998). The population figure for Europe was based on a population of 320 x $10^6$ persons (10 % eaters = 32 x $10^6$ persons).

According to the International Organization of the Flavour Industry (IOFI), the reported volume of AA use in flavourings in Europe increased from 77931 kg (in 1995) to 130479 kg (in 2004) (IOFI 2009). IOFI noted that the latter figure was obtained from the European Flavour and Fragrance Association (EFFA). These data demonstrate that the use of AA in Europe was 1.7-fold higher in 2004 than in 1995. However, the European population was also larger in 2004 than in 1995. Based on the reported volume of use of 130479 kg and on a population of 385 x $10^6$ persons in the EU-15 countries on 1 January 2005 (Eurostat 2006) (10 % eaters = 38.5 x $10^6$ persons), the MSDI would be 15.4 mg per person per day. Based on 459 x $10^6$ persons in the EU-25 countries on 1 January 2005 (Eurostat 2006) (10 countries joint the EU on 1 May 2004) (10 % eaters = 45.9 x $10^6$ persons), the MSDI would be 12.9 mg per person per day.

Theoretical Added Maximum Daily Intake (mTAMDI)

Considering that the MSDI model may underestimate the intake of flavouring substances by certain groups of consumers, the SCF recommended to take also into account the results of other intake assessments (SCF, 1999).

One of the alternatives to the MSDI estimates is the “Theoretical Added Maximum Daily Intake” (TAMDI) approach, which is calculated on the basis of standard portions and upper use levels (SCF, 1995) for flavourable beverages and foods in general, with exceptional levels for particular foods. This method is regarded as a conservative estimate of the actual intake in most consumers because it is based on the assumption that the consumer regularly eats and drinks several food products containing the same flavouring substance at the upper use level. One option to modify the TAMDI-approach is to base the calculation on normal rather than upper use levels of the flavouring substances. This “modified Theoretical Added Maximum Daily Intake” (mTAMDI) approach is less conservative (e.g., it may underestimate the intake of consumers being loyal to products flavoured at the maximum use levels reported (EC, 2000). However, it is considered as a suitable tool to screen and prioritise the flavouring substances according to the need for refined intake data (EFSA, 2004). As to the EU evaluation program for flavouring substances, the mTAMDI value was only used as a tool to screen and prioritise the flavouring substances according to the need for refined intake data (EFSA, 2004). The mTAMDI approach (like MSDI approach) takes into consideration the dietary exposure of a 60 kg adult.

The mTAMDI approach requires information on use levels of a flavouring substance for the 18 food categories according to Annex III of Commission Regulation EC (No) 1565/2000. Acetaldehyde was evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1999. According to Article 2 of Commission Regulation (EC) 1565/2000 acetaldehyde was not re-evaluated within the EU evaluation programme. Consequently, industry did not have to submit data on use levels for AA and thus, data required for (m)TAMDI calculations are not available to date.

## 4. Absorption, distribution, metabolism, elimination (ADME)

AA is an endogenous metabolite, resulting from oxidative decarboxylation of pyruvate or degradation of threonine in eukaryotic cells; it can also be produced by microorganisms in the oral cavity and intestine. AA is oxidized to acetic acid, which is further metabolized via the citric acid cycle; AA can also be reduced to ethanol. Taking into account an AA concentration in blood of 2.2 µmol/L and the AA clearance via liver (1,6 L/min.) and blood (0,95 L/min.), the endogenous AA formation has been estimated to be 5,6 µmol (0,25 mg) per minute (MAK 2008). Upon oral uptake, approx. 99 % of AA will be eliminated by a first pass effect in intestine and liver (Lindros 1978; Eriksson and Fukunga 1992; Matysiak-Budnik et al. 1996). Furthermore, the equilibrium resulting from the oxidation reaction of ethanol to AA is in favour of ethanol. Therefore, (a) oral uptake of AA at the level of MSDI (approx. 15 mg/person/day) is considered not to increase the blood level, (b) it appears unlikely that AA will exert systemic adverse effects and therefore (c) the upper aerodigestive tract tissues and esophagus are considered to be the relevant targets for potential adverse effects of AA upon oral uptake.

## 5. Toxicity

### 5.1. Genotoxicity studies

#### 5.1.1. Evaluation by MAK-Kommission

AA genotoxicity has recently been evaluated by the MAK-Kommission of the Deutsche Forschungsgemeinschaft (MAK 2008). The data available on AA genotoxicity comprise bacterial assays, SCE (human lymphocytes), comet assays (human lymphocytes, mucosa cells, CHO-cells), alkaline elution (human lymphocytes, bronchial cells, CHO cells), mouse lymphoma assays, chromosome aberration, micronuclei and hypoxanthine phosphoribosyltransferase-1 (HPRT1)-test (human lymphocytes) (MAK 2008, see Acetaldehyde Tab. 5 for *in vitro* and Tab. 6 for *in vivo* data). AA has also been found to cause DNA adducts (Fang and Vaca 1995, Vaca et al. 1995, 1998, Fränkel-Conrat and Singer 1988, Inagaki et al. 2003) and to interfer with DNA synthesis and repair (Obe et al. 1986; Dellarco 1988; Helander et al. 1991; Maffei et al. 2000, 2002; Matsuda et al. 1998). Furthermore, AA binds to the antioxidant glutathione as well as to proteins and may thereby cause structural and functional alterations of enzymes involved in DNA repair (Seitz and Stickel 2006).

