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MEETING REPORT

RISK ASSESSMENT OF FOOD ALLERGENS

PART 4: ESTABLISHING EXEMPTIONS FROM MANDATORY DECLARATION FOR PRIORITY FOOD ALLERGENS

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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
WORLD HEALTH ORGANIZATION
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The preparatory work and the convening of the Ad hoc Joint FAO/WHO Expert Consultation on Risk Assessment of Food Allergens required to generate this report was coordinated by the Secretariat.

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ABBREVIATIONS

CCFH	Codex Committee on Food Hygiene
CCFL	Codex Committee on Food Labelling
DBPCFC	double-blind, placebo-controlled food challenge
EFSA	European Food Safety Authority
EHC	extensively hydrolysed casein
ELISA	enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organization of the United Nations
FSANZ	Food Standards Australia New Zealand
ISP	Ice-structuring protein
MoE	margin of exposure
N/RBD	neutralized/refined bleached and deodorized
OFC	oral food challenge
p90, p95, p97.5	A per eating occasion food intake quantity derived from food consumption data/surveys and based on the 90th, 95th or 97.5th percentile of consumers
RfD	reference dose
USFDA	United States Food and Drug Administration
WHO	World Health Organization

DECLARATIONS OF INTERESTS

All participants completed a Declaration of Interests form in advance of the meeting. Three of the experts declared interest in the topic under consideration. Markus Lacorn and Eva Södergren declared significant interests connected with their employment and Clare Mills declared interests connected to investments that exceeded the FAO/WHO's threshold. It could not be excluded that the declared interests may be perceived as a potential conflict of interest. Therefore, while all three persons mentioned above had been invited to participate in the meeting, they had been excluded from the decision-making process regarding final recommendations and participated as technical resource people.

All remaining experts were not considered by FAO and WHO to have declared any interest that may be perceived as a potential conflict with regard to the objectives of the meeting.

All the declarations, together with any updates, were made known and available to all the participants at the beginning of the meeting.

All the experts participated in their individual capacities and not as representatives of their countries, governments, or organizations.

EXECUTIVE SUMMARY

A pro forma process (i.e. a flowchart, Figure 1) has been developed and tested against allergen derivatives previously granted exemptions in various countries or regions and was found to be effective for consideration of future exemption decisions.

After a succinct description of the derivative, including its source and composition (especially regarding protein from the allergenic source food), other key elements of the flowchart include the documentation of existing uses of the derivative, its safety and any reported adverse reactions, other compositional features, past exposure routes and amounts, and method of manufacture and processing. The information should include a specification for the derivative. The intended uses of the derivative and predicted exposure resulting from these uses should also be included. Predicted exposures should be expressed in mg total protein from the allergenic source.

The proposal for the exemption should assess the equivalence of any new derivative and its uses to any existing ingredient(s) of a similar type from similar sources, taking into account species of origin, total protein content, other critical compositional features, safety and any reported adverse reactions, and methods of manufacture.

For total protein quantification (flowchart Figure 1, Box 3), it is recommended to use more than one test method, each based on different principles, that are fit for purpose and may include total amino acid analysis as appropriate. Methods employing extraction should include assessments of recovery and precision of the protein content estimate. The choice of an appropriate calibrant is important, as well as are using appropriate sampling and sample preparation procedures.

Assessments of potential alterations in the allergenicity of the protein(s) in the derivative (flowchart Figure 1, Box 8) can be established using a weight of evidence approach based on data from:

- allergen profiling assays (e.g. mass spectrometry or allergen molecule-specific assays). These approaches could provide additional information to show how the allergen profile has been modified by the process used to manufacture a derivative. Also, protein/peptide size distribution through size exclusion chromatography or mass spectrometry or a combination thereof may be used to assess whether larger peptide fragments (e.g. with 15 amino acids or more) exist; and
- IgE-binding studies using sera from food-allergic individuals with a clinically relevant food allergy, confirmed using appropriate methods such as IgE-immunoblotting, IgE immunoassay (including inhibition assays) and effector cell assays.

Clinical evaluation (flowchart Figure 1, Box 10), when necessary, may require an oral food challenge study. Oral food challenge study design and assessment criteria should be determined on a case-by-case basis in consultation with relevant parties.

An exposure assessment is an essential component of the safety assessment process.

Inputs needed for the exposure assessment are:

> intended use levels of the derivative for relevant food product categories;

> consumption values for intended food product categories and relevant consumer groups on a per eating occasion basis; and

> analytical data or calculated equivalent concentration of total protein or total protein from the priority allergenic source.

The above inputs are combined into an estimation/calculation of exposure amounts, and if applicable, of exposures from a combination of multiple food categories consumed on a single eating occasion.

Existing dossiers and recommendations on exemption decisions have typically estimated exposures using:

> food consumption data based on the 90th, 95th or 97.5th percentile of consumers (a p90, p95 or p97.5 quantity of a single eating occasion), which may vary regionally; and

> maximum levels of intended use of the derivative(s).

Protein concentrations have typically been presented as ranges. Estimation/calculation of exposure amounts typically are presented using either the mean or maximum concentrations. This may vary depending on the applicant or the regulatory body doing the assessment.

The Expert Committee concluded that:

> for the current accepted exemptions, there is an established history of safe consumption;

> the exposure estimates in reasonable worst-case consumption scenarios, based on the scientific data considered for the exemptions approved to date (in the European Union [EU], Australia and New Zealand [ANZ], and the United States of America [USA]), lead to values (expressed in amounts of total protein from allergenic foods) around the relevant reference doses (RfD) established by the second meeting divided by 30 (RfD/30). Consequently, the RfD/30 appears to provide an adequate margin of exposure (MoE) for derivative safety assessment;[1]

[1] Discussions to develop this framework also considered a number of historical soy-related case studies. In Part 2 of the *Ad hoc Joint FAO/WHO Expert Consultation on Risk Assessment of Food Allergens* (FAO and WHO, 2022b), reference doses (RfDs) were recommended for the global priority allergens (FAO and WHO, 2022a), while an RfD for soy was not recommended as soy did not meet the criteria to be a global priority allergen. The "RfD" for soy used during discussions of soy-related exemptions in Meeting 4 was estimated (based on the principles elaborated at Meeting 2) and subsequentially confirmed during an additional fifth meeting which reviewed thresholds for a number of regional or national priority allergens (FAO and WHO, 2023b).

- suitable methods of analysis are available for protein levels based on the RfD/30; and
- a derivative that undergoes the weight of evidence risk assessment as outlined in this report and meets the criterion (RfD/30) may not require clinical studies to establish safety.

Based on these conclusions, the Expert Committee recommends that the process outlined in the flowchart (Figure 1) be used to guide any future development and evaluation of derivative exemptions. Establishment of safety based upon this weight of evidence approach is dependent upon consideration of data quality, outcome of the exposure assessment for all intended ingredient uses (specified for exemption) and review by competent authorities (as needed). When safety is established, exemption can be justified.

CHAPTER 1
INTRODUCTION

1.1 INTRODUCTION

At its 45th session in May 2019, the Codex Committee on Food Labelling (CCFL) requested FAO and WHO to provide scientific advice to validate, and if necessary, update the list of foods and ingredients in Section 4.2.1.4 of *General standard for the labelling of prepackaged foods* (GSLPF) (FAO and WHO, 2019). This request was addressed at the first meeting of the Ad hoc Joint FAO/WHO Expert Consultation on Risk Assessment of Food Allergens (30 November to 11 December 2020; 28 January 2021 and 8 February 2021) by first establishing the criteria for assessing additions and exclusions to the priority food allergen list, then evaluating the available evidence for foods of concern.

The Codex Committee on Food Hygiene (CCFH) has developed a code of practice (CoP) to provide guidance to food business operators and competent authorities on managing allergens in food production, including controls to prevent allergen cross-contact (FAO and WHO, 2020). In relation to this CoP, the 50th session of the CCFH requested FAO and WHO to provide scientific advice with respect to the list of priority allergens and the use of allergen threshold levels to inform allergen risk management for foods (FAO and WHO, 2018). In March 2021, the Expert Consultation reconvened to establish threshold levels for priority allergenic foods and recommend analytical methods for their detection in food and food processing environments. This second meeting addressed a part of the CCFH request by establishing recommended reference doses, based on health-based guidance values (FAO and WHO, 2022b).[2]

[2] In the second meeting, reference doses (RfDs) were recommended for the global priority allergens (FAO and WHO, 2022a), which included: walnut (and pecan), cashew (and pistachio), almond, peanut, egg, hazelnut, wheat, fish, shrimp, milk, and sesame. However, RfDs were not recommended for a number of regional or national priority allergens as they did not meet the criteria to be global priority allergens. An additional fifth meeting was held after the Codex Committee on Food Labelling (CCFL) indicated interest in potential RfD derivation for the following specific food allergens: specific tree nuts (Brazil nut, macadamia nut or Queensland nut, pine nut), soy, celery, lupin, mustard, buckwheat, and oats (FAO and WHO, 2023b).

The CCFL is also developing guidance on the use of precautionary allergen or advisory labelling (PAL) (FAO and WHO, 2021). In October 2021, FAO and WHO convened the Expert Consultation for a third meeting to review and evaluate the evidence in support of precautionary labelling (FAO and WHO, 2023a) to support the ongoing work of the CCFL.

The request from CCFL also sought advice as to:

> whether certain foods and ingredients, such as highly refined foods and ingredients, that are derived from the list of foods known to cause hypersensitivity can be exempted from mandatory declaration.

This request was not addressed at the three previous meetings of the Ad hoc Joint FAO/WHO Expert Consultation. The objective of the fourth meeting was to elaborate on the recommendations from the first meeting concerning derivatives of food allergens and establish a framework for evaluating exemptions for food allergens.

1.2 OVERVIEW OF THE PROCESS

A number of Codex member countries have already established lists of foods and ingredients derived from priority allergens that are exempted from allergen labelling. These were collated and considered by the committee (Annex 1). The committee noted that there is a high degree of concurrence between the jurisdictions about the exemptions, although the precise exemption criteria for the derivatives are often described differently in regulations being implemented. There are also a number of exemptions that are specific to individual jurisdictions. In most cases the Expert Committee had access to the assessment reports prepared by the regulatory authorities to justify the relevant exemption but not to the original data presented by the applicants.

The committee decided to examine the procedure necessary to evaluate a proposal to exempt a food or ingredient derived from a priority allergen from labelling. Three essential components were identified:

> characterization of the derivative, including source and composition, existing uses, safety and reported adverse events;

> analysis of proteins from the allergenic source; and

> exposure assessment from the proposed exempt uses for verification against an acceptable marker of safety.

The committee established three breakout groups to consider these stages and report back to the plenary session periodically. In order to test and refine the process, the committee selected a number of the current exemptions approved by countries or regions to use as case studies by the breakout groups.

Once the three breakout groups had completed their assigned tasks, the plenary sessions compiled a risk assessment-based pro forma process that could be used

either by CCFL or Codex members, to provide a standardized approach to evaluating proposals for allergen labelling exemptions. This could be used for the development of uniform criteria for exemptions either at the Codex level or in domestic regulations. The existing data available for the exemptions approved to date (in the European Union, Australia and New Zealand, and the United States of America) also enabled the committee to benchmark the apparent acceptable levels of exposure against the reference doses (RfD) established by the second meeting.[3]

[3] Discussions to develop this framework also considered a number of historical soy-related case studies. In Part 2 of the *Ad hoc Joint FAO/WHO Expert Consultation on Risk Assessment of Food Allergens* (FAO and WHO, 2022b), reference doses (RfDs) were recommended for the global priority allergens (FAO and WHO, 2022a), while an RfD for soy was not recommended as soy did not meet the criteria to be a global priority allergen. The "RfD" for soy used during discussions of soy-related exemptions in Meeting 4 was estimated (based on the principles elaborated at Meeting 2) and subsequentially confirmed during an additional fifth meeting which reviewed thresholds for a number of regional or national priority allergens (FAO and WHO, 2023b).

CHAPTER 2
ELEMENTS OF RISK ASSESSMENT OF DERIVATIVES FROM PRIORITY ALLERGENS

2.1 CHARACTERIZATION

Characterization of the derivative as part of the preparation of a regulatory or other dossier (e.g. third party supplier manufacturing) is critical in specifying the parameters within which any exemption from allergen labelling remains valid.

2.1.1 DESCRIBING THE DERIVATIVE

A clear, unambiguous description of the derivative should be provided, indicating the nature of the material/ingredient (e.g. glucose syrup, soybean oil) as well as referring to the priority allergenic source (e.g. wheat, barley, etc.), if not already included or implicit in the name. If the derivative is sourced from a genetically modified organism, then the priority allergenic source of the gene should be provided along with the recipient organism (e.g. nature-identical ice structuring protein from ocean pout fish produced in *Saccharomyces cerevisiae* using a synthetic gene adapted for yeast codon usage).

In some cases where the ingredient is primarily protein from an allergenic source (e.g. fish gelatin), the specific species of origin must be defined, where possible.

2.1.2 PROTEIN AND PROTEIN-DERIVED COMPONENTS OF DERIVATIVE SPECIFICATION

In evaluating potential allergenicity of the derivative, the most important element of the specification is the concentration of (total) protein from the priority allergenic source. Other relevant compositional features including protein from any other priority allergenic source (e.g. fermentation media, protein from any other sources, and characterizing components of the ingredient) should be detailed.

In some cases where the ingredient itself is primarily protein from an allergenic source and its functionality depends on the protein component (e.g. fish gelatin), the concentration of specific allergen proteins (e.g. parvalbumin in this instance) must be included, as measured using a validated, fit-for-purpose method.

In the case of derived ingredients manufactured in whole or in part by chemical hydrolysis, enzymatic hydrolysis, or fermentation of proteins sourced from a priority allergenic food, the peptide profile of the derivative should be characterized in terms of chain length and amino acid content.

The requirements in the preceding paragraph also apply in the case of ingredients manufactured in whole or in part by fermentation where the allergenic source protein is a component of the fermentation media, rather than the main substrate that undergoes hydrolysis.

2.1.3. METHODS OF MANUFACTURE

The method of manufacture of the derivative can play a critical role in the allergenicity of the final product. It therefore needs to be fully described to enable an assessment of whether it reduces allergenicity, leaves it unchanged, or even increases it.

The method of manufacture for the derivative should be described in sufficient detail to permit this assessment and should include evaluation of the contribution of individual unit operations and the related process parameters and their operational limits (see fully refining of edible oils as an example). This should include measurement of protein concentration and, if appropriate, characterization of the residual protein. The batch-to-batch reproducibility of the process should be determined. Characterization of residual proteins in the resulting derivative fraction of interest should be determined where necessary. Examples include highly refined soybean and peanut oils, glucose syrups from the starch fraction of wheat, and tocopherols from the deodorizer distillate fraction of soybean oil manufacturing.

2.1.4 ESTABLISHING A HISTORY OF SAFE USE

A comprehensive review of the safety of the derived ingredient should be conducted. This will usually start with an appropriately comprehensive search strategy, which should be fully described. It will normally include consideration of the population(s) exposed, together with any methods of preparation and use of the derivative and, if described, any observed misuse. Any adverse reactions observed should be described,

together with their frequency. Given that the primary concern is potential residual allergenicity, adverse reactions should be classified in terms of the likelihood that they have an allergic aetiology. The results of any known oral challenge trials with the derivative should be critically reviewed, particularly taking into consideration any knowledge of the specification of the materials used.

As part of the history of safe use, equivalence should also be determined to any existing derivative, involving a critical assessment of any differences and their relevance to possible differences in allergenicity.

2.1.5. PROPOSED USES OF THE DERIVATIVE

The proposed use(s) of the derivative should be provided as they will be critical for exposure assessments. Any statutory quantitative limits imposed on the use of the derivative in food products should be described (e.g. incorporation of phytosterols/phytostanols in margarines). In some cases, exemptions may only be sought for specific uses of an ingredient such as fish gelatine as a *carrier for vitamins*.[4]

2.2 ANALYSIS OF PROTEINS

The hazardous components in food that drive the immune-mediated adverse reactions to certain foods are almost entirely proteinaceous in nature. Derivatives intended to be used as an ingredient in foods can vary considerably in protein levels and composition. Highly processed ingredients, such as highly refined oils, may contain very low levels of protein whilst the protein fractions of other food ingredients vary in their complexity and include:

- complex mixtures representing the proteome of a tissue (such as meat) or a fraction (such as flour) comprising thousands of different types of protein molecules;
- protein fractions, such as fish collagen, which have a more limited repertoire of proteins; and
- almost pure, single proteins such as lactoferrin or certain types of whey-derived ingredients which may represent almost pure α-lactalbumin or β-lactoglobulin.

[4] Fish gelatine is comprised primarily of protein from a priority allergenic source, namely fish. As a result, the exemption of fish gelatine as a class would fail when using the proposed flowchart in this report. The exemption of fish gelatine as a class would also likely be impossible due to a number of factors including but not limited to: 1) the wide variety and mixtures of fish potentially used in gelatine production and the desire, in some jurisdictions, to define specific species of origin, where possible; 2) differing methods of manufacture which can play a critical role in the presence and levels of residual-specific potentially allergenic proteins in the final product and on the allergenicity of the final product; 3) differing methods of manufacture that may impact the applicability of different methods used to analyse for the presence and levels of specific potentially allergenic proteins in the derivative; and 4) differing potential intended/proposed uses of the derivative that will greatly impact exposure assessments. As such, a class-wide exemption application would fail the proposed flowchart in this report at multiple points (or even everywhere) due to the dependency on specific sourcing, manufacturing and exposure scenarios which cannot be determined for all types of products in a class within a single exemption dossier. However, the exemption of fish gelatine meeting certain criteria, such as the residual level of parvalbumin, the major allergenic fish protein, and intended for specific uses resulting in low levels of consumer exposure, such as vitamin encapsulation, could be considered for exemption provided that the inherent allergenicity of fish collagen in the population is considered to be a manageable risk.

The allergenicity of these different types of ingredients needs to be assessed in different ways. For highly refined ingredients with very low levels of protein, total protein analysis can be used in the risk assessment process (Stage 1), whilst for others that largely comprise protein, a profiling approach together with measurement of IgE-binding capacity are more relevant.

Irrespective of the methods used, an overarching consideration is extraction efficiency. Thus, buffers employing a combination of detergents, chaotropes and reduction will be necessary together with an effective combination of pH, homogenization, agitation, time-temperature combinations, and ratio of extractant to extraction buffer. These will inevitably vary depending on the material being extracted and the requirements of subsequent analysis – ionic detergents not being compatible with High Performance Liquid Chromatography (HPLC) methods whilst disruptive buffers are often not compatible with IgE-binding studies. As with any methodology, it must be fit for purpose and where compromises have to be made, these must be clear.

In addition, sampling and recovery need to be taken into account. Some matrices are highly complex, and target analytes are difficult to extract, therefore underestimation is possible. Also, sampling can be a challenge if the analyte is not homogeneously distributed in the matrix. In such cases, it is important to adapt the sample size or sampling techniques to obtain a representative sample. The same consideration will need to be applied to subsampling in the laboratory.

2.2.1 STAGE 1 CONSIDERATIONS FOR TOTAL PROTEIN DETERMINATION

Several methods can be used for the quantitation of proteins and peptides. Examples of commonly used methods for protein and peptide quantification are listed in Table 1.

For very low levels of proteins and peptides, several novel methods have been developed, some of them based on a combination of nanomaterials and ELISA.

