STATEMENT

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Human dietary exposure assessment to newly expressed proteins in GM foods

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Abstract

EFSA carries out the risk assessment of genetically modified organisms (GMOs) submitted under Regulation (EC) 1829/2003 and Regulation (EU) 503/2013. Dietary exposure is an essential element of the risk assessment on genetically modified (GM) foods. Dietary exposure estimations should cover average and high consumers across all the different age classes and special population groups and identify and consider particular consumer groups with expected higher exposure. This EFSA statement provides guidance on how human dietary exposure to newly expressed proteins in GM foods should be estimated using a deterministic model that makes use of the available information. Summary statistics of consumption of foods containing, consisting of and produced from crops relevant for the assessment of GMO applications are available in the EFSA website together with different factors to convert the reported consumption of processed foods into raw primary commodities. Guidance is also provided on how concentration data of newly expressed proteins, typically determined in raw primary commodities, should be used (materials to be analysed, growth stage, descriptive statistics to be used, etc.). An overview of the different uncertainties linked to the dietary exposure estimations is provided, informing on the strengths and limitations of the assessment. The document also describes the information applicants need to provide on human dietary exposure to allow EFSA doing an appropriate evaluation of the assessment provided as part of the application dossiers.

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1. Introduction

The European Food Safety Authority (EFSA) carries out the risk assessment of genetically modified organisms (GMOs) submitted under Regulation (EC) No 1829/2003.[1] Dietary exposure is an essential element of the risk assessment on genetically modified (GM) foods. As described in the scientific requirements (Annex 2) of Regulation (EU) 503/2013[2] and the EFSA Guidance for risk assessment of food and feed from genetically modified (GM) plants (EFSA GMO Panel, 2011), applicants are requested to estimate, by appropriate methods, the concentrations of newly expressed proteins, other new constituents and endogenous food and feed constituents, of which the levels have been altered as a result of the genetic modification. The expected intake of (exposure to) these constituents should consider the influences of processing, storage and expected use of the food and feed in question. In addition, applicants are also required to consider potential non-dietary exposure through different routes and sources (e.g. occupational exposure).

More generally, exposure assessment is defined as the qualitative and/or quantitative evaluation of the likely intake of biological, chemical or physical agents via food as well as other sources and routes, if relevant (FAO/WHO, 1997). When dealing with exposure assessment, it is important to distinguish between external and internal dose. The external dose refers to the amount of chemical potentially available for absorption after inhalation, dermal contact or ingestion; the internal dose, absorbed dose or systemic dose is the fraction of the external dose that has been absorbed and enters the general circulation (EFSA, 2016). When food is not considered, we refer to non-dietary exposure that typically occurs by at least one of the three exposure routes: inhalation, oral and dermal. When the focus is put on exposure through the diet to food chemicals that are unintentionally present in food or added for a technological purpose, we refer to dietary exposure. The term dietary intake is, instead, generally used to refer to the ingestion of nutrients, nutritive substances, novel foods, food ingredients, or biologically active substances, which have nutrition or health purposes. These definitions can be particularly confusing in GMO risk assessment since some constituents, such as newly expressed proteins, could be considered both nutrients and compounds that have been incorporated to food crops without nutritional or health purposes. Since in the case of newly expressed proteins the focus is on their safety assessment rather than on their nutritional relevance, the preferred option is to refer to dietary exposure assessment to newly expressed proteins.

Human dietary exposure assessments consider different durations of the exposure based on the outcome of the hazard characterisation (i.e. acute and/or chronic hazards). Chronic (long-term) exposure represents, in general, average daily exposure over years or the entire lifetime, while acute (short-term) exposure covers a period of up to 24 h. Although when estimating human dietary exposure attention is paid, in general, to the whole population (represented by the individuals participating in the survey), in certain occasions the assessment is extended to consumers only to cover exposure to compounds present in rarely consumed foods (USEPA, 2000; Boon et al., 2004).

2. Scope

This statement provides supplementary information for the risk assessment of foods containing, consisting of and produced from GM plants and submitted within the framework of Regulation (EC) No 1829/2003 and Regulation (EU) No 503/2013, and it also complements the Guidance Document on risk assessment of food and feed from GM plants (EFSA GMO Panel, 2011) and the EFSA statement on the use of the Comprehensive European Food Consumption database for estimating dietary exposure to GM foods (EFSA, 2015a). This document covers human dietary exposure assessments to newly expressed proteins as well as the consumption and concentration data used for the exposure estimations. Human dietary exposure in this statement refers to external dietary exposure (external dose), considering the different constituents at the moment the foods are ingested. The use of this statement to estimate dietary exposure to other GM constituents should be done on a case-by-case basis, following a careful evaluation of all available information (e.g. chemical characteristics of the constituents, information on processing, etc.).

[1] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. OJ L 268, 18.10.2003, p. 1–23.

[2] Commission Regulation (EU) No 503/2013 of 3 April 2013 on application for authorization of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 604/2004 and (EC) No 1981/2006.

3. Objective

The main aim of this statement is to provide guidance on how human dietary exposure to newly expressed proteins in GM foods should be estimated, and on how the available information on consumption (summary statistics) and constituent concentration should be used. Guidance is also given on the information applicants need to provide to EFSA on human dietary exposure to allow EFSA doing an appropriate evaluation of the assessment provided.

4. Transition period

Since applicants regularly provide dietary exposure assessments as part of GMO application dossiers submitted under Regulation (EU) 503/2013 making use of the data (consumption and concentration data) described and addressed in this statement, a general two-month transition period is granted for the implementation of the requirements described in this document after its publication. The requirements will be applicable for GM plant applications submitted after this transition period.