MAK concluded that AA is clastogenic, aneugenic, weakly mutagenic *in vitro* and clastogenic *in vivo*. LAV agrees with the MAK conclusions based upon the data set as used for the “Toxikologisch-arbeitsmedizinische Begründung von MAK-Werten” published in 2008.

Since March 2006 (closure of the MAK reference list) three new studies regarding the genotoxicity of acetaldehyde have been published. In the paper by Ishikawa et al. (2007) it has been shown that the lymphocytic micronuclei frequency was increased in non-smoking habitual (drinking > 3 times per week) and moderate drinkers (drinking < 60 g alcohol each time they consumed alcohol) with acetaldehyde dehydrogenase-2 (ALDH-2) gene polymorphism when compared to that of habitual and moderate drinkers having a wild-type ALDH-2 gene. This is the first evidence that an ALDH-2 gene polymorphism is associated with an enhanced genotoxicity in humans drinking habitually moderate amounts of alcohol. However, this association could not be observed in habitual heavy drinkers with a smoking habit. Speit et al. (2008) reported a concentration-related increase in the frequency of sister chromatid exchange (SCE) and micronuclei (MN) in cultured mammalian cells. In the very recent *in vitro* study by Kayani and Parry (2010) it has been shown that ethanol itself is genotoxic (through an aneugenic mechanism), whereas acetaldehyde is a clastogen. The results of these studies support the previous conclusion on AA genotoxicity.

### 5.1.2. Evaluation of acetaldehyde according to the EFSA guidance on the data required for the risk assessment of flavourings

Within the framework of the EU-evaluation programme, risk assessment of flavouring substances follows a tiered approach as outlined in Fig.1 (Overall strategy for the risk assessment of flavouring substances, EFSA 2010). One of the key elements of the risk assessment strategy for flavouring substances is the evaluation of their genotoxic potency. Genotoxicity of a substance results in safety concern. On the other hand, if genotoxicity can be excluded, a substance will be subjected to the further steps of the "Procedure" (EFSA 2010). Briefly, the "Procedure" again is a stepwise approach that integrates information on intake from current uses, structure-activity relationships, metabolism and, when needed, toxicity.

In the course of evaluating the use of **AA as flavouring substance**, it was checked whether the available data sets on AA genotoxicity fulfil the requirements for assessment of the genotoxic potential of flavouring substances as drafted by EFSA 2010. According to the guidelines for evaluation of flavouring substances *"the assessment should start with in vitro tests, covering all three genetic endpoints, i.e. gene mutations, structural and numerical chromosomal aberrations. The following three in vitro tests would normally be required:*

- *a test for induction of gene mutations in bacteria (Ames test; OECD Guideline 471);*
- *a test for induction of gene mutations in mammalian cells (preferably the mouse lymphoma tk assay with colony sizing; OECD Guideline 476);*
- *an in vitro chromosomal aberration test (OECD Guideline 473) or an in vitro micronucleus assay (Draft OECD Guideline 487).*

*There may be circumstances under which it may be justified to deviate from the above-mentioned core set. In such cases a scientific justification should be provided and additional types of considerations or mechanistic studies may be needed.*

*One or more positive in vitro tests normally require follow-up by in vivo testing, unless it can be adequately demonstrated that the positive in vitro findings are not relevant for the in vivo situation.*

