Stakeholder meeting I 6 February 2026

EFSA'S STAKEHOLDERS MEETING ON ACETALDEHYDE

EUROPEAN FOOD SAFETY AUTHORITY

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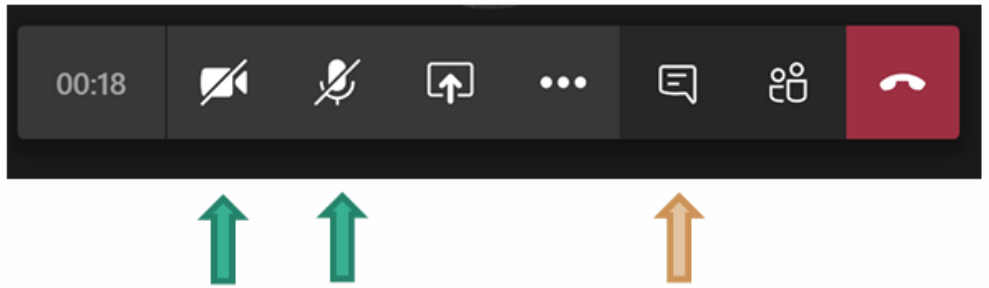
HOUSEKEEPING RULES



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- Use the **dedicated chat box** to write your questions and comments.
- Participants **may not intervene** without being granted the floor by the Chair.
- Recording or transcribing the meeting **is not permitted**.
- If you experience technical issue, please contact the **Meeting Moderator via the chat**.

GOOD PRACTICE OF CONDUCT FOR PARTICIPANTS

Q&A session

- Questions raised during the meeting will be addressed during the **Q&A session**
  - Write in the **dedicated chat box** or raise your hand if you have a question.
  - When invited to speak by the Chair, please **turn on your microphone and camera**.
- **Priority** will be given to questions submitted at the time of registration.
- **Questions posted in the dedicated chat box not answered** during the webinar, will be answered in written form after the webinar.
- **Questions outside the scope** of the webinar or EFSA's remit will not be addressed.

AFTER THE MEETING

- If you have **further questions** after the webinar, please submit them via:
  - **#AskEFSA** service on the EFSA website

  https://www.efsa.europa.eu/en/applications/askaquestion

- The **presentations** will be published on the EFSA website after the meeting:

https://www.efsa.europa.eu/en/events/stakeholders-meeting-acetaldehyde

PARTICIPANTS AFFILIATION

135 registered participants

External stakeholders

Private sector
Applicants
Art.36 Organisations
Registered Stakeholders
NGOs
Other

MS & Public institutions

MS Representatives
EU Institutions/Agencies
International Organisations
EFSA Staff

Academia

Universities/Public Research Institutes

OBJECTIVES OF THE MEETING

- To illustrate the background to the **mandate** received from the EC and the procedural steps applied by EFSA in addressing it.
- To present the **EFSA's preliminary considerations** on the existing information related to the genotoxicity of acetaldehyde.
- To **explore availability of relevant additional data** that could be provided by interested parties to support the weight of evidence.
- To **clarify the timeframe** within which these data could be provided to EFSA, in relation to the timelines for completing the scientific assessment.

AGENDA

Starting time 14:30

Welcome and Introduction

Valeriu Curtui

Terms of reference of the scientific mandate and preliminary considerations from EFSA

Carla Martino

Acetaldehyde (AA) Genotoxicity Data Evaluation & Study Plan

International Organization of the Flavor Industry (IOFI)

Q&A and discussion

All participants

Closing remarks

Valeriu Curtui

Ending time 16:30

TODAY'S MODERATORS AND SPEAKERS

Valeriu Curtui
Chair of the meeting
Head of Unit,
Food Ingredients and Packaging (FIP) Unit

Camilla Smeraldi
Moderator
Team Leader
Food Additives and Flavourings (FAF) Team

Carla Martino
Speaker
Senior Scientific Officer
Food Additives and Flavourings (FAF) Team

Maria Carfì
Moderator
Scientific Officer
Food Additives and Flavourings (FAF) Team

TODAY'S CONTRIBUTORS

Kevin Chipman
Chair of the
EFSA's
Scientific Committee
WG on Genotoxicity
and Member of the FAF WG on Flavourings

Laurence Castle
Chair of the EFSA's Food
Additives and Flavourings
(FAF) Panel

Gabriele Aquilina
Member of EFSA Panel
on Food Additives and Flavourings (FAF)
and Member of the FAF WG on Flavourings

Henriqueta Louro
Member of EFSA Panel
on Food Additives and Flavourings (FAF)
and Member of the FAF WG on Flavourings

Rainer Gürtler
Member of EFSA Panel
on Food Additives and Flavourings (FAF)
and Member of the FAF WG on Flavourings

Terms of reference of the scientific mandate and EFSA's preliminary considerations

Carla Martino

BACKGROUND

- In the context of the EFSA's renewal opinions on **smoke flavouring primary products** published in November 2023 concerns were raised about the genotoxic potential of several compounds present in the mixture, including **acetaldehyde.** It was concluded that **additional genotoxicity data are needed to rule out the potential concern for genotoxicity** for the substance:

  *"**Conclusion:** Based on the experimental data, **acetaldehyde is genotoxic in vitro and in vivo following intraperitoneal administration**. **These findings would require in vivo genotoxicity studies following oral administration.** These studies should address gene mutations and structural and numerical chromosomal aberrations **in particular at the site of contact**."*

- This conclusion was based on ECHA's evaluation of acetaldehyde under CLP Regulation (**ECHA, 2016**), leading to an EU harmonized classification of the substance as **Muta 2** (*Germ cell mutagenicity category 2: suspected of causing genetic defects*) and **Carc 1B** (*Carcinogenicity category 1B: may cause cancer*).
- Acetaldehyde is also an **authorised flavouring substance** in the EU [FL-no: 05.001] (Annex I of Regulation (EC) 1334/2008), based on a JECFA evaluation from 1999 (**JECFA, 1999**), where no health concern was found for acetaldehyde when used as flavouring substance.