An exception applies when plant material needs to be generated to produce concentration data needed to estimate human dietary exposure (e.g. levels of newly expressed proteins in pollen). In this case, the requirement to produce and make use of these concentration data will be applicable for GM plant applications submitted 24 months after the publication of this statement (EFSA, 2014a).

5. Background

The information on human dietary exposure provided by the applicants to EFSA in the context of the GMO application dossiers is very heterogeneous. The main differences refer to the use of sources of consumption data (i.e. Pesticide Residues Intake MOdel (PRIMo), Food Balance Sheets (FBSs), EFSA Comprehensive European food consumption database), the different strategies used to derive the concentration of constituents in processed foods, and the type of dietary exposure estimations reported, i.e. acute and/or chronic.

Overall, when performing dietary exposure to GM foods, the focus is on newly expressed proteins, e.g. proteins expressed in GM crops conferring insect resistance and/or herbicide tolerance. Examples of typical newly expressed proteins, among many others, are 5-enolpyruvylshikimate-3-phosphate-synthase (CP4-EPSPS/mEPSPS) enzyme that confers tolerance to glyphosate herbicides, phosphinothricin *N*-acetyltransferase (PAT) enzyme that confers tolerance to glufosinate (phosphinothricin) herbicides or crystal proteins (Cry) that are endotoxins produced by *Bacillus thuringiensis* conferring resistance to lepidopteran insects (EFSA GMO Panel, 2017a, 2018a). In certain occasions, dietary exposure assessment to endogenous food constituents is also performed to characterise potential risks associated, for instance, to significant increases in concentrations of *N*-acetyl amino acids in GM crops expressing glyphosate *N*-acetyltransferase (EFSA GMO Panel, 2013, 2017b,c).

As concerns newly expressed proteins, in most cases only acute exposure estimates are considered by applicants because toxic proteins are believed to mainly act through acute mechanisms of action (Sjoblad et al., 1992; Hammond et al., 2013). This is a concept widely accepted by the US Food Drug Administration (FDA) and followed by applicants; generally, acute exposure estimates are used in conjunction with acute/short-term toxicological studies for the risk characterisation of the acute exposure in applications for authorisation in Europe.

Regarding *N*-acetyl amino acids, these amino acid derivatives are well known in the mammalian metabolism. They are also present in relatively small quantities in conventional foodstuffs and thus consumed as part of a normal diet (Hession et al., 2008; van de Mortel et al., 2010a,b). *N*-acetyl-aspartic acid (NAA) and *N*-acetyl-glutamic acid (NAG) are *N*-acetyl amino acids which are found in much higher concentrations in some GM crops expressing glyphosate *N*-acetyltransferase (GAT) as compared to conventional crops. Unlike newly expressed proteins, in the context of GM plant applications, repeated-dose 90-day oral toxicity studies are available for some individual *N*-acetyl amino acids such as NAA (EFSA GMO Panel, 2013). These studies allow deriving health-based guidance values that can be subsequently combined with chronic dietary exposure estimations to characterise potential risks. Moreover, as also routinely submitted for newly expressed proteins, acute/short-term oral toxicity studies in rats are available for various *N*-acetylated amino acids.

6. Dietary exposure assessment to newly expressed proteins in GM food

Two types of data are required to estimate dietary exposure: occurrence or concentration data that provides information on the amount of a compound/s present in different food commodities, and consumption data that informs on the intake of these food commodities. By combining these two types of data and considering the body weight of the subjects, dietary exposure is estimated.

$$\textbf{Dietary exposure} = \sum[(\textbf{Concentration of compound}) \times (\textbf{Amount of food consumed})]/[\textbf{body weight} \times \textbf{number of days}]$$

In the next sections, the focus is put on how both the available concentration and consumption data should be used to estimate dietary exposure in a deterministic model. In the frame of the application dossiers submitted for the authorisation of GM crops, dietary exposure to newly expressed proteins is required for GM crops containing either a single transformation event or stacked events.[2] Newly expressed protein concentrations reported in the GM stack are the most appropriate to estimate the dietary exposure to each newly expressed protein following the consumption of foods containing, consisting of and produced from the GM stack. The estimated dietary exposure to a newly expressed protein in a GM stack crop may differ from the dietary exposure estimated to the same newly expressed protein in the context of the respective single-event GM crop applications: consumption data could be different as new dietary surveys became available, new foods could enter the market, and/or protein expression levels in the GM stack crop could differ from those in the single-event GM crop because the protein concentrations could be, for instance, the result of two events in the GM stack crop.

It is also important to mention that estimations of dietary exposure in this statement refer to external dietary exposure (external dose), considering the different constituents at the moment the foods are ingested. The internal dietary exposure to these constituents and/or the compounds generated following their absorption, distribution, metabolism and excretion (ADME) is out of the scope of this statement and therefore is not covered here. Irrefutable scientific evidence showing that the compounds under assessment are shortly degraded/removed during food processing or after ingestion without generating other compounds that may represent a hazard would indicate that dietary exposure estimations are not needed (EFSA GMO Panel, 2018b).

6.1. Concentration data

According to Regulation (EU) 503/2013, the concentrations of different constituents in GM crops are determined in raw primary commodities as these represent the main point of entry of the material into the food/feed chain production (e.g. in maize grains instead of popcorn). On a case-by-case basis, data on food constituents in selected processed commodities are available as part of the submitted dossiers or after request to applicants (EFSA GMO Panel, 2016). Regulation (EU) 503/2013 and the Guidance Document on risk assessment of food and feed from GM plants (EFSA GMO Panel, 2011) establish a minimum of three growing sites or one site over three seasons for the analysis of the expression levels of newly expressed proteins; this minimum requirement is usually implemented ensuring five replicates for each site/season. To minimise potential uncertainties and maximise the reliability and accuracy of dietary exposure estimates, it is fundamental to use the available concentration data in the most sound manner as further detailed below.