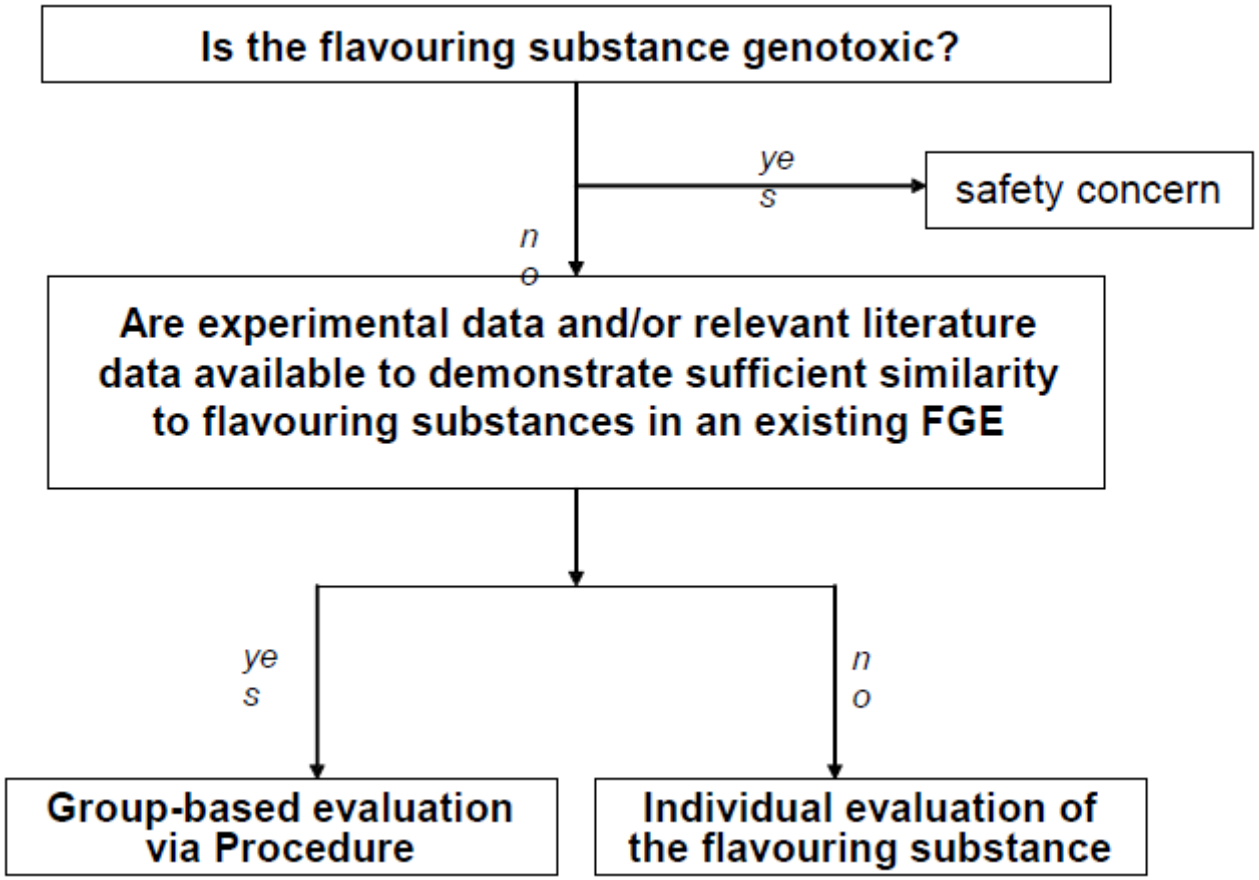


**Figure 1. Overall strategy for the risk assessment of flavouring substances. (**EFSA 2010)

*The choice of the appropriate in vivo test is critical, due to different sensitivities, different endpoints and other variables. It requires expert judgement based on all available information, to be applied case-by-case. For this reason, a flexible approach is preferable to a fixed decision tree.*

*Guidance for the follow-up on positive results from in vitro assays could be taken from a guidance document issued recently by the European Chemicals Agency (ECHA, 2008), which recommends that any of the following tests may be conducted:*

- *a rodent bone marrow or mouse peripheral blood micronucleus test (OECD Guideline 474) or a rodent bone marrow clastogenicity study (OECD Guideline 475);*
- *a Comet (single cell gel electrophoresis) assay;*
- *a test for gene mutations in a transgenic rodent model, e.g. using lacI, lacZ or cII as reporter gene;*

*According to this ECHA guidance (ECHA 2008), "the nature of the original in vitro response(s) (i.e. gene mutation, structural or numerical chromosome aberration) should be considered when selecting the in vivo study. For example, if the test substance showed evidence of in vitro clastogenicity, then it would be most appropriate to follow this up with either a micronucleus test or chromosomal aberration test or a Comet assay. However, if a positive result were obtained in the in vitro micronucleus test, the rodent micronucleus test would be appropriate to best address clastogenic and aneugenic potential. The rat liver UDS test may be appropriate for substances that appear preferentially to induce gene mutations, although the Comet and transgenic tests are also suitable (Kirkland and Speit, 2008). These latter test systems offer greater flexibility, most notably the possibility of selecting a range of tissues for study on the basis of what is known of the toxicokinetics and toxicodynamics of the substance. It should be realised that the UDS and Comet tests are indicator assays detecting putative DNA lesions. In contrast, the transgenic test measures permanent mutations."*

*A combination of the in vivo micronucleus assay and the Comet assay in a single study as suggested by Pfuhler et al. (2007) would also be acceptable.*

*Other studies (e.g. DNA adduct studies) could also be relevant in order to clarify the mechanism of genotoxicity.*

*It should also be taken into account that the sensitivity (ability to detect carcinogens as positive) and specificity (ability to give negative results with non-carcinogens) of such assays have recently been analysed by Kirkland and Speit (2008).*

*Studies should be conducted using internationally agreed protocols. Test methods described by OECD or in European Commission Directives are recommended. The most up-to-date edition of any test Guidelines should be followed. Studies should be carried out according to the principles of Good Laboratory Practice (GLP) described in Council Directives 87/18/EEC and 88/320/EEC and accompanied by a statement of GLP compliance. Use of any methods differing from internationally agreed protocols should be justified. An OECD Guideline is not yet available for the Comet assay. However, recommendations for an appropriate performance of the assay using OECD Guidelines for other in vivo tests have been published and a standard protocol and acceptance criteria for this assay have been developed through the International Workshop on Genotoxicity Working Parties and international Comet assay workshops (Tice et al., 2000; Hartman et al., 2003; Burlinson et al., 2007). Additional information could be taken from a website on the Comet assay (http://cometassay.com)".*

