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Practical Guidance on the Application of Food Allergen Quantitative Risk Assessment within Food Operations

REPORT

Commissioned by the Food Allergy Task Force

ILSI EUROPE

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PRACTICAL GUIDANCE on the Application of Food Allergen Quantitative Risk Assessment within Food Operations

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REPORT

COMMISSIONED BY: FOOD ALLERGY TASK FORCE

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Table of Contents

Acknowledgements 9

I. Foreword: Practical Guidance on the Application of Food Allergen Quantitative Risk Assessment within Food Operations 10

II. Layout of the Guidance Document 11

III. Links to highlighted sections of the Guidance 11

1. Introduction 12

1.1. What is QRA and the place of QRA within allergen management 13

2. Communication across supply chains 20

2.1. Global regulatory aspects (including variation in allergen requirements) 21

2.2. Information requirements to enable QRA across the supply chain 24

2.2.1. Nutritional/nutrient information 24

2.2.2. Allergen specific ingredients and proportions 25

2.2.3. Usage levels of ingredient in final product under assessment 27

2.3. How do you obtain the required information (Guide for questioning/obtaining info from suppliers)? 31

2.3.1. Transparent communication 31

2.3.2. Assessing potential cross-contact in supply chain 32

3. Management of Operations 35

Introduction to the chapter 35

3.1. QRA within Allergen Control Programs 35

3.2. Guide on QRA within Site Cross-Contact 36

3.2.1. Case Study – Example Foods - Case description of a process with homogeneous cross-contact. 38

3.2.2. The Role of GMP's in Allergen Management 39

3.2.3. Step 1: Hazard Identification: Assessing Unintended Allergen Presence (Cross-Contact) 41

3.2.4. Step 2: Chance of Occurrence 43

3.2.5. Step 3: Hazard Characterisation & Controls 44

3.2.6. Step 4: Validate control measures to minimise the risk of unintentional allergen presence 46

3.3. Validation & Verification of Cleaning 50

3.3.1. Guide on the application of QRA in Cleaning Validation 50

3.3.2. Approach to validation 51

3.3.3. Steps to take to validate cleaning 52

4. Management of incidents 59

4.1. Guidance on Incident Assessment 59

4.1.1. How to use the Incident Form 59

4.1.2. General Information & Assessment Summary (The following form may be downloaded and adapted for use) 60

4.1.3. Incident Flow Chart 61

4.1.4. Assessment Matrix 62
4.1.5. Guidance on the Assessment Matrix 67
4.1.6. Communication templates 72
4.1.7. Use of food allergy prevalence data in public health QRA 72
5. Core concepts 73
5.1. UAP Scenarios and characteristics of cross-contact 73
5.1.1. UAP Scenario & Chance of Occurrence 73
5.1.2. Characteristics of UAP 75
5.2. Amount of UAP in food 86
5.2.1. Allergen Sampling and Analysis 86
5.2.2. Data conversion guide 115
5.2.3. Carry-over calculation guidance 120
5.3. Guidance on food intake data for allergen risk assessment 123
5.4. Basic allergen QRA calculations 127
5.4.1. Exposure (mg) calculations 128
5.4.2. Calculation of an Action Level (mg/kg, ppm) 130
5.4.3. Sensitivity related to the uncertainty of assessment 131
5.4.4. Basic risk calculations 131
5.4.5. Public health risk assessment (basic) 133
6. Concluding remarks and future perspectives 134
7. Annexes 135
7.1. ANNEX: Definitions / Glossary 135
7.2. ANNEX: List of abbreviations 136
7.3. ANNEX: Guidance documents 137
7.4. ANNEX Examples Management of Operations 138
7.4.1. Case Study 2: Example Sesame in a Bakery 138
7.4.2. Case Study 3 Example – Milk in a Fruit Drink 140
7.5. ANNEX Examples of 'incidents' and their assessment 144
7.5.1. A Tier 4 upstream incident assessment concerning homogeneous UAP 144
7.5.2. A Tier 2 in-house incident assessment concerning particulate UAP 150
7.5.3. A tier 2 in-house incident assessment concerning homogeneous UAP 156
7.5.4. A Tier 4 downstream incident assessment concerning homogeneous UAP 162
7.6. ANNEX Food allergy prevalence data 168
7.7. ANNEX for sampling and analysis 170
7.7.1. Sampling and Analysis flow chart & data capture form 170
7.7.2. Sampling and Analysis References and useful links 174
7.8. ANNEX for protein content table used in data conversion 175
7.9. ANNEX for food intake section 176
7.9.1. Portion or serving size? 176
7.9.2. Data provided in national food consumption databases 176
7.9.3. Can I use data from the acute daily intake for a single day? 177