In the case of crops genetically modified to acquire herbicide tolerance, the analytical data from the GM-crop treated with the intended herbicides (expressed in fresh weight) should be used for dietary exposure estimations since these data are the most representative of the growing conditions of the GM crop entering the food chain once authorised. The analytical data should refer to those parts of the plant used for food purposes (grains, pollen, etc.) at the relevant growth stages. When information is provided on more than one growth stage that could enter the food chain (e.g. maize grains from R6 and senescent plants), dietary exposure should be estimated using the growth stage with the highest expression levels.

In estimating chronic dietary exposure, mean concentrations of the constituents under assessment expressed on a fresh weight basis should be used as they best represent the person's average dietary exposure over a long-term period. For acute dietary exposure estimations, the usual approach in other food domains is to use high compound concentrations (high percentiles, e.g. 95th percentile) of the most consumed food commodity (in some cases, for more than one food commodity) to represent worst-case situations. In the case of the GMO domain, the situation is different as concentration data are

usually only available for raw agricultural commodities. During the production of processed foods, raw primary commodities from different fields containing different concentrations of a target compound are typically blended (e.g. maize grains from different fields to produce cornmeal). Therefore, using high percentiles of concentration for acute dietary estimations would represent overly conservative scenarios. When working with raw primary commodities that are commonly consumed as processed blended commodities, the use of the mean concentrations represents the most adequate approach to estimate acute dietary exposure. Ad hoc scenarios might be needed when the concentration of one constituent differs among the different field sites; this could be indicative of certain environmental conditions affecting the concentration of the constituents. Under this scenario, the average concentration of the site where the highest concentration is reported should be used to cover a hypothetical worst-case scenario where processed foods are produced from GM crops cultivated in that site.

For both chronic and acute dietary exposure estimations, mean concentration should be derived by using all available samples. When individual analytical results for constituents are reported as below the limit of detection (LOD) or the limit of quantification (LOQ), these left-censoring limits should be used to derive the mean values used for dietary exposure estimations. The results below the LOD are replaced by the LOD and those below the LOQ by the value reported as LOQ; this is an upper-bound scenario (conservative) which relies on the substitution method commonly used in EFSA for the treatment of left-censored data (EFSA, 2010), and recommended in the 'Principles and Methods for the Risk Assessment of Chemicals in Food' (WHO/IPCS, 2009).

In the case concentration data are available on processed commodities (e.g. corn flakes, bread, etc.), the use of a value representing high concentration among those reported is the most appropriate approach to estimate a worst-case situation for acute dietary exposure. As an indication, the 95th percentile value of the distribution of the data is typically used; however, if the number of samples is lower than 60 this percentile is not considered statistically robust (EFSA, 2011a), and alternatively the average of the highest quartile can be used as indicative of samples with high concentration of the compound of interest.

The processing of raw primary commodities into different food commodities can impact the concentration of new and endogenous constituents; Regulation (EU) 503/2013 requires to evaluate the effects of processing on the new compounds, and in particular for the newly expressed protein: '. . .it shall be necessary to assess the extent to which the processing steps lead to the concentration or to the elimination, denaturation and/or degradation of these protein(s) in the final product'. As regards nutrients, composition databases habitually contain information on their presence in processed commodities and, in addition, there is a relatively good knowledge on how nutrients are affected by processing/cooking. Similar situation occurs for some antinutrients, such as for lectins present in legume seeds which are significantly reduced by heating processes due to their heat-sensitive nature while other processes such as soaking hardly decreases their levels (Shi et al., 2018). Concerning newly expressed proteins, it is well known that they are not present in certain processed commodities such as oil produced from different seeds/grains (maize, soybean, cotton, etc.), corn syrup and some distilled alcoholic beverages such as whiskey among others. However, for many other processed food commodities, more studies are needed to fully understand the fate of these proteins during processing and, above all, the effect of processing on the potential toxicity of the protein. Lacking this information, the preferred approach to assess consumer safety is to follow a conservative scenario considering that processing (e.g. baking, fermentation) does not affect the newly expressed proteins. It has to be also noted that, in specific cases, the concentration of certain constituents may increase in processed commodities as compared to the levels initially reported in the raw primary commodities (e.g. liposoluble compounds in oil or newly expressed protein levels in protein isolates).

Even if the knowledge on the fate of newly expressed proteins during food processing is limited, a direct link between the levels reported in the raw primary commodities and the consumption data on processed commodities should be avoided. In order to obtain more accurate and realistic dietary exposure estimations, two approaches can be used: one approach, already used in several GMO applications, is to use the ratio of total protein content between processed foods and raw primary commodity. Total protein content of raw primary commodities is generally provided as part of the application and the total protein content in processed foods can be easily retrieved from different food composition databases. To estimate the newly expressed protein concentrations in processed commodities, the concentration of the newly expressed protein reported in the raw primary commodity is multiplied by this ratio. This approach considers that all proteins present in processed commodities come from the GM crop and that no loss of newly expressed protein occurs during processing (conservative approach).