Data sets on AA genotoxicity *in vitro* match with the test battery suggested by EFSA as AA has been subjected to bacterial assays, mouse lymphoma *tk*-assay, chromosomal aberration

and micronucleus tests (MAK 2008; see Acetaldehyde Tab. 5). In addition, results of comet assays with human mucosa cells from stomach and colon are available. In summary, the bacterial assays with and without metabolic activation gave negative, all other tests for AA-genotoxicity gave positive results. Thus, there is sufficient evidence for genotoxicity of AA *in vitro* and this would require follow-up by *in vivo*-testing.

In those cases, in which the genotoxicity of AA was analyzed in rodents *in vivo*, either the intraperitoneal route (Morita et al.1997; Ma et al. 1985 cited in MAK 2008) or the intraamniotic application (Bariliak and Kozachuk 1983; IARC1999 cited in MAK 2008) were chosen. Furthermore, the genotoxicity of AA following oral uptake has only been tested in *Drosophila melanogaster* (via feed), providing negative and weakly positive results that, if at all, can provide some support to the *in vitro* data sets (MAK 2008). However, the present LAV opinion addresses the use of AA as flavouring substance and, therefore, data following oral uptake are considered to be the most relevant to evaluate AA genotoxicity.

### 5.1.3. LAV conclusion on genotoxicity of acetaldehyde

LAV concluded that AA genotoxicity upon oral uptake has not been adequately studied *in vivo*; however, a genotoxic action of AA upon oral administration may be expected. In this respect it should be pointed out that upon oral uptake AA is efficiently detoxified in the intestine and liver, and it appears unlikely that AA will become systemically available. Therefore, any *in vivo* genotoxic potential is considered to be limited to directly exposed tissues such as the upper aerodigestive tract (including mouth and esophagus), in analogy to previous conclusions drawn by BfR (2006) on formaldehyde.

## 5.2. Carcinogenicity studies

### 5.2.1. Animal studies

Inhalation studies in rats and hamsters revealed that AA caused nasal adenocarcinomas and squamous cell carcinomas; IARC classified AA as “possibly carcinogenic to humans” (IARC 71, 1999). The answer to the question addressed in the present opinion, however, would require carcinogenicity studies with oral AA administration. To date, only one study with oral AA application has been published (Soffritti et al 2002). This study has been used by Lachenmeier et al. (2009 a,b) for benchmark dose analysis and to derive a Margin of Exposure (MOE). However, for the reasons described in Appendix I in detail, LAV concludes that the study by Soffritti et al. (2002) cannot be used for these purposes and cancer risk assessment. Likewise, MAK 2008 as well as the risk assessment report on vinyl acetate, in which the study of Soffritti et al. (2002) on acetaldehyde was commented (ECB 2008), concluded: *“Soffritti and his colleagues (Soffritti et al., 2002) published the results of an oral carcinogenicity study that may correspond to the positive carcinogenicity bioassay that were announced by Maltoni et al. (1997). Acetaldehyde was administered to 50 male and 50 female Sprague-Dawley rats for 104 weeks in drinking water at concentrations of 0, 50, 250, 500, 1500, or 2500 mg/L. Increased rates of tumors at several organs were observed in treated groups. However, the effects were not dose-related and no clear conclusion could be drawn from this study.”*

### 5.2.2. Evidence for carcinogenic action of acetaldehyde in humans

In the context of studies on ethanol-induced human cancer, accumulating evidence suggests a significant role of AA, in particular in the pathogenesis of esophageal tumors. In 2007, IARC evaluated alcoholic beverages and classified ethanol as carcinogen to humans (group 1; IARC Vol. 96, 2007). In addition, IARC concluded that *"the substantial mechanistic evidence in humans deficient in aldehyde dehydrogenase indicates that acetaldehyde derived from the metabolism of ethanol in alcoholic beverages contributes to causing malignant esophageal tumours"* (IARC, Vol 96, 2007).
The mechanistic evidence indicating a causal relationship between AA exposure resulting from ethanol consumption (cf chapter 2, footnote2) and development of esophageal cancer is based upon ethanol metabolism and the polymorphism of the enzymes involved, namely alcohol dehydrogenase (ADH; oxidizing ethanol to acetaldehyde) and aldehyde dehydrogenase (ALDH; detoxifying AA to acetate; for review see Seitz and Stickel 2007).