EC MANDATE (LINK)

- In view of the conclusions reached by EFSA on acetaldehyde in the context of smoke flavourings, highlighting the need for additional genotoxicity data to conclude on the genotoxicity of the substance, the EC sent a mandate to EFSA in September 2025 with the following requests:

- **Terms of Reference**

  EFSA is requested to carry out an evaluation of the flavouring substance acetaldehyde [FL-no: 05.001] in relation to genotoxicity. In particular the EC requests EFSA:

  - *to issue a call for the relevant required data;*
  - *taking into account the data submitted, to evaluate the substance acetaldehyde [FL-no: 05.001] in relation to genotoxicity and advise on its safety when used as flavouring substance.*

- **Deadline:** *EFSA is asked to finalise the evaluation within **9 months after the completion of the "call for data" process foreseen by end of October 2026***

THE FAF PANEL (2024-2029)

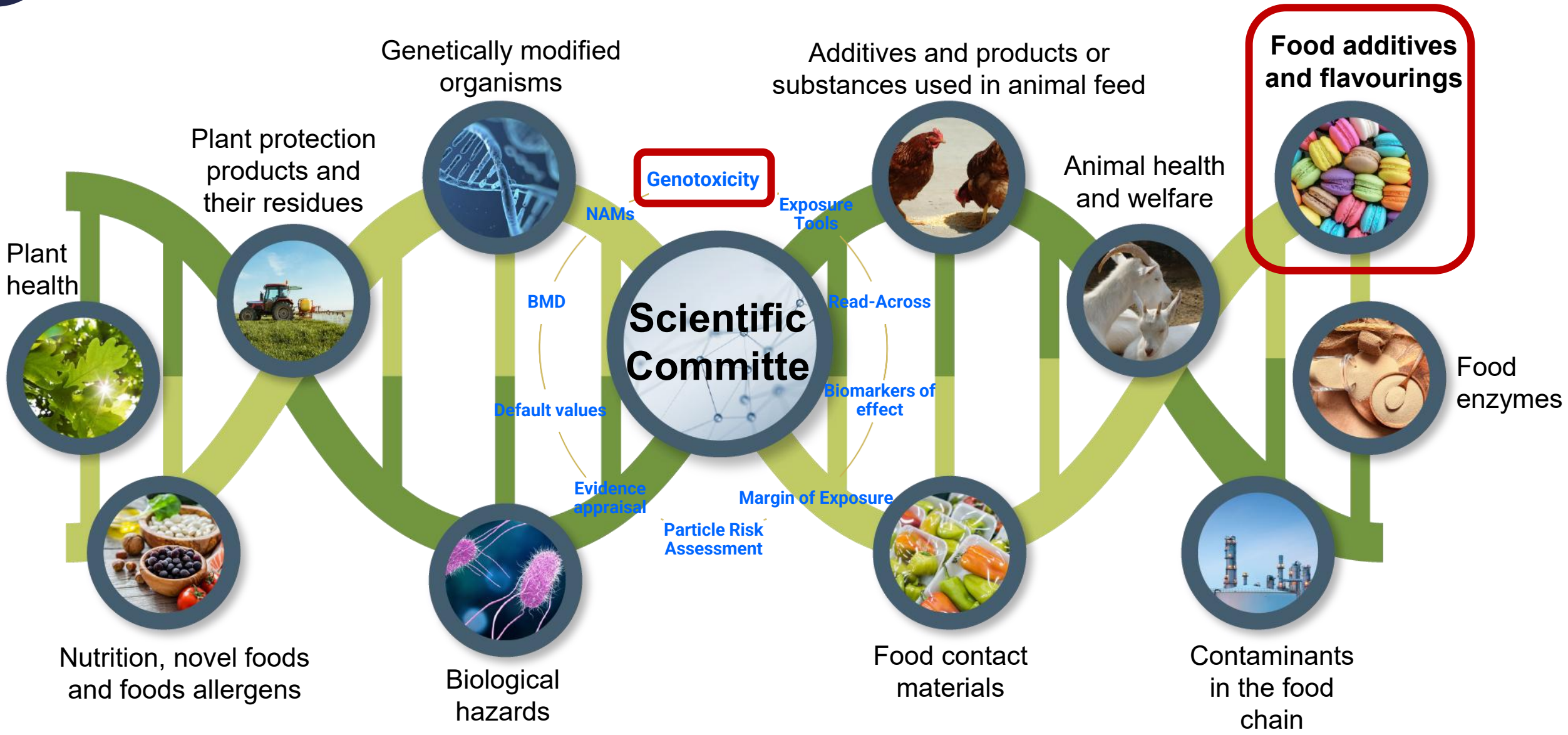
WG FOOD ADDITIVE APPLICATIONS

WG FA RE-EVALUATION & FOLLOW-UP

WG RE-EVALUATION OF SWEETENERS

WG FLAVOURINGS

SCIENTIFIC PANELS, SCIENTIFIC COMMITTEE AND ITS WORKING GROUPS



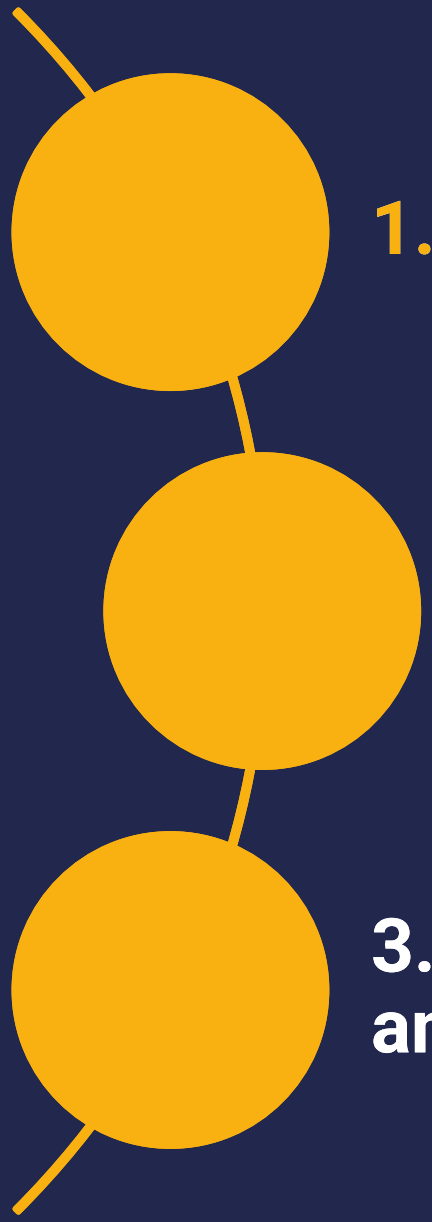
ONGOING ASSESSMENT

- EFSA started reviewing the **existing data** relevant for the genotoxicity assessment of acetaldehyde, in particular the focus was on the following documents:
  - ✓**Acetaldehyde as food flavouring substance: aspects of risk assessment** (Cartus et al., 2023)→ scientific review issued by the Senate Commission of Food Safety (SKLM) of the German Research Foundation (DFG) regarding the health risks associated with the use of acetaldehyde as flavouring substance in foods
  - ✓ **Background document to the ECHA RAC opinion proposing harmonized classification and labelling at EU level of acetaldehyde** (ECHA, 2016)
  - ✓**IARC Monograph** on the Evaluation of Carcinogenic Risks to Humans Volume 96 – **Consumption of Alcoholic beverages** (IARC, 2010)