However, each method has its own advantages and disadvantages, and each method must be evaluated for its fitness for purpose for each type of matrix and for each type of protein or peptide mix. It is crucial also to consider the impact of the extraction process when selecting the appropriate protein quantification method (i.e. see extraction comment in 2.2.).

TABLE 1 **COMMONLY USED METHODS FOR PROTEIN AND PEPTIDE QUANTIFICATION**

ASSAY NAME	METHOD	COMMENTS
Biuret assay (Beyer, 1983; Watters, 1978)	Based on the reaction of proteins with copper ions to form a violet-coloured complex	It may not be accurate for all proteins (e.g. highly basic proteins may not react well). It can be affected by contaminants such as detergents and reducing agents.
Bradford assay (Bradford, 1976; Harlow and Lane, 2006)	Based on the reaction of proteins with a dye called Coomassie Brilliant Blue G-250	It may not be accurate for all proteins (e.g. highly basic proteins may not react well). It can be affected by contaminants such as detergents and reducing agents.
Bicinchoninic acid (BCA) assay (Smith *et al.*, 1985; Wiechelman, Braun and Fitzpatrik, 1988)	Based on the reaction of proteins with BCA to form a purple-coloured complex	It may not be accurate for all proteins (e.g. highly basic proteins may not react well). It can be affected by contaminants such as detergents and reducing agents.
Lowry assay (Lowry *et al.*, 1951)	Based on the reaction of proteins with a reagent called Folin-Ciocalteu's reagent to form a blue-coloured complex	It may not be accurate for all proteins (e.g. highly basic proteins may not react well). It can be affected by contaminants such as detergents and reducing agents.
Fluorescent dyes (e.g. Coomassie Brilliant Blue) (Sedmak and Grossberg, 1977)	Based on the reaction of proteins with the fluorescent dye	It may not be accurate for all proteins (e.g. highly basic proteins may not react well). It can be affected by contaminants such as detergents and reducing agents.
Nitrogen analysis (e.g. Kjehldahl, Dumas) (Bradstreet, 1954; Kirk, 1950)	Based on determining the total nitrogen content of a sample, which can be converted to protein concentration using the conversion factor of 6.25 (as proteins contain about 16% nitrogen by weight) or, if known, a factor specific to the protein present	Requires the use of hazardous chemicals; melamine has been used to increase the apparent nitrogen content of protein mixtures fraudulently. Using appropriate nitrogen-to-protein conversion factors is key for correctly determining the total protein content (Shea and Watts, 1939; Maehre *et al.*, 2018; Charrondiére *et al.*, 2012).
Amino acid analysis (Kaspar *et al.* 2009; Zhang, L. and Denslow, 2000).	Based on hydrolysing the protein into its constituent amino acids and then quantitating the amino acids using various techniques such as chromatography or spectrophotometry	Requires the hydrolysis of proteins into individual amino acids.
Radioisotope analysis (Balcells *et al.*, 1999)	Based on labelling proteins with a radioisotope such as ^{14}C or ^{35}S and then measuring the radioactivity	The method requires the use of hazardous materials (radioisotopes). It requires specialized equipment.
Mass spectrometry (Van De Merbel, 2013; Trötschel and Poetsch, 2015; DeSouza and Siu, 2013; Pan *et al.*, 2009)	Based on breaking down the protein into its constituent peptides and then measuring the mass of the peptides to determine the protein concentration	The method requires specialized equipment. The accuracy of quantification may vary.

Note: Ordered to separate the foods with consensus and final RfD recommendations from those with values for risk management for clarity.
Source: See p. 48.

For example, the proteins might be degraded through hydrolysis or shear forces during the process, which may lead to an underestimation or overestimation of the (allergenic) proteins/peptides present.

Since each of the methods has its shortcomings, and because it may be difficult to establish which method is the best fit for purpose, it is highly recommended to use more than one method for protein quantification, ideally methods that are based on different principles (e.g. amino acid analysis and Bradford assay).

Another factor that will impact the accuracy of the result is the calibrant used. Some assays employ, for example, bovine serum albumin (BSA) as a calibrant, which may be a source of bias. This could be reduced by using calibrants based on relevant reference materials (if available) or matrix-matched materials.

2.2.2 STAGE 2 CONSIDERATIONS FOR ALLERGEN PROFILING

2.2.2.1 Molecular size characterization

Molecular size is an important consideration since it affects the ability of proteins, or derived fragments, to interact with the immune system to either sensitize or elicit an IgE-mediated adverse reaction. In order to sensitize an individual, T-cell epitopes must be present which are generally considered to be ~9 amino acids in length whilst longer peptides are required for B-cell activation which need to span both multiple B-cell epitopes and T-cell epitopes and need to be ~20 amino acids in length. This is illustrated by the observation that whey hydrolysates with peptides < 2 500kDa were unable to sensitize animal models (Bøgh, Barkholt and Madsen, 2015). Similarly for elicitation of an allergic reaction, a fragment that accommodates at least two IgE epitopes is required for elicitation although there is evidence that multimers and aggregates play an important role in stimulating effector cells (Bucaite *et al.*, 2019). Therefore, the initial step should be to assess the molecular size distribution of proteins and peptides in the food ingredient to characterize the peptide size distribution as has been done, for example, in characterizing hydrolysates for use in infant formula (EFSA, 2022). Appropriate methods that address this are gel-based and chromatographic molecular sieving techniques together with mass spectrometry.

2.2.2.2 Protein and peptide profiling

Where there is residual protein of sufficient size to be of concern, there is a need for a protein profile to identify whether the derivative has allergenic molecules. This might need to involve more than one complementary method such as proteomic analysis using gel-based or "shotgun" discovery proteomic approaches, antibody arrays of allergen molecule-specific assays. For example, an antibody preparation with a well-characterized specificity for a particular allergen molecule could be used in immunoblotting or immunoassays, SDS-PAGE analysis and in-gel digestion, proteomic analysis or N-terminal sequencing, or proteomic profiling using mass spectrometry.

The test methods need to be able to:

> identify allergenic proteins or derived peptide fragments in the protein profile of the derivative ingredient; and

> provide a relative or absolute quantitative analysis of the allergenic proteins or derived peptide fragment in the profile of the derivative ingredient compared to the starting material to demonstrate changes in levels of allergenic protein molecules.

2.2.2.3 Serum IgE-binding capacity

Where the presence of allergenic proteins or peptides >15 amino acids in length are identified in 2.2.2.1 and 2.2.2.2, these may need additional assessment to characterize their IgE-binding capacity. Building on existing approaches to assessing the IgE-binding capacity in food risk assessment (EFSA, 2010) and allergen extracts, these studies will require access to serum or plasma from individuals with a well-characterized allergy to the priority allergenic foods from which the derivative is prepared. Such individuals should have a clinically diagnosed food allergy not antedating blood sampling by more than five years. Patients should have clinical history and symptoms consistent with an IgE-mediated food allergy, evidence of sensitization to the specific foods and preferably (if available) a food allergy confirmed by food challenge or a history of severe reaction precluding a food challenge.

The heterogeneity in individual IgE responses means that individual sera should be checked even when serum pools are used. It may also be that the allergic individuals should be drawn from multiple centres, different ages and/or geographic locations to ensure the biological materials (sera) are fit for purpose. Serum samples from at least ten individuals should be used, although heterogeneity of individual responses may mean larger panels will sometimes be required (Platts Mills, Rawle and Chapman, 1985).

2.2.2.4 Case studies of protein analysis to support allergenicity risk assessment

Low protein derivative: One example of a derivative of an allergenic food source with a very low protein content is highly refined oil where a crude oil is degummed, neutralized with alkali, the resulting soap formed from fatty acids, phosphatides, residual protein and carbohydrate. Impurities are further removed by a bleaching and deodorization process, the resulting oil containing very low levels of protein (Rigby *et al.*, 2011). This process reduces the protein content of crude un-degummed soybean oil which contained from 86 000–87 900 ng/g of protein to between 62–265 ng/g oil (Rigby *et al.*, 2011), the refining process showing a similar level of reduction in peanut oils (Olszewski *et al.*, 1998). Such very low levels of protein require very sensitive methods for protein determination, from determination using amino acid analysis as well as fluorescence assays, such as those based on 3-(4-carboxybenzoyl) quinolone 2 carboxaldehyde. See case studies 3.2.4 soybean oil and 3.2.5 peanut oil for more information regarding how these analytical results have been utilized in prior exemption assessments.

Refined protein derivative: Derivatives may comprise a fraction of the protein in a raw commodity which may alter its allergenicity. One example of this is fish gelatine, which is produced by extraction and acid hydrolysis of collagen, often from fish skin. The approach taken for this product was to analyse for the presence of the major fish allergen parvalbumin, using an immunoassay which showed that the processing reduced the level of parvalbumin to no more than 0.15mg/g (Koppelman *et al.*, 2012). In a similar fashion, monoclonal anti-carp parvalbumin and polyclonal anti-cod parvalbumin immunoassays were utilized in an assessment to use fish gelatine as a formulation aid (carrier) in vitamin and carotenoid preparations (EFSA, 2007b). This is in contrast to another fish gelatine derivative produced from fish swim bladders, used as a fining agent and known as isinglass (Vriesekoop, 2021). In order to estimate the residual level of isinglass in beer, analysis of hydroxyproline as a marker of collagen content was used, which showed that it was undetectable in filtered beer (Chlup, Leiper and Stewart, 2006). An assessment of isinglass was provided, with data on residual parvalbumin suggesting that levels ranged from 1–35 mg/Kg, and it was undetectable in fined beer using an assay with a limit of detection of 9µg/L (EFSA, 2007). See case studies 3.2.8 fish gelatine and Annex 2 (estimated exposure details including gelatine and isinglass) for more information regarding how these analytical results have been utilized in prior exemption assessments.

Of note, one assessment the European Food Safety Authority rejected an application for was an exemption for a fish gelatine from allergen labelling due to a lack of data provided by the applicant, including a lack of information on residual levels of the major fish allergen parvalbumin in the fish gelatine preparations in question (EFSA, 2004b).

Purified protein: Derivatives comprising purified proteins are also used – one example being lysozyme which is used as a processing aid in the manufacture of cheese and wine. Residual lysozyme has been determined in food products using a variety of methods including several immunological methods employing lysozyme-specific antibodies, HPLC and mass spectrometry (Downs *et al.*, 2022; Rauch, Hochel and Kàš; 1990, Marchal *et al.*, 2000; Iaconelli *et al.*, 2008). These analyses indicated levels of lysozyme in wines ranging from 0.1–8.6mg/L (EFSA, 2011), and the exposure assessment was conducted assuming mean lysozyme concentrations of 250 mg/kg in cheese and 40 mg/L in wine. The EFSA Opinion consequently concluded that lysozyme, although considered a minor allergen in egg, could cause allergic reactions under expected conditions of exposure.

2.3 EXPOSURE ASSESSMENT

An exposure assessment is an essential component of the safety assessment process. The input parameters for an exposure assessment are: 1) the intended use levels of the derivative for relevant food products; 2) concentration values of protein in the derivative; and 3) consumption values for the intended food products. These are combined in the final exposure estimate.

Adherence to safety assessment principles means that decisions for exempting derivatives from labelling requirements should be based on reasonable worst-case scenarios to assure safety under all reasonably foreseeable situations. Reasonable worst-case scenarios may imply coinciding high values for input parameters.

2.3.1 INTENDED USE LEVELS OF THE DERIVATIVE FOR RELEVANT FOOD PRODUCTS

For a full assessment of the expected exposure, the range or maximum levels of intended uses of the derivative for each food application should be specified. Because actual levels may deviate from intended levels due to manufacturing process characteristics, information on variations from intended use levels should be considered. Further, information on possible technological aspects that limit the maximum concentration of the derivative in food products is of value. Information on analytical methods used and their suitability for quantifying the level of the derivative in food products is needed. Based on this information, an evaluation of estimated levels of the derivative in final food product(s) can be made.

2.3.2 CONCENTRATIONS OF PROTEIN IN THE DERIVATIVE

The second element for assessing the exposure is information on concentrations of proteins in the derivative. In the second meeting of this Expert Consultation (FAO and WHO, 2022b), this committee assessed the available information for characterizing the allergenic hazard of proteins from allergenic sources and identified the publications of Remington *et al.* (2020) and Houben *et al.* (2020) as the most comprehensive and best described sources available. These publications provide hazard characterization data for allergenic foods, i.e. eliciting dose values, expressed as amounts of total protein from the allergenic source. Therefore, for the assessment of the safety for allergic individuals of intended uses of ingredients derived from priority allergenic sources, information on concentrations of proteins in the derivative should be calculated and expressed as (maximum) levels of total protein from the allergenic source to allow comparison with the available hazard characterization data. Analytical data or a calculated equivalent concentration of total protein or total protein from allergenic source can be used in the calculation. Similar to the information on intended use levels of the derivative, information on variability, possible technological limits and analytical methods used and their suitability for quantifying the levels of proteins in the derivative should be specified.

2.3.3 CONSUMPTION VALUES FOR THE INTENDED FOOD PRODUCTS

For estimation of allergen exposure of allergic individuals, food intake values for single eating occasions (single meals) are to be used (EFSA, 2021; USFDA, 2015; FSANZ, 2016; Houben *et al.*, 2020). Such intake data can be based on general population food consumption surveys (Blom *et al.*, 2020). For a detailed assessment of intake scenarios, preferably at least the mean and 90th, 95th and 97.5th percentiles of intake for each final food product included in the intended uses would be available.[5] If applicable and possible, depending on the intended food applications, such data need to be differentiated for consumers of different ages, genders or ethnicity. In case multiple food applications are intended, integrated consumption levels should be calculated. It is recommended to consider several different consumption scenarios, if applicable.

2.3.4 EXPOSURE ESTIMATE

The above inputs are combined and calculated into an estimation of exposure to total protein from the allergenic source at single eating occasions. In case of possible combined exposures at single eating occasions from various food product applications, usually maximum values for derivative use levels in food products and maximum concentrations of protein in derivatives are assumed and combined with the 90th, 95th or 97.5th percentile of intake for each final food product. The chosen percentile may vary regionally (EFSA, 2021; FDA, 2015; FSANZ, 2016). The maximum or high percentile values from the input parameters may then, however, result in unrealistic high exposure estimates. More realistic estimates can then be provided by using the information on the variability in derivative use levels, protein concentrations in the derivative, and consumer food product intake frequencies and amounts. Probabilistic modelling may be applied for refining the exposure estimate based on variabilities in input parameters.

2.4 ACCEPTED LEVELS OF EXPOSURE TO UNLABELLED PRIORITY ALLERGEN DERIVATIVES

The committee made a number of observations drawn from the working group (WG) findings in relation to comparing the current allergen exemptions regarding the suitability of the proposed flowchart and to applying the reference doses (RfDs) established at the second meeting (FAO and WHO, 2022b):[6]

> The exposure assessment WG calculations indicate that the exemptions approved to date (in the European Union, Australia and New Zealand [ANZ], and the

[5] Of note, the consumption percentiles for use in an exemption dossier (90th, 95th or 97.5th percentile of intake) are different than the consumption percentiles used in the risk assessment for unintended allergen presence (UAP) or cross-contact. In Part 3 of the *Ad hoc Joint FAO/WHO Expert Consultation on Risk Assessment of Food Allergens*, the Expert Committee recommended using the 50th percentile as the consumption value for risk assessments of allergen cross-contact (FAO and WHO 2023a). If the 50th percentile is not available, the mean of the population distribution of the single-eating occasion intake of food would be a conservative alternative (FAO and WHO, 2023a).

[6] Discussions to develop this framework also considered a number of historical soy-related case studies. The "RfD" for soy used during discussions of soy-related exemptions in Meeting 4 was estimated (based on the principles elaborated at Meeting 2) and subsequentially confirmed during an additional fifth meeting (FAO and WHO, 2023b).

United States of America) may lead to exposures (expressed in doses of total protein from allergenic foods) around RfD/30 in reasonable worst-case consumption scenarios (Annex 2 and Annex 3).

> The analytical WG advised that there are suitable methods of analysis available for these protein levels, but analysis becomes problematic at lower thresholds such as RfD/60 or RfD/100.

> For many of the current allergen labelling exemptions, there appears to be a safe history of consumption in the countries and regions in which they have been applied.

The committee therefore determined that the RfD/30 provides a practical, useable and measurable safe margin of exposure for assessing suitability for allergen labelling exemptions. Higher margins of exposure appear to be overly precautionary and, in many cases, will be analytically unverifiable for monitoring or enforcement.

CHAPTER 3
RISK ASSESSMENT PROCESS FOR UNLABELLED PRIORITY ALLERGEN DERIVATIVES

3.1 FLOWCHART

A pro forma process (i.e. a flowchart, Figure 1) has been developed and tested against allergen derivatives previously granted exemptions in various countries or regions and found to be effective for consideration of future exemption decisions.

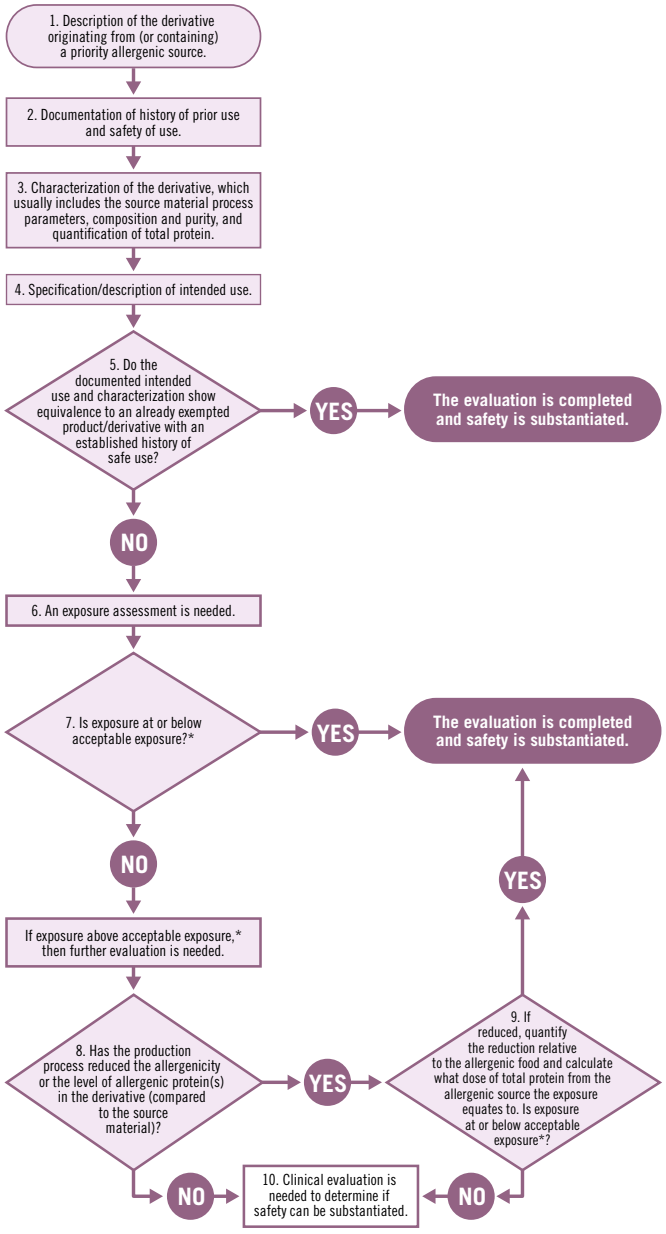
Boxes 1–5 of the flowchart (Figure 1) involve characterization of the derivative and will be critical for any risk assessment and evaluation of derivative exemption dossiers. See Sections 2.1 and 2.2 of this report for more information.

