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Recommendations for Assessing Human Dietary Exposure to Newly Expressed Proteins in Genetically Modified Crops

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Abstract

Risk assessment of genetically modified (GM) crops includes an evaluation of hazard and exposure to newly expressed crop constituents, exemplified herein by newly expressed proteins (NEPs). Guidance directing dietary exposure assessments (DEAs) is limited and/or globally inconsistent. Best practices for conducting DEAs are presented and include a preliminary problem formulation step to determine if a formal DEA is necessary to support the risk assessment. If a formal DEA is deemed necessary, the type of exposure (acute or chronic) and the availability of food consumption data relevant to the targeted population should be identified. Exposure should be estimated initially using a simple, straight-forward, and fit-for-purpose DEA (unrefined DEA) approach while ensuring relevance to the risk question. Unrefined assessments, which are very likely to overestimate exposure, provide a high level of protection to consumers of GM crops. If refinement of the DEA is necessary, then the simplest refinements that meet the needs of the risk assessment should be implemented. DEAs are not scientifically necessary for NEPs in GM crops if a hazard has not been identified, since risk is a function of both hazard and exposure. Nonetheless, dietary exposure assessments are sometimes required for regulatory purposes, and this publication outlines some key best practices and considerations for their conduct.

Keywords: dietary exposure assessment, genetically modified (GM) crop, best practices, risk assessment

Abbreviations: CARES-NG, Cumulative and Aggregate Risk Evaluation System Next Generation; DEA, dietary exposure assessment; DEEM-FCID, Dietary Exposure Evaluation Model - Food Commodity Intake Database; EFSA, European Food Safety Authority; ELISA, enzyme-linked immunosorbent assay; EPA, Environmental Protection Agency; EU, European Union; FAO, Food and Agriculture Organization of the United Nations; HT, herbicide tolerance; IR, insect resistance; GEMS, Global Environment Monitoring System; GM, genetically modified; LC-MS/MS, liquid chromatography coupled with tandem mass spectrometry; MRLs, maximum residue level; NEP, newly expressed protein; PRIMo, Pesticide Residue Intake Model; RAC, raw agricultural commodity; U.S., United States; WHO, World Health Organization

1. Introduction

Since first commercialized in the mid-1990s, the adoption of genetically modified (GM) crops continues to increase globally [31], and the technologies being employed continue to advance. The global area planted with GM crops increased ~113-fold since 1996, from 4.2 million acres in 1996 to 473 million acres in 2018, with 26 countries planting GM crops in 2018 [32]. Approximately 12 percent of global cropland produced GM crops in 2015 [29, 36].

Although herbicide tolerance (HT) has been the most dominant trait among approved GM crops [30, 36], in more recent years combined ("stacked") traits of insect resistance (IR) and HT are being increasingly grown due to their productivity and levels of stress tolerance. The IR traits include protection against specific targeted insect pests (e.g., insecticidal protein

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synthesis from modified *Bacillus thuringiensis* sources). Single and stacked IR and HT events are about 68 percent of all traits approved globally [30]. The portfolio of traits has been expanding to include additional modes of action for herbicide tolerance and insect protection traits, agronomic performance improvements, and other advancements such as enhanced nutrition and protection against viral, bacterial, and fungal diseases [1].

In countries that regulate GM crop import and cultivation, a comprehensive safety assessment of GM crops and foods derived from them is the foundation of the regulatory approval process. Codex Alimentarius principles and guidelines have served as a valuable standard delineating what should be included in a safety assessment, focusing on intended and potential unintended consequences that may result from the genetic changes or the expression of a new or altered constituent within the GM crop [5]. In the current agricultural environment, the intended new or altered constituent is most often a newly expressed protein (NEP); therefore, this paper focus on assessing dietary exposure to NEPs in GM crops. GM crop safety is supported by a comparison of the GM crop with its conventional counterpart, the characterization of the NEP and its coding DNA, an evaluation of potential hazards associated with the NEP, and, currently, a determination of the probability and extent of exposure of consumers to the NEP from the consumption of foods derived from the GM crop [5]. Much of the testing for product safety is delineated by regional regulatory guidance documents, but guidance describing human dietary exposure assessments (DEAs) is limited (e.g., [16]), and globally inconsistent.

The purpose of this paper is to propose a harmonized, stepwise approach to conduct human DEAs for NEPs in GM crops, not unlike recommended tiered approaches for DEAs in other food substances [10, 54, 8, 48, 49]. This suggested approach includes three steps (Figure 1), beginning with problem formulation to determine whether a formal DEA is necessary considering the needs of the larger risk assessment. If a formal DEA is determined to be needed following problem formulation, the second step is a simple, straight-forward, and fit-for-purpose DEA (unrefined DEA) to address relevant concerns regarding exposure. The third step would detail refinements, if a more comprehensive DEA (refined DEA) is needed. Descriptions of tools and methods needed to complete a DEA, and suggestions for best practices for using consumption data, are also described.