EtOH $\xrightarrow[ADH]{}$ Acetaldehyde $\xrightarrow[ALDH]{}$ Acetic acid

The IARC Working Group concluded that *"the variant allele ALDH2*2, which encodes an inactive subunit of the enzyme ALDH2, is dominant and highly prevalent in certain eastern-Asian populations (28–45 %), but rare in other ethnic groups. Most homozygous carriers of this allele (ALDH2*2/*2) are abstainers or infrequent drinkers, because the enzyme deficiency would cause a strong facial flushing response, physical discomfort, and severe toxic reactions. In heterozygous carriers (ALDH2*1/*2, with about 10 % residual ALDH2 activity) these acute adverse effects are less severe, but when they consume alcohol these carriers are at high risk for several alcohol-related aerodigestive cancers. For example, genetic epidemiological studies provide strong evidence that the heterozygous ALDH2*1/*2 genotype contributes substantially to the development of esophageal cancer related to alcohol consumption, with relative risks — compared with carriers of the homozygous ALDH2*1/*1 genotype, which encodes the active enzyme — of up to 12 for heavy drinkers. Compared with those with the ALDH2*1/*1 genotype, the heterozygous carriers have higher levels of acetaldehyde in blood and saliva after alcohol drinking, and in a recent study higher levels of acetaldehyde-related DNA adducts have been measured in their lymphocytes."* (IARC 2007).

LAV reviewed the literature on ALDH polymorphisms in the context of genotoxicity and cancer risk caused by AA, including studies published after the recent IARC classification (Secretan et al. 2009[4]). LAV recognized on the one hand that the epidemiological studies did not always reveal a significant enhancement of the relative risk (RR) or odds ratio (OR) for aerodigestive tract cancers or signs of genotoxicity associated with a specific genotype; on the other hand, in some cases it did, but only if very high ethanol consumption scenarios were considered. However, the data available on ALDH polymorphism consistently indicate a positive correlation between the ALDH2*1/2 genotpye and enhancement of esophageal cancer risk as well as an increased frequency of genotoxic events such as DNA adduct formation, sister chromatid exchange and micronuclei formation.

### 5.2.3. Mode of action underlying acetaldehyde-induced carcinogenesis

[4] Boccia et al 2009; Cui et al. 2009; Ding et al. 2009;Fang and Vaca 1995, 1997; Ishikawa et al. 2007; Lee et al. 2010;Li et al. 2008; Maehara 2010; Matsuda 2006; Morimoto et al. 1996; Morita et al. 2010; Oze et al. 2009; Takesita et al. 1997; Tanaka et al. 2010; Yang et al. 2010; Yokoyama et al. 1998, 2002, 2003, 2006; Zhang et al. 2010

Data on the mode of action underlying AA-induced carcinogenesis have mainly been collected from inhalation studies (MAK 2008). Tumor formation upon inhalative AA exposure is considered to include genotoxic events as well as AA-induced tissue damage as a consequence of irritation, similar to formaldehyde (MAK 2008, BfR 2006). At non-cytotoxic concentrations, AA appears to act as a weak initiator (Woutersen et al. 1985 as cited by MAK 2008). Cytotoxic AA concentrations, however, give rise to regenerative growth of affected epithelia, which substantially contributes to tumor development.
As in the case of AA resulting from alcohol consumption, AA used as flavouring substance is taken up orally and, consequently, epithelial cells of the upper aerodigestive tract (including mouth and esophagus) can be considered as the potential primary targets for AA:

- in case of ethanol exposure resulting from consumption of alcoholic beverages, AA could be formed either extracellularly by bacteria (e.g. in oral cavity) followed by AA resorption by epithelial cells (as would be the case if used as flavouring substance) or upon ethanol resorption, intracellularly by oxidation. Both pathways would particularly be affected by an ALDH gene polymorphism.
- AA as flavouring substance: AA resorption by epithelial cells, again ALDH gene polymorphism would affect the AA detoxification capacity.

No numeric values can be attributed to either conceivable pathway. However, LAV concluded that the mechanistic evidence for the role of AA as ethanol metabolite in esophageal cancer etiology also applies to direct AA exposure of epithelial cells of the upper aerodigestive tract (including mouth and esophagus) upon consumption of food or beverages flavoured with AA.

## 6. Conclusions

LAV concluded that additional studies are required for a final evaluation of AA as flavouring substance. There is sufficient evidence for genotoxicity of AA *in vitro*. However, under *in vivo* conditions AA genotoxicity upon oral uptake has not been adequately studied. Following oral uptake AA is anticipated to be efficiently detoxified in intestine and liver and it appears unlikely that AA will become systemically available. Therefore, any *in vivo* genotoxic potential is considered to be limited to directly exposed tissues such as the upper aerodigestive tract (including mouth and esophagus), in analogy to formaldehyde (BfR, 2006).

LAV is of the opinion that it should be possible to establish a safe limit for dietary exposure of AA, but the presently available data are not sufficient to support such an approach. Consequently, further studies should address the open questions regarding mechanistically relevant endpoints (e.g. concentration of AA in target tissues and their metabolic capacity, induction of cytotoxicity, long-term toxicity).

At present, the safety of acetaldehyde when used as flavouring substance cannot be finally evaluated.