7.9.4. Can I use data from one country for another country? 177
7.9.5. General population vs Population with food allergies 177
7.9.6. Frequency of consumption 177
8. References 179

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I. Foreword: Practical Guidance on the Application of Food Allergen Quantitative Risk Assessment within Food Operations

Allergen cross-contact and Unintended Allergen Presence (UAP) are a significant challenge for food operators.

The aim of this document is to translate the findings of the Expert Group on 'Food Allergen Quantitative Risk Assessment (QRA)' into a Guidance document which provides tools and approaches to help harmonize the data gathering process for food allergen risk assessments and therefore aid with their implementation. This Guidance aims to promote consistency in documentation, decision making and the application of allergen QRA.

The purpose of this Guidance is not to take an allergen labelling or risk management decision for the user, but rather is intended to help them decide when allergen QRA is appropriate or necessary, and how to decide if it can actually be performed and, if it is to be undertaken, what is the most suitable methodology.

Any resulting risk management measures or actions planned should be checked to assure compliance with local legislation before implementation.

As a part of the development of this Guidance and in order to strengthen the overall document, feedback was sought and received from stakeholders from the food industry, food safety authorities, academia, patients' organisations and other research organisations/ consultants (see Appendix A (Remington, Baumert et al. 2022)).

The intended audience is mainly industry wishing to understand and conduct food allergen risk assessments, and potentially QRA. However, it should be noted, that this guide could also be useful for others, including official control agencies.

Training videos for this Guidance will be made available after its publication and can be found here.

Finally, we appreciate comments and input on this Guidance from the users, which can be sent to: publications@ilsieurope.be. These communications will be taken on-board in future updates to the Guidance.

II. Layout of the Guidance Document

This Guidance provides an introduction to food allergen Quantitative Risk Assessment (allergen QRA), including when QRA methods may be appropriate and feasible within food business operations. Allergen QRA methods are described including input data and approaches to calculations. The Guidance is presented in 6 sections as follows:

- Introduction to allergen QRA
- Proactive assessments for food production under normal conditions, presented in 2 sections for the upstream supply chain and in the Management of Operations (MoO) within food production facilities
- Reactive assessments as part of an allergen incident response
- Core concepts that underly all allergen QRAs
- Supporting information and examples within an Annex

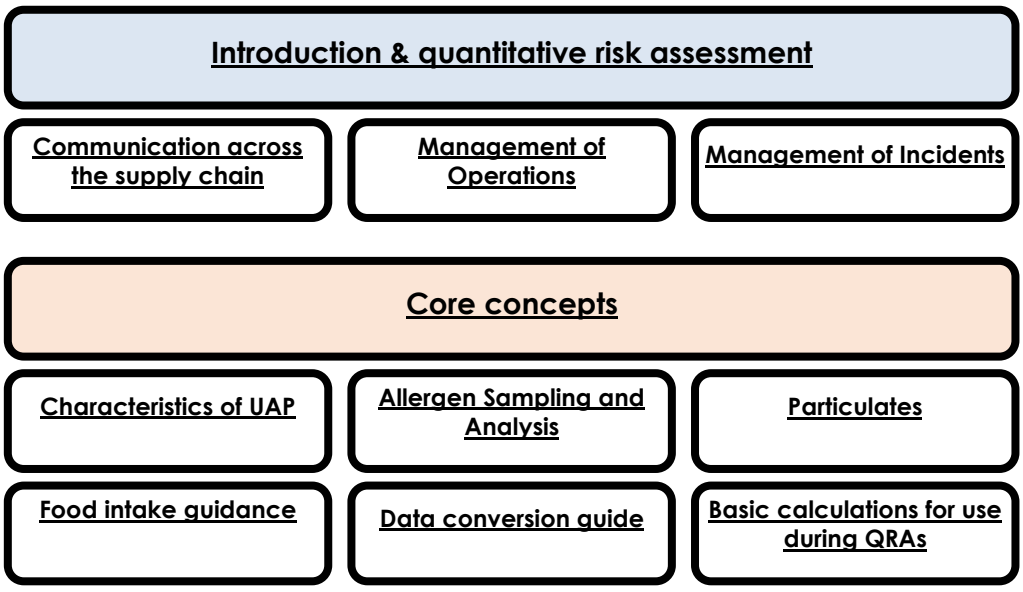
In the core concepts section, the concepts which are common to all allergen risk assessments including QRA are discussed. These overarching core concepts detailed below are:

- Unintended Allergen Presence (UAP) scenarios and understanding the associated characteristics of cross-contact
- The amount of UAP in food
- Guidance on food intake data for allergen risk assessments
- Exposure assessment inputs and basic QRA calculations

These core concepts will be used to a large extent in any allergen (Q)RA, but the specific core concepts utilized will vary with the needs of each assessment. The core concepts are intended to be brief reference materials that should be used in combination with other sections of the Guidance. As such, the Guidance requires the user to link from one section to another for information on a specific topic. Additionally, training videos for different sections of this Guidance will be made available after its publication and can be found here.

III. Links to highlighted sections of the Guidance

It is recommended that the reader/user starts with the Introduction chapter and the links here provide a shortcut for the reader to sections of interest. Additionally, the links in the footnotes on each page return the reader to the full Table of Contents or the abbreviated links here.



1. Introduction

Allergen Quantitative Risk Assessment (QRA) is a tool that complements allergen management practices by enabling the risk presented to allergic consumers due to an UAP in a food to be estimated. It thereby can provide useful information as input into risk management decision-making, such as whether Precautionary Allergen Labeling (PAL) is appropriate. However, risk assessors tasked with QRA for allergens will encounter various forms of questions and assessments that all fall under the guise of QRA, each with their own requirements.

The Guidance provides an introduction to allergen QRA and an overview of inputs potentially needed for different QRA methods, when QRA has been deemed appropriate and feasible . Areas of focus include proactive assessments for food production under normal conditions to understand cross-contact[1,2] hazards and appropriate risk mitigation, both in the upstream ingredient supply chains and in the Management of Operations (MoO) of food production facilities under the control of the user. Also included are reactive risk assessments as a part of responding to an allergen incident wherein product previously produced and possibly at market is implicated with new information thereby requiring an allergen assessment.

The aim of this Guidance document is to provide tools and approaches to help harmonize both the data gathering and methodologies used for food allergen risk assessments and therefore aid with the implementation of allergen QRA. The intended audience is mainly industry wishing to understand and conduct food allergen risk assessments, and potentially QRA. However, it should be noted, that this guide could also be useful for others, including official control agencies. This Guidance aims to promote consistency in decision making on the application of allergen QRA and thereby in the risk management outcomes. The purpose of this Guidance is not to provide an allergen labelling or risk management decision for the user, but rather is intended to enable a decision to be taken on when allergen QRA is appropriate or necessary, and how to decide if it is feasible to perform, and if it is to be undertaken how it can be performed.

In theory all sources of allergen cross-contact and unanticipated incidents, illustrated in **Figure 1,** are amenable to QRA. However, QRA will only be necessary when the risk presented to consumers is not immediately clear, and it will only be feasible based on the quality of available data or time available.

[1] Throughout this guidance, the term 'cross-contact' will be used in alignment with terminology used by the US FDA (2018). "Draft Guidance for Industry: Hazard Analysis and Risk-Based Preventive Controls for Human Food. Available at: https://bit.ly/31Lq1ru." and the Codex Code of Practice on Food Allergen Management for Food Business Operators Codex Alimentarius (2020). "Code of practice on food allergen management for food business operators. CXC 80-2020. Adopted in 2020. Available at: https://bit.ly/3OvAQpK." We have chosen 'cross-contact' for consistency but the terms can be viewed as interchangeable.

[2] We also use the abbreviation 'UAP' to mean 'unintended allergen presence' within a foodstuff due to either cross-contact, or incorrect labelling or ingredient use.