2. Step-Wise Approach of Human DEA

2.1. Step 1: Problem Forumulation to Determine if a Formal DEA is Needed

The safety of NEPs in GM crops, including those with pesticidal activities, is fully considered throughout product development. NEPs chosen to confer a GM trait may, for example, share amino acid similarity with a common edible protein with a history of safe use, or may function through a mode of action that is not relevant to humans. Most dietary proteins can be safely consumed, but because a small number of natural proteins are known to be toxic or allergenic, a risk assessment of NEPs in GM crops must also consider whether the NEP could be potentially toxic or allergenic [6].

Risk is a function of hazard and exposure. As a first step in assessing risk, problem formulation considers both components and helps frame the risk assessment in terms of evaluation of existing data and identification of data gaps. A structured problem formulation approach has been described in Sauve-Ciencewicki et al. [43]. By means of problem formulation, an assessor can determine whether conducting a formal DEA is necessary and/or sufficient to meet the objectives of a risk assessment of a GM crop. If a weight of evidence [17] supports a conclusion of low or negligible hazard associated with consumption of a GM crop, then a formal DEA may not be needed to assess risk. Such a weight of evidence might be generated in Tier 1 hazard identification testing, as described in Delaney et al. [6], and may include a number of factors such as amino acid sequence homology to a commonly consumed protein; history of safe use of a similar protein; a non-relevant mode of action in mammals, such as those of Cry proteins [33, 38, 42, 45, 18]; and a lack of potential toxicity and allergenicity of the NEP (with in vivo toxicity testing considered in Tier 2 only) [26, 35, 7, 27]. In addition, degradation or deactivation of the NEP due to standard food processing, cooking or digestion [25] reduces the potential exposure to an active NEP. In comparison, regulatory guidance in the related agricultural pesticide industry indicates a formal, acute DEA is not needed to evaluate a product's safety in the absence of an acute hazard [17, 21]. There are many substances that do not have acute reference doses [51, 56].

Conversely, the problem formulation step may demonstrate that, because of minimal or no exposure to a given NEP (for example, absence of the NEP in the consumed seed oil fraction), hazard testing would not be necessary. Typically, NEPs in GM crops have been considered first from a hazard-led approach, but there is increasing interest in assessing risk using an exposure-led framework [40]. Evidence for limited exposure includes degradation of the NEP or, in some cases, loss of activity during processing of the GM crop into food commodities, something relevant for proteins with a toxic mode of action (i.e. insecticidal toxins [25]), and the digestibility of the NEP. If a lack of exposure can be demonstrated, then prescribed hazard assessment studies may not be necessary for risk assessment. This exposure-led approach would ultimately save time and resources by preventing unnecessary testing, and is consistent with an ethical imperative to reduce the number of animals used in experimental testing.

In summary, if the NEP has been demonstrated to be a low or negligible hazard (e.g., it is non-toxic, unlikely to be allergenic, and digested or degraded), there is no need for a dietary exposure assessment.

2.2. Step 2: Conducting an Unrefined and Conservative Dietary Exposure Assessment

When problem formulation dictates a need for a formal DEA, a basic, straight-forward approach (unrefined DEA)