The second approach is the use of factors resulting from combining standard recipes that allows deriving the amount of GM crop-derived ingredients in processed foods, with the reverse of the yield efficiency when converting raw primary commodities into these ingredients. EFSA has recently published a technical report describing the conversion of the consumption data contained in the EFSA Comprehensive European food consumption database (EFSA consumption database) into consumption data of raw primary commodities creating the RPC Consumption Database (EFSA, 2019). The combined factors are applied to the newly expressed protein concentrations reported in raw primary commodities or alternatively to the consumption data retrieved from the EFSA consumption database before estimating dietary exposure. As an example, to estimate the dietary exposure to a particular newly expressed protein through the consumption of maize bread, the first step would be to disaggregate this processed commodity into its different ingredients to find how much GM crop-derived ingredients are used. For maize bread, a standard recipe describes that 26.4 g of maize flour is used to produce 100 g of bread (together with water, wheat flour, etc.). The yield efficiency of milling maize is around 0.82 (i.e. the milling of 100 g of maize grains produces approximately 82 g of flour). Multiplying the reverse of this yield efficiency (1.22) by the amount of maize flour per gram of bread (0.264) results in a factor of 0.32 that should be applied to the newly expressed protein concentration reported in the maize grains (raw primary commodity) to estimate the concentration of newly expressed protein in maize bread. Alternatively, the amounts of maize bread reported in the EFSA consumption database could be converted into maize grains considering that 32.3 g of maize grain are used to produce 100 g of maize bread; then the 32.3 g of maize grain could be directly linked to the newly expressed protein concentration reported in the maize grains. This approach assumes that no loss of newly expressed protein occurs during processing, i.e. the concentration of newly expressed proteins is only based on the amount of raw agricultural commodity used to produce the processed food.

6.2. Consumption data

Given the lack of specific consumption data on GM foods and primarily aiming to maximise consumer protection, a conservative scenario with full replacement of the consumption of conventional foods by their GM counterparts is followed.

Traditionally, as source of consumption data, GM plant applications provided Food Balance Sheets (FBSs) that contain data collected by the Food and Agriculture Organization of the United Nations (FAO) on raw or semi-processed commodities.[3] These data reflect food availability rather than food consumption, as they are based on production of commodities, imports, exports and stocks. As the losses due to cooking or processing are not easy to assess, it is assumed that, overall, the use of FBSs tend to overestimate about 15% the consumption as compared to national dietary surveys (Héraud et al., 2013). As they refer to the average availability of foods per person, FBSs can be used to estimate chronic dietary exposure in the average population but they are not adequate for high consumers, particular groups of populations or for acute estimations (Héraud et al., 2013). Another source of consumption data habitually used in GM plant applications is the PRIMo. This model contains summary statistics of food consumption expressed as raw primary commodities for children and adults provided by Member States (EFSA, 2007b, 2018). One of the limitations of these consumption data refer to the different methodologies used in each country to convert the consumed foods into raw primary commodities. Another limitation is the fact that it is not possible to identify the different processed commodities initially reported in the dietary survey and then disaggregated into raw primary commodities. This implies that the total amount of raw primary commodity could have been derived from consumed processed foods that are not relevant for the exposure to a particular constituent (e.g. maize oil when estimating exposure to a particular newly expressed protein); this last aspect also affects to the data from FBSs.

EFSA published in 2015 a statement on the use of the EFSA consumption database to estimate dietary exposure to GM foods (EFSA, 2015a). In its 2015 statement, EFSA encourages applicants to use summary statistics of consumption derived from the EFSA consumption database to estimate dietary exposure. Following the publication of this EFSA statement, the EFSA consumption database became the habitual source of consumption data used in GM plants applications. When first launched in 2010, the EFSA consumption database contained the most recent national dietary surveys on consumption by individual consumers as provided by different EU Member States (EFSA, 2011a), together with food consumption data for children (Huybrechts et al., 2011). The EFSA consumption

[3] http://www.fao.org/economic/ess/fbs/en/

database is periodically updated with new dietary surveys from the EFSA's ongoing EU Menu project that aims to provide standardised information on consumption in all countries and regions across the EU (EFSA, 2014b). A total of 60 dietary surveys from 25 different Member states accounting for almost 120,000 subjects and more than 12 million consumption records are available at the moment (last update: April 2018).

Summary statistics of the EFSA consumption database can be accessed via the EFSA website using a business intelligent tool called MicroStrategy and easily downloaded in different formats (xls, xlm, pdf). The summary statistics contain data on chronic and acute consumption of different food commodities divided by different age classes (from infants to adults aged 75 years or older) and European countries (considering the total population and consumers only).[4] In addition, consumption data on particular groups of population such as 'Pregnant women' and 'Lactating women' are also available. Consumption data are codified under FoodEx, the EFSA Food classification and description system for exposure assessment (EFSA, 2011b) and under the most updated version of this system called FoodEx2. The data codified under FoodEx2 provides higher level of details as the foods can be classified up to a maximum of seven different levels; consumption data codified under FoodEx2 may allow in certain cases more accurate estimations of dietary exposure as compared to data codified under FoodEx (EFSA, 2015b).

The GMO unit has made available in the EFSA website[5] different Microsoft Excel™ worksheets containing specific summary statistics of consumption of foods containing, consisting of and produced from crops relevant for the assessment of GMO applications; these statistics have been extracted from the EFSA consumption database using MicroStrategy and are regularly updated when new dietary surveys become available. The consumption data are codified under the first version of FoodEx, with 20 main food categories that are further divided into subgroups up to a maximum of four levels, comprising about 1,700 different end-points (food names) (EFSA, 2011b). The degree of accuracy and the level of details as provided by the consumption data codified under the first version of FoodEx are considered sufficient for estimating dietary exposure in the area of GMO risk assessment. These summary statistics from the EFSA consumption database should be used by applicants to estimate chronic and acute dietary exposure to constituents present in GM foods. Together with the summary statistics of consumption, the GMO unit has also published information for each relevant GM crop (e.g. maize, soybean, rapeseed) regarding the use of factors to convert the reported consumption of processed foods into raw primary commodities (as described in Section 6.1, second approach). Summary statistics as well as information on conversion factors will be updated as needed; therefore, applicants are asked to visit EFSA website regularly before estimating human dietary exposure to ensure the use of the most recent data and information available.