- In October and November 2025, the cross-cutting Scientific Committee WG on Genotoxicity and the FAF WG on Flavourings were consulted **on the type of additional data** required to assess the genotoxicity of acetaldehyde when used as a flavouring substance via oral exposure

EFSA PRELIMINARY VIEW

- Both WGs reviewed the available information and considered that **the existing information was sufficient to proceed with the evaluation of the genotoxicity of acetaldehyde without requesting additional data**
- This advice was communicated to the FAF Panel who concurred with this view and considered that there is **no need to request additional data by means of a public call**
- One of the purposes of this stakeholder meeting is to explain the reasons behind these **EFSA's preliminary considerations**

RATIONALE FOR NOT REQUESTING ADDITIONAL DATA ON ACETALDEHYDE

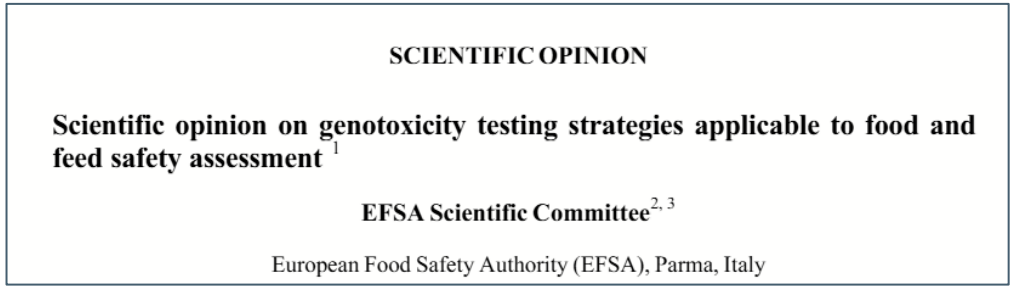


1. How EFSA evaluates genotoxicity

2. Influence of ALDH2 genetic polymorphism on acetaldehyde detoxification and its potential genotoxicity

3. Additional considerations on existing *in vitro* and *in vivo* genotoxicity data

1. GENOTOXICITY IN VIVO IS AN ADVERSE EFFECT PER SE



SCIENTIFIC OPINION

Scientific opinion on genotoxicity testing strategies applicable to food and feed safety assessment [1]

EFSA Scientific Committee[2, 3]

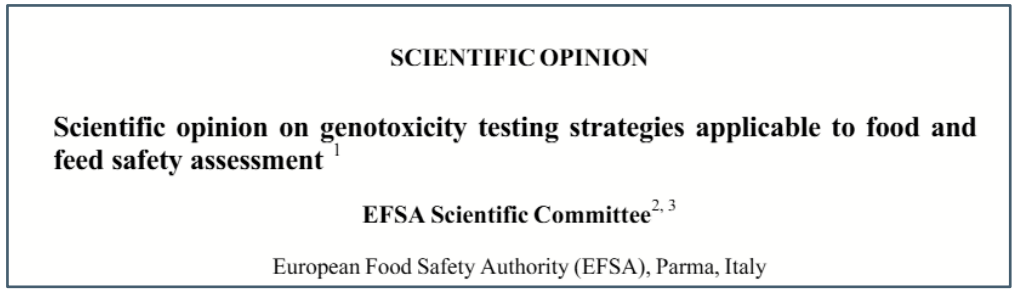
European Food Safety Authority (EFSA), Parma, Italy

https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2011.2379

- **Genetic alterations in somatic and germ cells are associated with serious health effects**, which in principle may occur even at low exposure levels.
- Mutations in **somatic cells** may cause **cancer** (…) and they are responsible for a **variety of genetic diseases**. Accumulation of DNA damage in somatic cells has also been proposed to play a role in **degenerative conditions** such as accelerated aging, immune dysfunction, cardiovascular and neurodegenerative diseases.
- Mutations in **germ cells can lead to spontaneous abortions, infertility or heritable damage to the offspring** and possibly to the subsequent generations.