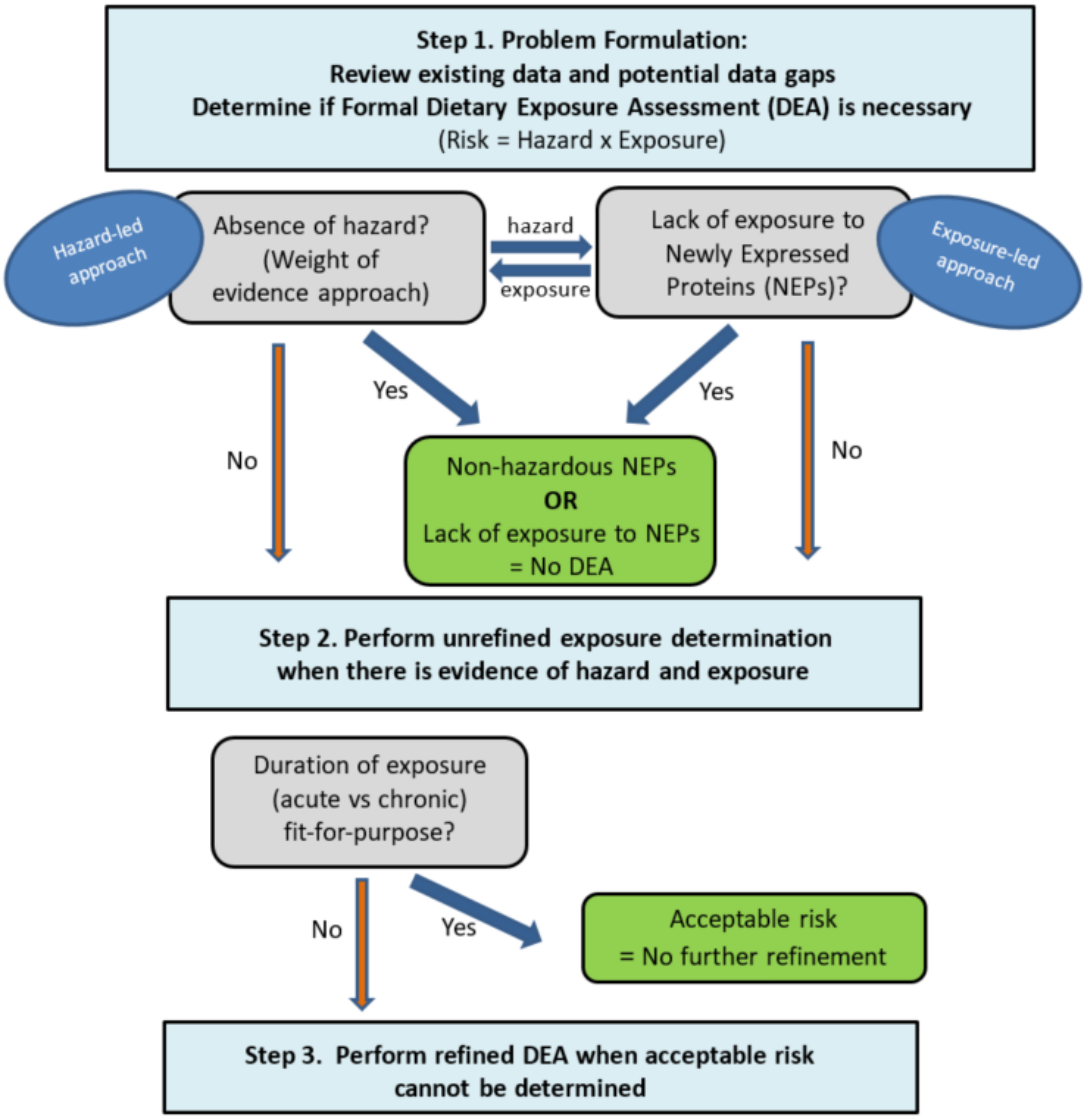

Figure 1: Approach for conducting Dietary Exposure Assessments (DEAs) for newly expressed proteins (NEPs) in genetically modified (GM) crops.

should be considered first, with the duration of exposure determined by the hazard identification and characterization. Acute exposures (also termed short-term intakes) are single exposure events or exposures that occur within a short period of time (typically 24 hours); chronic exposures are exposure events that occur over a longer time period (for example, repeated intake over a year). Chronic exposures tend to be substantially lower on a daily basis than acute exposures.

Exposures to the NEP are most often estimated as acute exposures because most toxic proteins are acutely toxic [46]. Although repeated exposures to NEPs could theoretically occur, acute exposure estimates tend to be more conservative, and therefore, more protective of consumers. In addition, NEPS are generally susceptible to digestion, as demonstrated using surrogate digestibility assays, which shortens the duration of exposure. Based on these factors, acute DEAs that assume high-end exposures are usually considered sufficient for evaluation of potential risks from exposures to the NEP, and chronic DEAs are often considered unlikely to add value to the risk assessment.

If a DEA indicates negligible risk associated with dietary exposure using an unrefined exposure estimate, further refinements are not required. Unrefined exposure estimates tend to be more conservative than refined estimates and will in most cases utilize 'worst-case' NEP expression and consumption data. An unrefined acute exposure assessment could, for example, assume a relatively high concentration of the NEP, such as the maximum concentration measured in the crop commodity combined with high raw agricultural commodity (RAC) or food intake values. In theory, environmental conditions could impact the NEP concentration, but in practice, growing conditions do not lead to biologically relevant variations in NEP concentration values [57, 4]. Unrefined assessments are very likely to overestimate exposure and provide a high level of protection to consumers. A simple, unrefined, conservative DEA would

suffice for many current GM crops without the need for further refinement. Parameters involved in estimating dietary exposure are discussed in further detail in Step 3.

2.3. Step 3: Refining the Dietary Exposure Assessment

A refined DEA might be necessary to determine acceptable risk in cases in which, (a) the NEP is associated with a hazard; (b) a simple, unrefined, and conservative estimate does not provide a level of exposure supporting acceptable risk; or (c) a regulatory authority requests a refined DEA. In these cases, a more detailed exposure assessment should be conducted with refinements to further characterize the exposure and inform the risk assessment. For example, it may be appropriate to apply refinements such as the impact of processing and/or cooking on NEP integrity, or the use of variety-specific NEP data (e.g., protein expression data), market share, or alternative modelling types. Such refinements serve to provide more realistic quantitative estimates of human exposure, while still ensuring protection to sensitive populations of consumers. These refinements are discussed in more detail in Section 5.

3. Estimating Dietary Exposure

Estimating dietary exposure to a NEP requires two components: the known concentrations of the NEP in edible crop fractions, and the known amounts of the relevant crop commodity (or foods prepared with the relevant crop commodity) consumed by the target population. Each component is described in detail in the following subsections.

3.1. Measuring NEP Concentration

A DEA of the NEP in a GM crop requires a quantitative measure of the NEP in the RAC or, in certain cases, in the derived processed food(s) to be consumed. The DEA is dependent on the quality of the protein expression data (study design, sample handling, methodology, reproducibility, and the like). GM crop exposure assessments typically reflect measurements of the NEP in edible tissues from crop samples collected in multiple field trials conducted in representative cultivation areas and under a variety of environmental conditions. Although it is possible that factors such as environmental conditions, growing seasons, and germplasms could impact NEP concentration, in practice, major variations in NEP concentration values have not been observed [57, 4]. There is likely to be more variability in DEA values based on the assumptions made concerning exposure (e.g., assuming 100 percent market penetration or lack of protein degradation due to processing), rather than owing to reproducibility of the measured expression data. Other factors such as relevance for food and feed use, developmental stage, moisture content of the plant tissues, as well as any other factors that could affect the expression of the protein, should also be considered. Typically, units of concentration of the NEP are provided on fresh weight (as opposed to dry weight) measurements to facilitate comparison to intake data expressed as fresh weight in food consumption databases.