6.2.1. How to use summary statistics of consumption data

The Microsoft Excel™ worksheets contain summary statistics on chronic and acute food consumption for the whole population/all days and for consumers only/consumption days only, expressed in grams/kg body weight per day, codified under the first version of FoodEx.

For chronic dietary exposure estimations, dietary surveys with only 1 day are excluded from the Microsoft Excel™ worksheets since they are considered not adequate to assess chronic exposure because the number of assessed days affects the distribution of consumption, particularly the upper tails (EFSA, 2007a). For acute dietary exposure estimations, all dietary surveys are considered.

In a first step, only those consumed foods considered as potential contributors to the dietary exposure to the constituent under assessment should be selected; e.g. in the case of dietary exposure estimations to newly expressed proteins, protein-free commodities should be disregarded (e.g. oil). If concentration data for processed commodities are also available, the link with the consumption data can be directly done to estimate exposure. In the case concentration data are reported for raw primary commodities, the consumption data for processed commodities should be converted into amounts of raw primary commodity using the available factors before estimating dietary exposure (see Sections 6.1 and 6.2).

The approach to estimate dietary exposure in average and high consumers is based on that described in the original Food Additives Intake Model (FAIM)[6] produced in 2012 by EFSA to estimate dietary exposure to food additives using summary statistics from the EFSA consumption database. As

[4] https://www.efsa.europa.eu/en/food-consumption/comprehensive-database
[5] http://www.efsa.europa.eu/en/applications/gmo/tools
[6] https://www.efsa.europa.eu/sites/default/files/assets/faimtemplateinstructions.pdf

stated in the 2015 EFSA statement on the use of the EFSA consumption database to estimate dietary exposure to GM foods (EFSA, 2015a), the calculated exposure estimates using this approach are comparable to those obtained by using the food consumption data at the individual level (EFSA, 2014c). Recently, a comprehensive European dietary exposure model (CEDEM) was developed based on the summary statistics from the EFSA consumption database and the FAIM model; the exposure estimates were very close to those reported using individual consumption data in EFSA Scientific Opinions of food additives (Tennant, 2016).

Chronic dietary exposure

Chronic dietary exposure in the average population, within a particular country, dietary survey and age class/special population group, is estimated as the sum of the exposure from each relevant food which is obtained by multiplying the average consumption of each food in the whole population (at the highest level of detail) by the mean concentration value reported in the GM crop/processed commodity for the compound of interest (e.g. newly expressed protein).

Chronic dietary exposure in high consumers, within a particular country, dietary survey and age class/special population group, is estimated by adding the high percentile dietary exposure estimates from the dominant food category[7] from consumers only (using the 95th percentile consumption) to the mean dietary exposure estimates from all the other foods in the whole population (using the mean consumption), in both cases using the mean value reported for the compound of interest in the GM crop. This approach assumes that an individual might be a high consumer of only one food commodity since it is considered very unlikely that individuals are high consumers of more than one food category when a limited number of food categories are used.

Acute dietary exposure

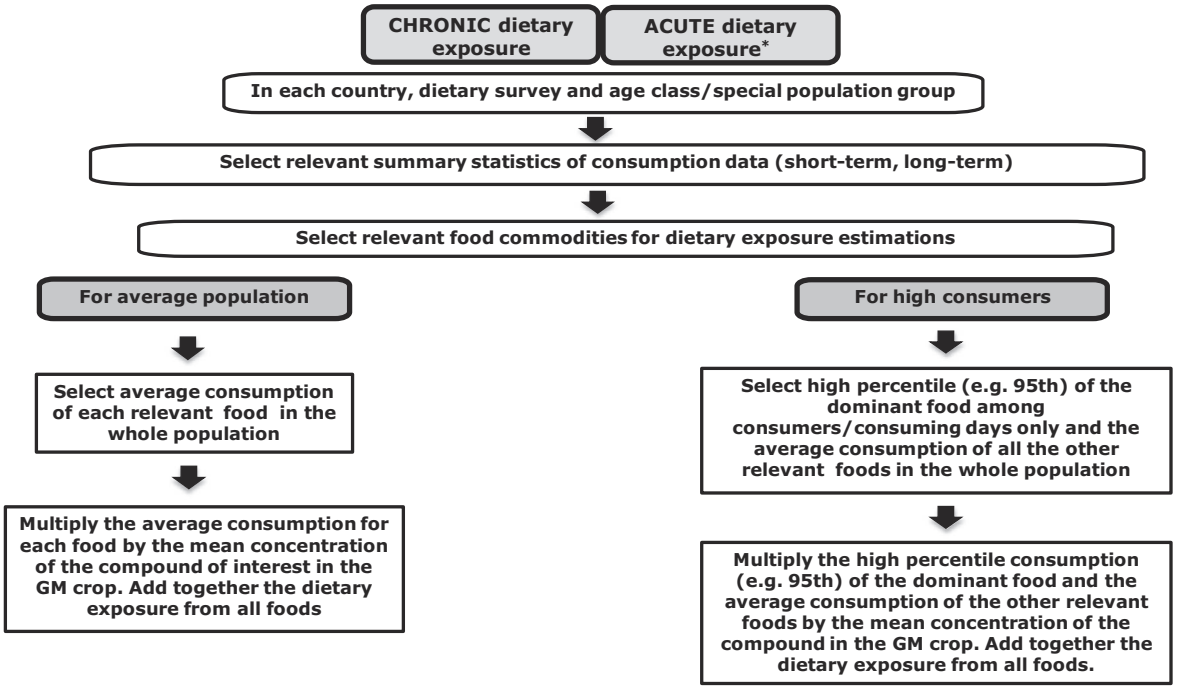
To estimate acute dietary exposure in the average population and in high consumers, the approach to follow should be the same as used to estimate chronic dietary exposure, with the difference that summary statistics for acute consumption also include one-day dietary surveys.