If the documented intended use and characterization of the derivative being evaluated demonstrate equivalence to an already exempted product/derivative with an established history of safe use, then the evaluation is completed, and safety is substantiated.

If this "equivalence" criterion is not met, then an exposure assessment will be needed (flowchart Figure 1, Boxes 6–7). Exposure assessment is an essential component of the safety assessment process. See Section 2.3 for more information on exposure assessment.

At this point in the flowchart, safety could be substantiated if the results of the exposure assessment show that protein exposure is at or below an "acceptable exposure." Acceptable exposure in the context of assessing an exemption application can be derived by applying a margin of exposure (MoE) to the reference dose (RfD) proposed in the second meeting of this Expert Consultation (i.e. RfD divided by MoE; RfD/MoE).

FIGURE 1. **OUTLINE OF THE PROCESS FOR CONSIDERATION OF LABELLING EXEMPTIONS FOR FOODS AND INGREDIENTS DERIVED FROM PRIORITY ALLERGENIC SOURCES**



*Acceptable exposure in the context of assessing an exemption application can be derived by applying a margin of exposure (MoE) to the reference dose (RfD) proposed in the second meeting of this Expert Consultation (i.e. RfD divided by MoE; RfD/MoE). The RfD/30 appears to provide an adequate MoE for derivative safety assessment.[7] For comparison with the acceptable exposure, protein exposure should be calculated and expressed as the equivalent dose of total protein from the priority allergenic source.

Note: Establishment of safety based upon this weight of evidence approach is dependent upon consideration of data quality, outcome of the exposure assessment and review by competent authorities (as needed). When safety is established, a labelling exemption can be granted.

[7] Discussions to develop this framework also considered a number of historical soy-related case studies. The "RfD" for soy used during discussions of soy-related exemptions in Meeting 4 was estimated (based on the principles elaborated at Meeting 2) and subsequentially confirmed during an additional fifth meeting (FAO and WHO, 2023b).

The RfD/30 appears to provide an adequate MoE for derivative safety assessment.[8] For comparison with the acceptable exposure, protein exposure from the allergenic source should be calculated and expressed as the equivalent dose of total protein from the priority allergenic source. If exposure is above an acceptable exposure, then further evaluation is needed.

If exposure is above an acceptable exposure, it is pertinent to investigate whether the production process has reduced the allergenicity or the level of allergenic protein(s) in the derivative (compared to the source material) (flowchart Figure 1, Box 8). See Section 2.2.2 for considerations regarding assessments of potential alterations in the allergenicity of the protein(s) in the derivative. If the allergenicity or the level of allergenic protein(s) in the derivative is reduced, the exposure assessment should be amended as appropriate and the resulting exposure compared to the acceptable exposure (flowchart Figure 1, Box 9).

If the production process has not reduced or not adequately reduced the allergenicity or the level of allergenic protein(s) in the derivative, then clinical evaluation (flowchart Figure 1, Box 10) will be needed to determine whether safety can be substantiated.

Clinical evaluation, when necessary, may require an oral food challenge study. Oral food challenge study design and assessment criteria should be determined on a case-by-case basis in consultation with relevant parties. However, the Expert Committee noted that if clinical evaluation is needed, ideally this should include a representative population of subjects, adults and children as necessary, who have different clinical phenotypes (e.g. sensitized to specific protein[s] from the allergenic source of the derivative) and have been documented to have clinical reactivity to this protein source. While the United States Food and Drug Administration (USFDA) guidance (USFDA, 2015) does give some indication of what information might be recorded during clinical testing, the Expert Committee also noted that there is a data gap with regard to guidance for designing appropriate clinical evaluations with sufficient statistical power in this area.

3.2 SUMMARY OF CASE STUDIES

As noted in Section 2.4 above, the committee made a number of observations drawn from the working group findings in relation to comparing the current allergen exemptions regarding the suitability of the proposed flowchart and to applying the reference doses (RfDs) established at the second meeting (FAO and WHO, 2022b).[9]

This section summarizes prior allergen exemption "case studies" as well as some cases where an exemption was not granted. Observations from the Expert Consultation

[8] Discussions to develop this framework also considered a number of historical soy-related case studies. The "RfD" for soy used during discussions of soy-related exemptions in Meeting 4 was estimated (based on the principles elaborated at Meeting 2) and subsequentially confirmed during an additional fifth meeting (FAO and WHO, 2023b).

[9] Discussions to develop this framework also considered a number of historical soy-related case studies. The "RfD" for soy used during discussions of soy-related exemptions in Meeting 4 was estimated (based on the principles elaborated at Meeting 2) and subsequentially confirmed during an additional fifth meeting (FAO and WHO, 2023b).

are included for each case study. It should be noted that these observations are not meant to be recommendations or endorsement for the exemption of a derivative from required allergen labelling on a global basis. These observations are made for the purpose of discussing the suitability of the proposed flowchart and of the potential application of the RfD/30 as a practical, useable and measurable safe margin of exposure for assessing suitability for allergen labelling exemptions. It should also be noted that the Expert Consultation did not aim to reassess the conclusions reached in each assessment.

3.2.1 GLUCOSE SYRUPS (WHEAT)

Wheat-based glucose syrups (including dextrose) are purified and concentrated aqueous solutions of saccharides derived from wheat by hydrolysis of a wheat starch solution. Hydrolysis is followed by treatment with activated charcoal to remove undesired components including proteins. The EFSA Opinion on glucose syrups derived from barley indicate that similar procedures also apply to preparations of glucose syrups from that source (EFSA, 2007f, 2007g).

Eleven putatively food-allergenic molecules are listed in the IUIS Allergen Nomenclature database, although two of those are hypothetical proteins. Several of those wheat proteins are gliadins or glutenins, but the only one of possible relevance to glucose syrups is a beta-amylase (Tri a17), although it clearly is not, by mechanism, a starch synthase, which is the main constituent of the residual proteins in glucose syrups. Wheat proteins also include other allergens which are associated with respiratory allergy (bakers' asthma). These proteins are not relevant to consideration of the potential allergenicity of wheat-based glucose syrups as respiratory allergy (bakers' asthma) is quite distinct from wheat food allergy. Analytical methods for wheat protein have focused on the detection of gluten (Report 2), and those are the types of methods used in the exemption dossier.

History of safe use: Wheat-based glucose syrups have a long history of use and form part of many food products. According to the EFSA Opinion supporting exemption, wheat-based glucose syrups such as dextrose are used for confectionery, jams and fruit preparations, dairy ice-cream, beverages and fruit syrups, dairy desserts and biscuits, infant foods, bakery products, and also for dietetic and medicinal products for oral use. They are ingredients in the production of food additives such as sorbitol, xylitol, mannitol, maltitol, caramel colouring, ascorbic acid and lactic acid among others. No known allergic reactions have been attributed to glucose syrup. Two challenge studies in wheat-allergic individuals were considered inconclusive by the EFSA (although this appears to be because of participant selection issues).

Characterization: Glucose syrups are made using a standardized, well-defined process, supported by Good Manufacturing Practices (GMP) protocols. The material for which exemption was sought from the EFSA showed low amounts of residual gluten and peptides by mass spectrometry and high-pressure liquid chromatography analysis in wheat starch glucose syrups including dextrose (0.3–1.4 mg/kg).

Exposure: Assessment is needed and can be based on total protein content of glucose syrups because wheat is the sole source of protein. Further details for exposure estimates by FSANZ, EFSA and additional calculation by the Expert Committee can be found in Annex 2 (exposure estimated details). These estimates all determined that exposure levels greater than 1 mg of wheat protein per eating occasion was possible.

Clinical studies: No clinical studies have been performed to support exemption.

EFSA Opinion:

> Taking into account all the scientific information provided and in particular the levels of wheat proteins reported in glucose syrups including dextrose, the Panel considers that it is not very likely that this product will trigger a severe allergic reaction in susceptible individuals (EFSA, 2007f, p. 6).

The derivative is now exempt from required allergen labelling as specified in Regulation (EU) No 1169/2011.

Expert Consultation observations (regarding the suitability of the proposed flowchart and to the potential application of the RfD/30): Exposures for the intended use could be in the range of or above the wheat RfD/10. Still, wheat-based glucose syrups have a long history of use and this observation provides further information that the RfD/30 provides a practical, useable and measurable safe margin of exposure for assessing suitability for allergen labelling exemptions. For future applications, within the proposed flowchart, if process parameters and intended uses are equivalent, then safety can be substantiated; no further evaluation required.

3.2.2 SOY PHYTOSTEROLS/TOCOPHEROLS

The derivatives "vegetable oils-derived phytosterols and phytosterol esters from soybean sources" and "tocopherols from soybean sources" are both produced from the vegetable oil deodorized distillate (VOD) that results from the final step in the production of highly refined soybean oil (EFSA, 2007d, e). The VOD is subject to a series of processing steps to remove unwanted by-products, including fatty acids, di- and triglycerides, waxes, fatty acid esters and others. The processing steps include distillation, filtration and crystallization techniques (Thomas, 2004). The process results in the almost complete removal of protein as it is not volatile.

The major allergens in soybean seeds are the storage proteins: conglycinin (Gly m 5) and glycinin (Gly m 6). Minor soybean allergens include the 2S albumin (Gly m 8) and a PR-10 protein known as SAM-22.

History of safe use: Soy phytosterols have a long history of use, which pre-dates their more recent history as cholesterol-lowering ingredients in a variety of products, including margarines, milk, yoghurts, etc. Tocopherols are used largely as antioxidants in foods at comparatively low concentrations (50mg/kg), while phytosterol levels are higher in order to deliver an appropriate cholesterol-lowering amount with a nominal portion of the food. A literature search did not reveal any evidence of allergic reactions to phytosterols or tocopherols, including in soybean-allergic individuals.

Characterization: The manufacturing process is fully described and standardized, being part of the soy oil refining process. As a derivative of soy, there is a possibility of soy protein residues. No lipophilic or hydrophilic proteins were detected at the limit of detection in the vegetable oil distillate (100 or 1µg/g) or the phytosterols used to prepare them (10–20 µg/g – ELISA). Data presented for tocopherols relied on assays with a higher limit of detection (LoD), but given the starting materials, it would be reasonable to infer that similar levels were achieved for those materials.

Clinical studies: Clinical data were obtained by testing soybean-allergic participants with the products. Thirty-two subjects with clinically confirmed soybean allergy were recruited. Those participants were skin-prick tested with a commercial soy extract, soy isolate/soy milk and the phytosterols blend. Twenty-two had a positive skin reaction (soy isolate: 16; soy [extract]: 6). None of the participants had a positive skin-prick test (SPT) to the phytosterols blend. All 32 participants underwent an open challenge with 3 g of phytosterols. Of those, 29 subjects tolerated the challenge and three subjects experienced mild symptoms (EFSA, 2007d). One of those three subjects was reported in a previous study on soy thresholds to have reacted with oral allergy symptoms (OAS) to 4 g of soy protein without developing systemic symptoms after additional challenges to higher doses (Ballmer-Webber *et al.*, 2007). All three subjects subsequently underwent a phytosterols double-blind, placebo-controlled food challenge (DBPCFC), where two subjects had no reactions and mild OAS was reported by the subject who previously reported OAS to 4 g of soy protein. Additional *in vitro* IgE-binding studies for this one reactive individual were negative to samples of phytosterols (EFSA, 2007d).

Intended use (tocopherol): Vitamin and antioxidant use of Tocopherol – vitamin E is equivalent to history of safe use. Phytosterols are a functional ingredient and are nutraceutical, and were authorized as a novel food under Regulation (EC) 258/97 in 2000 (European Commission, 2000). History of safe use by 2007 (EFSA, 2007e) was approximately 5 years.

Exposure (tocopherol): An exposure assessment was deemed to be needed, and these estimates found a daily intake of up to 41 µg of soy protein from tocopherols. Further details can be found in Annex 2 (exposure estimated details).

Need analysis of total soy protein levels in tocopherol and phytosterol ingredients (Rigby *et al.*, 2011).

Intended use (phytosterols): Phytosterols are used as a cholesterol-lowering functional ingredient in a variety of products including margarines, milk and yoghurts and are incorporated in products at a concentration sufficient to deliver the amount required for optimal activity in a nominal portion. In the European Union, intake is aimed to be limited to 3 g/person per day, controlled through concentration in the final product.

Deodorizer distillate fraction is separated from soy protein fraction by solvent extraction, degumming, and bleaching steps; the final distillation process further lowers protein residues.

Exposure (phytosterols): A daily intake of up to 30 µg of soy protein from phytosterols was estimated, depending on analytical results. Further details can be found in Annex 2 (exposure estimated details).

EFSA Opinion:

> Considering the information provided by the applicant regarding the starting material, the subsequent production process, and the demonstration of low residual protein content, the Panel considers that it is unlikely that [natural mixed tocopherol/D-alpha tocopherols] *or* [vegetable oils derived phytosterols and phytosterol esters] from soybean sources will trigger a severe allergic reaction in susceptible individuals (EFSA, 2007d, p. 7, 2007e, p. 7).

The derivative is now exempt from required allergen labelling as specified in Regulation (EU) No 1169/2011.

Expert Consultation observations (regarding the suitability of the proposed flowchart and to the potential application of the RfD/30): Assuming this was a new submission and not equivalent to an already exempted product, exposures for the intended use will likely be below the soy RfD/30 acceptable exposure. For future applications, within the proposed flowchart, if process parameters and intended uses are equivalent, then safety could be substantiated; no further evaluation required.

3.2.3 SOYBEAN OIL

The derivative neutralized/refined bleached deodorized (N/RBD) soybean oil is the highly refined edible vegetable oil derived from soy. The oil is first separated from crushed soybeans by solvent (hexane) extraction, followed by degumming, neutralization/refining (United States of America), bleaching and deodorization. The process results in very low residual levels of protein (EFSA, 2007a).

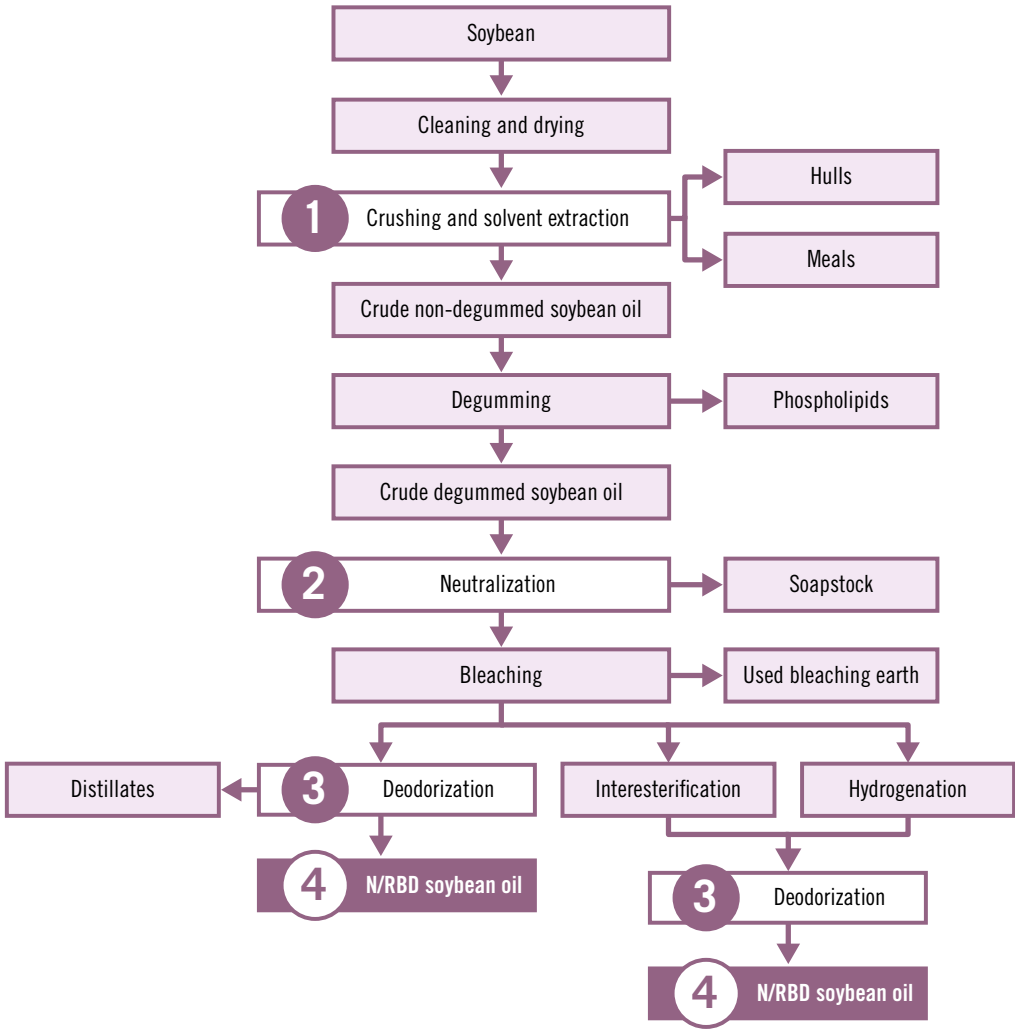
History of safe use: N/RBD soybean oil is an important edible vegetable oil, used both on its own and as part of edible vegetable oil blends. There is a long history of use in many categories of products at comparatively high levels, with few reports of allergic reactions attributed to RBD soy oil.

Characterization: The process is fully described and standardized. **Figure 2** illustrates the different stages and associated components and side products.

The process is supported by Codes of Practice to ensure that the resulting product is consistent in terms of safety and quality (e.g. FEDIOL [2020] Code of Practice on vegetable oil and fat refining for food purposes in the European Union).

Analysis shows very low levels of residual protein in N/RBD soybean oil. The reduction of protein content through the refining process was clearly demonstrated with a residual protein concentration of 265 ng/g with crude, non-degummed soybean oil compared to the corresponding crude oil (average 87 250 ng/g) (Rigby *et al.*, 2011). Additional data on immunochemical identification of proteins supported these conclusions.

FIGURE 2. SIMPLIFIED ILLUSTRATION OF THE PRODUCTION PROCESS FOR NEUTRALIZED/REFINED BLEACHED AND DEODORIZED (N/RBD) SOYBEAN OIL



Source: Authors' own elaboration.

Intended use: Widespread use both as an ingredient in food products and on its own.

Intended use and specification were equivalent to existing history of safe use.

Exposure: Assessment shows very low soy protein exposure (less than 60 µg in a worst-case scenario). Further details can be found in Annex 2 (exposure estimated details).

Analysis conducted using suitable method (Rigby *et al.*, 2011)

Clinical studies: Bush *et al.* (1985) did not observe any reactions following ingestion of three different types of soybean oil in seven individuals with well-documented reactions to soy.

Two studies (unpublished) were undertaken to support the exemption dossier submitted to the EFSA (EFSA, 2007a). In the first study, 30 individuals (18–57 y, 13 males) with a history of exquisite peanut food allergy, confirmed by double-blind placebo-controlled challenge, were recruited at each of two participating clinics (University clinics in Berlin and Utrecht) experienced in conducting double-blind placebo-controlled food challenges. They consumed increasing doses of soybean (12, 24 and 48 ml) or placebo oil mixed in a mashed potato vehicle (up to 400 g in total) up to a dose representing the worst-case intake for a single eating occasion (84 ml).