Clear evidence of genotoxicity in somatic cells *in vivo* has to be considered an adverse effect *per se*, even if the results of cancer bioassays are negative, since genotoxicity is also implicated in other somatic diseases than cancer

1. THRESHOLDS AND NON-LINEAR DOSE RESPONSE RELATIONSHIPS FOR GENOTOXICITY



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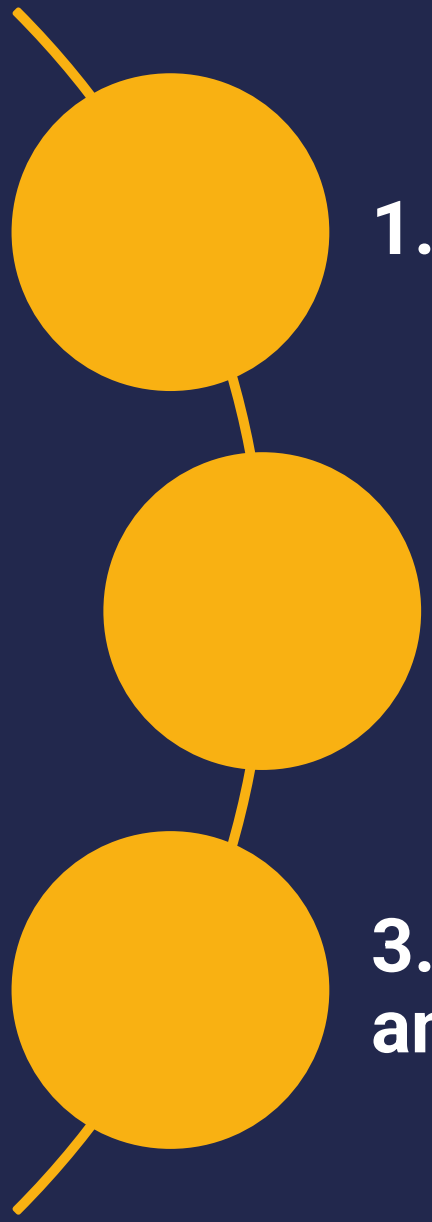
- There is a consensus on the **existence of a threshold for genotoxic substances that interact with molecular targets other than DNA** (e.g. DNA polymerases, topoisomerases, spindle proteins).
- In the case of some **DNA-reactive alkylating agents**, a non-linear dose-response relationship is experimentally observable, while other substances display a linear dose-response relationship. (...) Therefore, the possibility of **adopting a threshold model for alkylating chemicals should be considered with caution**, with consideration of the genetic heterogeneity of human species and the possible occurrence of susceptible individuals and **evaluated on a case-by-case basis**.
- For many other **DNA-reactive agents, no experimental evidence of thresholds has yet been found**. In these cases, a precautionary approach suggests the **adoption of a linear dose-response model**.
- The practical consequence of this approach is that **no exposure level to these agents would be considered without risk**.
- Many chemical mutagens (…) do not act via a single mode of action but **through different concomitant mechanisms, with or without a threshold**. Therefore, a **simplistic model based on a single prevalent mode of action could underestimate the actual risk for human health**.

RATIONALE FOR NOT REQUESTING ADDITIONAL DATA ON ACETALDEHYDE



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2. METABOLISM OF ACETALDEHYDE

- Acetaldehyde is metabolised predominantly by **aldehyde dehydrogenase (ALDH) enzymes**
- There are two main types of ALDHs:
  - **ALDHA1 (cytosolic)**
  - **ALDH2 (mitochondrial)** mainly responsible for detoxifying acetaldehyde in humans
- ALDH2 is encoded by the *ALDH2* gene, for which two different alleles, ***ALDH2*1*** and ***ALDH2*2*,** are known.
- The *ALDH2*2* allele differs from the normal *ALDH2*1* allele by a nucleotide substitution in the *ALDH2* gene, which **results in the ALDH enzyme being inactive**.
- Three ALDH2 genotypes:
  - *ALDH2*1/*1*, active (100% activity) ALDH2
  - *ALDH2*1/*2*, inactive (<10% activity) ALDH2
  - ***ALDH2*2/*2*, inactive (0% activity) ALDH2**
- **About 40% of the Eastern Asians** carry the ALDH2*2/*2 allele in the heterozygous form resulting in a **markedly reduced or inactive form of mitochondrial ALDH2.** This would correspond to at least **540 million ALDH2-deficient individuals in the world**, representing approximately 8% of the population (Brooks et al., 2009)

REVIEW

Molecular Nutrition & Food Research
www.mnf-journal.com

Acetaldehyde as a Food Flavoring Substance: Aspects of Risk Assessment

*Alexander T. Cartus, Dirk W. Lachenmeier, Sabine Guth, Angelika Roth, Matthias Baum, Patrick Diel, Gerhard Eisenbrand, Barbara Engeli, Michael Hellwig, Hans-Ulrich Humpf, Hans-Georg Joost, Sabine E. Kulling, Alfonso Lampen, Doris Marko, Pablo Steinberg, Wim Wätjen, Jan G. Hengstler, and Angela Mally**