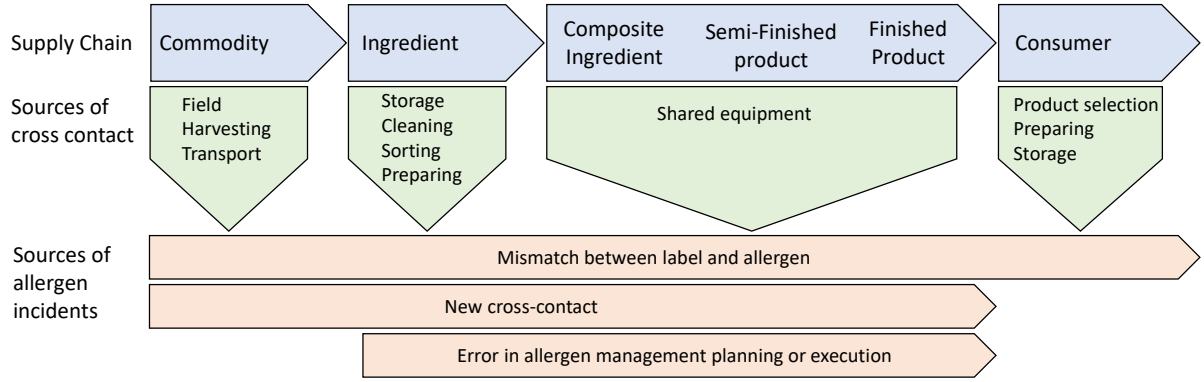


***Figure 1**. Example situations of allergen incidents across a supply chain in which allergen QRA may provide useful information on risk presented to allergic consumers.*

1.1. What is QRA and the place of QRA within allergen management

UAP in a food can result from allergen incidents or cross-contact throughout the supply chain, including agricultural practices, storage, transportation and production processes (Codex Alimentarius 2020).

Food allergen Risk Assessment (RA) is the use of information on the characteristics of UAP or cross-contact to estimate the degree of risk of allergic reactions within the group of consumers that have food allergy, related to a specific exposure scenario to an allergenic food substance in a consumed final product. However, due to varying definitions and standard assumptions within an assessment, it is important to clearly define the risk being presented and discussed.

QRA for allergens exists in different forms with different requirements that need to be considered by the risk assessor depending on the question that needs to be answered. ***Figure 2*** illustrates four general levels of allergen (Q)RA, with an increasing level of detail required for the input parameters as the assessments are refined from 1) screening approaches to 2) deterministic or Reference Dose (RfD, Ref Dose) approaches [in the at-risk population], 3) probabilistic approaches [in the at-risk population] and 4) public health-based assessments [covering the at-risk population within the context of the overall population] (Remington, Baumert et al. 2022).

At the most basic level of QRA, a deterministic food allergen QRA will compare either 1) the exposure to allergen to an appropriately protective RfD for that allergen based on the relevant allergic population or 2) the concentration of allergen in a food to a derived Action Level for that food. For more information and key references regarding the fundamentals of how allergen RfDs are derived from oral food challenge data and subsequent dose-distribution models, as well as what might constitute an appropriate RfD see **Box Reference Doses**.

When QRA is appropriate and feasible it can be used as one of the inputs into risk management. However, QRA cannot substitute for compliance with Good Manufacturing Practices (GMPs) and prerequisite programs (PRPs). Allergen QRA is an additional tool to enable decision making that should complement established practices that seek to identify and mitigate sources of cross-contact.