## 7. References

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# APPENDIX I

## Comment on the publication of Lachenmeier et al. (2009)

### 1. Description of the study by Soffritti et al. (2002) used for BMD analysis by Lachenmeier et al. (2009a, 2009b)

Acetaldehyde was administered to 50 male and 50 female Sprague-Dawley rats beginning at six weeks of age at concentrations of 2500, 1500, 500, 250, 50, or 0 mg/L. Animals were kept under observation until the last animal died in week 161. The authors concluded that acetaldehyde led to an increase in the total number of malignant tumors in the treated groups and showed specific carcinogenic effects on various organs and tissues (Soffritti et al. 2002). Data on tumor-bearing animals as well as tumors of the oral cavity are plotted in Fig I,1 (taken from Soffritti et al. 2002, Table 4, 5 and 6).

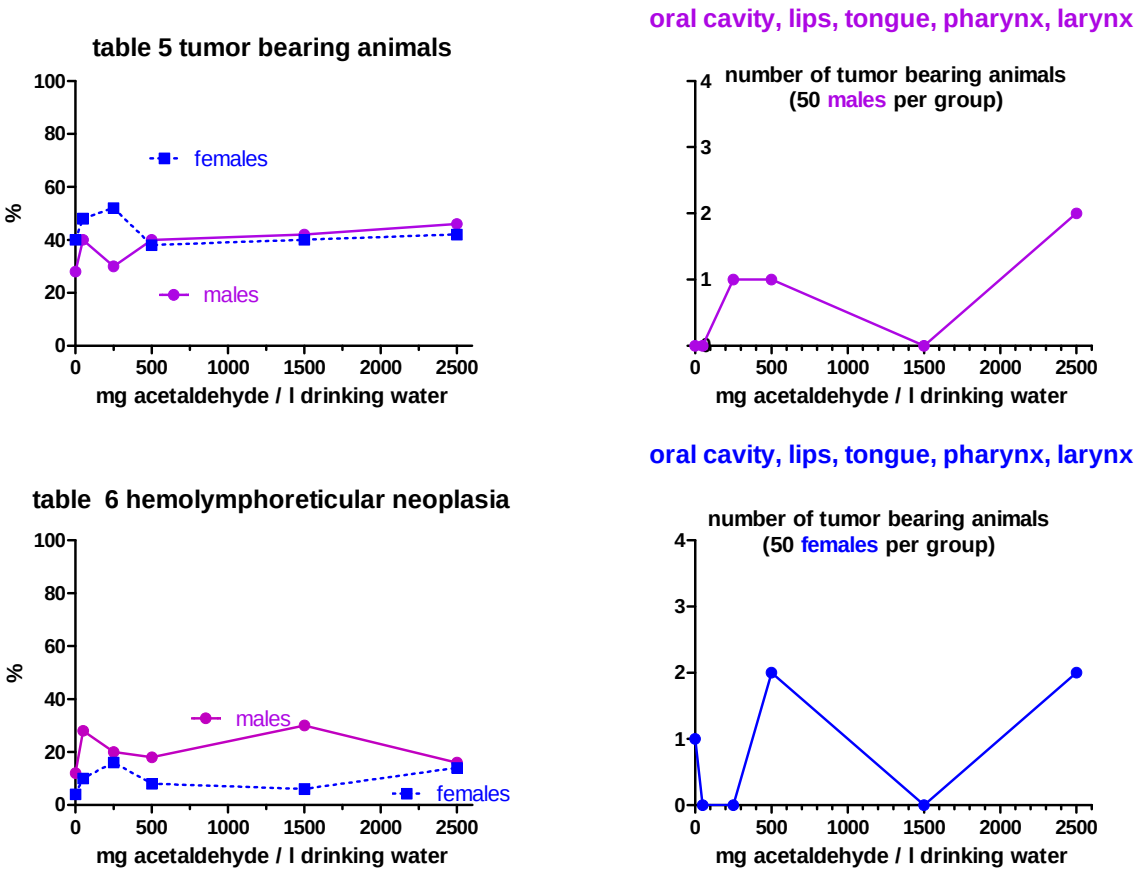


Fig I,1

## 2. Evaluation

### 2.1. Design of the study performed by Soffritti et al. (2002) and relevance of endpoints for assessment of cancer risk resulting from oral uptake of acetaldehyde

***Study design and report of data***

The life-span carcinogenicity bioassay design used in the study on AA does not meet the study duration as recommended in OECD Test Guideline 451: the study ended with the death of the last animal in week 161; 13 individuals of the middle dose-group bearing haemolymphoreticular neoplasia died on average after 71 weeks, whereas 15 animals of the highest dose groups survived until approx. week 104; 8 animals of the control group survived until week 113. Soffritti et al. (2002) considered that extending the treatment period until the natural death of rats increased the sensitivity of the assay. The disadvantages of this approach were addressed in detail by BfR (2006) when evaluating the formaldehyde chapter of this study. Likewise, an expert group of EFSA (2006a) evaluated a study on aspartame performed by the same research institution and noted that this approach includes an increase in background pathology as well as a higher probability of autolytic changes and consequently, might limit the validity of the data and the conclusions that can be drawn (EFSA 2006a).

Furthermore, no data were presented on the incidence of tumour-related mortalities and deaths associated with other non-neoplastic causes (i.e. unexpected infections or high mortality rates in controls).