In the second study, 32 individuals (12–62 y, 10 males) with demonstrated soy allergy, confirmed by challenge, were recruited at each of three participating clinics (University clinics in Berlin, Utrecht and Zurich). All patients were challenged under the same conditions and using the same doses as in the first study.

Clinical data on 32 subjects with clinically confirmed soybean allergy who were fed phytosterols by open challenge are also relevant to the safety of soybean oil, given that the starting material for phytosterol production is a by-product (distillate from the deodorization stage) of soybean oil refining (and other oils where appropriate). Twenty-nine subjects tolerated the challenge and three subjects experienced mild symptoms. The three subjects underwent DBPCFC, and two had no reactions while one subject had mild OAS. IgE-binding studies to phytosterols for this one individual were negative (EFSA, 2007d; Ballmer-Weber, 2007).

EFSA Opinion:

> Based on the data submitted, EFSA (2007a, p. 2) concluded that "it is not very likely that N/RBD soybean oils will trigger a severe allergic reaction in susceptible individuals under the conditions of production and use stated by the applicant". The derivative is now exempt from required allergen labelling as specified in Regulation (EU) No 1169/2011.

Expert Consultation observations (regarding the suitability of the proposed flowchart and to the potential application of the RfD/30): Assuming this was a new submission and not equivalent to an already exempted product, exposures for the intended use will likely be below the soy RfD/30 acceptable exposure. For future applications, within the proposed flowchart, if process parameters and intended uses are equivalent, then safety can be substantiated; no further evaluation required.

3.2.4 PEANUT OIL

The derivative neutralized/refined bleached deodorized (N/RBD) peanut oil is the highly refined edible vegetable oil derived from peanut. The oil is first separated from crushed peanuts by solvent (hexane) extraction, followed by degumming, neutralization/refining (United States of America), bleaching and deodorization. The process results in very low residual levels of protein.

History of safe use: N/RBD peanut oil is a common edible vegetable oil, used both on its own for its culinary properties and taste and as part of edible vegetable oil blends. There is a long history of use at comparatively high levels, with few reports of allergic reactions attributed to N/RBD peanut oil.

Characterization: The process is fully described and standardized – please refer to the soybean oil process for further details. Adherence to appropriate Codes of Practice, such as that of FEDIOL (2020) should assure very low levels of residual peanut protein.

Available analytical data are equivocal. In a review on edible oil allergenicity, Crevel *et al.* (2000) found a range of values from 6.47 to 220 mg/kg reported for crude peanut oil, while highly refined peanut oil concentrations ranged from < 0.0003mg/kg to 48 mg/kg, depending on the method and extraction process used. Thus, confirmation of true values may be needed.

Clinical studies: Two studies have been reported using commercially available N/RBD peanut oils, as well as crude peanut oil. In the first, Taylor *et al.* (1981) administered up to 8 ml of encapsulated peanut oil in a DBPCFC to ten volunteers. None reacted. The volunteers were also skin prick-tested with crude peanut oil and were positive, as were their radioallergosorbent test (RAST) results.

In the second study, Hourihane *et al.* (1997) administered up to 16 ml of highly refined and crude peanut oil using a DBPCFC protocol to 58 individuals with challenge-confirmed peanut allergy. None reacted to the refined peanut oil, but six did to the crude peanut oil. This result confirmed the results of the earlier smaller study on ten peanut-allergic individuals of which none reacted (Taylor *et al.*, 1981).

Exposure: More complete exposure assessment is needed, based on appropriate analytical methods, for example, the Rigby *et al.* (2011) method, established in soybean oil.

Exposure assessment with additional calculations by the Expert Committee shows peanut protein exposures of less than 100 µg in a worst-case scenario. Further details can be found in Annex 2 (exposure estimated details).

EFSA Opinion:

> Clinical studies had previously confirmed that the very low levels of protein in highly refined oils do not cause reactions in oral challenges in soybean (Taylor *et al.*, 2004) and peanut allergic subjects (Taylor *et al.*, 1981; Hourihane *et al.*, 1997). The European Food Safety Authority concluded for peanut oil that the data submitted by the applicant were insufficient to predict the likelihood of reactions in peanut-allergic individuals. They considered that more clinical information was required with regard to the effects of highly refined peanut oil on individuals with a severe peanut allergy, which would need to be based on further clinical studies (EFSA, 2004a).

Expert Consultation observations (regarding the suitability of the proposed flowchart and to the potential application of the RfD/30): In the United States of America, all highly refined oils, including peanut oil are exempt from required allergen labelling (FALCPA, 2004). While the United States Congress and the USFDA have not defined "highly refined", it is generally recognized as an oil that has undergone refining, bleaching and deodorizing (RBD) processing. Exposures for the intended use of peanut oil could be in the range of or above the peanut RfD/30 acceptable exposure. Still, there is a long history of use at comparatively high levels for N/RBD peanut oil, and this observation provides further information that the RfD/30 provides a practical, useable and measurable safe margin of exposure for assessing suitability for allergen labelling exemptions.

3.2.5 SOY LECITHIN

The derivative Soy lecithin is derived from soybean by separation from the solvent-extracted oil fraction at the degumming stage.

History of (safe) use: Soy lecithin has a long history of use in many categories of products at comparatively high or low levels, including processing aid use. A few isolated case reports of allergic reactions have been attributed to ingestion of soy lecithin. In the first of these case reports, a 3-year old male suffered intestinal symptoms including diarrhea and emesis on exposure to foods containing soy lecithin (Renaud, Cardiet, and Dupont, 1997). A double-blind, placebo-controlled food challenge (DBPCFC) with an unknown dose of soy lecithin elicited diarrhea in the child. The lecithin used in the challenge was reported to have a protein content of 4.31 g/100 g as determined by catharometric analysis, but this protein level seems far too high and possibly erroneous. The catharometric analysis method is not referenced and is not a known method for protein analysis of lecithin. In the second case report, the DBPCFC with 100 mg of lecithin was positive, with the appearance of an erythematous rash on the jaw 1 h after ingestion in a 15-month-old female. Protein assay of soy lecithin by the Kjeldahl method revealed a level of 3.5 percent (35 000 ppm); however, this assay method is inappropriate for use on soy lecithin because it does not distinguish between protein and phospholipid nitrogen content (Palm *et al.*, 1999). In a more recent case report, a 9-year-old female suffered a fixed food eruption that was reproducible on oral challenge with 0.5 g soy lecithin that caused lip and chin swelling (de-Andrés-del-Rosario, 2022).

Characterization: Variable process between manufacturers also affects protein residue levels. An analytical survey of the protein content of multiple lots of commercial soy lecithins is needed to characterize the range of protein concentrations. From the analysis of a limited number of commercial samples of various types of soy lecithin, protein levels can range from 163 mg/kg to 1 338 mg/kg (Martín-Hernández, Bénet and Marvin-Guy, 2005). The presence of IgE-binding soy proteins in commercial soy lecithins has been identified in several publications (Awazuhara *et al.*, 1998; Müller *et al.*, 1998; Gu *et al.*, 2001; Martín-Hernández, Bénet and Marvin-Guy, 2005; see above), but insufficient data are available to characterize

the full range of concentrations. Protein levels are difficult to quantify in the lecithin matrix as the choline moiety tends to interfere with protein assay methods. To some extent, the protein content of soy lecithins can be partially characterized by hexane-insoluble solids content as protein residues would be expected to be found in the hexane insoluble solids fraction.

Clinical studies: No clinical studies have been performed to support exemption.

Intended use: There are many uses, with the highest exposure associated with uses as an emulsifier and in food supplements. Only one use of soy lecithin has been granted exemption. This was for release agent use for specific soy lecithins and was granted for two petitions (FALP 003 and 004), based on exposure assessment by the USFDA (2013, 2017).

Exposure: The FALP 003 petitioner estimated an exposure < 100 µg soy protein (< 0.1 mg soy protein) per serving while the FALP 004 petitioner provided worst-case conservative exposures per eating occasion of 0.04–0.17 mg (P90), 0.05–0.231 mg (P95), 0.07–0.333 mg (P99) and 0.07–0.866 mg (maximum) hexane insoluble material, the material from which soy lecithin is derived. Further details can be found in Annex 2 (estimated exposure details).

USFDA assessment outcome: The FDA considered these soy lecithins, when used as release agents, to present a "negligible risk to soy allergic individuals" (USFDA, 2013, 2017).

Expert Consultation observations (regarding the suitability of the proposed flowchart and to the potential application of the RfD/30): Assuming this was a new submission and not equivalent to an already exempted product, exposures for the intended use will likely be below the soy RfD/30 acceptable exposure with only a few maximum consumption scenarios reaching above the RfD/30. For future applications, within the proposed flowchart, if process parameters and intended uses are equivalent, then safety can be substantiated; no further evaluation required. Different soy lecithins and/or other uses would require evaluation of exposure in relation to thresholds of reactivity.

3.2.6 WHEY ETHANOL

Ethanol can be derived from the whey fraction of milk after fermentation with suitable yeast strains capable of the conversion of lactose to ethanol, followed by distillation. Typical distillates made from whey include gin, genever, pastis, ouzo, anis, aquavit, vodka, jagertee, advocaat, slivovice and similar spirit drinks (EFSA, 2007c).

History of (safe) use: There is a long history of use of ethanol and ethanol-based beverages made from whey. There have been no reported cases of allergic reactions to whey-derived ethanol, documented in the EFSA Opinion by a comprehensive literature search (up to 2006).

Characterization: Production of the distillate starts with the separation of the whey from cheese by ultrafiltration, followed by fermentation of the resulting permeate

with yeast, followed by distillation. The distillation process should remove protein residues and other non-volatiles, if properly conducted, and data cited in the EFSA Opinion confirms this for model distillation using pure protein solutions, as well as for neutral alcohol distillates produced by different companies and sites.

Intended use: Whey distillate is currently used as a solvent as well as the basis of a number of alcoholic beverages. New applications are unlikely to differ and will therefore be equivalent to the established history of safe use and not require further studies.

Exposure: In the dossier examined by the EFSA, proteins, peptides and lactose are not carried over into the distillate during a properly controlled distillation process (based on LoD of 0.5 mg/L for total protein using the Bradford Analysis Microassay). This was also confirmed using a commercial ELISA for the whey protein β-lactoglobulin (LoD 0.5 mg/L) in alcohol distillates, although the EFSA noted that there was no evidence that denatured β-lactoglobulin was detectable. In the FSANZ document, analytical data confirm that distilled alcohol from whey and wheat produced under proper controls contains no detectable protein (i.e. < 1 mg/kg), and an EFSA Opinion came to similar conclusions for residual proteins in nut distillates.

These analytical estimates lead to a range of exposure estimates (0.3–248 µg protein) for different product categories (e.g. flavour carrier, pure alcohol, spirits, alcoholic drinks – alcohol above or below 15 percent) and different usage levels of distillates in final consumed products. Further details for exposure estimates by FSANZ, the EFSA and additional calculations by the Expert Committee can be found in Annex 2 (exposure estimated details).

EFSA Opinion:

> Based on the data submitted by the applicant, the Panel notes that proteins, peptides and lactose are not carried over into the distillate during a properly controlled distillation process, at least not above 0.5 mg/L for proteins and 0.04 mg/L for lactose. The Panel considers that distillates made from whey are unlikely to trigger a severe allergic reaction in susceptible individuals (EFSA, 2007c, p. 1).

The derivative is now exempt from required allergen labelling as specified in Regulation (EU) No 1169/2011.

Expert Consultation observations (regarding the suitability of the proposed flowchart and to the potential application of the RfD/30): Exposures for the intended use could be in the range of or above the milk RfD/10 or RfD/30. Additionally, similar exposure ranges can be estimated for alcohol distillates from wheat, hazelnut and other nuts (see Annex 2 for exposure estimated details). Still, even with these potential exposure levels, there is a long history of use of ethanol and ethanol-based beverages made from whey, nuts and cereals, and this observation provides further information that the RfD/30 provides a practical, useable and measurable safe margin of exposure for assessing suitability for allergen labelling exemptions.

3.2.7 FISH GELATINE

The derivative is fish gelatine for use as a formulation aid (carrier) in vitamin and carotenoid preparations (EFSA, 2007b). It is derived from fish, but the raw material is primarily fish skins and bones, with some fish muscle likely adherent to skins.

History of (safe) use: The derivative has a long history of use as an encapsulating agent for vitamin A and carotenoids, as well as other substances. Gelatine is made by denaturing collagen. Use of fish skins and bones likely lessens exposure to known fish allergens, but fish collagen itself is reported as an allergenic protein in some fish-allergic patients in Japan (Kobayashi *et al.*, 2016). Levels of parvalbumin, the main fish allergen, can be lowered by extensive washing (Koppelman *et al.*, 2012), but it is not known whether all manufacturers use this process step or, if they do, the extent to which it is standardized.

Characterization: The starting material can vary in terms of the species of fish from which it is sourced and their proportions. The production process is possibly variable. More information is needed on both starting material and process. One supplier, washes the product extensively to reduce parvalbumin levels, which are the primary allergenic concern. Fish gelatine is 100 percent protein from fish; therefore, there always remains a theoretical residual allergenicity from collagen.

Intended use: Fish gelatine has many uses. The conclusions outlined here only apply to fish gelatine for use as a formulation aid (carrier) in vitamin and carotenoid preparations. Any proposed use for similar purposes and involving similar exposure could be exempted after consideration of possible cumulative exposure from these uses. Uses which are not equivalent to those would require further evaluation.

Clinical studies: A fish gelatine challenge study was conducted on 30 codfish-allergic patients. None reacted up to a cumulative dose of 3.6 g of fish gelatine (extensively washed fish gelatine was used). There was one subjective reactor at a cumulative dose of 7.61 g, giving a no observed adverse effect level (NOAEL) of 3.3 g (Hansen *et al.*, 2004).

Exposure: The EFSA Opinion (EFSA, 2007, p. 1) on fish gelatine reports that data provided in the dossier reviewed indicated that:

> daily fish gelatine intake from vitamin preparations intended for use in food supplements, colourings and beverages is in the low milligram range. Estimation of the highest concentration of fish gelatine in vitamin-containing preparations available on the market, indicates a concentration of 30mg per litre, or 7.5mg per 250 ml serving. Assuming a parvalbumin content in gelatine of 0.04µg/g, the estimated intake of parvalbumin with one serving will be 0.0003µg.

Limit of detection for cod parvalbumin in fish gelatine in the ELISA used was 0.04 µg/g. Calculations performed by the Expert Committee estimated that an exposure to 0.0003 µg parvalbumin would equate to an exposure to 0.048 µg of total fish protein per serving (carrier in vitamin, EFSA, 2007b dossier). For exposure levels up to 1 g gelatine (EFSA, 2004c), assuming a parvalbumin content in gelatine of 0.04 µg/g and a parvalbumin content in muscle tissue of 6.25 mg/g, an exposure up to 6.4 µg

of total fish protein could be expected. Further details can be found in Annex 2 (estimated exposure details).

EFSA Opinion (EFSA, 2007, p. 1):

> Taking into account the information available, the Panel considers that it is unlikely that fish gelatine used as a formulation aid (carrier) in vitamin and carotenoid preparations will trigger an adverse allergic reaction in susceptible individuals under the conditions of production and use specified by the applicant.

The derivative is now exempt from required allergen labelling as specified in Regulation (EU) No 1169/2011.

Expert Consultation observations (regarding the suitability of the proposed flowchart and to the potential application of the RfD/30): Assuming this was a new submission and not equivalent to an already exempted product, exposures for the intended use will likely be below the fish RfD/30 acceptable exposure. For future applications, within the proposed flowchart, if process parameters and intended uses are equivalent, then exposures are likely to be below the RfD/30 acceptable exposure, depending on analytical results.

3.2.8 ICE-STRUCTURING PROTEIN (ISP) PREPARATION

Ice-structuring protein (ISP) is a so-called antifreeze protein present in the blood of certain fish living in very cold, deep-sea waters that would otherwise freeze most aqueous systems. The ISP derivative is a nature-identical protein first identified in ocean pout (*Macrozoarces americanus*), a species of fish related to eels. The priority allergenic origin of the protein is, therefore, fish. As the protein is produced by fermentation of the yeast *Saccharomyces cerevisiae* in which the ISP gene has been inserted, the preparation does not contain any other protein from fish.

Fish are known to contain several proteins with allergenic activity. A muscle protein, parvalbumin, is the major allergen present in many fish species and responsible for allergic cross reactivity among most fish species for susceptible consumers. Other known allergens from fish include enolase, triose phosphate isomerase, and collagen. Ice-structuring protein (ISP) bears no sequence homology or biological relationship to any of these known fish allergens.

History of safe use: Ocean pout has been fished in an area of the Northeast coast of the United States of America and consumed locally. Limited information exists on the history of its use owing to the small size of the fishery. However, a literature search revealed no known reactions to ocean pout or to any antifreeze protein (previous designation of ISP) or fish ISP. Saccharomyces is food grade, and yeast proteins are not considered priority allergens.

Characterization: The functional attribute of ISP (modifying ice crystal formation) is dependent on the integrity of its structure, starting with its primary sequence. Ice-structuring protein (ISP) preparation, which contains the active ISP, has a detailed specification, supported by reproducibility data over several batches and contains 4.8–6.2g/L of ISP, representing 26.2–36.6 percent of the total Kjeldahl protein. The balance of proteins includes the inactive, glycosylated form of ISP, as well as common yeast proteins resulting from metabolic activity during culture.

Intended use: The intended use of ISP preparation is in frozen desserts, edible ices (EFSA [EFSA, 2008] and EU Union authorization as a novel food [EC, 2000]) and other frozen products levels not exceeding 0.01 percent by weight; exposure can occur from multiple food categories.

As the use of ISP preparation is novel, equivalence to an existing product cannot be demonstrated.

Exposure: An exposure assessment is therefore required, and further details can be found in Annex 2 (exposure estimated details). These exposure estimates (up to 40 mg ISP per eating occasion) indicate that ISP would be expected to be above an acceptable intake as discussed in Section 2.4.

Clinical studies: Clinical studies were deemed to be required and were performed. No evidence of IgE-binding was observed in fish-allergic patients by skin prick testing and immunoblotting, demonstrating that ISP was not an allergenic protein of fish. Furthermore, feeding studies showed that development of sensitization in human volunteers did not take place over eight weeks of daily exposure to the determined acceptable daily intake, followed by four weeks with no further exposure.

EFSA assessment outcome (EFSA, 2008, p. 2):

> No adverse reactions were reported in countries where the ISP is authorised. Human studies were performed and the ISP preparation did not provoke a skin prick test reaction in, or bind IgE from, individuals allergic to fish. On the basis of these results the risk of an allergenic reaction in fish-allergic individuals or the population at large is very unlikely.
>
> From 2003 to 2007 more than 470 million ISP-containing edible ice products have been sold in the USA and 47 thousand litres of ISP containing ice cream has been sold in Australia/New Zealand. There have been no reported safety issues.
>
> With regard to the potential of adverse allergic reactions against yeast allergens, the Panel considers it is unlikely that such reactions would occur after ingestion of the ISP-containing products.
>
> The Panel concludes that the use of the ISP type III HPLC 12 preparation at a maximum level equivalent to 0.01 % ISP type III HPLC 12 in edible ices is safe subject to adherence to the specification and production practices described by the applicant.

