Health risk assessment of ethylene oxide residues in sesame seeds

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In products including sesame seeds from India, such as bars, snacks or salad toppings, the German regional authorities have detected residues of the substance ethylene oxide in some cases. These products could not be marketed because ethylene oxide residues are not permitted in the EU. The affected products were taken off the market and, at the same time, the public was informed by the rapid alert system operated between EU food safety authorities.

In the EU, ethylene oxide is prohibited from any use in plant protection products. In biocidal products, ethylene oxide may be used as an active substance for disinfection but food contact is not allowed. Ethylene oxide is mutagenic and carcinogenic. Accordingly, the substance has no safe health-based guidance value. The BfR has therefore derived a figure for an intake of low concern: at lower quantities, the additional risk of contracting cancer from a lifelong intake is less than approx. 1:100,000. The BfR has calculated this intake of low concern for ethylene oxide as 0.037 micrograms per kilogram of body weight/day (μg/kg body weight/day). This calculation has been based on the ‘Large Assessment Factor’ approach from the European Food Safety Authority (EFSA).

More recent analyses from the regional authorities have shown that ethylene oxide was almost completely converted into 2-chloroethanol in the samples investigated. To date, the EU has assessed the metabolite 2-chloroethanol together with ethylene oxide. The EU’s maximum allowed residue level of 0.05 milligrams of ethylene oxide per kilogram of sesame is based on the analytical detection limit and also relates to the sum of ethylene oxide and 2-chloroethanol, expressed as ethylene oxide. The BfR has reviewed and confirmed the methodology used to date. Indications of mutagenic activity by 2-chloroethanol have also been provided by animal studies and there is not enough data available to exclude the possibility of the substance having carcinogenic effects with sufficient certainty. From the perspective of consumer health protection, the joint assessment of ethylene oxide and 2-chloroethanol as sum parameters is acceptable until new data are provided, since there are no indications that the degradation product 2-chloroethanol might produce stronger mutagenic or carcinogenic effects than ethylene oxide.

The BfR has reviewed how residues at the maximum residue level of 0.05 mg/kg sesame should be assessed from a health risk perspective, in cases where the intake of low concern of 0.037 μg/kg body weight/day and consumption quantities from available consumption studies can be applied. In the case of average consumption over a longer period of time, neither children nor adults exceed the intake of low concern. Since ethylene oxide is both mutagenic and carcinogenic, however, residues in food are to be avoided.

1 Subject of the assessment

The German Federal Institute for Risk Assessment was asked by the Federal Ministry of Food and Agriculture (BMEL) to assess the toxicity of ethylene oxide and 2-chloroethanol, and especially in terms of the possibility of deriving toxicological threshold values as well as information about the metabolism of ethylene oxide.
The BMEL also requested information about the acute and chronic toxicity of processed products containing sesame seeds with ethylene oxide concentrations above the maximum residue level.

This request follows similar enquiries made to the BMEL by German states about various original and follow-up notifications on ethylene oxide in sesame seeds from India and goods made from these.

2 Results

Since ethylene oxide is a genotoxic carcinogen, deriving a health-based reference value without risk is not possible as a threshold for the effect cannot be set. However, the "large assessment factor approach" from EFSA permits the calculation of an intake of low concern, which is associated with an additional cancer risk of approx. 1:100,000 following lifelong exposure. This intake of low concern has been determined as 0.037 µg/kg body weight/day for ethylene oxide and can be applied as a basis for risk management decision-making.

When considering the ethylene oxide degradation product 2-chloroethanol, the available data are inconsistent and partially incomplete. A reliable statement about the carcinogenic properties of 2-chloroethanol cannot be made at this time. While there are numerous indications for genotoxic activity, the existence of a potential threshold value and in vivo relevance have not been definitively clarified. As yet, there have been no indications that 2-chloroethanol has a greater toxicity than ethylene oxide. The BfR therefore considers the risk assessment for 2-chloroethanol to be the same as for ethylene oxide.

Actual results from investigations of sesame samples were not submitted to the BfR for assessment. For this reason, indicative values for exposure in children and adults were calculated for an ethylene oxide residue (corresponding to the sum of ethylene oxide and 2-chloroethanol, expressed as ethylene oxide) equivalent to the limit of quantification and applicable maximum residue level of 0.05 mg/kg in sesame.

For children consuming a quantity of 23.4 g of sesame per day (equivalent to the 'large portion' determined in consumption studies), the intake of low concern was exceeded even with an ethylene oxide residue of just 0.05 mg/kg. For adults, the intake for a large portion of 39.6 g per day was below the intake of low concern. However, if one considers average consumption over a prolonged period of time, this level is exceeded neither by children nor by adults.