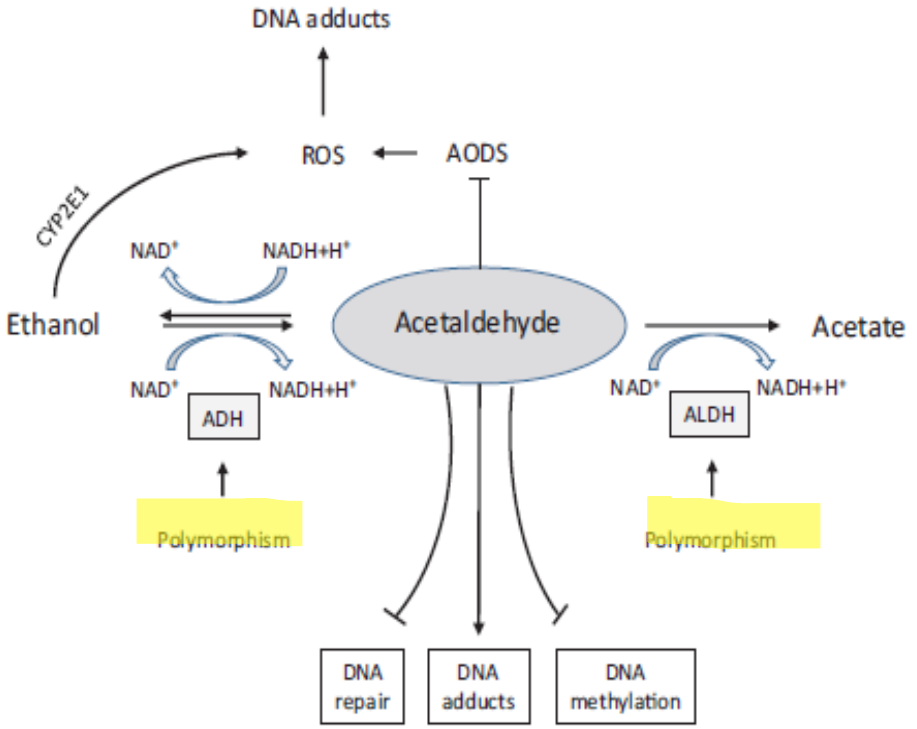

Figure 2. Metabolism of acetaldehyde (modified from refs. [73, 78]). ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; AODS, antioxidative defense system; CYP2E1, cytochrome P450 2E1; NAD^+, oxidized form of the co-enzyme nicotinamide adenine nucleotide; $NADH/H^+$, reduced form of the co-enzyme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

2. TOXICOKINETICS

REVIEW

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- Toxicokinetic data from laboratory animals and humans indicate that acetaldehyde **is systemically available after oral and inhalation exposure** and it is then **rapidly and efficiently metabolized**
- **Systemic exposure** is expected to be **low and to decrease quickly after the end of oral/inhalation exposure.** This is due to the rapid metabolism as indicated by **the half-time values in blood of 3 minutes** (in rats after repeated exposure by inhalation and in mice after single i.p. administration)
- A **transient increase in salivary acetaldehyde concentrations** (from < 20µM before administration to 0.06-1mM after ingestion) was observed within 30 sec in human volunteers sipping alcoholic beverages with high acetaldehyde content (210 µM to 15.8 mM) (Lachenmeier et al., 2011), suggesting a **transiently increased local acetaldehyde exposure of the oral cavity and the upper digestive tract when consuming foods with a high acetaldehyde content**
- This increase is more substantial in **ALDH-2 deficient individuals**: an approximately **2-fold** increase in the **concentration of acetaldehyde in saliva** and **5-fold increase in the gastric juice** have been observed in ALDH2-deficient individuals compared to individuals with active form of ALDH2 enzyme after ethanol ingestion (Maejima et al, 2015)

2. TISSUE DISTRIBUTION OF ALDH2

IARC Monographs on the Evaluation of Carcinogenic Risks to Humans

VOLUME 96

Alcohol Consumption and Ethyl Carbamate

- ***ALDH2* mRNA** is expressed in a variety of human tissues particularly in the liver
- Some of the first sites of contact tissues, such as the **mouth, oesophagus,** and **salivary glands**, express **low or no ALDH2 mRNA transcripts**
- The low ALDH2 expression in these tissues suggests a limited detoxification capacity at first contact sites tissues, **increasing their vulnerability to acetaldehyde-induced DNA damage**.

Figure 4.2. Tissue distribution of aldehyde dehydrogenase (*ALDH*) transcripts reflected by the abundance of expressed sequence tags

Tissue	*ALDH1A1*	*ALDH2*	*ALDH1B1*	*ALDH9A1*
Adipose tissue	360	504	72	432
Adrenal gland	1506	384	29	324
Blood	123	53	23	169
Bone	27	55	55	41
Bone marrow	306	0	20	102
Brain	360	119	22	185
Cervix	103	20	0	228
Colon	272	198	59	59
Connective tissue	326	34	6	217
Eye	231	115	14	106
Heart	178	133	33	156
Kidney	648	84	75	338
Larynx	65	65	0	0
Liver	1439	376	14	138
Lung	437	138	8	115
Lymph	0	134	22	22
Lymph node	0	83	10	20
Mammary gland	81	35	23	245
Mouth	477	57	28	159
Muscle	78	34	0	95
Nerve	119	239	0	119
Oesophagus	156	0	104	156
Ovary	65	150	0	28
Pancreas	182	91	9	54
Pharynx	351	43	0	329
Placenta	84	40	3	90
Prostate	135	65	35	175
Salivary gland	48	0	0	97
Skin	217	95	74	127
Small intestine	5103	112	22	474
Spleen	813	18	18	302
Stomach	1047	264	48	97
Testis	733	60	37	266
Thymus	193	0	0	296
Thyroid	90	200	54	345
Tonsil	0	116	0	0
Trachea	2784	0	20	329
Urinary bladder	725	65	0	32
Uterus	928	58	62	150
Vascular	533	59	19	197

The number given for each tissue is the abundance of the expressed sequence tag in terms of transcripts/million.
This Figure is compiled from information publicly available at the National Center for Biotechnology Information (NCBI) (see http://www.ncbi.nlm.nih.gov/unigene)