A variety of analytical methods can be utilized to quantify NEPs extracted from plant tissues, including plate-based enzyme-linked immunosorbent assays (ELISA), micro-bead based immunoassays, and electrophoresis and blotting with antibody-based detection methods [44, 19, 23]. More recently, methods have been developed using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) that can simultaneously perform multiple analyses with high sensitivity and high selectivity to quantify NEPs [2, 28, 44, 19, 23]. The particular analytical method used should be validated and appropriate for the NEP.

3.2. Estimating Food Consumption

To estimate dietary exposure, the amount of relevant foods consumed by the population of interest must be known. Many countries, national organizations, and international bodies have developed and made available databases for estimations of food consumption. Food consumption data include the types and amounts of food(s) consumed, as well as the frequencies of consumption. Since the consumption patterns of a GM RAC are, in a vast majority of cases, expected to be the same as those of a RAC derived from a conventional crop, existing food consumption databases are appropriate to use in estimating intake for the purpose of conducting a DEA of a NEP. Other considerations can be made, on a case-by-case basis, such as for a nutritionally-enhanced crop that may fall into a different category based on the nature of its intended modifications.

3.3. Comparison of Different Dietary Food Consumption Data Collection Methods

The quality of a DEA is also dependent on the quality of the data in the food consumption database. Food consumption data can vary from simple gross population figures to detailed information about a single individual's consumption patterns. The use of robust databases that accurately reflect the eating habits of the relevant population(s) under consideration is important for obtaining accurate estimates of dietary exposure. In principle, four different types of food consumption data can be used: food supply data; data from household consumption surveys; data from dietary surveys among individuals; and data generated by the collection of duplicate diets. Each data type corresponds to a different stage in the food chain and is obtained by different methods [34], and each food consumption data type has associated advantages and disadvantages.

Food supply data generated by population-based methods such as food balance sheets, are given in terms of raw or semi-processed commodities, such as the Food and Agriculture Organization of the United Nations (FAO) Food Balance Sheets, for example [55, 58, 20]. These food production statistics reflect food availability rather than actual consumption. Food balance sheets typically do not fully account for food losses (e.g., cooking, processing, spoilage, or other sources of waste), and therefore tend to report approximately 15 percent higher consumption relative to data from household or individual surveys [54]. However, because food balance sheet consumption data are provided for the entire population or at the per capita level,

but not for specific population subgroups, there is a chance they may not account for highest exposure scenarios. These estimates may also be less realistic. For example, since food balance data provide average consumption for the entire population, they cannot distinguish non-consumers of a food and may underestimate high consumers. Furthermore, such population-based methods provide an estimate of average daily consumption and are used for chronic exposure assessments, but cannot provide information describing consumption levels on days of higher consumption, such as would be needed in an acute exposure assessment. An advantage of food balance sheets, however, is that they are easier and cheaper for a country to produce than actual consumption data, and therefore, more countries may be represented.

Food consumption databases may also be populated with data generated by individual and household consumption surveys, which report the actual consumption of specific food items and allow for estimation of differences between populations, and differences between average daily consumption and high-end consumption, such as chronic versus acute [50, 14]. However, the quality of data in such databases can be impacted by survey type and ease of use, consumer recall, seasonal variations in the diet, and the statistical robustness of the sample size. Additionally, families or households included in the survey are not always a representative sample of the whole population.

Two commonly employed survey approaches, food frequency questionnaires and 24-hour recall, illustrate different benefits and potential sources of error in estimation of food consumption [22]. In a food frequency questionnaire, a subject is surveyed regarding the frequency of consumption of various foods over a set period of time (e.g., one year). This method is more economical in terms of time and resources than some other survey methods, but does not allow for estimation of variations in the diet over time and is subject to errors in recollecting consumption practices. In a 24-hour recall survey, the subject is asked only to recall the specific foods consumed over the past 24 hours; multiple surveys of 24-hour recall per subject are needed in order to understand food intake over a period of time. This type of survey method is more resource-intensive but provides greater information on the day-to-day variation in food consumption. The quality and reliability of survey results are improved by large numbers of survey participants, by trained survey facilitators who ask follow-up questions meant to fill in gaps or omissions in reported consumption, and by memory aids that help in more accurately estimating portion sizes. Public availability of the raw survey data (rather than summary statistics) aids in proper evaluation of data quality and in using the data for different purposes. Thus, higher confidence may be placed in surveys when individual survey responses are available for public review and usage.

Duplicate diet studies in which samples are physically collected and analyzed for all food that a participant consumes over a period of time are very realistic but also resource-intensive, and data tend to be limited to a small cohort; therefore, they are not representative of the population. This type of realistic total dietary study is rarely used in regulatory assessments; however, it can be useful as a reality check for regulatory assessment results.