European Commission decision authorizing ISP on the market as a novel food: "The designation of the novel food ingredient authorized by this Decision on the labelling of the foodstuff containing it shall be 'Ice Structuring Protein'" (European Commission, 2009, Article 2).

Expert Consultation observations (regarding the suitability of the proposed flowchart and to the potential application of the RfD/30): Assuming this was a new submission and not equivalent to an already exempted product, based on protein levels alone, exposures for the intended use will be above the fish RfD/30 acceptable exposure, and more information will likely be needed regarding the allergenicity of the protein. Clinical studies could be needed to substantiate safety and establish exemption.

3.2.9 HYPOALLERGENIC INFANT FORMULA (EXTENSIVELY HYDROLYSED CASEIN [EHC])

The derivative extensively hydrolysed casein (EHC) has been the subject of two notifications (FALN 001 and 002) in the United States of America (USFDA, 2005a and 2005b).

History of (safe) use: Extensively hydrolysed casein (EHC) has a long history of use as the sole source of nutrition for infants who have a milk allergy and therefore cannot tolerate ordinary formula. Such formulae meet the standard for hypoallergenicity agreed by the American Academy of Pediatrics (AAP). This means that clinical studies have established with 95 percent confidence that 90 percent of infants with milk allergy will not react adversely to the product. There are, however, well-documented published reports of reactions, including anaphylaxis to EHC formula in milk-allergic infants, as would be expected, based on the hypoallergenicity standard.

Characterization: The starting material is the casein fraction of cow's milk, which is enzymatically hydrolysed, following which the enzyme used is inactivated, and the hydrolysate is filtered by diatomaceous earth filtration or microfiltration (USFDA, 2005b). Previously it was observed that most casein is expected to be completely hydrolyzed to amino acids, but 1.7 percent of the resulting peptides are reported to have molecular masses of 1 200–1 500 Daltons (Cordle *et al.*, 1991).

Intended use: It is the sole source of nutrition in infants with allergy to milk-based formulae.

Clinical studies: Clinical trials demonstrated safe administration of formula in 29 infants with milk allergy, who showed no reactions, thus the trials support the hypoallergenicity standard, although they are of insufficient statistical power on their own to confirm compliance.

Animal studies: FALN 001 presented data on reactivity to EHC in a rabbit model hyperimmunized with casein, showing considerable reduction in reactivity compared to intact casein. However, this is a model of effect on IgG response, not the IgE response observed in milk-allergic human beings. They also presented data showing attenuation of responses to casein in a guinea-pig model (USFDA, 2005a).

Animal studies by other researchers showed that, while EHC provoked smaller responses than intact casein, some immunogenic activity was retained. Some *in vivo* human data from skin prick tests (SPT) showed that EHC directly binds IgE (Sampson *et al.*, 1991; Oldaeus *et al.*, 1991). Also, history of use data showed that consumption of formula may cause objective reactions, some severe, in milk-allergic populations.

Clinical data: Case reports of anaphylactic reactions have occurred after consumption of such formula (Lifshitz *et al.*, 1988; Saylor and Bahna, 1991; De Boissieu, Matarazzo and Dupont, 1997; Ragno *et al.*, 1993). Because infant formula is the sole source of nutrition of infants, the exposure level is quite high. The hypoallergenic definition used by the American Academy of Pediatrics indicates a high probability that 90 percent of milk-allergic infants will tolerate casein hydrolysate formulae. However, the very definition implies that some milk-allergic infants will experience adverse reactions to ingestion of these formulae. Also, animal model data and other biological studies are not sufficient evidence of safety compared to human consumption data.

Exposure: 1.7 percent of casein peptides were present at the size range of 1 200–1 500 Daltons in 960 ml of daily formula consumption (estimate high mg amounts per meal consumption) according to applicants notifying FDA. Exposure assessment estimates that very high mg levels of casein/milk protein/peptides may be consumed (grams of protein), and further details can be found in Annex 2 (exposure estimated details).

USFDA assessment outcome: The USFDA objected to the notifications by both applicants on the grounds that they had not demonstrated evidence of absence of allergenic protein within the meaning of FALCPA, nor details of the analytical methods to demonstrate such absence. The agency noted that both applications also relied heavily on assertions that the hypoallergenicity standard was met, but emphasized that demonstrating hypoallergenicity in accordance with the AAP standard did not demonstrate absence of allergenic protein or that the derivative could not provoke allergic reactions harmful to human health within the meaning of FALCPA. Lack of characterization of the derivative and its source as well as incomplete specifications were also highlighted as important gaps in the data. Furthermore, the applications failed to discuss adverse reactions to EHC-based formulae described in the published literature, including some reactions to formulae made by the applicants, even though they were cited (USFDA, 2005a and 2005b).

Expert Consultation observations (regarding the suitability of the proposed flowchart and to the potential application of the RfD/30) assessment outcome: Based on protein levels alone, exposures for intended use (grams of protein) will be grossly above the milk RfD/30 acceptable exposure for intended use and more information would be needed regarding the allergenicity of the protein. Due to the large amount of protein present, it is likely that clinical studies will be needed if attempting to substantiate safety.

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CHAPTER 4
CONCLUSIONS

A pro forma process (i.e. a flowchart, Figure 1) has been developed and tested against allergen derivatives previously granted exemptions in various countries or regions and found to be effective for consideration of future exemption decisions.

After a succinct description of the derivative, including its source and composition (especially regarding protein from the allergenic source food), other key elements of the flowchart include the documentation of existing uses of the derivative, its safety and any reported adverse reactions, other compositional features, past exposure routes and amounts, and method of manufacture. The information should include a specification for the derivative. The intended uses of the derivative and predicted exposure, expressed in mg total protein from the allergenic source, resulting from these uses should also be included.

The proposal for the exemption should assess the equivalence of any new derivative and that of its uses to any existing ingredient(s) of a similar type from similar sources, taking into account species of origin, total protein content, other critical compositional features, safety and any reported adverse reactions, and methods of manufacture.

For total protein quantification, (Figure 1, Box 3), it is recommended to use more than one test method, each based on different principles, that are fit for purpose and may include total amino acid analysis as appropriate. Methods employing extraction should include assessments of recovery and precision. The choice of an appropriate calibrant is important, as well as using appropriate sampling and sample preparation procedures.

Assessments of potential alterations in the allergenicity of the protein(s) in the derivative (flowchart Figure 1, Box 8) can be established using a weight of evidence approach based on data from:

> allergen profiling assays (e.g. mass spectrometry or allergen molecule-specific assays). These approaches could provide additional information to show how the allergen profile has been modified by the process used to manufacture a derivative. Also, protein/peptide size distribution through size exclusion chromatography or mass spectrometry or a combination thereof to assess if larger peptide fragments (e.g. with 15 amino acids or more) exist may be used; and

> IgE-binding studies using sera from relevant food-allergic individuals with a clinically confirmed food allergy using appropriate methods such as IgE-immunoblotting, IgE immunoassay (including inhibition assays) and effector cell assays.

Clinical evaluation (flowchart Figure 1, Box 10), when necessary, may require an oral food challenge study. Oral food challenge study design and assessment criteria should be determined on a case-by-case basis in consultation with relevant parties.

Exposure assessment is an essential component of the safety assessment process.

Inputs needed for the exposure assessment are:

> intended use levels of the derivative for relevant food product categories;

> consumption values for intended food product categories and relevant consumer groups on a per eating occasion basis; and

> analytical data or calculated equivalent of concentration of total protein or total protein from the priority allergenic source.

The above inputs are combined into an estimation/calculation of exposure amounts, and if applicable, of exposures from a combination of multiple food categories consumed on a single eating occasion.

Existing dossiers and recommendations have typically estimated exposures using:

> food consumption data based on the 90th, 95th or 97.5th percentile of consumers (a p90, p95 or p97.5 quantity of a single eating occasion), which may vary regionally; and

> maximum levels of intended uses of the derivative(s).

Protein concentrations have typically been presented as ranges. Estimation/calculation of exposure amounts are typically presented using either the mean or maximum concentrations. This may vary depending on the applicant or the regulatory body doing the assessment.

The Expert Committee concluded that:

> for the current accepted exemptions, there is an established history of safe consumption;

> the exposure estimates in reasonable worst-case consumption scenarios, based on the scientific data considered for the exemptions approved to date (in the European Union, Australia and New Zealand [ANZ], and the United States of America), lead to values around the relevant reference doses (RfD) established by the second meeting[10] divided by 30 (RfD/30). Consequently, the RfD/30 appears to provide an adequate margin of exposure (MoE) for derivative safety assessment;

[10] Discussions to develop this framework also considered a number of historical soy-related case studies. The "RfD" for soy used during discussions of soy-related exemptions in Meeting 4 was estimated (based on the principles elaborated at Meeting 2) and subsequentially confirmed during an additional fifth meeting (FAO and WHO, 2023b).

- suitable methods of analysis are available for protein levels based on the RfD/30; and
- a derivative that undergoes the weight of evidence risk assessment as outlined in this report and meets the threshold criterion (RfD/30) may not require clinical studies to establish safety.

Based on these conclusions, the Expert Committee recommends that the process outlined in the flowchart (Figure 1) may be used as a guide for future development and evaluation of derivative exemptions. Establishment of safety based upon this weight of evidence approach is dependent upon consideration of data quality, outcome of the exposure assessment for all intended ingredient uses (specified for exemption), and review by competent authorities (as needed). When safety is established, exemption can be justified.

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ANNEX 1
OBSERVATION OF MANDATORY ALLERGEN LABELLING EXEMPTIONS

A number of Codex member countries have already established lists of foods and ingredients derived from priority allergens that are exempted from allergen labelling. These were collated into Table A1.1 by the committee for further consideration.

TABLE A1.1 **PREVIOUSLY ESTABLISHED LISTS OF EXEMPTIONS FROM ALLERGEN LABELLING**

ALLERGEN	AUSTRALIA & NEW ZEALAND	EUROPEAN UNION	UNITED KINGDOM OF GREAT BRITAIN AND NORTHERN IRELAND	UNITED STATES OF AMERICA	CANADA	ARGENTINA	PARAGUAY	CHILE	MEXICO	CHINA	JAPAN
CEREALS CONTAINING GLUTEN	Beer and spirits	Glucose syrups based on barley; cereals (where) used for making distillates or ethyl alcohol of agricultural origins for spirit drinks and other alcoholic beverages			The source of the glucose syrup doesn't have to be identified (no residual protein).	Glucose syrups based on barley; cereals (where) used for making distillates or ethyl alcohol of agricultural origins for spirit drinks and other alcoholic beverages	Glucose syrups based on barley; cereals (where) used for making distillates or ethyl alcohol of agricultural origins for spirit drinks and other alcoholic beverages	Glucose syrups based on barley	No exemption	Starch and dextrin	No exemption
WHEAT	Glucose syrups made from wheat starch that: (1) (i) have been subject to a refining process that has removed gluten protein content to the lowest level that is reasonably achievable; and (ii) have a gluten protein content that does not exceed 20 mg/kg; or (2) alcohol distilled from wheat	Wheat-based glucose syrups including dextrose; wheat-based maltodextrins			The source of the glucose syrup doesn't have to be identified (no residual protein).	Wheat-based glucose syrups including dextrose; wheat-based maltodextrins	Wheat-based glucose syrups including dextrose; wheat-based maltodextrins	Wheat-based glucose syrups including dextrose; wheat-based maltodextrins	No exemption	Starch and dextrin	No exemption
FISH	Isinglass derived from swim bladders and used as a clarifying agent in beer and wine Unilever recombinantly expressed ice-structuring protein (ISP) (Not classed as a fish protein in ANZ when produced from yeast). Fish allergen labelling does not apply (FSANZ, 2005).	Fish gelatine used as carrier for vitamin or carotenoid preparations Fish gelatine or isinglass used as fining agent in beer and wine Unilever recombinantly expressed ice-structuring protein (ISP). (Authorized as a novel food ingredient and designated on the label of food products as "Ice Structuring Protein"). Fish allergen labelling does not apply (EU, 2009).		Unilever recombinately expressed ice-structuring protein (USFDA, 2003)		Isinglass derived from swim bladders and used as a clarifying agent in beer and wine	Isinglass derived from swim bladders and used as a clarifying agent in beer and wine	Fish gelatine used as carrier for vitamin or carotenoid preparations	No exemption		No exemption

TABLE A1.1 **PREVIOUSLY ESTABLISHED LISTS OF EXEMPTIONS FROM ALLERGEN LABELLING** (continued)

ALLERGEN	AUSTRALIA & NEW ZEALAND	EUROPEAN UNION	UNITED KINGDOM OF GREAT BRITAIN AND NORTHERN IRELAND	UNITED STATES OF AMERICA	CANADA	ARGENTINA	PARAGUAY	CHILE	MEXICO	CHINA	JAPAN
CRUSTACEANS & MOLLUSCS						No exemption	No exemption	No exemption	No exemption		No exemption
TREE NUTS	Coconut	Nuts used for making distillates or ethyl alcohol of agricultural origin for spirit drinks and other alcoholic beverages				Nuts used for making distillates or ethyl alcohol of agricultural origin for spirit drinks and other alcoholic beverages	Nuts used for making distillates or ethyl alcohol of agricultural origin for spirit drinks and other alcoholic beverages	No exemption	No exemption		No exemption
MILK	Alcohol distilled from whey	Whey (where) used for making distillates or ethyl alcohol of agricultural origin for spirit drinks and other alcoholic beverages; Lactitol				Whey (where) used for making distillates or ethyl alcohol of agricultural origin for spirit drinks and other alcoholic beverages; Lactitol	Whey (where) used for making distillates or ethyl alcohol of agricultural origin for spirit drinks and other alcoholic beverages; Lactitol	Lactitol	No exemption		No exemption
SOY	Oil that has been degummed, neutralized, bleached and deodorized; tocopherols or phytosterols	Fully refined soybean oil and fat; natural mixed tocopherols (E306), natural D-alpha tocopherol, natural D-alpha tocopherol acetate, and natural D-alpha tocopherol succinate from soybean source: vegetable oil derived phytosterols and phytosterol esters from soybean sources; plant stanol ester produced from vegetable oil sterols from soybean sources		All highly refined oils, including soy oil (U.S. Congress and the USFDA have not defined “highly refined”); however, it is recognized as an oil that has undergone refining, bleaching and deodorizing (RBD) processing. Specific soy lecithin products are exempt but only when these lecithin products are applied as release agents to food contact surfaces (USFDA, 2013, 2017).	All highly refined (i.e. degummed, neutralized, bleached and deodorized) oils derived from food allergen sources - except peanut oil - are exempt.	Fully refined soybean oil and fat; natural mixed tocopherols (E306), natural D-alpha tocopherol, natural D-alpha tocopherol acetate, natural D-alpha tocopherol succinate from soybean source: vegetable oil derived phytosterols and phytosterol esters from soybean sources; plant stanol ester produced from vegetable oil sterols from soybean sources	Fully refined soybean oil and fat; natural mixed tocopherols (E306), natural D-alpha tocopherol, natural D-alpha tocopherol acetate, natural D-alpha tocopherol succinate from soybean source: vegetable oil derived phytosterols and phytosterol esters from soybean sources; plant stanol ester produced from vegetable oil sterols from soybean sources	Fully refined soybean oil and fat; natural mixed tocopherols (E306), natural D-alpha tocopherol, natural D-alpha tocopherol acetate, natural D-alpha tocopherol succinate from soybean source: vegetable oil derived phytosterols and phytosterol esters from soybean sources; plant stanol ester produced from vegetable oil sterols from soybean sources	Soybean oil (not specified “refined” or “highly refined”)	Soybean oil (refined) phospholipid	No exemption

TABLE A1.1 **PREVIOUSLY ESTABLISHED LISTS OF EXEMPTIONS FROM ALLERGEN LABELLING** (continued)

ALLERGEN	AUSTRALIA & NEW ZEALAND	EUROPEAN UNION	UNITED KINGDOM OF GREAT BRITAIN AND NORTHERN IRELAND	UNITED STATES OF AMERICA	CANADA	ARGENTINA	PARAGUAY	CHILE	MEXICO	CHINA	JAPAN
PEANUT				All highly refined oils, including peanut oil (the United States Congress and the USFDA have not defined "highly refined"); however, it is recognized as an oil that has undergone refining, bleaching and deodorizing (RBD) processing.	Highly refined peanut oil not exempt	No exemption	No exemption	No exemption	No exemption	Peanut oil (refined)	
SULPHITES	Naturally occurring sulphites; added sulphites < 10 mg/kg	< 10 mg/kg		< 10 mg/kg	Naturally occurring sulphites; added sulphites < 10 mg/kg	< 10 mg/kg	< 10 mg/kg	< 10 mg/kg	< 10 mg/kg		

REFERENCES IN ANNEX 1

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ANNEX 2
EXPOSURE ESTIMATES FOR CURRENT EXEMPTIONS OR NOTIFICATIONS

This Annex provides exposure estimate details for the case studies presented in Section 3.2 as well as additional exposure estimates for exemption dossiers of interest (Table A2.1).

Full list of exposure estimates detailed in Annex 2, and clarification if the exposure assessment is also part of case study presented in section 3.2.

TABLE A2.1 **FULL LIST OF EXPOSURE ESTIMATES**

CASE STUDIES IN SECTION 3.2	ADDITIONAL EXPOSURE ESTIMATES PROVIDED IN THIS ANNEX
- Glucose syrups (wheat) - Soy phytosterols/tocopherols - Soybean oil - Peanut oil - Soy lecithin - Whey ethanol - Fish gelatine - Ice structuring protein (ISP) preparation - Hypoallergenic infant formula (extensively hydrolysed casein [EHC])	- Wheat-based maltodextrins - Alcohol distillates from cereals, nuts and whey - Isinglass used as a clarifying agent in wines and beers - Lactitol

A2.1 GLUCOSE SYRUP DERIVED FROM WHEAT STARCH (FSANZ AND EFSA)

FOOD STANDARDS AUSTRALIA NEW ZEALAND (FSANZ) ASSESSMENT

Based on the Food Standards Australia New Zealand (FSANZ) document (P1031-AppR-SD1) (FSANZ, 2016), the main use of glucose syrup in Australia and New Zealand is in ice-cream, confectionery and in chocolate filling.

There is no direct exposure assessment provided in the document P1031-APPR-SD1; however, assessments were done regarding the amount of product needed to be consumed under different usage levels to reach an exposure amount of 1 mg (1 000 µg) wheat protein. These consumption requirements were compared to reported consumption survey data from Australia and New Zealand.

The report concludes that reducing gluten in all glucose syrup samples to as low as technically and practically feasible would ensure that the dietary exposure of most consumers does not exceed 1 mg (1 000 µg) of wheat protein in one single meal.