2. IN VIVO GENOTOXICITY, IMPORTANCE OF ALDH2 (ECHA, 2016)

- The effect of **ALDH2 genetic polymorphism associated with the inactive form of the enzyme** on the potential genotoxicity of acetaldehyde was investigated by **Kunugita et al (2008)**
- The study authors used ***Aldh2* knock-out (*Aldh2* -/-) mice as a model of ALDH2-deficient humans**
- **Wild type and knock-out (*Aldh2* -/-) mice** were exposed to acetaldehyde following **oral exposure or inhalation** for 2 weeks. Induction of gene mutations in T-lymphocytes (spleen) and of MN in reticulocytes (bone marrow) was studied in both wild type and knock-out animals

ORAL EXPOSURE

Table 13 Summary of in vivo mutagenicity studies (animal studies)

Method	Animal	Exposure conditions	Results	Klimisch(15) score*	References
Somatic cell mutagencicity					
Gene mutation and micronuclei	Wildtype and knock-out mice with inactive ALDH2 gene; micronuclei determined in reticulocytes; mutations were deter-mined by TCR gene mutation assay	Oral administration, 0 and 100 mg/kg bw, daily, once a day for two weeks; 5 – 10 animals/group	*Micronuclei*: + in knock-out mice ($p<0.05$); - in wild-type mice. *Mutation* (TCR mutant frequency): + in knock-out mice ($p<0.05$); - in wild-type mice	2	Kunugita et al. 2008(46)

2. IN VIVO GENOTOXICITY, IMPORTANCE OF ALDH2 (ECHA, 2016)

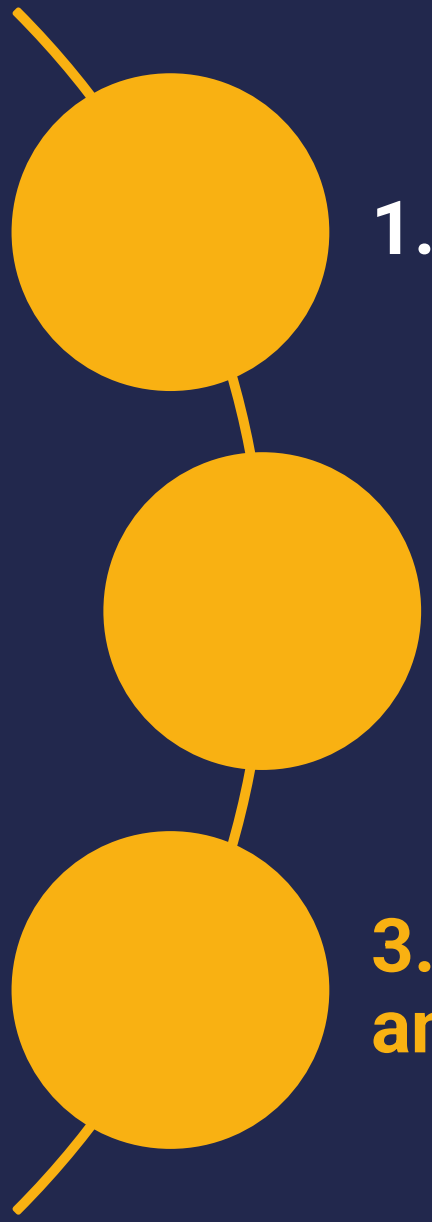
Table 14 Induction factors of micronuclei and TCR gene mutations in knockout mice (Kunugita et al 2008).(46)

Exposure route	Exposure level	Micronuclei in reticulocytes	Mutant frequency in T-cell receptor gene
Knock-out mice (Aldh2 -/-)			
Inhalation	0 (control)	-	-
	225 mg/m^3	1.8 *	Not determined
	900 mg/m^3	1.9/unspecified **/***	1.7**
Oral administration	0 (control)	-	-
	100 mg/kg bw	2/1.7 **/***	2.4/1.6 **/***
Wildtype mice (Aldh2 +/+)			
Inhalation	0 (control)	-	-
	225 mg/m^3	-	-
	900 mg/m^3	-	-
Oral administration	0 (control)	-	-
	100 mg/kg bw	-	-

* compared to Aldh2 +/+ control mice ($p<0.05$); ** compared to Aldh2 +/+ control mice ($p<0.01$); *** compared to Aldh2 -/- control mice ($p<0.05$).

- Irrespective the route of exposure, **in ALDH2 knockout mice**, the **number of micronuclei** positive cells, and the frequency of TCR (T-cell receptor) **gene mutations** in lymphocytes was **statistically significantly increased** compared to the respective controls.
- In **wildtype animals**, acetaldehyde **did not cause any effects** on these endpoints.
- These data show that the **absence of ALDH2 and the consequent reduction in acetaldehyde detoxification led to *in vivo* genotoxic effects following oral or inhalation exposure.**

RATIONALE FOR NOT REQUESTING ADDITIONAL DATA ON ACETALDEHYDE



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3. IN VITRO GENOTOXICITY (ECHA, 2016)

- Acetaldehyde **induces gene mutations in mammalian cells**, while it does not induce gene mutations in bacteria (ECHA, 2016)
- There is evidence that the substance is mainly **clastogenic *in vitro*.** Kinetochore analysis proved that most of the MN induced by acetaldehyde were originated by a clastogenic mechanism (Kayani and Parry, 2010)
- Acetaldehyde induces also **DNA-strand breaks, DNA-adducts, DNA-protein crosslinks**, in both rodent and human cells (the latter were mainly lymphocytes).
- ***In vitro* Comet assays** showed positive results. However, Speit et al (2008) reported **negative** results in an *in vitro* Comet assay at concentrations inducing increase in the frequency of SCE and of MN. Using the comet assay modification with **proteinase K**, slightly enhanced DNA migration that was measured in comparison to conventional Comet results.
  - These results suggests that the **Comet assay has a low sensitivity for the detection of acetaldehyde induced DNA damage**.