3.4. Food Consumption Data Organization by Foodstuffs and Subpopulations

In all food consumption databases, consumed foods are sorted into categories or food groups (such as cereals or legumes). Listing specific foods is often not practical, since the number of foods in a given region may be vast, and the foods consumed may change frequently as new or improved foods are introduced or with seasonal fluctuation. In addition, processed foods may not be easily categorized. Pizza, for example, may contain wheat flour in the crust, tomato in the sauce, and cheese as a topping, none of which would likely be grouped together. Therefore, the most appropriate food consumption data for DEAs of GM crops are values collected from the RACs. Furthermore, the concentrations of the NEPs are determined in RACs to meet regulatory requirements, and thus, grouping of foods by RACs is a best practice which provides consistency in DEAs across regions.

However, in some cases the analogous RAC consumption data are not available or regulatory agencies request the use of specific or regional food consumption databases that categorize food differently, such as foods as eaten (see for example [50] and [11]). In such cases, the consumption of a food item should not be combined with the concentration of the NEPs in the RAC to estimate exposure because under- or over-estimations can easily occur. For example, exposure to a NEP may be overestimated if the RAC concentration is used for the soybean food categories soy sauce or soy drink. If needed, the concentration of the NEP in a food product should be estimated based on standard recipes or on the ratio of the total protein content in the RAC and the total protein content of the respective food product derived from the RAC. The protein contents of many foods can be found in published sources, such as the Centers for Disease Control and Prevention [3] and Public Health England [41].

Food consumption patterns can vary across and within populations, leading to different levels of exposure for various subpopulation groups. A key parameter that impacts dietary exposure is the age of the consumers. Small children consume the most food on a per-kg body weight basis, and therefore, on this basis, have potentially the highest exposure to substances in food. Consequently, many food consumption databases provide specific consumption data for adults and younger populations, as well as data for high, low or average volume consumers. Consumption patterns can also vary across cultures, such that survey data from one group may have limited applicability to members of another cultural group. The appropriate type of consumption data will depend on the context of the assessment. One approach to capture this wide variability in consumption is to estimate consumption for both the general population and a range of subpopulations to accurately determine the most highly exposed subpopulation.

3.5. Comparison of Acute Versus Chronic Exposure Scenarios

Food consumption databases can also offer options to estimate either acute or chronic exposures to food components.

Acute exposure estimates are calculated to account for high-end, short-term (e.g., one meal or one day) exposures. Methods used to estimate acute exposure vary globally, but often incorporate food consumption data from high-end consumers (e.g., 95th or 97.5th percentile), and often leverage a maximum concentration of the NEP in that food to estimate exposure (or a mean or median concentration for commodities that would be blended during storage, such as corn and soybeans).

For the evaluation of chronic dietary exposure, mean or median concentrations of the NEP are combined with average consumption values to be representative of actual consumption over time. As a result, large differences may be observed between acute and chronic exposure to food commodities.

The choice of exposure scenario can significantly impact a DEA. In a risk assessment context, the choice of using acute or chronic exposure information is driven both by the characteristics of the potential exposure (i.e., whether it is brief, periodic or extended), and the toxicological data. Therefore, it is important to consider the specific test substance being evaluated to determine whether an acute or chronic exposure assessment needs to be conducted.

3.6. Consumption Databases Available for Use in Assessing Dietary Exposure

Several consumption databases have been reviewed for use in dietary exposure assessments and can be downloaded free of charge from the websites of governmental agencies or other institutions. The level of detail provided in each database will determine its potential application in an assessment. For example, databases that provide consumption data at the food nutrient level are necessary to assess exposure at the nutrient level, such as in the case of nutritionally-enhanced crops. Although databases may include some considerations in estimating the exposure to pesticide residues that may not be applicable to the exposure assessments for GM crops, such as variability factors for pesticide residues, consumption data can still be extracted from these models for use in calculating estimates of exposure to NEPs in GM crops. An overview of the main food consumption databases available for use for GM crop exposure assessments is given in Table 1. Four key regulatory databases are described in more detail below, although many other databases and models are available for use in human DEAs.

3.6.1. Global Environment Monitoring System (GEMS/Food)

The World Health Organization (WHO) GEMS/Food program [53] supports exposure assessments at international and national levels. Both acute and chronic exposure assessments can be performed from the data provided, which include consumption data for the country reporting the highest consumption of each food item for each population group, and surveys of countries with varied food consumption patterns. Therefore, it may be useful for evaluating worst-case acute exposures in countries where individual-level acute consumption data are not available.

For chronic exposure assessments, consumption data are available for over 400 different food items and 17 different cluster diets (composed of two to as many as 30 countries with comparable diets), representing a total of 179 countries [53, 47]. The GEMS/Food Cluster Diets were established based on FAO food balance sheets (http://www.fao.org/faostat/en/#data/FBS), and provide average long-term consumption for whole populations without distinguishing between age groups. In addition to chronic consumption data for the RAC, the 17 cluster diets also include consumption data for some processed commodities. The GEMS/Food consumption database may be particularly helpful if DEAs are needed in countries that have not published their own food consumption data, because exposure can be estimated using an existing database from a country with similar eating habits and/or consumption patterns.