***Relevance of endpoints for assessment of cancer risk resulting from oral uptake of AA (qualitative aspect)***

BfR and EFSA expert groups expressed major concerns regarding the validity of cancer studies performed with rat colonies kept at the European Ramazzini Foundation for Oncology, resulting from the predominance of hemolymphoreticular neoplasias (histiocytomas, lymphomas, leukaemia) upon formaldehyde and aspartame treatment (BfR 2006, EFSA 2006a,b).

BfR 2006: "*The statistical significance of the tumor response at high dose levels and its dose relationship would strongly support that formaldehyde was associated with systemic carcinogenicity in Sprague-Dawley rats. But, for interpretation of the results from Soffritti's studies some aspects should be considered which might limit the validity of the data:*

*A major limit are the new data from Soffritti's own reevaluation of his early study (Soffritti et al., 2002a). A review of the same tissues revealed marked excess in tumor rates in control and treated rats. The tumor incidences of hemopoietic neoplasias and of those in the gastrointestinal tract were nearly twice compared to the original publication. Since no explanation (such as extended evaluations by higher numbers of tissue sections examined) were given, the extraordinary excess of tumors raises some concern on the validity of the study reevaluation and also on the credibility of the originial study."*

BfR (2006) concluded on hemopoietic neoplasias (HPN) as follows*: "In a carcinogenicity study on Sprague-Dawley rats oral administration of formaldehyde increased the rates of tumors of the hemopoietic system (Soffritti et al., 1989, 2002a). Due to the limitations of this study and the strong contrast of the findings to the negative results of another valid carcinogenicity study in the Wistar rat, a firm conclusion on a potential for formaldehyde- associated induction of hemopoietic neoplasias in experimental animals can not be drawn.*

*The contradictory response in the Soffritti studies might be attributable to a strain-specific response in the Sprague Dawley rat or to the unusual study design. If so, this needs further confirmation or support by other data to investigate strain specificity. In a reliable inhalative carcinogenicity study in rats no increase in tumors of the hemopoietic system has been observed. Thus, this does not support the concern from the Soffritti group. No oral carcinogenicity studies were available for the mouse as a second test species.*

*Overall, the weight of evidence that formaldehyde has the potential to induce neoplasias of the hemopoietic system in experimental animals is insufficient."*

EFSA 2006b: "*However, the lack of data on background incidence of chronic inflammatory changes in the lungs of treated rats does not allow to evaluate the influence of chronic respiratory diseases in the development of lymphomas/leukemias, a well known inducing factor (EFSA, 2006). Indeed, the lack of appreciation of this infectious parameter was identified as a major confounder in a recent review of a longterm study from the same laboratory using the same rat colony (EFSA, 2006). Moreover, data presented in Soffritti et al. (2002) would come from its own re-evaluation of a previous study on formaldehyde carried out by the authors in which the incidence of haemopoietic neoplasias were nearly half those reported in 2002 (BfR, 2006). The lack of explanation for this discrepancy raised concerns on the study's validity and credibility (BfR, 2006)."*

Furthermore, a fundamental aspect in the assessment of the cancer risk resulting from oral uptake of AA is whether its target sites *in vivo* are limited to directly exposed tissues or not. Studies on AA induced carcinogenesis in the respiratory tract upon inhalative exposure suggest a combined action of AA genotoxicity along with cytotoxicity resulting in epithelial tissue regeneration and tumor promotion (IARC 1999; MAK 2008). Due to the high reactivity and efficient cellular metabolism of acetaldehyde, both effects appear to be limited to directly exposed epithelia of the respiratory tract and may not be readily observed in distant tissues (MAK 2008). Hemolymphoreticular neoplasias induced by oral uptake of AA would imply its systemic carcinogenic action. However, as outlined above, a systemic carcinogenic effect of orally administered AA is not to be expected and, therefore, the predominance of this tumor type upon AA treatment, as reported by Soffritti and colleagues, remains unexplained at the present time.

LAV concluded that the concerns put forward by BfR (2006) on the formaldehyde data set also apply to the AA part of the same study. Furthermore, based upon the mode of action underlying AA carcinogenicity in the respiratory tract, it appears unlikely that AA (like formaldehyde, aspartame) predominantly induce hemolymphoreticular tumors. Notably, esophageal cancer was not addressed in the study by Soffritti et al. (2002). Taken together, no conclusion relevant for risk assessment of AA upon oral uptake can be drawn from this study.

### 2.2. Benchmark Dose (BMD) analysis by Lachenmeier et al. (2009)

The data by Soffritti et al. (2002), as used by Lachenmeier et al. for BMD analysis, were also examined regarding the recommendations described in the BMD Methodology document published by EPA (http://www.epa.gov/NCEA/bmds/bmds_training/methodology/intro.htm). Two questions are addressed:

**1. Is the endpoint as selected by Lachenmeier et al. 2009, namely the number of tumor bearing animals, of relevance to evaluate the carcinogenicity of acetaldehyde upon oral uptake (qualitative aspect)?**

Based on the limitations of the study outlined in the previous section, LAV concluded that the BMD analysis, as performed by Lachenmeier et al. (2009 a,b), is based on a non-relevant endpoint for risk assessment of AA upon oral uptake.