3. IN VIVO GENOTOXICITY, IP ADMINISTRATION (ECHA, 2016)

Table 13 Summary of in vivo mutagenicity studies (animal studies)

Method	Animal	Exposure conditions	Results	Klimisch(15) score*	References
Somatic cell mutagencicity					
Micronuclei; multi-substance study	Male SD and F344 rats, bone marrow erythrocytes and peripheral blood erythrocytes	250 mg/kg bw, intraperitoneal injection. Highest dose tested was maximum tolerated dose; at least four animals/group	+ (both cell types)	2; only highest dose tested	Wakata et al. 1998(47)
Micronuclei	5 male CD-1 mice	0 – 400 mg/kg bw, Intraperitoneal injection, three dose levels; tests on acute toxicity performed	+ (dose-related increase)	2	Morita et al. 1997(48)
Micronuclei	Male Han rats, 5 animals/group	Single intraperitoneal injection of 125 or 250 mg/kg bw; blood samples collected after 0, 24, 48 and 72 hours	+ (at 24 and 48 hours), dose-related increase; no data at 72 hours due to toxicity	2	Hynes et al. 2002(49)

- **In vivo MN assays via intraperitoneal administration** → in all these studies **acetaldehyde induced increase of MN frequency in bone marrow and blood cells** after i.p. injection in mice and rats

- Despite the i.p. administration is considered a non-physiological route of administration, these data can be used as **supportive evidence**, **demonstrating a genotoxic effect of the unmetabolized substance in the bone marrow**

IN SUMMARY

Existing evidence considered by EFSA for not requesting additional genotoxicity data on acetaldehyde:

- ✓ **ALDH2** is the main enzyme responsible for acetaldehyde detoxification *in vivo*. It has a **high degree of genetic polymorphism in humans**, that gives rise to differences in the catalytic activity of the enzyme, where the **genotype *Aldh2*2/*2* shows little or no activity**. Approximately **8% of human population is ALDH2-deficient**.
- ✓ The effect of **ALDH2 genetic polymorphism** on the potential genotoxicity of acetaldehyde was investigated by Kunugita et al (2008), who used *Aldh2 -/-* knock-out mice as a model of ALDH2-deficient humans and showed that **acetaldehyde is genotoxic *in vivo* in *Aldh2 (-/-)* knock-out mice after oral exposure**. **These results highlight the importance of ALDH2 polymorphism on acetaldehyde detoxification and its relevance to the potential genotoxicity of the substance, following oral exposure.**
- ✓ The positive results observed in the ***in vivo* MN studies via i.p. administration** can be used as **supportive evidence,** showing a genotoxic effect of the **unmetabolized substance in the bone marrow.**

IN SUMMARY

Cont'd

- ✓ Information on **ALDH2 tissue distribution,** as reported in IARC 2010, **reinforces the concern on the potential genotoxicity at the first site of contact after oral exposure**. The **low levels of ALDH2 expression in the upper aerodigestive tract** (e.g. oesophagus, salivary glands) may increase the risk for acetaldehyde-associated genotoxicity in these tissues, following oral exposure.
- ✓ The **DNA-protein cross-links induced by acetaldehyde may affect the outcome of a Comet assay (Speit et al, 2008)**. For this reason, an **in vivo Comet assay (OECD TG 489) is not recommended**. Even if proteinase K is used to remove DNA-protein crosslinks with the aim to obtain an adequate DNA migration, the risk of false negative results remains high.

Acetaldehyde - Genotoxicity Data Evaluation & Study Plan

International Organization of the Flavour Industry (IOFI)

Questions & Answers

All participants

Q1 – WHY DOES THE FAF PANEL DIVERGE FROM SMOKE FLAVOURING OPINIONS

- **Givaudan International SA -** Why did EFSA's Expert Panel diverge from its previous opinion on smoke flavourings and believes that new data - despite the explicit mandate - is not needed for the evaluation?

REPLY TO QUESTION 1

- In 2023, in the context of EFSA's renewal opinions on smoke flavorings, the FAF Panel reviewed the **ECHA RAC evaluation of germ cell mutagenicity of acetaldehyde** (ECHA, 2016) and considered that **in most of the *in vivo* studies assessed by ECHA** the **animals were administered via i.p. injection** (i.e. *in vivo* MN assays in rodents).
- This route of administration was considered **nonphysiological**, since it may overwhelm detoxification mechanism and **it may not reflect responses to oral administration**.
- The **effect of ALDH2 genetic polymorphism** on the potential genotoxicity of acetaldehyde was not discussed at that time.
- For this reason, the Panel concluded that **these findings would require *in vivo* genotoxicity studies following oral administration, investigating the site of contact tissues.**
- Following the adoption of the smoke flavouring opinions, the publication of **Cartus et al. (2023)** highlighted the **importance of ALDH2 polymorphism on acetaldehyde detoxification and its relevance to the potential genotoxicity of the substance** — an aspect **consistent with the findings of Kunugita et al. (2008)**.
- In the smoke flavouring opinions, the Panel put the focus mainly on the outcome of the ECHA evaluation and the studies on which it was mainly based but did not discuss all details.

Q2 - WHY A CALL FOR DATA WAS NOT LAUNCHED FOR ACETALDEHYDE?

- **Franck Atienzar:** Can you explain why a call for data was not launched for acetaldehyde? Does EFSA consider that there is enough information particularly for the in vivo genotoxicity evaluation via the oral route?
- **Coca-cola Services:** It would be great to be able to understand why a call for data has not been considered by EFSA.
- **PepsiCo International:** Why does the Panel consider that an in vivo genotoxicity study by the oral route is not required?
- **Silesia G. Hanke GmbH & Co. KG:** Why does EFSA restrain from a call for data? Why are restrictions of use necessary assuming that evaluation can be finalized in a few months?
- **International Flavors & Fragrances NEDERLAND BV:** Based on what arguments did EFSA decide that a call for data (mandate) is not needed and how does this relate to Art 19 of Reg 1334/2008?
- **Nestlé Research:** What is the weight of evidence for acetaldehyde's genotoxicity and carcinogenicity across in vitro, in vivo, and human data? What points of departure are suitable for risk for oral and inhalation route?