3.6.2. Pesticide Residue Intake Model (PRIMo)

The European Food Safety Authority (EFSA) has developed the PRIMo [12] to estimate chronic and acute dietary consumer exposure to pesticide residues in the risk assessments of European Union (EU) maximum residue levels (MRLs). Acute exposure is currently based on information from 37 diets from 13 EU Member States, covering various age groups. For the chronic exposure assessment, 36 different diets are provided (EU-specific national and sub-population diets, GEMS/Food Cluster diets relevant to Europe). The consumption data within PRIMo are provided as deterministic summary statistics, which represent the consumption of a given food commodity and the concentration of the compound in that commodity by a single numerical value. PRIMo supports a few additional features for refined intake calculations, and has been used by registrants of GM crops for the purposes of assessing exposure to NEPs. Variability factors are applied in some pesticide models to account for composite sampling in residue trials, and therefore are not applicable outside of pesticide exposure assessments. However, because variability factors are typically embedded into spreadsheet models, it can be important to remove the inappropriate factor prior to use of a model.

3.6.3. Dietary Exposure Evaluation Model - Food Commodity Intake Database (DEEM-FCID)

The Dietary Exposure Evaluation Model - Food Commodity Intake Database (DEEM-FCID) [50] was developed as a risk-based tool to compare pesticide exposure estimates against hazard endpoints determined in toxicology studies. The database, which is based on "What We Eat In America" from the U.S. Center for Disease Control and Prevention National Health and Nutrition Examination Survey [3], is currently supported by the U.S. Environmental Protection Agency (EPA) and is publicly available. It uses recipe files to decode the foods as eaten to the individual RAC [52].

Both chronic and acute assessments can be performed using the U.S. food consumption data for population subgroups such as infants and children. The chronic model provides deterministic estimates of exposure, while the acute model can provide either deterministic exposure estimates or fully probabilistic models for exposure considering the distribution of consumption data across the population, as well as the distribution of different concentrations of the NEP being assessed.

Database	**Exposure Duration**	**Population Groups Represented**	**Consumption Data**	**Comments**
WHO GEMS/Food [a]	Acute	Population groups in 17 countries	RAC only	Global database
	Chronic	Considers whole population, 179 countries	RAC and processed commodities (415 different food items, 17 different cluster diets)	Global and regional databases (cluster diets); Cluster diet data (chronic exposure) are based on food balance sheets
DEEM-FCID [b]	Acute and chronic	U.S. population and key sub-populations (e.g., infants, children)	RAC and consumed commodities	Deterministic and probabilistic assessments, and modeling
PRIMo [c]	Acute	EU population and subpopulations	Database contains 17 diets	Deterministic summary statistics
	Chronic	EU population and subpopulations	Database contains 27 diets	Variability factors do not apply to GM crops
EFSA database [d]	Acute and chronic	EU population and subpopulations	Processed food commodities for individual countries and population sub-groups	Raw data not available for many subpopulations; limited data for some food commodities; many data not statistically robust
Chinese National Nutrition and Health Survey [e]	Chronic	General population, 31 provinces	RAC and processed food commodities	Publicly available; limited sub-populations
Taiwan National Nutrition and Health Survey [f]	Acute and chronic	Male and female populations; different age groups; Taiwan	Consumption for 19 crops/crop grouping	Some groups are very large categories (e.g., grain which is divided into rice, wheat, dry bean and pulses); no specific entries for corn or soybeans
Australia/New Zealand Harvest [g]	Acute and chronic	Subpopulations in Australia and New Zealand; includes age, gender, consumers	RACs and processed food commodities	Not publicly available; 24-hour recall surveys
Japan National Nutrition and Health Survey [h]	Chronic	Male and female populations from Japan; different age groups	RACs and processed food commodities	Not publicly available

Table 1: Overview of currently available consumption databases.

[a] https://www.who.int/foodsafety/areas_work/chemical-risks/gems-food/en/
[b] https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/deem-fcidcalendex-software-installer
[c] http://www.efsa.europa.eu/en/applications/pesticides/tools
[d] https://www.efsa.europa.eu/en/applications/gmo/tools
[e] https://www.cpc.unc.edu/projects/china
[f] https://www.hpa.gov.tw/EngPages/Detail.aspx?nodeid=1077&pid=6201
[g] http://www.foodstandards.gov.au/science/exposure/Pages/fsanzdietaryexposure4439.aspx
[h] https://www.mhlw.go.jp/bunya/kenkou/eiyou/dl/h28-houkoku.pdf

Consumption data for both the RAC and several food types are available. In addition, food forms at various cooking stages (baked, boiled, fresh, etc.) are also available. DEEM-FCID can be used without modification to estimate exposures only, or it can be used as a risk assessment model in the unlikely event that relevant adverse effect levels are established for a NEP. DEEM-FCID is unique in that it can employ software to perform "critical commodity analyses" to identify individual food items that drive exposure estimates. It is anticipated that in the future DEEM-FCID will be replaced by Cumulative and Aggregate Risk Evaluation System Next Generation (CARES-NG; https://caresng.org/), which is presently being evaluated by the EPA.