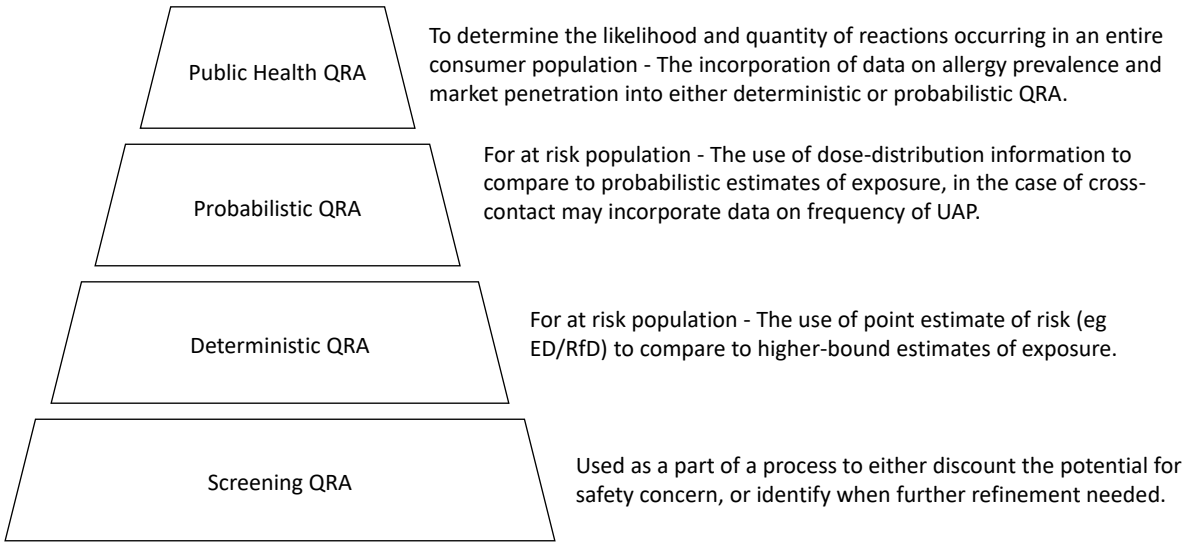


***Figure 2**. Sliding scale of (Q)RA definitions, with increasingly more data inputs required near the top of the scale. Although it is not often necessary to deploy more sophisticated RA methods, such as Probabilistic QRA calculations, it could be the case that the required expertise for these methods may not be immediately available in-house and external expertise is needed. [reprinted from peer-reviewed publication (Remington, Baumert et al. 2022).*

The application of allergen QRA is inherently more complex compared to the historical approach of risk assessment, which relies only on determining whether or not there was a possibility that cross-contact allergen could be present and if so, passing that information down the supply chain until it reached the consumer in the form of PAL. As such any guidance on allergen QRA needs to balance between oversimplification and overcomplication in order to be effective and achieve the objective of supporting the application of the QRA approach (ILSI Europe Digital Event report).

Allergen QRA attempts to understand the degree of risk presented to consumers, to better inform risk management action. It should not be seen just as a calculation tool. As the conduct of allergen QRA requires the best information available, it should be seen within the context of refinement to better understand the reality of risk. That includes refinement in the understanding of the probability of cross-contact, and the characteristics of such cross-contact. Allergen QRA is as such set within the context of a more informed understanding of cross-contact situations, and thus can be a part of improving the information that travels with food. **Figure 3** shows the common steps in considering whether to apply allergen QRA.

It is required in many countries to indicate on the label of foods that use ingredients or processing aids derived from allergenic foods that are considered to cause the majority of allergic reactions, these regulatory requirements vary per country (For more information, see section **2.1**). The Codex Alimentarius General Standard For The Labelling of Prepackaged Foods may be considered as an inter-governmental consensus (Codex Alimentarius 2018). However, current regulations do not cover the management of UAP that can occur because of unintentional cross-contact during production processes across supply chains. Furthermore, such 'trace amounts' are not defined in regulations, with Japan and Switzerland being the notable exceptions (Allen, Turner et al. 2014, Soon and Manning 2017), however these regulatory schemes do not appear to be based on contemporary scientific evidence. As such, given the residual risk to consumers with food allergy and the obligation placed on food businesses to provide safe food, precautionary allergen labelling (PAL) and its various phraseologies (e.g., may contain, not suitable for, manufactured on shared equipment, manufactured in a shared facility) are found in the vast majority of jurisdictions but vary widely in both wording and application (For more information, see **Box PAL**). PAL remains largely unregulated, voluntary, and inconsistently applied while stakeholders desire transparent decision-making criteria for the application PAL that bears a relationship to the actual risk (DunnGalvin, Chan et al. 2015, Soon and Manning 2017, Allen and Taylor 2018, Gupta, Kanaley

et al. 2021). Unfortunately, international guidance surrounding the risk assessment needed to inform the potential application of PAL is currently lacking in a number of areas, particularly in the area of allergen QRA to support the risk management decision making process (Remington, Baumert et al. 2022).