In the unusual situation when constituent levels in processed commodities are available, high percentiles of the concentration data should be used to assess worst-case scenarios instead of average values as used when levels are reported in the raw primary commodities. Accordingly, the acute dietary exposure in the average population should be estimated by adding the mean dietary exposure of the dominant food, obtained by multiplying the average consumption in the whole population by the 95th percentile concentration in the processed commodity, to the mean dietary exposure estimates from all the other foods also in the whole population using mean concentration values. The acute dietary exposure in high consumers should be estimated by adding the high percentile dietary exposure estimates (e.g. 95th percentile) from the dominant food, obtained by multiplying high consumption (95th percentile) in consuming days only by the 95th percentile concentration in the processed commodity, to the mean dietary exposure estimates from all the other foods in the whole population using mean concentration values.

Special attention should be paid to the centiles in the consumption data since its statistical robustness is very much affected by the number of subjects available and, therefore, wherever possible, the highest centile supported by the data should be selected. Hence, when the number of subjects/days is lower than 60, the 95th percentile should not be used for the dietary exposure estimations as it may not be statistically robust; instead, the mean consumption in consumers only should be used (EFSA, 2011a).

Figure 1 shows the different steps to be considered when using summary statistics of consumption data to estimate both acute and chronic dietary exposure for the average population and high consumers.

[7] Dominant food commodity refers to the food that will lead to the highest exposure among all consumed foods.



*: If processed food commodities are available, see Section 6.2.1 for details on how to use concentration data to estimate dietary exposure.

Figure 1: Steps to follow to estimate dietary exposure to GM food constituents using the available summary statistics of the EFSA consumption database (http://www.efsa.europa.eu/en/applications/gmo/tools)

In estimating dietary exposure using summary statistics, the estimates are considered sufficiently conservative (in particular for chronic). When initial dietary exposure estimations using summary statistics represent a possible health concern (e.g. estimates close to or above health-based guidance values, if these values are available), applicants are advised to revisit the dietary exposure estimations considering different scenarios (e.g. the 100% replacement scenario for the consumption data) and/or additional information (e.g. information on levels in processed foods). If needed, more accurate and precise exposure estimates can also be provided by EFSA by making use of the raw individual data available in the EFSA consumption database.

6.3. Ad hoc dietary exposure scenarios

Regulation (EU) 503/2013 requires to identify and consider particular groups of the EU population with an expected higher exposure and consider this higher exposure within the risk assessment; ad hoc scenarios are used for this purpose. These scenarios are usually focused on consumers only and calculated and reported separately from the dietary exposure estimations provided for the whole population. As an example, ad hoc scenarios could be pertinent for food commodities recently introduced in the market as novel foods (e.g. rapeseed protein isolates) or food supplements consumed by particular groups of populations (e.g. pollen supplements). If data are not available on processed commodities, the concentration values for the GM food constituents under assessment can be derived from experimental/literature data (if available), otherwise using the concentrations reported for raw primary commodities (e.g. rape seeds) in field trials, considering the potential effect of processing when possible.

A tiered approach can be followed to estimate dietary exposure in the ad hoc scenarios. For soybean and rapeseed protein isolates where no specific consumption data are available, a conservative scenario could make use of reported average and high protein intake in different age classes (EFSA NDA Panel, 2012) assuming that all consumed protein is derived from the protein isolates. If under this scenario a safety concern is identified, more refined scenarios as those using consumption data of food commodities where the protein isolates are intended to replace similar commodities should be considered (e.g. replacing soy protein isolates in meat imitates, bakery

products, protein supplements, etc.). Similarly, for pollen supplements a 100% replacement scenario assuming that the supplements contain uniquely pollen from the GM plant under assessment should be initially used.

6.4. Uncertainties in dietary exposure estimations

The identification of the sources of uncertainties associated with dietary exposure estimations is pivotal for a reliable estimation of the overall risk and, at the same time, informs on the strengths and limitations of the assessment.

Some of the uncertainties are linked to the 'point estimate' (deterministic) approach described here to estimate dietary exposure, i.e. when a fixed value for food consumption (average or high consumption value) is combined with a fixed value of concentration of a particular constituent (e.g. mean value) and the exposures from each food are summed to estimate the total dietary exposure. Although these exposure models are intended to be sufficiently conservative, a comprehensive evaluation of their inherent uncertainties is needed to confirm such a level of confidence on the outcome of the assessment. Together with the uncertainties linked to the exposure model, other relevant sources of uncertainties are related to the data used (consumption and concentration data); the main uncertainties are listed in Table 1 indicating the direction of their contribution on the outcome of the dietary exposure assessment. A more detailed description of all uncertainties and limitations typically linked to the use of consumption data and to the use of the EFSA Consumption Database has been already provided (EFSA, 2011a).