The following was stated in the document P1031-APPR-SD1 (FSANZ, 2016, p. 24):

> Ice-cream is consumed in larger quantities than either chocolate or confectionery in both Australia and New Zealand with little difference between children and adults: the estimated 97.5th percentile amount for consumers of ice-cream was 165 g/day for Australian children aged 2-4 years, 276g/day for Australian children aged 5-14 years and 348 g/day for New Zealand children aged 5-14 years. For populations aged 15 years and over the estimated 97.5th percentile amount for consumers of ice-cream was 348 g/day in Australia and 305g/day in New Zealand.
>
> The amount of chocolate (bars of chocolate and filled chocolates) estimated to be consumed by high consumers (97.5th percentile) was 100 g/day for Australian children aged 2-4 years, 183g/day for Australian children aged 5-14 years and 100g/day for New Zealand children aged 5-14 years. For populations aged 15 years and over the estimated 97.5th percentile amount for consumers of chocolate was 190g/day in Australia and 180g/day in New Zealand.
>
> For confectionery, the patterns of consumption were different for the Australian and New Zealand populations, with approximately double the amount of confectionery being consumed in New Zealand compared to Australia for children and adults, however the proportion of consumers of these products was similar. The amount of confectionery estimated to be consumed by high consumers (97.5th percentile) was 52 g/day for Australian children aged 2-4 years, 100g/day for Australian children aged 5-14 years and 232g/day for New Zealand children aged 5-14 years. For populations aged 15 years and over the estimated 97.5th percentile amount for consumers of confectionery was 125g/day in Australia and 240g/day in New Zealand.
>
> For ice cream, chocolates and confectionery containing glucose syrup with 10 mg/kg gluten all populations have estimated food consumption amounts lower than the maximum amount of food that can be consumed before the threshold level of 1 mg wheat protein is reached, the one possible exception being New Zealand children aged 5-14 years if it is assumed confectionery has 50% glucose syrup, which is not the case for most of these products (p. 26).
>
> Analytical data from Australian produced glucose syrup shows that in samples taken from daily batch testing over 10 months, 90% of syrups contained less than 10 mg/kg gluten and the remaining 10% were below 20 mg/kg. Minimising gluten in all glucose syrup samples to as low as technically and practically achievable,

> would ensure that dietary exposure for **most**[11] consumers does not exceed 1 mg (1000 µg) of wheat protein in a single meal. The risk assessment concluded that based on the available evidence, consumption of wheat-derived glucose syrup that had been purified and prepared as described in Appendix 2 would present negligible risk to the majority of wheat allergic individuals; such syrups would also be suitable for those with coeliac disease (p. ii).

Of note, document P1031-APPR-SD1 finds that the dietary exposure for **most** consumers does not exceed 1 mg (1 000 µg) of wheat protein in a single meal, but review of the document does find that there are multiple categories where exposures greater than 1 mg (1 000 µg) could be predicted. Additional calculations done by the Expert Committee for this report find wheat protein exposures in multiple food and age combinations that are estimated near or above the RfD/10 (500 µg wheat protein) for 97.5th percentile consumptions for all levels of gluten (10, 15 or 20 mg/kg) and near or above the RfD/30 (167 µg wheat protein) for the mean consumption levels for all levels of gluten (Table A2.2).

EFSA ASSESSMENT

In Europe, the EFSA reported in 2007 that starches from wheat were not found to contain any detected gluten at levels higher than 25.3 mg/kg in glucose syrups and dextrose (starch hydrolysate) for 2005 and 2006 samples (EFSA, 2007m). One glucose syrup sample had a gluten content of 39.6 mg/kg, but this was assumed to be through accidental contamination. In another survey, the EFSA reported that of 21 European samples (14 wheat glucose syrups, 3 crystalline dextrose, 4 glucose syrups) which had undergone a comprehensive purification scheme, the total protein concentration measured by high-pressure liquid chromatography ranged from only 0.3–1.4 mg/kg (EFSA, 2007m). After the 2007 EFSA assessment, Dostálek *et al.* (2009) reported residual gluten content to be < 3 mg/kg in all syrup samples tested (n=9) in Europe.

The exposure assessment in the EFSA opinion was as follows (EFSA, 2007m, p. 3):

> A new study analysing dietary exposure to gluten from wheat starch hydrolysates has been conducted by TNO Nutrition and Food Research and provided by the applicant. Main sources of exposure were soft drinks, dairy desserts, yoghurt drinks, candy and canned food, soups and savoury sauces. This study was designed to collect data from The Netherlands, Italy and Ireland (representative sample of Dutch population including children, Italian students living in the district of Rome, Irish adults aged 18-64 years) based on food consumption data from these countries and on gluten content in glucose syrups and dextrose from wheat starch hydrolysates of 10-20 mg/kg (mass spectrometry). According to the applicant, exposure to gluten from glucose syrups and dextrose was less than 3.5 mg per day for 95% of the adult Dutch men [3500 µg gluten per day]. All other population subgroups had lower exposure.

[11] Expert Committee emphasis.

TABLE A2.2 **SELECT PRODUCT USAGE LEVELS, AGE GROUP CONSUMPTION DATA AND GLUTEN CONTENT IN TOTAL WHEAT PROTEIN (75 PERCENT) ASSUMPTION FROM DOCUMENT P1031-APPR-SD1. EXPOSURE ESTIMATE GENERATED BY EXPERT COMMITTEE**

FOOD CATEGORY	COUNTRY/AGE GROUP	CONSUMPTION VALUE	GRAMS/DAY	GLUTEN mg/kg	INCLUSION RATE	WHEAT PROTEIN EXPOSURE ESTIMATE (µg – micrograms)
ICE CREAM	Australia 5–14 years	97.5th percentile consumption	276	10–20	10%	368–736
ICE CREAM	Australia 15 years and above	97.5th percentile consumption	348	10–20	10%	464–928
CHOCOLATE	New Zealand 5–14 years	97.5th percentile consumption	100	10–20	30%	400–800
CHOCOLATE	New Zealand 15 years and above	97.5th percentile consumption	180	10–20	30%	720–1440
CONFECTIONARY	Australia 15 years and above	97.5th percentile consumption	125	10–20	30%	500–1000
CONFECTIONARY	New Zealand 5–14 years	97.5th percentile consumption	232	10–20	30%	928–1856
CONFECTIONARY	New Zealand 14 years and above	97.5th percentile consumption	240	10–20	30%	960–1920
ICE CREAM	Australia 5–14 years & Australia 15 years and above	Mean	113	10–20	10%	151–301
CHOCOLATE	New Zealand 5–14 years	Mean	35	10–20	30%	140–280
CHOCOLATE	Australia 5–14 years	Mean	40	10–20	30%	160–320
CONFECTIONARY	Australia 15 years and above	Mean	30	10–20	30%	120–240
CONFECTIONARY	New Zealand 14 years and above	Mean	45	10–20	30%	180–360

These values are before any potential corrections for assumptions regarding gluten content in total wheat protein, which would lead to an estimated exposure of total wheat protein greater than 3 500 µg per day.

Finally, as stated by EFSA (2007m, p. 6):

> Taking into account all the scientific information provided and in particular the levels of wheat proteins reported in glucose syrups including dextrose, the Panel considers that it is not very likely that this product will trigger a severe allergic reaction in susceptible individuals.

Wheat protein exposures are predicted above the RfD/10 (500 µg wheat protein) in the EFSA assessment (EFSA, 2007m).

A2.2 WHEAT-BASED MALTODEXTRINS

As stated by the EFSA in the 2007 Opinion regarding the permanent exemption of wheat-based maltodextrins from required allergen labelling, the exposure assessment was detailed as follows (EFSA, 2007l, p. 3):

> A new study analysing dietary exposure to gluten from wheat starch hydrolysates has been conducted by TNO Nutrition and Food Research and provided by the applicant. Main sources of exposure were soft drinks, dairy desserts, yoghurt drinks, candy and canned food, soups and savoury sauces. This study was designed to collect data from The Netherlands, Italy and Ireland (representative sample of Dutch population including children, Italian students living in the district of Rome, Irish adults aged 18-64 years) based on food consumption data from these countries and on gluten content in maltodextrins from wheat starch hydrolysates of 20-40 mg per kg (mass spectrometry). According to the applicant, exposure to gluten from maltodextrin was less than 1 mg per day for 95% of the adult Dutch men [1000 µg gluten per day]. All other population subgroups had lower exposure.

Finally, as stated by the EFSA (2007l, p. 6):

> Taking into account the scientific information provided and in particular the levels of wheat proteins reported in wheat-based maltodextrins, the Panel considers that it is not very likely that this product will trigger a severe allergic reaction in susceptible individuals.

Wheat protein exposures are predicted above the RfD/10 (500 µg wheat protein) in the EFSA assessment (EFSA, 2007l).

A2.3 PHYTOSTEROLS/PHYTOSTEROL ESTERS, TOCOPHEROLS/TOCOPHEROL ESTERS AND PLANT STANOL ESTERS

As stated by the EFSA in the 2007 Opinion regarding the permanent exemption of tocopherols from soybean sources from required allergen labelling (EFSA, 2007e, p. 4):

> The applicant states dietary supplements found on the European market limit the maximum recommended additional daily intake to 830 mg (ERNA, 2003). This would result in 41 µg soy protein when taking into account 50 mg/kg residual protein in the tocopherol fraction.
>
> The applicant states that mixed tocopherols are used as a food antioxidant in concentrations of about 50 mg/kg (referring to the fat fraction of the specific food). Assuming a fat intake of 60 - 80g/day, this could result in a dose of 3 (- 4 mg) tocopherols per day, which corresponds to 0.03 µg of protein (based on 10 µg/g residual protein in tocopherol). This amount of protein, likely to be spread over three meals a day, is considerably below levels at which clinical allergic reactions have been reported (NDA, 2004).

> The applicant also states that D-alpha tocopherol-succinates are used as food supplements, assuming additional daily doses of up to 830 mg/day as recommended by food supplement producers would result in a dose of 8.3 µg protein (based on the 10mg/kg residual protein in the tocopherol fraction as a worst-case assumption).
>
> Considering the information provided by the applicant regarding the starting material, the subsequent production process, and the demonstration of low residual protein content, the Panel considers that it is unlikely that natural mixed tocopherol/D-alpha tocopherols from soybean sources will trigger a severe allergic reaction in susceptible individuals (p. 7).

As stated by the EFSA in the 2007 Opinion regarding the permanent exemption of vegetable oil derived phytosterols and phytosterol esters from soybean sources from required allergen labelling (EFSA, 2007d, p. 4):

> Phytosterols and phytosterol esters may be added to selected foods to help reduce intestinal cholesterol absorption and as a consequence lower blood low-density lipoprotein cholesterol. The EU regulations limit exposure to a maximum of 3 grams per day of phytosterols through labelling requirements and maximum concentrations in certain food categories in order to avoid intakes above the recommended limits from multiple sources of intake (Commission Regulation 608/2004/EC).
>
> The in vitro analytical data (described in Section 3.1) demonstrates 1 - 10 µg/g of detectable residual soy proteins in phytosterols. Taking this into account, a daily intake of 3 grams of phytosterols would be equivalent to 3 - 30 µg of soy protein. This amount of protein is below levels at which clinical allergic reactions have been reported (NDA 2004). Consumption of phytosterols from multiple sources may result in a higher intake. Further, the Panel notes the uncertainty with regard to the lowest allergen dose triggering a clinical reaction.
>
> Considering the information provided by the applicant regarding the starting material, the subsequent production process, and the demonstration of low residual protein content, the Panel considers that it is unlikely that vegetable oils derived phytosterols and phytosterol esters from soybean sources will trigger a severe allergic reaction in susceptible individuals (p. 7).

As stated by the EFSA in the 2007 Opinion regarding the permanent exemption of plant stanol esters produced from soybean oil sterols from required allergen labelling (EFSA, 2007f, p. 3):

> Plant stanols are present in some functional foods and added to products such as margarines, spreads and salad dressings. The EU regulations limit exposure to a maximum of 3 grams per day of plant sterols through labelling requirements and maximum concentrations in certain food categories in order to avoid intakes above the recommended limits from multiple sources of intake (Commission Regulation 608/2004/EC). The applicant's estimate daily intake of stanols from commercial products is 2-2.3g plant stanols (equivalent to about 3.2-3.7g of plant stanol esters).

> The applicant provides new analytical data and results of a clinical study. The analytical study involved analysis of the amino acids obtained after hydrolysis and further processing of the sample. The content of individual amino acids in the soy-based sterol and stanol samples analysed were below the limit of detection (1mg/kg). In the clinical study with 33 participants, no participant reported immediate symptoms following the double-blind placebo-controlled food challenge with plant stanol esters (p. 1).
>
> Taking into account the information provided regarding the starting material and the production process, the Panel considers that it is unlikely that plant stanol ester produced from soybean oil sterols will trigger a severe allergic reaction in soy allergic individuals under the conditions of use stated by the applicant (p. 1).

No further exposure estimates were done in the dossier for plant stanol esters (EFSA, 2007f).

No exposure estimates were done for "Phytosterols/phytosterol esters and Tocopherols/tocopherol esters" in the FSANZ document P1031-APPR-SD1 (FSANZ, 2016).

Exposures up to 41 µg soybean protein in phytosterols/phytosterol esters and tocopherols/tocopherol esters dossiers (EFSA, 2007d, e) would be less than the RfD/30 (333 µg soybean protein) or the RfD/50 (200 µg soybean protein) and are in the range of the RfD/250 (40 µg soybean protein).

A2.4 SOYBEAN OIL

As stated by the EFSA in the 2007 Opinion regarding the permanent exemption of edible neutralized (alkali refined) bleached and deodorized (N/RBD) soybean oils from required allergen labelling (EFSA, 2007a, p. 4):

> The main four applications for soybean oil are margarine, salad dressing, mayonnaise, and frying oil. The applicant states that there is no standardised serving size for food products in the EU. The applicant has used several sources such as US Food and Drug Administration reference amount and manufacturer's information to determine the potential exposure of soybean protein in the main four food products. The applicant states that the average serving size in Europe is as follows:
>
> - Margarine: 10g (8g of NRBD soybean oil)
> - Salad dressing: 15ml (15ml of NRBD soybean oil)
> - French fries: 200g (40g of NRBD soybean oil)
> - Mayonnaise: 25ml (17.5ml of NRBD soybean oil)
>
> The applicant assumes a mean protein concentration of 150µg/kg for the N/RBD oil (calculated concentration of protein in N/RBD oil used for the clinical studies, section 6). The calculation of the soy protein dose ingested is based

> on the maximum possible fat content in each food product and with this fat made exclusively from N/RBD soybean oil. Considering this and assuming a mean protein concentration of a meal consisting of the four food items above will lead to a total intake of about 12.1µg soy protein.

Building on the EFSA exposure assessment, FSANZ (2016) utilized the 97.5th percentile consumptions, a mean concentration of 150µg/kg and an upper estimate of 500 µg/kg soybean protein in their exposure assessments. These values led to an estimated range of exposures of 10.5–41.0 µg soy protein.

Additional information available to the Expert Committee for this report found levels up to 700 µg/kg soybean protein in N/RBD oils (Rigby *et al.*, 2011). Recalculation of the FSANZ assessment with this new upper level would estimate a range of exposures of 10.5–57.4 µg soy protein.

Exposure to soybean protein in N/RBD would be less than the RfD/30 (333 µg soybean protein) or the RfD/50 (200 µg soybean protein) and are in the range of the RfD/175 (57 µg soybean protein) to the RfD/950 (10.5 µg soybean protein).

A2.5 PEANUT OIL

In a 2004 notification to the Directorate General for Health and Consumer Protection of the European Commission (DG SANCO) regarding the potential temporary exemption of edible neutralized (alkali refined) bleached and deodorized (N/RBD) peanut oils from required allergen labelling, the applicant performed the exposure assessment as follows (FEDIOL and IMACE, personal communication, 2004):

> The calculation of the peanut protein dose ingested in a portion represents the worst-case scenario for each food product. It is based on the maximum possible fat content in each application and this fat will be made exclusively from N/RBD peanut oil. The maximum level of residual protein in the N/RBD peanut oil used in this calculation is 0.4 µg/g. This is based on the highest level of protein reported in refined soybean oil in the most recent analytical studies (p. 25).
>
> [There were no measurements of protein in peanut oil within the notification].[12]
>
> Due to its high cost, fully refined peanut oil is typically used in specific applications in food preparation e.g. frying oil, and not utilised as extensively as e.g. soybean oil, which is described in the Notification for the Temporary Labelling Exemption of Fully Refined Soybean Oil and Fat. Thus, estimating level of exposure based on a meal consisting of food products that may contain peanut oil - as done for soybean oil - may not present a realistic picture to the actual practice (p. 26).

[12] Expert Committee insertion.

Estimates per food were given as follows:

- Potato chips: 40 g (12 g of N/RBD peanut oil) = 4.8 µg peanut protein
- French fries: 200 g (40 g of N/RBD peanut oil) = 16 µg peanut protein
- Salad dressing: 15 ml (15 ml of N/RBD peanut oil) = 6 µg peanut protein

These lead to an estimated range of exposure of 5–17 µg peanut protein for individual foods and 11–22 µg peanut protein (peanut oil, 0.4 µg/g concentration) in combined meal of chips or fries with salad dressing as part of the meal (and in extreme 27 µg if multiple potato products eaten) (combined exposure calculated by the Expert Committee).

Additional information available to the Expert Committee for this report (personal communication) found mean levels of 0.8 mg/kg peanut protein in N/RBD oils and up to 1.8 mg/kg peanut protein in N/RBD oils. Recalculation of the applicant's assessment with this new upper level would estimate a range of exposures of 10–32 µg peanut protein for individual foods and 22–42 µg peanut protein (peanut oil, 0.8 µg/g concentration) in a combined meal of chips or fries with salad dressing as part of the meal, of 22–72 µg peanut protein for individual foods, and 49–99 µg peanut protein (peanut oil, 1.8 µg/g concentration) in a combined meal of chips or fries with salad dressing as part of the meal.

Exposure to peanut protein in N/RBD would be less than the RfD/10 (200 µg peanut protein) and depending on the scenario, in the range of the RfD/30 (67 µg peanut protein) and the RfD/50 (20 µg peanut protein).

A2.6 SOY LECITHIN

Solae soybean lecithin as a release agent (USA food allergen labelling petition [FALP] 003) was estimated by the petitioner to have an exposure < 100 µg soy protein per serving (as hexane insoluble matter [HI]), with an overall maximum daily exposure < 3mg/day (USFDA, 2013).

In their response letter, the USFDA (2013, p. 2) stated:

> FDA then evaluated the estimated levels of exposure to soy protein that would result from consumption of the food products that typically use the petitioner's soy lecithin products. These exposure estimates used the information provided in the petition on the estimated levels of usage of the soy lecithin products for the specified applications, as well as information on consumption levels for the food products described in the petition. Finally, FDA compared the estimated exposure to soy protein from the petitioner's soy lecithin products to the assessment dose level that FDA calculated to evaluate whether a particular exposure to the petitioner's soy lecithin would cause an allergic response that poses a risk to human health. FDA did not consider an exposure below the assessment dose level to cause an allergic response that poses a risk to human health.

ADM soybean lecithin as a release agent (USA FALP 004) was estimated by the petitioner to have an exposure < 231 µg soybean protein (as HI) per serving (Table 4, 12–18 years, others, p95 consumption), and the petition stated that, "In all age groups and at all eating occasions, the 99th percentiles of the HI intakes are <0.334 mg and the maximal intakes are <1 mg per eating occasion" (USFDA, 2017, p. 6).

Of note, the petition (USFDA, 2017, p. 3) states that:

> In this estimation, we used a 15% lecithin content release agent formula as the highest lecithin content noted in a commercial release product. Thus, the highest possible quantity of soy protein intake would be obtained from the lecithin in foods that contacted the release agents. A formulation of 15% lecithin once used in small or semi-automated bakeries and applied by hand (brush), are no longer common industry practices or available for home use. To maintain the function as an effective release agent, each formula is optimized for special applications with the majority of commercial formulas containing 1-3% lecithin.

The exposure estimates up to 0.334 mg or 1 mg per eating occasion use a lecithin content of 15 percent and are conservative, and likely an overestimate by a factor of five.

