Bundesinstitut für Risikobewertung

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Health risk assessment of ethylene oxide residues in sesame seeds

Updated BfR Opinion No 024/2021 issued 01 September, 2021*

German regional authorities have detected residues of the substance ethylene oxide in products containing sesame seeds from India. The affected products comprised various categories such as bars, snacks or salad toppings and were taken off the market. At the same time, the public was informed by means of the EU's rapid alert system.

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 is not subject to a safe health-based guidance value and residues in food are generally undesirable. The BfR has therefore derived a so-called "intake level of low concern" based on the "large assessment factor approach" method of the European Food Safety Authority (EFSA). The approach principally serves as a tool for risk management for prioritising risk reduction measures with regard to scope and urgency. The approach calculates the amount of substance for which even in case of lifelong intake the additional risk of contracting cancer is unlikely to exceed 1:100,000. For ethylene oxide the BfR has calculated this "intake of low concern" to be as low as 0.037 micrograms per kilogram of body weight/day (µg/kg body weight/day). It should be noted, however, that in agreement with EFSA, this approach is not used to decide whether active substances or plant protection products can be authorised nor for setting maximum residue levels. Under no circumstances should the approach be used for determining the marketability of foodstuffs containing ethylene oxide residues, nor should it lead to a general abandonment of the requirement for the minimisation of genotoxic carcinogens without threshold.

Recent analyses from the regional authorities show that in the sesame samples investigated ethylene oxide was almost completely converted to 2-chloroethanol. Currently the EU jointly assesses both substances together, that is ethylene oxide and its metabolite 2-chloroethanol. The EU's maximum allowed residue level of 0.05 milligrams of ethylene oxide per kilogram of sesame is based on the respective analytical detection limit and relates to the sum of ethylene oxide and 2-chloroethanol. The subsumed values are reported as ethylene oxide. The BfR supports this approach given the indications of mutagenic activity by 2-chloroethanol in animal studies. Currently there is not enough data as to exclude with sufficient certainty the possibility of 2-chorethanol not having carcinogenic effects. However, there are no indications that the degradation product 2-chloroethanol might produce stronger mutagenic or carcinogenic effects than ethylene oxide. Further notice pending it is hence recommended to evaluate the genotoxicity and carcinogenicity of the metabolite 2-chloroethanol in line with that of ethylene oxide.

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 opinion replaces No 056/2020 of 23 December 2020

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. Any substance residues in food are therefore considered undesirable. The present data for the carcinogenic potential, i.e. the dose-response relationship for ethylene oxide, offer the possibility of an orientation assessment of the residues associated with cancer risk. Using EFSA's "Large Assessment Factor" approach[1], it can be determined whether exposure to the respective food is of high or low concern.

This approach defines the level of low concern as the amount resulting from the application of a safety factor of 10,000 to a dose leading to an increase in tumour frequency by 10 % in animal experiments. This amount may be associated over lifelong intake with a possible additional cancer risk of about 1:100,000 and can assist risk management in prioritising risk mitigation measures with regard to scope and urgency. For ethylene oxide the level of low concern was determined at 0.037 µg/kg bw/day.

In accordance with EFSA (2005), the BfR does not use this approach to decide on the eligibility of plant protection products for authorisation, the determination of maximum residue levels or the suitability for approval of active substances used in plant protection products. The approach should also not be used for determining the marketability of foodstuffs with ethylene oxide residues by state authorities. As stated by EFSA (2005), the BfR considers that the approach is suitable of estimating the level of risk for exposed persons, as well as assisting risk management to prioritise risk mitigation measures with regard to scope and urgency. In accordance with EFSA, the BfR was and is of the opinion that residues of carcinogenic plant protection products without threshold values, such as ethylene oxide in food, are undesirable regardless of the result of the "Large Assessment Factor" method.