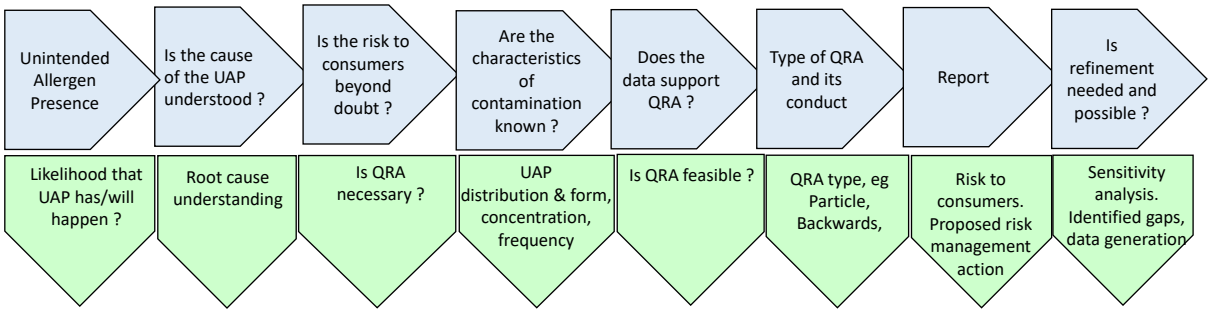


Figure 3. *Common steps in the process of allergen risk assessment.*

In response to this gap in knowledge, the European branch of the International Life Sciences Institute (ILSI Europe) formed an Expert Group (EG) to attempt to achieve consensus on the methodologies needed for allergen QRAs, and their implementation by food business operators (Remington, Baumert et al. 2022). An October 2020 electronic workshop with representatives from across food allergy and allergen stakeholder groups identified that a summary of current best in class approaches should be developed to improve the management of allergen cross-contact risks and mitigation (ILSI Europe Digital Event report). This should in particular seek the harmonization of allergen QRA as it is applied within food operations, as this would be beneficial to consistent decision making across the sector on the application of PAL and therefore better inform allergic consumers. As such, this Guidance was developed with the assistance of a wide stakeholder community.

Box Reference Doses. *Key references regarding the fundamentals of how allergen RfDs are derived from oral food challenge data and subsequent dose-distribution models, as well as what might constitute an appropriate RfD.*

Fundamentally, allergen QRA is the comparison between an exposure to an amount of protein from an allergen, to a suitable 'Reference Dose' (RfD, Ref Dose).

RfDs are amounts of total protein from the allergenic food source that reflect an exposure without appreciable health risk. RfDs have been developed for many regulated allergens, these being the only ones for which the volume and quality of dose-distribution data are adequate (VITAL Scientific Expert Panel Recommendations 2019, FAO/WHO 2021, Joint FAO/WHO Expert Consultation on Risk Assessment of Food Allergens 2021). RfDs form a critical part of risk characterisation, and therefore play a key role in allergen risk management and subsequent QRAs.

RfDs are commonly derived from allergen dose-distribution modelling, in which data from oral food challenge studies performed in allergy clinics with many allergic patients are combined to predict the proportion of the allergic population that will react at a given dose of allergenic protein (Westerhout, Baumert et al. 2019, Houben, Baumert et al. 2020, Remington, Westerhout et al. 2020). This includes analysis to understand the proportion of the population that will react to low doses. These low doses, such as the 'eliciting dose 1%' or 5%, so-called ED01 or ED05, are the doses expressed as mg of total protein from the allergen, which are predicted to respectively result in no more than 1% or 5% of allergic consumers experiencing an objective reaction. The ED01 or ED05 have previously been recommended as appropriate RfDs to use in allergen QRA (VITAL Scientific Expert Panel Recommendations 2019, Joint FAO/WHO Expert Consultation on Risk Assessment of Food Allergens 2021). Available data indicates that these modelled ED values do not underpredict the proportion of reactions, and reactions experienced at these doses have not been shown to be severe (Patel, Adelman et al. 2021, Turner, Patel et al. 2022).

It must be clear that any use of a specific RfD within this Guidance is for example purposes and not an endorsement or rejection of any specific risk management system.