In their response letter and Finding of No Significant Impact (FONSI), the USFDA (2017, p. 2) stated:

> No new uses of lecithin are authorized as a result of the requested labeling exemption; soy is already being used in industrial food processing as a release agent on food-contact surfaces. The effect of this action would be to exempt industry from the requirement to label products processed with soy lecithin release agents as containing soy. Based on information contained in the FALP, FDA has determined soy lecithin, when used as release agents, presents negligible risk to soy allergic individuals.

If an RfD for soybean was to be set following the principles in Report 2 (FAO and WHO 2022), an RfD of 10 mg soybean protein could be expected. While it is accepted that use of HI as an assumption for soy protein is an overestimate, the exact amount of protein remaining in the HI material in the petitions is not known. As such, exposure to soybean protein in soy lecithin used as a release agent, as described in the submitted petitions, would be expected to be less than the RfD/30 (333 µg soybean protein) and possibly than the RfD/50 (200 µg soybean protein), but this is not known for certain.

A2.7 ALCOHOL DISTILLATES

As stated by FSANZ (2016) in the document P1031-APPR-SD1:

> Distilled alcohol derived from cereals and from whey is commonly used in alcoholic beverages and for use as a solvent in the formulation of flavours and other food ingredients. Distilled alcohol may be further processed to produce vinegar (p. 28).

> Alcohol distilled from wheat and whey is produced in Australia and New Zealand for use in alcoholic beverages and flavour carriers. There is general scientific agreement that non-volatile substances such as sugars (e.g. lactose from whey) and proteins, are unlikely to be found in the distillate. Reported analytical data, confirm that distilled alcohol from whey and wheat produced under proper controls, contain no detectable protein (i.e. <1 mg/kg). The data also confirmed the absence of detectable whey proteins in vinegar derived from whey alcohol (p. 30).

As stated by the EFSA in the 2007 Opinion regarding the permanent exemption of nuts used in distillates for spirits from required allergen labelling (EFSA, 2007g, p. 1):

> The applicant provided information regarding the addition of almonds, almond oils, and nuts to an alcohol distillation process where they act as natural flavouring agents of the final alcoholic distillate, supplementing information submitted to obtain temporary exemption.
>
> Based on the data submitted by the applicant, the Panel notes that proteins and peptides are not carried over into the distillate during a properly controlled distillation process, at least not in amounts above 1 mg/L. Although the analytical evidence is derived from experiments that were performed predominantly with almonds, the Panel considers that distillates made from nuts are unlikely to trigger a severe allergic reaction in susceptible individuals.

As stated by the EFSA in the 2007 Opinion regarding the permanent exemption of whey used in distillates for spirits from required allergen labelling (EFSA, 2007h, p. 1):

> Distillates made from whey include gin, genever, pastis, ouzo, anis, aquavit, vodka, jagertee, advocaat, slivovice and similar spirit drinks.
>
> Based on the data submitted by the applicant, the Panel notes that proteins, peptides and lactose are not carried over into the distillate during a properly controlled distillation process, at least not above 0.5 mg/L for proteins and 0.04 mg/L for lactose. The Panel considers that distillates made from whey are unlikely to trigger a severe allergic reaction in susceptible individuals.

As stated by the EFSA in the 2007 Opinion regarding the permanent exemption of cereals used in distillates for spirits from required allergen labelling (EFSA, 2007i, p. 1):

> The applicant provided further information regarding distillates made from cereals which include whisky, Kornbrand, gin, vodka and “made wine” produced using vodka, liqueur and similar beverages.
>
> Based on the data submitted by the applicant, the Panel notes that proteins and peptides are not carried over into the distillate during a properly controlled distillation process, at least not in amounts higher than 1 mg/L for total proteins and 0.4 mg/kg for gluten. The Panel considers that distillates made from cereals are unlikely to trigger a severe allergic reaction in susceptible individuals.

No formal exposure estimates for fish protein of concern were done in these three dossiers (three EFSA files + FSANZ P1031-APPR-SD1 again).

TABLE A2.3 **SELECT PRODUCT USAGE LEVELS, AGE GROUP CONSUMPTION DATA AND PROTEIN CONTENT IN ALCOHOL DISTILLATES. EXPOSURE ESTIMATE GENERATED BY EXPERT COMMITTEE**

FOOD CATEGORY	COUNTRY/AGE GROUP	CONSUMPTION VALUE	GRAMS/ EATING OCCASION	mg/kg	INCLUSION RATE (% alcohol/ volume)	PROTEIN EXPOSURE ESTIMATE (μg – micrograms)
PURE ALCOHOL	N/A	Typical[a]	30	0.5–1	100%	15–30
			60	0.5–1	100%	30–60
			90	0.5–1	100%	45–90
			120	0.5–1	100%	60–120
			150	0.5–1	100%	75–150
SPIRITS	N/A	Typical[a]	30	0.5–1	40%	6–12
			60	0.5–1	40%	12–24
			90	0.5–1	40%	18–36
			120	0.5–1	40%	24–48
			150	0.5–1	40%	30–60
ALCOHOLIC DRINKS, ALCOHOL ABOVE 15%	United States of America[b]	97.5th percentile consumption	990	0.5–1	15%–25%	74–248
	Netherlands (Kingdom of the)[c]	97.5th percentile consumption	329.1	0.5–1	15%–25%	25–82
	European Union – combined Birot[d]	90th percentile consumption	120	0.5–1	15%–25%	9–30
ALCOHOLIC DRINKS, ALCOHOL BELOW 15%	United States of America[b]	97.5th percentile consumption	947.2	0.5–1	5%–15%	24–142
	Netherlands (Kingdom of the)[c]	97.5th percentile consumption	657.3	0.5–1	5%–15%	16–99
	European Union – combined Birot[d]	90th percentile consumption	420	0.5–1	5%–15%	11–63
FLAVOUR CARRIER	N/A	Generic[a]	100	0.5–1	0.5%–5%	0.3–5
			250	0.5–1	0.5%–5%	0.6–13
			500	0.5–1	0.5%–5%	1–25
			1 000	0.5–1	0.5%–5%	3–50

Notes:

[a] The Expert Committee used a combination of "generic" consumption amounts and recipe formulations for pure alcohol distillates, spirits and flavour carriers for a broad overview of potential, conservative exposure estimates.

[b] While the United States of America has not exempted alcohol distillates from required allergen labelling, consumption estimates were used here due to their availability on a per eating occasion basis in a comparison of USA and Dutch consumption habits (Meima *et al.*, 2021). The USA data is in line with European data from the EFSA Comprehensive European Food Consumption Database (available on an acute grams per day consumption basis). For example, the 97.5th percentile consumption of "Cocktail drink" by adults ranged from 500–1 384 grams per day in data from five EU Member States; the 97.5th percentile consumption of "Spirits" by adults ranged from 120–700 grams per day in data from 11 EU Member States; and the 97.5th percentile consumption of "Vodka and vodka-like spirits" by adults ranged from 183–1 000 grams per day in data from ten EU Member States.

[c] Meima *et al.*, 2021.

[d] Birot *et al.*, 2018.

Additional calculations done by the Expert Committee for this report utilized concentrations of 0.5–1 mg/kg for total proteins in alcohol distillates and flavour carriers. As no formal exposure assessment estimates had been previously done, the Expert Committee used a combination of "generic" consumption amounts and recipe formulations for pure alcohol distillates and flavour carriers, as well as consumption data from population surveys in available peer-reviewed literature (Table YY).

Protein exposures in multiple food categories were estimated as follows:

- Wheat: near or above the RfD/30 (167 µg wheat protein), and near or above the RfD/50 (100 µg wheat protein)
- Hazelnut: near the RfD/10 (300 µg hazelnut protein), near or above the RfD/30 (100 µg hazelnut protein), and near or above the RfD/50 (60 µg hazelnut protein)
- Milk: near or above the RfD/10 (200 µg milk protein), near or above the RfD/30 (67 µg milk protein), and near or above the RfD/50 (40 µg milk protein)
- Other nuts (walnut, pecan, cashew, pistachio, almond): near or above the RfD/10 (100 µg other nut protein), near or above the RfD/30 (33 g other protein), and near or above the RfD/50 (20 µg other nut protein)

A2.8 FISH GELATINE AS A CARRIER FOR VITAMIN OR CAROTENOID PREPARATIONS

As stated by the EFSA in the 2007 Opinion regarding the permanent exemption of fish gelatine for use as a formulation aid (carrier) in vitamin and carotenoid preparations from required allergen labelling (EFSA, 2007c, p. 1):

> Allergens of concern are residual amounts of parvalbumin, and gelatine itself. The information provided by the applicant indicates that the production process of gelatine from fish skins [primarily cod, pollock and haddock][13] for this particular purpose is well standardized. A monoclonal [anti-carp][14] and a polyclonal [anti-cod][15] ELISA assay for measuring parvalbumin in fish gelatine have been developed, with a limit of detection of 1µg/g and 0.04µg/g, respectively. None of the assays detected parvalbumin in ten commercial lots of gelatine [tested with both ELSIA tests].[16]According to the applicant, daily fish gelatine intake from vitamin preparations intended for use in food supplements, colourings and beverages is in the low milligram range. Estimation of the highest concentration of fish gelatine in vitamin containing preparations available on the market, indicates a concentration of 30mg per litre, or 7.5mg per 250ml serving. Assuming a parvalbumin content in gelatine of 0.04µg/g, the estimated intake of parvalbumin with one serving will be 0.0003µg.

[13] Expert Committee addition to text in block quote.
[14] Expert Committee addition to text in block quote.
[15] Expert Committee addition to text in block quote.
[16] Expert Committee addition to text in block quote.

> Taking into account the information available, the Panel considers that it is unlikely that fish gelatine used as a formulation aid (carrier) in vitamin and carotenoid preparations will trigger an adverse allergic reaction in susceptible individuals under the conditions of production and use specified by the applicant.

Fish gelatine was provisionally exempt from required allergen labelling when "used as carrier for vitamins and flavours" (European Commission, 2005, p. 2), but the final adoption of the permanent exemption changed to, "fish gelatine used as carrier for vitamin or carotenoid preparation (European Union, 2011, p. 43)." As stated by the EFSA in the 2004 Opinion regarding the permanent exemption of fish gelatine used as carrier for flavour from required allergen labelling (EFSA, 2004, p. 1):

> The major allergen of fish is the muscle protein parvalbumin. Gelatine is made by denaturation of collagen. Fish gelatine is used in foods and pharmaceuticals, and the present application concerns use of fish gelatine for a flavour encapsulation carrier system.
>
> Gelatine for the present application is produced from cold and warm water fish skins. No analytical data regarding possible residual levels of the major fish allergen parvalbumin in the fish gelatine preparation are provided. Typical levels of fish gelatine in industrially processed foods are indicated to be up to around 1000 mg/kg. Levels of intake of fish gelatine under the conditions of use specified by the applicant are likely to be ranging from tens to hundreds of mg per day.
>
> The applicant provides the following indicative values for calculated ranges of fish gelatine in the flavoured foods (mg/kg) in commercial practice: processed vegetables 15-20, dry soups 8-194, extruded snacks 40-731, sauces 7-120, biscuits and cakes 14-971, chewing gum 864-1356, marinade 950-1000, and fats and margarines 57-1122. Based on these concentrations, exposure levels are expected to be up to 1 g per day (p. 3).
>
> The scientific data provided by the applicant are insufficient to predict the likelihood of adverse reactions in fish allergic individuals. Nevertheless, taking all the information into account the Panel considers that it is not very likely that fish gelatine, under the conditions of use specified by the applicant, will cause a severe allergic reaction in the majority of fish allergic individuals (p. 1).

The exposures in the two EFSA dossiers were expressed in units of gelatine or parvalbumin. Koppelman *et al.* (2012) found a parvalbumin content in cod muscle tissue of 6.25 mg/g or 0.625 percent.

Calculations performed by the Expert Committee estimated that an exposure to 0.0003 µg parvalbumin would equate to an exposure to 0.048 µg of total fish protein per serving (carrier in vitamin) (EFSA, 2007c dossier). For exposure levels up to 1 g gelatine (EFSA, 2004), assuming a parvalbumin content in gelatine of 0.04 µg/g and a parvalbumin content in muscle tissue of 6.25 mg/g, an exposure up to 6.4 µg of total fish protein could be expected.

Fish protein exposures from fish gelatine as a vitamin encapsulating agent and as a flavour carrier are both predicted to be below the RfD/100 (50 µg fish protein) in the EFSA assessment and in the range of the RfD/750 and the RfD/100 000.

A2.9 ICE-STRUCTURING PROTEIN (ISP)

As summarized in the European Food Safety Authority (EFSA) opinion regarding the safety of "Ice Structuring Protein" (ISP):

> Average daily ice cream intakes for consumers only have also been estimated for the Netherlands using the Dutch National Food Consumption Survey (DNFCS - 3, 1997-98). Using these data it is adults who have the highest potential ice cream intake of 100 g/day at the 95th percentile. If all this ice cream were to contain ISP at the maximum proposed level of 0.01 % by weight this would equate to 10 mg ISP/day (EFSA, 2008, p. 10).

Additional information regarding usage levels states, "ISP is proposed to be used in products at levels not exceeding 0.01 % by weight and more commonly less than 0.005%" (EFSA, 2008, p. 9)

In their 2002 ISP GRAS Notification filing to the United States Food and Drug Administration (US FDA GRN No. 117), Unilever stated mean intake levels frozen novelty desserts to be 58 g/eating occasion and 164 g/eating occasion at the 90th percentile of intake (USFDA, 2003). These intakes at levels not exceeding 0.01 percent by weight and more commonly less than 0.005 percent would equate to 3–6 mg ISP/eating occasion and 8–16 mg ISP/eating occasion.

Newer consumption data reports an ice cream intake of 150 g/eating occasion at the 90th percentile (Birot *et al.*, 2018) across the Kingdom of the Netherlands, Denmark and France and an ice cream intake in the 97.5th percentile ranging from 203–400 g/eating occasion in the United States of America and the Kingdom of the Netherlands (Meima *et al.*, 2021), Australia and New Zealand (FSANZ, 2016). Using 0.005–0.01 percent ISP usage levels, these ice cream intakes would equate to ISP exposures of 7.5–15 mg ISP per eating occasion and 10–40 mg ISP per eating occasion.

Ice structuring protein (ISP) is a purified protein, derived from fish and produced in yeast. The estimated exposure level to ISP is greater than the RfD for fish (5 mg total fish protein). Based on exposure alone, ISP would not pass the flowchart (fail at box 7 and box 9 –> box 10). More information will likely be needed regarding the allergenicity (or lack thereof) of the protein. Clinical studies could be needed to substantiate safety and establish exemption.

A2.10 ISINGLASS USED AS A CLARIFYING AGENT

WINES

As stated by the EFSA in the 2007 Opinion regarding the permanent exemption of fish products (isinglass) from Winemakers' Federation of Australia (WFA) and the Australian Wine Research Institute (AWRI) used in the manufacture of wine from required allergen labelling (EFSA, 2007j, p. 4):

> Different batches of wine need different amounts of isinglass. According to the applicant, bench trials are performed and wine samples tested in the laboratory to determine an optimal amount of isinglass to be added to the specific production batch of wine. Typical usage is indicated by the applicant to be 10-25 mg/L of isinglass in white wine. Isinglass is assumed to be less often used with red and rosé wines. Of the 23 isinglass fined wines used in laboratory studies reported in the current application, the lowest amount added was 0.1 mg/L, the average amount was about 18 mg/L, one wine had added about 25 mg/L, three wines 50 mg/L, and one wine nearly 120 mg/L. Isinglass is added usually after fermentation is complete, is often used in conjunction with bentonite which aids settling of the isinglass-phenolic compound complex, and is removed by sedimentation and filtration.
>
> According to the applicant, there are no published reports available on the concentration of isinglass in finished wine, nor are there published assays for measurement of its concentration in wine. A laboratory study of two white wines is reported by the applicant, based on the partial purification of collagen from the test samples followed by SDS-PAGE technique. A similar technique had been used for beer, where concentrations of collagen as low as 0.02 mg/L had been detected, according to the applicant. The wine samples in question had been fined with 0.42 and 4.4 mg/L of isinglass. No collagen bands were detected in the two wine samples after sedimentation and filtration, whereas collagen residues could be recovered at a "spiked" concentration of 1.0 mg/L, which indicates a limit of detection for the assay of less than 1.0 mg/L. The applicant concludes that the concentration of residual isinglass in the wines tested was less than 1 mg/L. The Panel notes the limitations inherent in this approach.
>
> The applicant states that the average wine consumption is 79 g/day, and for wine consumers the highest consumption rate is 312 g/day for 45-64 year old males, based on a national nutrition survey of foods eaten by Australians (Australian Bureau of Statistics, 1999). However, intake during a single occasion rather than average daily dose is the relevant dose in relation to food allergic reactions. No information has been found about this dose distribution, which generally would range from one small glass (125 ml) and upwards. If an isinglass content of 1 mg/L is assumed (cf. above), one 125-ml glass of wine would give an intake of 0.125 mg isinglass, and one bottle of wine (750 ml) an intake of 0.750 mg isinglass.
>
> The data submitted do not allow the Panel to assess the likelihood that isinglass used as fining agent in wine will trigger an allergic adverse reaction in susceptible individuals under the conditions of use stated by the applicant (p. 6).

As stated by another EFSA 2007 Opinion regarding the permanent exemption of fish gelatine or isinglass from Deutscher Weinbauverband (DWV) and the Office National Interprofessionnel des Fruits, des Légumes, des Vins et de l'Horticulture (VINIFLHOR) used as fining agents in wine from required allergen labelling (EFSA, 2007k, p. 4):

> Isinglass is first of all used for clarification of white wines, most commonly with a dosage between 10 to 25 mg/L, and for rosé wines, but also for some red wines, then at typical doses from 30 to 50 mg/L. However, the dose of isinglass used may vary ten-fold or more depending on the desired properties of the wine.
>
> Two potentially quantitative immunochemical tests, a competitive ELISA and a sandwich ELISA, were developed. However, there were matrix effects that caused problems with the competitive assay used for the five German wines. Therefore the larger part of the analytical work was performed with a sandwich ELISA using French wines. The sandwich ELISA also exhibited accuracy problems when used with wine samples, so that the assay could not be used to measure quantitatively the amount of fining agent residues. Instead, the sandwich ELISA was used as a qualitative test to determine the presence of isinglass, with a threshold for positivity corresponding to average absorbance of the unfined control wines plus two standard deviations. The Panel expresses concerns with this methodology. Among 400 commercial French wines, most of them with unknown fining agents, 17 tested positive in the sandwich ELISA. Whether the wine was red, rosé or white was not predictive of isinglass presence. The highest percentage of positive tests was found among the organic wines, some of which are known not to be filtered after the fining operation. Of 28 wines fined with isinglass, two tested positive for the fining agent. In the light of these findings, the Panel finds it difficult to understand the statement made by the applicant in the introductory part of the application that "no residuals were detected" (p. 5).
>
> The data submitted do not allow the Panel to assess the likelihood that isinglass used as fining agent in wine will trigger an adverse reaction in susceptible individuals under the conditions of use stated by the applicant (p. 8).