3.6.4. Comprehensive European Food Consumption Database

The Comprehensive European Food Consumption Database was compiled by EFSA and to date contains acute and chronic food consumption data from 25 Member States and 60 dietary surveys representing nine different population groups [14]. Consumption data for each crop and its associated processed foods, such as maize grain, maize starch, cornmeal porridge, etc., are reported for each Member State, survey and consumer group at different consumption levels. A comprehensive review of the database was composed by the Crop Life International Working Group on the DEA of GM crops (C. Du Marchie Sarvaas, personal communication, September 2013; B. Spaeth, personal communication, May 2014), which highlighted the challenges of using an earlier version of this database for estimation of exposure to NEPs, such as the statistical insufficiencies of the available national surveys that are fragmented into sub-populations of individual countries, and the lack of recipes that translate the food survey data to the RACs. Some of these limitations were recognized in an EFSA guidance [9], and appear to be addressed in the current database version [13]. Further guidance on utilizing the database for human dietary exposure assessments of NEPs in GM crops was provided [15].

3.6.5. Other Databases

In addition to the four consumption databases discussed above, many individual countries such as China, Japan, Taiwan, and Australia/New Zealand have conducted country-specific dietary consumption surveys (Table 1). These data are preferred by some regulatory authorities because the data reflect consumption surveys within their own countries. A disadvantage with these data is that the majority of country-specific consumption databases report only chronic consumption. Furthermore, some country-specific databases are not publicly available or may not be available in English. Where local or regional consumption databases are not available, the GEMS/Food models can serve as a surrogate database.

4. Best Practices for Calculating Dietary Exposure to NEPs

The simplest approach to estimating exposure that meets the needs of the risk assessment (which is often the most conservative approach) should be applied. Typically, NEPs are digested into amino acids and small peptides in surrogate assays and are therefore not thought to bioaccumulate in the blood or other tissues. For assessment of such NEPs, an acute exposure assessment will often suffice, as it represents the highest level of possible exposure and can be considered a reasonable worst-case for many NEPs. In such cases, a chronic DEA would not add value to the risk assessment. For NEPs associated with a hazard, an acute exposure assessment may also be appropriate because most known protein toxins act through acute mechanisms of action [24, 39, 46].

Acute dietary exposure estimates calculated using the maximum concentration of the NEP in the RAC and high percentile consumption data from consumers (e.g., 97.5th or 95th percentile) are highly conservative. For foods such as grains, where a high level of blending occurs between the time of harvest and human consumption (including blending between GM commodity and non-GM commodity), use of a mean or median concentration level of the NEP is likely to more accurately represent consumption of the food and is more appropriate for use in a DEA than the highest measured value [37, 48, 54].

Once the amount of the food commodity consumed and the amount of NEP in that food are known, exposure can be calculated by simply multiplying the two together. Using estimates of daily food consumption, a calculation for determining an acute exposure is demonstrated in the equation 1:

$$AE = D_{high} \times C_{NEP}$$

where

- AE = acute exposure in mg/kg bw in a single day,
- D_{high} is the consumer only high percentile daily consumption of a food commodity containing the NEP in g/kg bw, and
- C_{NEP} is the concentration of the NEP in a given food commodity, in mg/g.

If a database provides consumption data for multiple derived foodstuffs from the same RAC, rather than the RAC itself, there is an alternative approach to perform an acute DEA with which a more realistic estimate of exposure can be obtained. As it is unlikely that a person would be a high consumer of all foodstuffs derived from the RAC, it is appropriate to estimate total exposure to the NEP based on the foodstuff with the highest intake value plus average consumption values of the other foodstuffs.

A chronic risk assessment may be important in some cases to capture repeated exposures over longer periods of time, for example, if repeated exposure causes tissue injury without opportunity for recovery. Rare cases may occur in which the assessment of effects of extended exposure to a NEP would be indicated. Situations that could indicate the need for a DEA based on chronic exposure include:

1. An indication of stability of the intact, active NEP in the presence of digestive enzymes;

2. relevant sequence or structural homology to toxic proteins known to exert effects after prolonged or repeated exposures;

3. structural or functional similarity to lectins or other proteins that, upon repeated exposure, cause sub-chronic adverse effects related to intestinal inflammation and/or damage; and

4. an observation of one or more adverse effects of the NEP following toxicological testing, prompting further investigation.

In cases in which an estimate of chronic exposure is required, NEP concentrations are based on the mean or median NEP concentration levels determined from representative samples for both blended and non-blended commodities. The evaluation of chronic dietary exposure may include consumers and non-consumers, with average consumption data for each sub-population group being typically considered. The contributions of all relevant food commodities are included in the estimate of exposure. Because chronic consumption represents an average consumption over time, it is likely to be substantially lower than an estimate of acute consumption. Equation 2 illustrates the calculation of chronic exposure:

$$CE = \sum_{t=1}^{n} [D_{\text{avg}} \times C_{\text{NEP}}]_{commodity\ i}$$

where

- CE = chronic exposure in mg/kg bw/day;
- D_{avg} = total population (consumers and non-consumers) average daily consumption of a food commodity containing the NEP in g/kg bw;
- C_{NEP} = is the mean or median concentration of the NEP in a given food commodity, in mg/g;
- i = the first food commodity under consideration;
- n = the total number of food commodities; for GM crops; and
- t = 1 (the single event for which the DEA is being conducted).