**2. Are the data appropriate for a BMD analysis (quantitative aspect)?**

Irrespective of the inappropriate endpoint used, the data set on the number of tumor bearing animals was examined on the basis of the US-EPA Benchmark Dose Software (BMDS) Methodology (http://www.epa.gov/ncea/bmds/bmds_training/index.htm; see also EFSA 2009). According to the US-EPA recommendations, the following constraints should be applied on data sets to be used for BMD calculations:

a) *"There must be at least a statistically or biologically significant **dose-related trend** in the selected endpoint; and*
b) *The data set should contain information relevant to dose-response for modeling. A determination of the amount of information about the dose-response that is available need not be quantitative or technical. For example: A data set in which all non-control doses have essentially the same response level provides limited information about the dose-response, since the complete range of response from background to maximum must occur somewhere below the lowest dose: the BMD may be just below the first dose, or orders of magnitude lower. When this situation arises in quantal data, especially if the maximum response is less than 100 %, it is tempting to use a model like the Weibull with no restrictions on the power parameter, because such models reach a plateau of less than 100 %. This situation can result in seriously distorted BMDs, because the model predictions jump rapidly from background levels to the maximum level. In principle, other models could be found that force the BMD to be anywhere between that extreme and the lowest administered dose. Thus the BMD computed here depends solely on the model selected, and goodness of fit provides no help in selecting among the possibilities."*

As shown in Figure I.1., neither the total number of tumor-bearing animals nor tumors found in oral cavity, lips, tongue, pharynx, and larynx exhibited a dose-response pattern[5]. As to the total number of tumor-bearing animals, most strikingly all non-control doses have essentially the same response level and, thus, the complete range of responses (from background to maximum) must occur somewhere below the lowest dose: the BMD may be just below the first dose, or orders of magnitude lower. This holds true for relevant endpoints to evaluate AA carcinogenicity such as tumours of oral cavity, esophagus, stomach. Furthermore, the number of these tumors (1-2 per 50 animals) does not have sufficient statistical power.

[5]**BfR 2008:** In der Studie zeigten sich erhöhte Inzidenzen maligner Tumoren bei allen behandelten Gruppen außer bei männlichen Ratten der 250 mg/L-Gruppe. Die Gesamtzahl der Tumoren pro 100 Tiere war bei der höchsten Dosis bei männlichen und weiblichen Tieren und bei den weiblichen Tieren der niedrigsten Dosis statistisch signifikant erhöht. Die Inzidenz für Osteosarkome war bei männlichen Tieren der höchsten und der niedrigsten Dosisgruppe erhöht. In der höchsten Dosisgruppe war das statistisch signifikant. Erhöhte Tumorinzidenzen, die jedoch statistisch nicht signifikant waren, wurden auch bei folgenden Organen beobachtet: Zymbaldrüse, Ohrkanal, Nasen- und Mundhöhle, Brustdrüse, Uterus, Magen, Darm und Lunge. Insgesamt ist eine eindeutige Dosis-Effekt-Beziehung nicht erkennbar. Das BfR ist der Auffassung, dass diese Studie (Soffritti et al. 2002) keinen sicheren Beleg für eine kanzerogene Wirkung von Acetaldehyd nach oraler Exposition liefert. Das BfR stimmt insofern mit der MAK-Kommission überein, die zu dem Schluss kam, dass die Studie einen Hinweis auf eine kanzerogene Wirkung von Acetaldehyd bei Applikation über das Trinkwasser zumindest für die höchste Dosisgruppe (ca. 125 mg/kg KG und Tag) liefere, jedoch wegen der fehlenden Dosisabhängigkeit nicht verwertbar sei (MAK 2008).

LAV concluded that the data of the study by Soffritti et al. (2002) do not meet the requirements for BMD analysis.

## 3. Conclusion

The study on the carcinogenicity of AA upon oral administration revealed no dose-related effects. Therefore, no valid conclusion can be drawn from the study by Soffritti et al. (2002). Consequently, this study *per se* cannot be used for benchmark dose analysis and calculation of MOE. Furthermore, the BMD-analysis, as performed by Lachenmeier et al. (2009a), is based upon the total number of tumor bearing animals, which is a non-relevant endpoint for risk assessment of AA upon oral uptake. Rationale: using “total number of tumors” implies a systemic adverse effect which, however, appears unlikely for AA upon oral uptake (see sections 4 and 5.1. of this Statement). As to the predominance of hemolymphoreticular neoplasias, BfR and EFSA expert groups expressed major concerns on the validity of cancer studies performed with rat colonies kept at the European Ramazzini Foundation for Oncology because of chronic inflammatory changes in the lungs of rats of this colony (BfR 2006, EFSA 2006a,b). Moreover, LAV noted that relevant endpoints recorded in the AA study, such as tumors of the oral cavity, larynx and pharynx, neither exhibited a dose-response pattern nor had sufficient statistical power (see. Fig. I 1).

## 4. References


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