3 Rationale

3.1 Regulatory background

In the EU, ethylene oxide is prohibited from any use in plant protection products. The substance was formerly used as a fumigant. Applications of biocidal products containing ethylene oxide are permitted in the EU for disinfection – but without food contact.

As regards maximum residue levels, the sum total of ethylene oxide and 2-chloroethanol, expressed as ethylene oxide, applies for all foods. In the EU, maximum residue levels are specified for all foods at the level of the respective limit of quantification. In sesame seeds, Regulation (EU) 2015/868 lowered this maximum level from 0.2* mg/kg to 0.05 mg/kg. The use of '*' indicates that the maximum level is set to the respective analytical limit of quantification.
Sesame seeds and products that contain sesame seeds can be marketed if ethylene oxide (corresponding to the sum total of ethylene oxide and 2-chloroethanol, expressed as ethylene oxide) cannot be quantified while applying the analytical limit of quantification.

3.2 Toxicological assessment of ethylene oxide and 2-chloroethanol

Ethylene oxide
In the EU, ethylene oxide has recently been assessed as a biocidal agent. This reassessment confirmed that ethylene oxide, as a mutagenic carcinogen, should be considered as having no threshold value: it is therefore impossible to define a health-based guidance value without a health risk. However, the available data permits the derivation of intake quantities below which one may assume a minimal additional cancer risk and which are therefore typically considered to be tolerable as part of a risk management strategy.

In the biocide assessment procedure, DMELs of 3 ppb for workplace exposure and 0.06 ppb for the general population were determined on the basis of findings from a chronic inhalation study on bronchioalveolar adenomas and carcinomas in female mice. These values were derived according to established ECHA/REACH guidelines. A DMEL (derived minimum effect level) as defined for e.g. workers or the general population is a calculated level of exposure with minimum effect, and is associated with an additional lifetime cancer risk of 1:100,000 (workers) and 1:1,000,000 (general population). The DMEL values from the biocide procedure for ethylene oxide are based on inhaled air, however, and are therefore not suitable for assessing residues in food. This applies for the same reason for other reference values that were determined based on cancer incidence in inhalation studies.

A similar approach is described in the EFSA document ‘Opinion of the Scientific Committee on a request from EFSA related to A Harmonised Approach for Risk Assessment of Substances Which are both Genotoxic and Carcinogenic’¹. This approach has been recommended by the BfR previously for the assessment of unavoidable concentrations of mutagenic and/or carcinogenic substances in food². With this approach, the application of an extrapolation factor of 10,000 to the relevant BMDL₁₀ value from a suitable animal experiment or from epidemiological surveys can be used to estimate an intake of low concern. The BMDL₁₀ value describes the calculated lower confidence limit of the dose that causes an increase in tumour incidence of 10 percent following lifelong intake. The extrapolated intake quantity is associated with an additional cancer risk of approx. 1:100,000 following lifelong exposure. The decision as to whether or not the residual health risk can be considered to be tolerable is in any event a question for risk management and should not exclude the implementation of further measures for risk mitigation. The approach described here is referred to as the ‘large assessment factor approach’ from the EFSA and has been recommended by the BfR on several occasions.

For ethylene oxide, a 150-week study in rats is available that is suitable for estimating the cancer risk following oral intake (Dunkelberg, 1982)³. The study authors describe a dose-dependent increase in tumours of the gastrointestinal tract. This matches the expected profile for

² BfR Opinion no. 029/2005, dated 18 May 2005: Risk assessment of genotoxic and carcinogenic substances to be harmonised in the EU.
the direct effects of the comparatively chemically reactive ethylene oxide. In the abovementioned study on carcinogenicity after inhalation, a marked increase in tumours in the directly exposed lungs was also observed – although not as an exclusive event. The findings from the study by Dunkelberg (1982) were recently reassessed in terms of the dose-response relationship according to the state-of-the-art approach described by EFSA (2017) using benchmark dose modelling4. The details of this re-evaluation were reviewed by the BfR and are considered to be plausible. A BMDL\textsubscript{10} of 0.37 mg/kg body weight/day was determined for the increase in tumour incidence in the forestomach and stomach for female animals following lifelong exposure. The BMDU/BMDL ratio describes the width of the confidence interval, which is 3.7 in this case and is considered to be within an acceptable range. An application of the TD\textsubscript{50} – i.e. the dose at which 50 percent of laboratory animals developed tumours – is no longer the method of choice and the BMDL10 provides the preferred starting for quantitative risk assessment.