REPLY TO QUESTION 2

- **ALDH2 is the key enzyme for acetaldehyde detoxification in vivo** - it shows high genetic polymorphism, with the Aldh2*2/*2 genotype displaying little or no activity.
- **ALDH2 polymorphism affects the efficiency of acetaldehyde detoxification and plays a key role in affecting individual vulnerability to genotoxicity** - Kunugita et al. (2008) demonstrated that Aldh2-/- knockout mice, modelling ALDH2-deficient humans, exhibit in vivo genotoxicity after oral exposure to acetaldehyde.
- **In vivo micronucleus (MN) studies via intraperitoneal (i.p.) administration provide supportive evidence of acetaldehyde's genotoxicity -** unmetabolized acetaldehyde can exert genotoxic effects in the bone marrow.
- **Low ALDH2 expression in upper aerodigestive tract tissues** (e.g., oesophagus, salivary glands) suggests **higher susceptibility to DNA damage at first-contact sites** following oral exposure.
- Acetaldehyde forms **DNA–protein cross-links**, which **can reduce the sensitivity of the Comet assay**, leading to possible false negatives - in vivo Comet assay (OECD TG 489) is not recommended.
- The Working Groups and the Panel did not identify a specific study that might be used to overrule the results from the Kunugita et al. (2008) study. Taking also animal welfare into account, the Panel did therefore not see a good scientific justification or even a need to request additional data.

Q3 – NATURAL OCCURRENCE IN FOOD OF ACETALDEHYDE

- **SRA Consulting:** Acetaldehyde is not only a flavouring agent but also occuring naturally in foods. How will this be considered in the risk assessment?
- **Matran Irina:** Are there differences in genotoxicity between synthetic and natural acetaldehyde? What is the LD50/kg body weight? The amounts of flavors added to food are very small.
- **Alpla Alwin Lehner GmbH Co&KG:** Acetaldehyde is a major component of almost every natural food product, would you like to receive typical values?

REPLY TO QUESTION 3

- EFSA ongoing assessment relates to the **genotoxicity assessment of acetaldehyde when used as flavouring substance**, as requested in the EC mandate.
- Therefore, the focus of the EFSA assessment is on the flavouring substance acetaldehyde, which is **deliberately added to food**.
- **Natural occurrence data on acetaldehyde in food may be reported in the opinion for information**, but it will not be taken into consideration in the evaluation of genotoxicity of the flavouring substance acetaldehyde.
- **Exposure data could be considered**, however, as explained in the EFSA presentation, for most DNA-reactive substances (such as acetaldehyde) **no experimental evidence of thresholds has been found and therefore a linear dose-response model is applied**. As a consequence, no exposure level would be considered without risk.

Q4 – ASSESSMENT OF ACETALDEHYDE AS PART OF NATURAL EXTRACTS

- **Sluys International NV:** Acetaldehyde is a naturally occurring chemical compound/molecule, which exists in many fruits. Would there be any impact when reviewing its safetyness for natural extracts, e.g. essential oils, etc.

REPLY TO QUESTION 4

- When acetaldehyde is part of natural extracts such as essential oils, the principles described in the **SC Statement on genotoxicity of mixtures** (EFSA SC, 2019) apply:
  - ✓Concentrations of the components of the mixture should be provided and the genotoxic potential of these components should be assessed individually.
  - ✓If the mixture contains one or more components that have been assessed to be genotoxic *in vivo* via a relevant route of administration, then the mixture raises a concern for genotoxicity and the risk to human health related to this identified hazard needs to be considered in the risk assessment.
  - ✓For **unavoidable contaminants and impurities** it might be possible to conclude that human exposure is likely to be of low concern from a public health perspective. Such a conclusion maybe reached based on a **Margin of Exposure (MOE) approach** (EFSA, 2025) **when respective carcinogenicity data are available**.

Q5 –ACETALDEHYDE DETOXIFICATION AND ITS IMPACT ON SYSTEMIC GENOTOXICITY

- **Kerry** How to factor the efficiency of detoxification into its assessment of systemic genotoxic risk from oral exposure to acetaldehyde?

REPLY TO QUESTION 5

- As explained in the presentation from EFSA, **ALDH2 genetic polymorphism affects the efficiency of acetaldehyde detoxification and has an impact on the potential genotoxicity of acetaldehyde.**
- It is acknowledged that, due to its rapid metabolism following oral intake, **systemic exposure to acetaldehyde is expected to be low** and to decrease quickly. However, **the efficiency of this process is impaired in individuals with an ALDH2 deficiency,** resulting in increased exposure to acetaldehyde.
- **Genotoxic effect of the unmetabolized substance at the first site of contact tissues are of primary concern.**

OTHER QUESTIONS

- **ToxStrategies, LLC:** What prompted the re-evaluation of acetaldehyde? What is the timeline for evaluation and draft opinion? Will stakeholders be engaged through the process? Will a hazard protocol be published for review?

Reply: The terms of reference requesting EFSA to evaluate the genotoxicity of acetaldehyde has been described in the EFSA presentation, including the expected timelines for evaluation. Publication of a protocol is not envisaged.

- **Cybelle Fiallos (consultant):** Will acetaldehyde will be delisted during 2026?

Reply: this is a risk management decision, following EFSA's evaluation.

- **proFagus GmbH:** Will there be reviews about the policy in the EU Risk Management that individual components of flavour formulations are sufficient to eliminate entire and complex product formulations?

Reply: The genotoxicity assessment of chemical mixtures is not a risk management choice but is an integral part of risk assessment and is performed according to the latest EFSA Scientific Committee Guidance. In any case this concept is not relevant to the present discussion about the assessment of acetaldehyde when used as a flavouring substance.

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