Table 1: Main sources of uncertainty (qualitative evaluation) and their impact on the dietary exposure estimations to newly expressed proteins using a point-estimate (deterministic) approach

Sources of uncertainty	Direction[(a)]
Using high percentile (e.g. 95th) of the most consumed food among only consumers and the average consumption of all the other foods in the whole population to estimate chronic and acute dietary exposure[(b)]	+
Extrapolation from food consumption surveys of few days to estimate long-term (chronic) exposure in high consumers[(c)]	+
Use of 100% replacement scenario due to the lack of consumption data on GM foods	+
Concentration data (representativity of the samples, measurement uncertainty of analytical results)	+/−
Conversion model used to make use of the measured concentrations in raw primary commodities to estimate dietary exposure (processing/recipe factors, total protein concentration, stability of the constituents, etc.).	+

(a): + = uncertainty with potential to cause over-estimation of exposure; − = uncertainty with potential to cause under-estimation of exposure;
(b): Kettler et al., 2015; EFSA, 2011a.
(c): EFSA, 2011a.

Overall, the impact of the uncertainties from all the above mentioned sources would lead to an overestimation of the dietary exposure to GM food constituents, thus providing conservatives estimates.

6.5. Reporting dietary exposure estimations

When reporting human dietary exposure estimations, the descriptive information should allow an appropriate assessment and address the impact of the uncertainties associated to the different assumptions taken. The following bullet points describe the minimum information requirements and recommend how to report such information:

- The source of consumption data should be reported (e.g. EFSA consumption database) together with a list of the EU countries, dietary surveys and age classes/special population groups covered.
- Information on the source of concentration data (i.e. raw primary commodities, processed commodities) used in the exposure estimations should be reported indicating the number of samples analysed with the analytical data expressed in fresh weight whenever possible. If the sources of data are raw primary commodities, further information such as the part of the plant,

growth stage, and the number of field sites used should be included together with any other information considered relevant for the assessment (e.g. average concentration in each field site). Analytical data should be produced from GM-crops treated with the intended herbicides.

- Any factor used on the original consumption/concentration data (e.g. based on reverse yield factors and/or recipes, protein content) should be reported and the related assumptions described. Any other assumption (e.g. 100% replacement based on the absence of consumption data of GM foods) should be reported and discussed in terms of impact on the outcome of the exposure estimations.
- Dietary exposure estimates should cover both short-term and long-term consumption and should be reported for average and high consumers; the approaches used to estimate dietary exposure should be described in each case, explaining and justifying any possible deviation to the exposure model described in this statement.
- The report on dietary exposure (chronic and acute) should contain tabulated exposure estimates presented by country, dietary survey and age class/special population group for average and high consumers.
- If ad hoc scenarios are used to estimate dietary exposure, these scenarios as well as the assumptions made should be thoroughly described.

7. Future directions

EFSA is continuously working to improve the scientific tools used for its risk assessment activities. Part of this effort is put on dietary exposure assessment, and some of the work recently developed in EFSA together with some on-going projects may have a direct impact on the way dietary exposure assessment to GM food constituents will develop in the future.

The dietary exposure to GM constituents can vary depending on the levels of the constituent in GM foods, the arrival of new GM products on the market, changes in processing and/or variation in consumption data (consumption habits, new consumption data available). These variables need to be regularly monitored to understand their possible impact on the dietary exposure and, consequently, on the outcome of the risk assessment (e.g. conclusions on the safety of newly expressed protein and, therefore, on the safety of the GM foods).

7.1. Raw primary commodity (RPC) model

EFSA recently published a technical report describing the conversion of the consumption data contained in the EFSA consumption database into consumption data of raw primary commodities creating the RPC Consumption Database (EFSA, 2019).To carry out this work, EFSA considered different reverse yield factors as well as recipes from different sources to estimate the amounts of raw primary commodities in each reported processed foods consumed. Processed foods are initially disaggregated into its different ingredients using the recipes, and these ingredients are then further converted into the raw primary commodities using the reverse yield factors (see example in Figure 2). The use of the same methodology across all dietary surveys during the conversion of the processed commodities into raw primary commodities is an added value that guarantees consistency in the estimates.

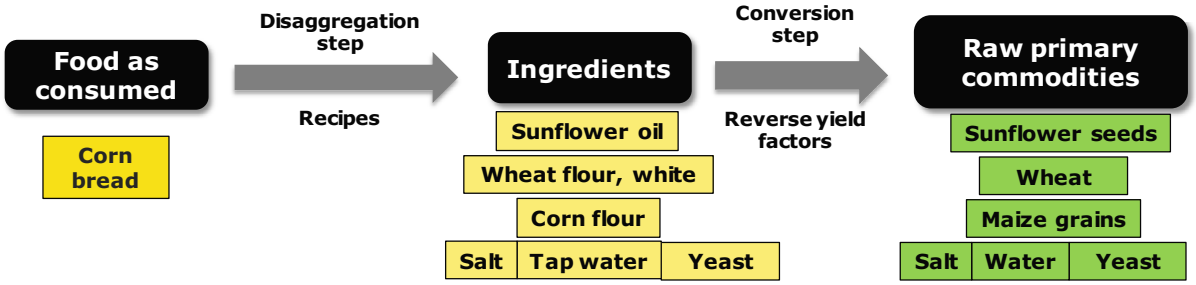


Figure 2: Two-step approach used to estimate the amount of raw primary commodities from processed foods as reported in the EFSA Comprehensive European food consumption database

The future use of this database will allow a direct linking between the consumption data and the concentration values reported for the different GM food constituents in raw primary commodities (maize grains, soya beans, etc.). Summary statistics by country, dietary survey and age class based on

the consumption of raw primary commodities will be produced selecting the relevant GM crops and the relevant processed foods depending on the constituent under assessment.