2-chloroethanol
Due to a lack of relevant data, a reliable statement about the carcinogenic properties of 2-chloroethanol cannot be made at this time. While there are numerous indications for genotoxic activity, the existence of a potential threshold value and in vivo relevance have not been fully clarified.

In terms of mutagenicity, the available data are partially conflicting. Although 2-chloroethanol was assessed as genotoxic in the Ames test submitted as part of the EU biocide assessment of ethylene oxide, an internal evaluation of an article by Pfeiffer and Dunkelberg (1980)5 did not yield clear findings of genotoxicity. Instead, the results were considered to be unclear or indicating only weak genotoxicity at best. Unfortunately, neither the original data nor a repeat test conforming to OECD TG 471 are available. However, data from the National Toxicology Programme (NTP) do substantiate mutagenicity in the Ames test following metabolic activation, as well as clastogenicity in an in vitro chromosomal aberration test in CHO cells (Tennant et al., 1987)6. Two mechanisms can be identified for the mutagenicity of 2-chloroethanol. Alkylhalogenides are electrophiles that are capable of direct DNA alkylation. As a consequence, they are often mutagenic in the presence and absence of S9 mix in the Ames test – especially in the TA100 and TA1535 strains. This substance class is active in vivo especially in the transgenic rodent mutation assay, which would also be the preferable follow-up test here. Notably, the formation of glutathione adducts in vivo has been demonstrated for 2-chloroethanol. This is typical for electrophile alkylating agents and highlights the plausibility of a direct mutagenic mechanism of action without a threshold. As a primary alcohol, 2-chloroethanol is also oxidised to carboxylic acid, whereby the protein- and DNA-reactive aldehyde is created as an intermediate. Since this biotransformation of 2-chloroethanol to 2-chloro acetaldehyde has also been demonstrated (Grunow and Altmann, 1982)7, mutagenic activity is considered plausible overall.

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5 Pfeiffer and Dunkelberg (1980) Mutagenicity of ethylene oxide and propylene oxide and of the glycols and halohydrins formed from them during the fumigation of foodstuffs. Food and Cosmetics Toxicology 18 (2), 115-118. doi.org/10.1016/0015-6264(80)90062-0.
In the REACH dossier, 2-chloroethanol is also assessed as generally genotoxic in terms of mutagenicity in bacteria. To date, the information from the REACH dossier has not been subjected to an independent substantive review by an EU Member State or the ECHA, and merely represent summaries of information that cannot be verified without access to the original data. Information is also available from in vitro studies that provides indications of chromosomal damage.

The relevance of positive results from in vitro genotoxicity studies can be further investigated in suitable in vivo studies. Of the 16 in vivo genotoxicity studies reported on in the REACH dossier, 5 were adjudged to be unreliable or not capable of assessment in terms of their reliability by the registrant. Of the 11 remaining studies, two were performed in Drosophila (fruit flies), while two further studies investigated germ cell mutagenicity. One test concerned an in vivo UDS assay. Following a comprehensive analysis conducted by the OECD, this type of study is now no longer considered to be sufficient in terms of its sensitivity. This leaves 6 studies of relevance. Of these, only Shelby et al. (1993) was identified as a ‘key study’ by the registrant. The study summary did not stand up to critical scrutiny when verified against the published article. Although the registrant did not single out any deviations from the benchmark OECD testing guideline 474, serious deviations that significantly limit study reliability can be identified. These include: the counting of only 1,000 instead of 4,000 cells per animal, the omission of a second observation time and the exceedance of the recommended time to sampling, as well as a failure to provide evidence of bone marrow exposure with the simultaneous absence of an effect on the PCE/NCE ratio and with the limit dose envisaged in the testing guideline being undershot by a factor of 10. The in vivo data on the genotoxicity of 2-chloroethanol therefore offer no useful basis for a full refutation of the genotoxic findings in vitro.

Kitchin et al. (1992) predict carcinogenic properties for 2-chloroethanol on the basis of a series of genetic and biochemical parameters from in vivo short-term tests. The carcinogenicity study available with dermal exposure is also unsuitable for use as a basis for resolving existing concerns based on the data as presented. As yet, however, there have been no indications that 2-chloroethanol has a greater toxicity than ethylene oxide. Several authorities have derived occupational exposure limit values for handling 2-chloroethanol as an industrial chemical. These values relate to dermal exposure or exposure by inhalation and are not suitable for assessing residues in foods.

From a toxicological perspective, the risk assessment of 2-chloroethanol should be conducted - pending further clarification - as for ethylene oxide, so as to offer optimum protection to health and to guard against underestimations.