For exposure estimates, residual isinglass in wine was considered less than 1 mg/L, and one 125 ml glass of wine would give an intake of 0.125 mg isinglass. One bottle of wine (750 ml) would estimate an intake of 0.750 mg isinglass (EFSA, 2007j). However, the limitations in assuming residual isinglass concentrations of less than 1.0 mg/L were noted (EFSA, 2007j), and the Expert Committee also performed exposure estimations with the highest usage level of isinglass noted in the dossier, 120 mg/L, which estimates a very conservative intake up to 90 mg isinglass.

Assuming a (worst case scenario) parvalbumin content in isinglass[17] of 0.5 µg/g, fish protein exposures could be estimated up to 0.72 µg fish protein in one bottle of wine containing 120 mg/L isinglass (see Table A2.4).

[17] Koppelman *et al.* (2012) (REF) found a parvalbumin content in cod muscle tissue of 6.25 mg/g or 0.625 percent, while the skins contained 0.4 mg/g. Washing of the skins, a common industrial procedure during the manufacturing of fish gelatine, reduced the level of parvalbumin to 0.5 µg/g (ppm). From 95 commercial lots of fish gelatine (Koppelman *et al.*, 2012), 73 are below 0.02 ppm parvalbumin and from the other 22 lots, the one with the highest concentration contained 0.15 ppm of parvalbumin. It is noted that some fish species other than cod can be used to produce fish gelatine by manufacturers (such as haddock, pollock) without any major impact on reactivity (Regenstein *et al.*, 2010; Koppelman *et al.*, 2012).

Fish protein exposures in wine (up to 0.72 µg fish protein) which used isinglass as a fining or clarifying agent are predicted to be below the RfD/100 (50 µg fish protein) and in the range of the RfD/7 000.

TABLE A2.4 **SELECT PRODUCT USAGE LEVELS, AGE GROUP CONSUMPTION DATA AND PARVALBUMIN CONTENT IN TOTAL FISH PROTEIN (6.25 PERCENT) ASSUMPTION FROM KOPPELMAN *ET AL.* (2012). EXPOSURE ESTIMATE GENERATED BY EXPERT COMMITTEE FOR FISH PROTEIN IN WINE WHICH USED ISINGLASS AS A FINING AGENT**

FOOD CATEGORY	CONSUMPTION VALUE[a]	ISINGLASS INTAKE (mg)	PARVALBUMIN CONCENTRATION IN ISINGLASS (µg/g, mg/kg)	FISH PROTEIN EXPOSURE ESTIMATE (µg – micrograms)
WINE	1 glass (1 mg/L residual isinglass)	0.125	0.5[b]	0.001
	1 bottle (1 mg/L residual isinglass)	0.750	0.5	0.006
	1 bottle (50 mg/L isinglass usage, all remains in final product)	37.5	0.5	0.300
	1 bottle (120 mg/L isinglass usage, all remains in final product)	90	0.5	0.720

Notes:

[a] The Expert Committee used a combination of "generic" consumption amounts to represent wine consumption as done in the EFSA dossiers.

[b] 0.5 µg/g equates to 0.0005 µg/mg.

BEERS

As stated by the EFSA in the 2007 Opinion regarding the permanent exemption of isinglass from Brewers of Europe and Brewing Food and Beverage Industry Suppliers Association (BFBi) used as a clarifying agent in brewing from required allergen labelling (EFSA, 2007b, p. 5):

> Parvalbumin exposure must be calculated from isinglass residue data because the applicant was unable to measure parvalbumin directly in beer (see below). According to the applicant's calculations, the maximum concentration of parvalbumin in beer could range from 0.001 µg/L (bottle and can beer) to 0.005 µg/L (cask conditioned beer) based on measurement data with the new GMP isinglass. With the use of traditional commercial isinglass the estimate would be about ten-fold higher. Taking measurement uncertainties into account and making certain assumptions, e.g. that parvalbumin is not eluted from the isinglass into the beer, the highest parvalbumin concentration (new GMP code) derived for cask beer is 0.02 µg/L according to the applicant's estimates.
>
> However, the Panel notes the uncertainties about the accuracy of the measurements and assumptions regarding the parvalbumin concentration in beer, as well as the uncertainty with regard to the lowest dose of parvalbumin that can trigger an allergic reaction.

> Two new double-blind placebo-controlled food challenge studies with isinglass are reported, in which none of 21 fish allergic patients experienced any adverse effects (p. 1).
>
> Isinglass was given with mashed potatoes at cumulative doses of 50.5 mg over two hours (0.5 mg, 5 mg, 15 mg, 30 mg), corresponding to about 10 litres of cask conditioned beer or 50 litres of brewery conditioned beer. None of fifteen patients subjected to this challenge protocol in Denmark and Switzerland reacted to the challenges (p. 8).
>
> In a study in France employing a different protocol, a cumulative dose of 20 mg isinglass was given corresponding to the equivalent of four litres of beer. Inclusion criteria were "allergic patients" age 14 to 18 years but are not further specified in the dossier. None out of six patients challenged (2/6 skin test positive to fish) had a reaction (p. 8).
>
> On the basis of the data provided, the Panel considers that it is not very likely that isinglass used as clarifying agent in beer will trigger a severe allergic reaction in susceptible individuals under the conditions of production and use specified by the applicant (p. 8).

Using the highest estimated concentration of parvalbumin in beer (0.02 µg/L), additional calculations performed by the Expert Committee estimated an exposure up to 1.38 µg of total fish protein (Table A2.5) after consumption of more than 4 litres of beer.

TABLE A2.5 **SELECT PRODUCT USAGE LEVELS, AGE GROUP CONSUMPTION DATA AND PARVALBUMIN CONTENT IN TOTAL FISH PROTEIN (6.25 PERCENT) ASSUMPTION FROM KOPPELMAN *ET AL.* (2012). EXPOSURE ESTIMATE GENERATED BY EXPERT COMMITTEE FOR FISH PROTEIN IN BEER WHICH USED ISINGLASS AS A CLARIFYING AGENT**

FOOD CATEGORY	COUNTRY/AGE GROUP	CONSUMPTION VALUE	GRAMS/EATING OCCASION	Mg/kg[d]	PROTEIN EXPOSURE ESTIMATE (µg – micrograms)
BEER	United States of America[a]	97.5th percentile consumption	4 320	0.000001–0.00002	0.07–1.38
	Netherlands (Kingdom of the)[b]	97.5th percentile consumption	3 600	0.000001–0.00002	0.06–1.15
	European Union – combined Birot[c]	90th percentile consumption	990	0.000001–0.00002	0.02–0.32

Notes:

[a] While the United States of America has not exempted isinglass used as a clarifying agent from required allergen labelling, consumption estimates were used here due to their availability on a per eating occasion basis in a comparison of USA and Dutch consumption habits (Meima *et al.*, 2021). The USA data is in line with European data from the EFSA Comprehensive European Food Consumption Database (available on an acute grams per day consumption basis). For example, the 97.5th percentile consumption of "Beer, regular" by adults ranged from 990–6 248 grams per day in data from 17 EU Member States and the 97.5th percentile consumption of "Beer, strong" by adults ranged from 750–8 000 grams per day in data from eight EU Member States.

[b] Meima *et al.*, 2021.

[c] Birot *et al.*, 2018.

[d] 0.02 µg/L equates to 0.00002 mg/L or mg/kg.

Fish protein exposures in beers (up to 1.38 µg of total fish protein) which used isinglass as a fining or clarifying agent are predicted to be below the RfD/100 (50 µg fish protein) and in the range of the RfD/3 600.

A2.11 LACTITOL

Based on a 2007 EFSA opinion (EFSA, 2007l) regarding the permanent exemption from required allergen labelling for lactitol, lactitol is mainly used in solid food products such as cakes and biscuits but also in chewing gum. It also may be used in yoghurt. Assuming a lactose content in lactitol of less than 0.2 percent and a daily intake of lactitol of 10–20 g, the intake of lactose would be 0.02–0.04 g, which is lower than the dose of 10 g generally tolerated in people with lactose intolerance.

Regarding milk-allergic individuals, the source reports (EFSA, 2007l, p. 1):

> The applicant bases the evidence that lactitol preparations do not trigger cow's milk allergic reactions on analytical data regarding the residual content of the two major milk proteins in lactitol preparations (up to 3.2mg/kg for casein and 9.7mg/kg for β-lactoglobulin).
>
> The applicant assumes a daily intake of lactitol of 10g; higher daily intakes are possible from consumption of chocolate and cakes. An intake of 10g would lead to a combined maximum daily intake of 130µg casein and β-lactoglobulin. This may be an underestimation due to the decrease in detectability of the native proteins by the ELISA test due to thermal processing (p. 4).

Considering a daily intake of lactitol of 10–20 g, this would lead to a combined maximum daily intake of 130–260 µg casein and beta-lactoglobulin.

Additionally, while casein and beta-lactoglobulin constitute the majority of milk proteins, these exposure estimates are before any potential corrections for assumptions regarding their content in total milk protein. This correction would lead to an estimated exposure of total milk protein greater than 130–260 µg per day.

As stated by the EFSA (2007l, p. 6):

> taking into account the data submitted, the Panel considers that it is not very likely that lactitol will trigger adverse reactions in cow's milk allergic individuals under the conditions of use specified by the applicant.

These exposures are estimated near or above the RfD/10 (200 µg milk protein) and above the RfD/30 (67 µg milk protein).

A2.12 HYPOALLERGENIC INFANT FORMULA (EXTENSIVELY HYDROLYZED CASEIN [EHC])

Extensively hydrolyzed casein (EHC) in hypoallergenic infant formula is purified product, intended to provide the dietary/nutritional requirements of a growing infant/baby. The USFDA has objected to a number of notifications for EHC-related products which were attempting to be exempted from required food allergen labeling (USFDA 2005a, 2005b). According to their objection letters:

> [The notifications do not][18] contain scientific evidence (including the analytical method used) that demonstrates that EHC (as derived by the method specified in the notification) does not contain allergenic protein as required by section 403(w)(7) of the Act (USFDA, 2005a, p. 1, 2005b, p. 1).

In calculations done by the Expert Committee, the estimated exposure level to casein peptides (1.7 percent in EHC [USFDA 2005b]) in 960 mL of daily EHC consumption is 16.32 grams [16 320 mg, 1 6320 000 µg] of casein peptides per day. A 30 mL (roughly 1 fl oz) consumption would have an estimated exposure of 0.51 grams [510 mg, 510 000 µg] of casein peptides per 30 mL of hypoallergenic infant formula.

The estimated exposure level to casein peptides is in extreme excess of the RfD for milk (2 mg total milk protein). Based on exposure alone, EHC in hypoallergenic infant formula would not pass the flowchart (fail at 7b and 9 –> box 10), and clinical investigations would be required for considerations regarding exemption from required allergen labelling.

[18] Expert Committee clarification.

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ANNEX 3
COMPARISON OF THE EXPOSURE ESTIMATES

TABLE A3.1 **COMPARISON OF THE EXPOSURE ESTIMATES IN CURRENT ALLERGEN EXEMPTIONS TO THE REFERENCES DOSES (RfDs) EITHER ESTABLISHED AT THE SECOND MEETING (FAO AND WHO, 2022) OR, FOR THE NON-PRIORITY ALLERGENS, ESTIMATED AND SUBSEQUENTLY CONFIRMED AT THE FIFTH MEETING (FAO AND WHO, 2023). EXPOSURES ESTIMATES FOR CURRENT EXEMPTIONS ARE BASED ON p95 OR p97.5 CONSUMPTION AMOUNT AND MAXIMUM USE LEVELS IN PRODUCTS. REFERENCE DOSES ARE LISTED IN UNITS OF TOTAL PROTEIN FROM THE ALLERGENIC SOURCE, I.E. mg OR µg TOTAL PROTEIN FROM THE ALLERGENIC SOURCE**

	RfD	RfD/10	RfD/30	RfD/50	EXPOSURES ESTIMATES FOR CURRENT ALLERGEN EXEMPTIONS
ALMOND; CASHEW (& PISTACHIO); WALNUT (& PECAN)	1 mg (1 000 µg)	100 µg	33 µg	20 µg	< 0.3–5 µg of protein (0.5%–5% usage level of nut alcohol distillate as flavour carrier, 100 g eating occasion) < 0.3–25 µg of protein (0.5%–5% usage level of nut alcohol distillate as flavour carrier, 100–500 g eating occasion) < 6–60 µg of protein (nut alcohol distillate in 30–150 g of 40% alcohol spirits drink) < 11–142 µg of protein (nut alcohol distillate in category alcoholic drinks, alcohol below 15%) < 15–150 µg of protein (nut alcohol distillate in 30–150 g of 100% pure alcohol drink) < 9–248 µg of protein (nut alcohol distillate in category alcoholic drinks, alcohol above 15%)
COW'S MILK; EGG; PEANUT; SESAME	2 mg (2 000 µg)	200 µg	67 µg	40 µg	< 0.3–5 µg of protein (0.5%–5% usage level of whey alcohol distillate as flavour carrier, 100 g eating occasion) < 0.3–25 µg of protein (0.5%–5% usage level of whey alcohol distillate as flavour carrier, 100–500 g eating occasion) 11–22 µg peanut protein (peanut oil, 0.4 µg/g concentration) 22–42 µg peanut protein (peanut oil, up to 0.8 µg/g concentration) 49–99 µg peanut protein (peanut oil, up to 1.8 µg/g concentration) < 6–60 µg of protein (whey alcohol distillate in 30–150 g of 40% alcohol spirits drink) < 11–142 µg of protein (whey alcohol distillate in category alcoholic drinks, alcohol below 15%) < 15–150 µg of protein (whey alcohol distillate in 30–150 g of 100% pure alcohol drink) < 9–248 µg of protein (whey alcohol distillate in category alcoholic drinks, alcohol above 15%) < 130–260 µg combined casein and β-lactoglobulin (lacitol, may be underestimate)
HAZELNUT	3 mg (3 000 µg)	300 µg	100 µg	60 µg	< 0.3–5 µg of protein (0.5%-5% usage level of nut alcohol distillate as flavour carrier, 100 g eating occasion) < 0.3–25 µg of protein (0.5%-5% usage level of nut alcohol distillate as flavour carrier, 100–500 g eating occasion) < 6–60 µg of protein (nut alcohol distillate in 30–150 g of 40% alcohol spirits drink) < 11–142 µg of protein (nut alcohol distillate in category alcoholic drinks, alcohol below 15%) < 15–150 µg of protein (nut alcohol distillate in 30–150 g of 100% pure alcohol drink) < 9–248 µg of protein (nut alcohol distillate in category alcoholic drinks, alcohol above 15%)

TABLE 3.1 **COMPARISON OF THE EXPOSURE ESTIMATES IN CURRENT ALLERGEN EXEMPTIONS TO THE REFERENCES DOSES (RfDs) EITHER ESTABLISHED AT THE SECOND MEETING (FAO AND WHO, 2022) OR, FOR THE NON-PRIORITY ALLERGENS, ESTIMATED AND SUBSEQUENTLY CONFIRMED AT THE FIFTH MEETING (FAO AND WHO, 2023). EXPOSURES ESTIMATES FOR CURRENT EXEMPTIONS ARE BASED ON p95 OR p97.5 CONSUMPTION AMOUNT AND MAXIMUM USE LEVELS IN PRODUCTS. REFERENCE DOSES ARE LISTED IN UNITS OF TOTAL PROTEIN FROM THE ALLERGENIC SOURCE, I.E. mg OR µg TOTAL PROTEIN FROM THE ALLERGENIC SOURCE.** (continued)

	RfD	RfD/10	RfD/30	RfD/50	EXPOSURES ESTIMATES FOR CURRENT ALLERGEN EXEMPTIONS
FISH; WHEAT	5 mg (5 000 µg)	500 µg	167 µg	100 µg	0.001–0.72 µg fish protein (isinglass in 1 glass–1 bottle of wine, calculated by Expert Committee) 0.02–1.38 µg fish protein (isinglass in beer, calculated by Expert Committee) < 0.048 µg of total fish protein per serving (fish gelatine as a carrier in vitamin) < 6.4 µg of total fish protein per day (fish gelatine as a carrier in flavoured foods – was provisionally exempted but not granted a permanent exemption) < 0.3–5 µg of protein (0.5%–5% usage level of wheat alcohol distillate as flavour carrier, 100 g eating occasion) < 0.3–25 µg of protein (0.5%–5% usage level of wheat alcohol distillate as flavour carrier, 100–500 g eating occasion) < 6–60 µg of protein (wheat alcohol distillate in 30–150 g of 40% alcohol spirits drink) < 11–142 µg of protein (wheat alcohol distillate in category alcoholic drinks, alcohol below 15%) < 15–150 µg of protein (wheat alcohol distillate in 30–150g of 100% pure alcohol drink) < 9–248 µg of protein (wheat alcohol distillate in category alcoholic drinks, alcohol above 15%) **Glucose syrup derived from wheat starch FSANZ and the EFSA** < 1 000 µg wheat protein/eating occasion (glucose syrup derived from wheat starch FSANZ) < 1 000 µg gluten per day (AAC on wheat-based maltodextrins EFSA) < 3 500 µg gluten per day (glucose syrups and dextrose EFSA) **Glucose syrup derived from wheat starch FSANZ – recalculated by Expert Committee** < 368–928 µg wheat protein/eating occasion (ice cream, 97.5th percentile intake) < 400–1 440 µg wheat protein/eating occasion (chocolate, 97.5th percentile intake) < 500–1 856 µg wheat protein/eating occasion (confectionary, 97.5th percentile intake) **Calculations by Expert Committee with mean intake instead of 97.5th percentile used by FSANZ** < 120–360 µg wheat protein/eating occasion (confectionary, mean intake) < 140–320 µg wheat protein/eating occasion (chocolate, mean intake) < 151–301 µg wheat protein/eating occasion (ice cream, mean intake)
SOY	10 mg (10 000 µg)	1 000 µg	333 µg	200 µg	10.5–41.0 µg soy protein (soybean oils) 10.5–57.4 µg soy protein (soybean oils with updated analytical information) 3–41 µg soy protein (pytosterols, tocopherols) < 100 µg soy protein per serving (Solae soy lecithin RA) < 55–231 µg Hexane Insoluble (ADM soy lecithin RA, per eating occasion, 95th pecentile) < 70–334 µg Hexane Insoluble (ADM soy lecithin RA, per eating occasion, 99th pecentile)
SHRIMP	200 mg (200 000 µg)	20 000 µg	6 667 µg	4 000 µg	n/a

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RISK ASSESSMENT OF FOOD ALLERGENS
PART 4: ESTABLISHING EXEMPTIONS FROM MANDATORY DECLARATION FOR PRIORITY FOOD ALLERGENS

MEETING REPORT

The Codex Committee on Food Labelling (CCFL) requested scientific advice as to whether certain foods and ingredients, such as highly refined foods and ingredients, that are derived from the list of foods known to cause hypersensitivity can be exempted from mandatory declaration. The objective of this fourth meeting was to expand on the recommendations from the first meeting concerning derivatives of food allergens and establish a framework for evaluating exemptions for food allergens.

A pro forma process has been developed and tested against allergen derivatives previously granted exemptions in various countries or regions and found to be effective for consideration in future exemption decisions. The Expert Committee recommends that the process outlined in the pro forma process be used to guide any future development and evaluation of derivative exemptions. Establishment of safety based upon this weight of evidence approach is dependent upon consideration of data quality, outcome of the exposure assessment for all intended ingredient uses (specified for exemption) and review by competent authorities (as needed). When safety is established, exemption can be justified.

FOOD SYSTEMS AND FOOD SAFETY - ECONOMIC AND SOCIAL DEVELOPMENT
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