7.2. Dietary exposure tools for dietary exposure estimations

In past months, EFSA launched different user-friendly tools that allow applicants, risk assessors and risk managers to estimate dietary exposure; among these tools are the FAIM[8] and the Food Enzyme Intake Model (FEIM).[9] FAIM allows calculation of exposure to new food additives or to additives that are already authorised but for which a new use is proposed. FAIM is based on the individual food consumption data collected by Member States for different population groups and present in the EFSA consumption database; the use of individual food consumption data increases the accuracy of exposure estimates. In the case of the FEIM, consumption data are first disaggregated to the raw primary commodities or minimally processed commodities (e.g. flour) and then combined with use levels of the food enzyme in different processing (baking, brewing).

8. Conclusions

Dietary exposure is an essential element of the risk assessment on GM foods. This statement describes how dietary exposure to newly expressed proteins in GM foods should be estimated and how the available information on consumption (summary statistics) and constituent concentration should be used. Guidance is also given on the information applicants need to provide on the dietary exposure estimations to these GM food constituents. Estimations of dietary exposure (chronic and acute) should cover average and high consumers across all the different age classes and special population groups for which consumption data are available. Particular consumer groups of the EU population with an expected higher dietary exposure should be identified and considered during the assessment. As occurs in each of the steps of the risk assessment, dietary exposure assessment is also surrounded by different sources of uncertainty that need to be considered when interpreting the results and concluding on the presence/absence of health concerns.

Dietary exposure estimates using deterministic methods are sufficiently conservative. In addition, the different assumptions taken (e.g. replacement scenario for the consumption data) result, overall, in even more protective scenarios. When initial dietary exposure estimations represent a possible health concern (e.g. estimates close to or above health-based guidance values), the exposure scenario should be revisited considering the assumptions taken and/or seeking for additional information when possible (e.g. information on levels in processed foods). Additionally, and if needed, more accurate and precise exposure estimates can also be provided by EFSA by making use of the raw individual data available in the EFSA consumption database.

Further revisions and updates of this statement will be provided in the future as appropriate, after considering new scientific and regulatory developments in the risk assessment of GMO.

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[8] https://www.efsa.europa.eu/en/press/news/171204-1
[9] https://www.efsa.europa.eu/en/press/news/180704-0

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Abbreviations

ADME	absorption, distribution, metabolism and excretion
CEDEM	comprehensive European dietary exposure model
CP4-EPSPS/mEPSPS	5-enolpyruvylshikimate-3-phosphate-synthase
FAO	Food and Agriculture Organization of the United Nations
FAIM	Food Additives Intake Model
FBS	Food Balance Sheet
FDA	Food Drug Administration
FEIM	Food Enzyme Intake Model
GAT	glyphosate *N*-acetyltransferase
GM	genetically modified
GMO	genetically modified food
LOD	limit of detection
LOQ	limit of quantification
NAA	*N*-acetyl-aspartic acid
NAG	*N*-acetyl-glutamic acid

PAT	phosphinothricin *N*-acetyltransferase
PRIMo	pesticide residues intake model
RPC	raw primary commodity
WHO/IPCS	World Health Organization/International Programme on Chemical Safety

Appendix A – Completeness check

Applicants should fill in and provide the completeness checklist found in the online version of this output as part of the submission when submitting a GMO application to EFSA (to be provided in Microsoft Word® format): https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2019.5802.

Human dietary exposure assessment of newly expressed proteins
Application number:
Events:
Newly expressed proteins/constituents:

	Applicants to fill in	**EFSA to check**	**Comments/actions**
Chronic dietary exposure estimates provided for each of the newly expressed proteins present in the treated (with the intended herbicide) GM crop. A study report should be provided as part of the dossier. (APDESK to check)	☐ Yes ☐ No ☐ Partially	☐ Yes ☐ No ☐ Partially ☐ Unclear	
Acute dietary exposure estimates provided for each of the newly expressed proteins present in the treated (with the intended herbicide) GM crop. A study report should be provided as part of the dossier. (APDESK to check)	☐ Yes ☐ No ☐ Partially	☐ Yes ☐ No ☐ Partially ☐ Unclear	
Tabulated exposure estimates presented by country, dietary survey and age class for average and high consumers. (APDESK to check)	☐ Yes ☐ No ☐ Partially	☐ Yes ☐ No ☐ Partially ☐ Unclear	
EFSA Comprehensive consumption database used for dietary exposure estimations, including vulnerable groups (e.g. lactating women, pregnant women). (GMO unit to check)	☐ Yes ☐ No ☐ Partially	☐ Yes ☐ No ☐ Partially ☐ Unclear	
Concentration data used as described in EFSA statement [appropriate descriptive statistics, appropriate growth stage, expression of results (fresh weight), etc.]. (GMO unit to check)	☐ Yes ☐ No ☐ Partially	☐ Yes ☐ No ☐ Partially ☐ Unclear	
Factor & recipes used as described in EFSA statement. (GMO unit to check)	☐ Yes ☐ No ☐ Partially	☐ Yes ☐ No ☐ Partially ☐ Unclear	
Exposure model used as described in EFSA statement. (GMO unit to check)	☐ Yes ☐ No ☐ Partially	☐ Yes ☐ No ☐ Partially ☐ Unclear	
Ad hoc dietary exposure scenarios provided (if needed). (GMO unit to check)	☐ Yes ☐ No ☐ Partially	☐ Yes ☐ No ☐ Partially ☐ Unclear	
Description of uncertainties linked to human dietary exposure estimates. (GMO unit to check)	☐ Yes ☐ No ☐ Partially	☐ Yes ☐ No ☐ Partially ☐ Unclear	