### 3.3 Behaviour of ethylene oxide residues in foods

Formerly, 2-chloroethanol was used as a starting material for ethylene oxide synthesis. Modern production methods utilise the oxidation of ethylene with a metal catalyst, however – a process that does not produce 2-chloroethanol. The BfR has not identified any studies investigating ethylene oxide metabolism in plants. Ethylene oxide is reactive, however. In the presence of chloride, ring cleavage takes place with the formation of 2-chloroethanol that, in spices and sesame seeds treated with ethylene oxide, has often been detected at higher
concentrations than in the parent substance (ANZFA, 2000)\(^{10}\). While it is to be expected that residues of ethylene oxide in food will be comparatively low, due to the high vapour pressure and high reactivity – and that these residues are further reduced by the heating processes that occur during food processing – this is not equally true of 2-chloroethanol residues in food.

### 3.4 Risk assessment for ethylene oxide in sesame

The BfR generally agrees with the findings of the Dutch risk and exposure assessment\(^{11}\) concerning ethylene oxide in sesame seeds.

For residues of ethylene oxide (corresponding to the sum of ethylene oxide and 2-chloroethanol, expressed as ethylene oxide) equivalent to the limit of quantification and applicable maximum residue level of 0.05 mg/kg in sesame, the BfR has calculated the short-term intake quantity for children and adults with the German NVS II model\(^{12}\).

#### Exposure assessment using the NVS II national consumption model

<table>
<thead>
<tr>
<th>Food</th>
<th>Population group</th>
<th>Per-centile</th>
<th>Large portion (g/day)</th>
<th>Residue (mg/kg)</th>
<th>Intake (mg/kg body weight/day)</th>
<th>BMDL(_{10}) (mg/kg body weight/day)</th>
<th>Margin of exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sesame (processed)</td>
<td>Children, 2–4 years (140 person days)</td>
<td>97.5</td>
<td>23.4</td>
<td>0.05</td>
<td>0.00007</td>
<td>0.37</td>
<td>5286</td>
</tr>
<tr>
<td>Sesame (raw)</td>
<td>General population (15 person days)</td>
<td>90</td>
<td>39.6</td>
<td>0.05</td>
<td>0.00003</td>
<td>0.37</td>
<td>12,333</td>
</tr>
<tr>
<td>Sesame (processed)</td>
<td>General population (135 person days)</td>
<td>97.5</td>
<td>29.7</td>
<td>0.05</td>
<td>0.00002</td>
<td>0.37</td>
<td>18,500</td>
</tr>
</tbody>
</table>

According to EFSA PRIMO, children and the general adult population in Germany are also respectively the population groups with the greatest exposure to sesame at EU level. EFSA PRIMO (version 3.1), which includes both German consumption data as well as the corresponding data for consumer groups from other EU Member States, therefore provides the same results. It should be noted, however, that some data for EU consumer groups have not yet been implemented in PRIMO (such as data for Greek or Cypriot population groups, for example, which presumably exhibit higher levels of sesame consumption).

For children consuming a quantity of sesame per day equivalent to a high intake in the nutrition studies (‘large portion’), the intake quantity classified as an intake of low concern (0.037 µg/kg body weight/day, equating to a margin of exposure of 10,000 based on the BMDL\(_{10}\) was exceeded even with an ethylene oxide residue of just 0.05 mg/kg. For adults, the intake remained below this level. Mutagenicity as the critical effect is essentially considered to be an acute effect occurring after a single exposure. Secondary effects such as tumour formation occur later on, however. Since mutagenic effects are effectively irreversible and accumulate during the course of a lifetime, a risk assessment based on average exposure over


\(^{11}\) RIVM and Universität Wageningen, Front Office Food and Product Safety (2020) Risk Assessment of ethylene oxide in sesame seeds. 25/10/2020

longer periods of time is thus more meaningful. As a result of this irreversibility and the high probability of secondary effects such as carcinogenicity, single high intakes in childhood/adolescence are of particular concern and should generally be avoided.

If one considers average consumption over a prolonged period of time, the intake of low concern is exceeded neither in children (0.0005 µg/kg body weight/day) nor in adults (0.00001 µg/kg body weight/day) if compliance with the maximum residue level of 0.05 mg/kg is assured.

Further information on the subject from the BfR website

Biocides
https://www.bfr.bund.de/en/a-z_index/biocides-129802.html

Consumer safety and plant protection product residues

About the BfR

The German Federal Institute for Risk Assessment (BfR) is a scientifically independent institution within the portfolio of the Federal Ministry of Food and Agriculture (BMEL) in Germany. The BfR advises the Federal Government and the States (‘Laender’) on questions of food, chemical and product safety. The BfR conducts its own research on topics that are closely linked to its assessment tasks.