Both EFSA (2005) and the BfR (2005, 2020) have noted that the derivation of a health-based guidance value without an associated health risk is not possible for genotoxic carcinogens lacking a threshold. Therefore, risk assessment recommends a comprehensive reduction of such substances in food and consumer products according to the ALARA Principle ("AS Low AS Reasonably Achievable"). Foodstuffs with relevant residues above the quantification limit or the legally binding maximum residue level should also, in principle, not be marketable even if a "low level of concern" has been determined.

Likewise, both the BfR and EFSA (2005) recognise that an assessment of the actual health risk due to the specific residue findings may be required to provide information about the risk

[1] EFSA (2005): 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. The EFSA Journal (2005) 282, 1-31. doi: 10.2903/j.efsa.2005.282

to exposed groups and to offer risk management guidance in determining urgency, prioritisation and scope of the necessary measures. However, the “Large Assessment Factor Approach” should not be used to deviate from the rule to minimise genotoxic carcinogens lacking a threshold value (BfR, 2005).[2]

When considering the ethylene oxide degradation product 2-chloroethanol data is inconsistent and partially incomplete. Therefore no reliable regulatory conclusion can be drawn on the carcinogenic properties of 2-chloroethanol. While there are numerous indications for genotoxic activity, clarification of the *in vivo* relevance or the existence of a potential threshold value are pending. According to the standards of Regulation (EC) No 1107/2009 and subordinate regulations for plant protection products including stock protection, for 2-chloroethanol no safe level of intake can therefore be derived. Yet there are no indications that 2-chloroethanol has a higher toxicity than ethylene oxide. In order to ensure the highest possible health protection and to prevent underestimations the risk assessment of 2-chloroethanol should therefore be carried out in the same way 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 according to Regulation (EC) No 396/2005. 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.

[2] BfR (2005) BfR Expert Opinion No. 029/2005 of May 18, 2005: Risk assessment of genotoxic and carcinogenic substances to be harmonized in the EU.

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 is a mutagenic carcinogen which 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 only a minimal additional risk for cancer. In the context of risk management this allows to prioritise risk mitigation measures with regard to scope and urgency.

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 bronchioloalveolar 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*'[3]. This approach has also been recommended by the BfR previously for the assessment of unavoidable concentrations of mutagenic and/or carcinogenic substances in food[4]. With this approach, the application of an extrapolation or safety factor of 10,000 to the relevant $BMDL_{10}$ value from a suitable animal experiment or from epidemiological surveys can be used to estimate an intake of low concern. The $BMDL_{10}$ value describes the calculated lower confidence limit of the dose that causes an increase in tumour incidence of 10 % following lifelong intake. The extrapolated intake quantity can hence be associated with an additional cancer risk of approx. 1:100,000 following lifelong exposure. The decision as to which measures and what urgency with regard to the residual health risk are deemed necessary is in any event a question for risk management. The approach described here is referred to as the '*large assessment factor approach*' from the EFSA and has been recommended as a tool for risk management 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)[5]. The study authors describe a dose-dependent increase in tumours of the gastrointestinal tract. This matches the expected profile for the direct effects of the comparatively chemically reactive ethylene oxide. In the above-mentioned 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

[3] The EFSA Journal (2005) 282, 1-31. doi: 10.2903/j.efsa.2005.282

[4] BfR Opinion no. 029/2005, dated 18 May 2005: Risk assessment of genotoxic and carcinogenic substances to be harmonised in the EU.

[5] Dunkelberg H (1982) Carcinogenicity of ethylene oxide and 1,2-propylene oxide upon intragastric administration to rats. Br. J. Cancer, 46, 924-933.

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 modelling[6]. The details of this re-evaluation were reviewed by the BfR and are considered to be plausible. A $BMDL_{10}$ 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_{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)[7] 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)[8]. 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)[9], mutagenic activity is considered plausible overall.

[6] RIVM and WFSR (2020) Risk Assessment of ethylene oxide in sesame seeds. Project No. V/093130. https://www.rivm.nl/sites/default/files/2020-11/FO%20beoordeling%20ethyleenoxide%20in%20sesamzaad_final_20201025_anon.pdf

[7] 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.