Although it is conceivable that multiple GM crops may contain the same NEP, the exposure to a NEP is generally estimated for a specific transformation event in a single crop. For an unrefined DEA, it is typically assumed that 100 percent of the crop commodity consumed is derived from the GM crop of interest because the degree of blending is not known and a conservative estimate can be obtained.

5. Refinements of the DEA

If refinement of the DEA is necessary, then the simplest refinements that meet the needs of the risk assessment should be implemented. For example, it may be appropriate to evaluate the impact of processing and/or cooking on NEP integrity; to consider variety-specific NEP data (e.g., protein expression data), to take the market share into account, or to apply alternative modelling types.

The processing of the RAC into foodstuffs, which can reduce or concentrate the level of a NEP compared to its original concentration in fresh crop tissues, can also be considered when refining the exposure estimate. A more realistic assessment can be performed by taking into account the concentration of the NEP with a factor calculated from the ratio of the total protein content in the RAC and the respective food product derived from the RAC. Although this approach is a crude estimate, it is helpful in cases when the consumption database only reports data on food products as eaten and does not also include recipes or conversion factors that would enable a link between the consumed food group/category and the raw commodities. For GM crops that are eaten almost exclusively as cooked or otherwise processed commodities, such as field maize and soy, and are consequently exposed to conditions such as high temperature or low pH that are known to denature proteins, the potential for exposure to intact, functionally active NEP is often negligible [25]. In addition, the NEP concentration in the processed fractions could be measured directly, if needed, to further refine the DEA.

Considerations of the specific crop variety or varieties can refine an exposure assessment to support more realistic scenarios. When different varieties of a crop are cultivated for different purposes, a NEP conferring a new trait may not be commercialized in all crop varieties. For example, a trait conferred by a NEP in field corn may not be introduced in sweet corn or popcorn. While sweet corn and popcorn are intentionally cultivated for direct human consumption, field corn is not typically consumed by humans in the unprocessed form of the RAC. Another example is that consumption data for food-grade, vegetable-type soybeans do not need to be considered in a human DEA for oil-crop types of soybean varieties.

An additional refinement might consider the true fraction of market share represented by the GM RAC containing the NEP. Refinements taking true market share into account can produce more realistic assessments, since there are several non-GM and GM products available to be consumed and it is very unlikely that consumers are only exposed to one single GM product. For example, if a GM crop is approved for importation and consumption but not cultivation in a given country, then the identification of crop commodities being imported can be used to form the basis of the DEA in that country. In such cases, information about the relative proportion of imported and domestic crops could serve a similar role as market share in refining exposure estimates. It is important to note, however, that fluctuations in market share and import/export statistics do occur and use of these refinements are reflective of a given timeframe but may not be applicable over extended periods of time.

Finally, deterministic exposure estimates may be replaced by probabilistic modeling, which was developed for DEAs of chemical pesticides because consideration of the variability in the frequency and magnitude of pesticide residue values can be used to deliver more accurate exposure estimates. Currently, the use of probabilistic models is largely limited to the United States and Canada, due to the lack of comprehensive dietary surveys and consumption databases in other parts of the world. As dietary surveys and probabilistic models are developed for other regions, probabilistic models could be useful for GM DEAs if relatively complex refinements are needed. Those assessing the safety of NEPs should consider whether such refined exposure estimates add value to the overall risk assessment.

6. Conclusions

Both hazard and dietary intake contribute to the understanding of the potential risk from exposure to a NEP in a GM crop. Available data should guide problem formulation to identify data gaps that could critically impact the risk assessment. Using problem formulation ensures that the approach to the overall risk assessment will be transparent, scientifically robust, justifiable, and protective of consumers. Problem formulation could result in the conclusion that a formal DEA for a NEP is unnecessary to assess risk, especially if existing data support negligible exposure and/or hazard.

There is increasing interest in assessing risk using an exposure-led framework prior to the evaluation of hazard [40]. If a weight-of-evidence approach supporting lack of exposure is demonstrated, then all prescribed hazard assessment studies may not be necessary for risk assessment. Thus, an exposure-led approach could ultimately save time and resources, and would be consistent with an ethical imperative to reduce the number of animals used in experimental testing. When a formal DEA is needed, a step-wise approach should be taken, using the most straight-forward method that addresses risk concerns. Several international and regional agencies also recommend a tiered approach for conducting a DEA of substances in food [10, 8, 54, 48, 49]. Strengths and limitations of available databases should be considered, and the exposure duration selected should be relevant to the NEP. Refinements should be applied only when necessary to meet the needs of the assessment. While the focus of this paper is on human DEAs, the same principles could be applied to DEAs of GM crops for animal species.

In conclusion, while formal DEAs may not be scientifically necessary for the risk assessment of NEPs in GM crops, key best practices and considerations following a tiered approach are presented for cases in which formal DEAs are required to inform the risk assessment or to fulfill regulatory requirements.

7. Declaration of Conflicting Interest

Employment affiliation of the authors is given on the first page. C. Mathesius, A. Sauve-Ciencewicki, J. Anderson, P. Bauman, C. Cleveland, C. Fleming, G. Frierdich, L. Goodwin, M. Grunenwald, F. Laporte, E. Lipscomb, R. Oberdoerfer, and J. Petrick are employed in the agricultural biotechnology industry. The research discussed in this paper did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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