[8] Tennant, Margolin, Shelby, Zeiger, Haseman, Spalding, Caspary, Resnick, Stasiewicz, Anderson, et al. (1987) Prediction of chemical carcinogenicity in rodents from *in vitro* genetic toxicity assays. Science 236(4804):933-41. doi:10.1126/science.3554512.

[9] Grunow and Altmann (1982) Toxicokinetics of Chlorethanol in the rat after single oral administration. Arch Toxicol 49, 275-284. ASB2009-6059

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)[10] 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)[11] 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.

On the basis of the available data no safe intake can be derived for 2-chloroethanol according to the standards of Regulation (EC) No 1107/2009 and subordinate regulations for plant protection products, including stock protection.

[10] Shelby MD, Erexson GL, Hook GJ, Tice RR. Evaluation of a three-exposure mouse bone marrow micronucleus protocol: results with 49 chemicals. Environ Mol Mutagen. 1993;21(2):160-79. doi: 10.1002/em.2850210210.

[11] Kitchin, Brown, Kulkarn (1992) Predictive assay for rodent carcinogenicity using *in vivo* biochemical parameters: operational characteristics and complementarity. Mutat Res, 266(2):253−272

Assessments by the US EPA[12] the SCF[13] and the MAK[14] Commission differ in their legal remits as well as their respective data requirements. Hence, they are transferable only to a limited extent.

According to the particularly strict criteria for the assessment of plant protection products in the EU the BfR concludes that the available *in vivo* data are not suitable to waive the genotoxic effects observed *in vitro* with sufficient certainty. The existence of a possible threshold value associated with saturation of the cellular detoxification capacity is also not supported by current data.

Based on the available data the BfR concludes that the genotoxic and carcinogenic potency of 2-chloroethanol is not expected to exceed that of ethylene oxide after oral intake. Therefore, the BfR based its assessment on the respective value of 0.037 µg/kg bw/day.

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)[15]. 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[16] 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

[12] United States Environmental Protection Agency (US EPA) (2012) Provisional Peer-Reviewed Toxicity Values for 2-Chlorethanol. EPA/690/R-12/007F

[13] Scientific Committee on Food (2002) Opinion of the Scientific Committee on Food on Impurities of1,4-dioxane, 2-chloroethanol and mono- and diethylene glycol in currently permitted food additives and in proposed use of ethyl hydroxyethyl cellulose in gluten-free bread. SCF/CS/ADD/EMU/198 Final

[14] A. Hartwig (2019) 2-Chlorethanol - MAK Value Documentation in German language. The MAK Collection for Occupational Health and Safety 2019, Vol 4, No 2, DOI: 10.1002/3527600418.mb10707d0067

[15] Australia New Zealand Food Authority (ANZFA) (2000) Full assessment report MRL for ethylene oxide in herbs and spices. Application a412. 29/11/2000

[16] RIVM and Universität Wageningen, Front Office Food and Product Safety (2020) Risk Assessment of ethylene oxide in sesame seeds. 25/10/2020

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[17].

Exposure assessment using the NVS II national consumption model

Food	Population group	Percentile	Large portion (g/day)	Residue (mg/kg)	Intake (mg/kg body weight/day)	$BMDL_{10}$ (mg/kg body weight/day)	Margin of exposure
Sesame (processed)	Children, 2–4 years (140 person days)	97.5	23.4	0.05	0.00007	0.37	5286
Sesame (raw)	General population (15 person days)	90	39.6	0.05	0.00003	0.37	12,333
Sesame (processed)	General population (135 person days)	97.5	29.7	0.05	0.00002	0.37	18,500

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 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.

[17] http://www.bfr.bund.de/cm/343/bfr-berechnungsmodell-zur-aufnahme-von-pflanzenschutzmittel-rueckstaenden-nvs2.zip

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
https://www.bfr.bund.de/en/consumer_safety_and_plant_protection_product_residues-197980.html

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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.