However, as of June 2022 it should be noted that:

- Endorsement or rejection by authoritative bodies of RfDs in general or specific RfDs and their application in allergen risk assessment can vary by country and region (and subsequent Action Levels calculated from them).
- If endorsed, the applicable RfDs endorsed can vary by country or region and may be subject to (rapidly) changing views of relevant authorities.

With that in mind:

- This Guidance has been written to be globally applicable.
- This Guidance has been written in a way that it will still be relevant long after its publication.
- The purpose of this Guidance document is to provide tools and approaches to harmonize the data gathering process for food allergen risk assessments, and potentially for quantitative risk assessments. The purpose of this ILSI Guidance is NOT to take an allergen labelling or risk management decision for the user.
- However, there are many ongoing [international] discussions (FAO/WHO Codex, different national authorities) regarding the topic/use/acceptance/nonacceptance of RfDs and risk-based Precautionary Allergen Labelling (PAL).
- **This is a "rapidly" changing area and users should update themselves regularly on this topic**

Finally, if conducting a risk assessment for an allergen where a RfD has not been established, it should again be noted that the recommended approach taken may vary regionally for legal purposes and users should update themselves regularly on what is best for their situation. In some cases, it may be in the best interests of a company to:

- Take a 'read across' approach by selecting a suitable RfD based on RfDs defined for closely-related allergen(s).
- Take a 'read across' approach by selecting the lowest RfD from closest taxonomically matching allergen.
- Select the lowest available RfD from the regulated allergens which has RfDs available.
- Proceed with an option not based on RfDs or allergen QRA.

Keep in mind that choosing the incorrect RfD option could place an unexpected liability on a company.

If there is any uncertainty regarding RfDs as to what is best for the user/reader, it is recommended to contact the relevant food safety authority or a recognized allergen expert for more advice.

References in this box

Summary of the 2019 VITAL Scientific Expert Panel Recommendations: https://allergenbureau.net/resources/allergen-bureau-resources/

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Westerhout, J. et al., 2019. Deriving individual threshold doses from clinical food challenge data for population risk assessment of food allergens. J. Allergy Clin. Immunol. 144, 1290–1309. https://doi.org/10.1016/j.jaci.2019.07.046

Box PAL. *The regulatory status of Precautionary Allergen Labelling (PAL) and Labelling phraseology for Unintentional Allergen Presence (UAP)*

- The purpose of this Guidance document is to provide tools and approaches to harmonize the data gathering process for food allergen risk assessments, and potentially for quantitative risk assessments. The purpose of this ILSI Guidance is NOT to take an allergen labelling or risk management decision for the user.

- Currently (June 2022) there are no generally recognized international regulatory frameworks for the application or management of PAL. As such, there are a large variety of local regulations and guidances that differ considerably in their risk assessment and risk management decision making process for products with or without PAL declarations. In some countries, voluntary PAL declarations are common, while other countries do not allow for them. Therefore, regardless of the outcome of the QRA, PAL declarations may still differ on the same product depending on the country where the final food is sold.

- PAL can be recognized by its commonly used wordings such as 'may contain…', 'may be present…', 'made in a factory that also manufactures…' etc., see the (Codex Committee on Food Labelling. CX/FL 19/45/8 2019).

- Risk communication for UAP must always follow a risk assessment that is as thorough as possible, and should serve as the last resort as an allergen risk mitigation measure.

Guidance published by the UK Food Standards Agency in 2007 states that "Advisory labelling [PAL] should only be used when, following a thorough risk assessment, there is a demonstrable and significant risk of allergen cross-contamination [*now referred to as cross contact]*" (FSA 2020). It should neither be used as a substitute for poor GMPs and allergen control or a default position based on a lack of substantive evidence. Further to this, the decision on whether to apply a precautionary label can be informed by QRA when it is appropriate and possible. The recent Ad hoc Joint FAO/WHO Expert Consultation on Risk Assessment of Food Allergens (2021, available here) and the PAL guidelines published by FoodDrinkEurope (2021, available here) came to similar conclusions. For more information on this topic, see the (Codex Committee on Food Labelling. CX/FL 19/45/8 2019) specifically the section on "Precautionary allergen or advisory labelling". If more information regarding PAL is desired, a more detailed review of PAL and how adoption of quantitative limits (based on Reference Doses) could form part of an EU-wide approach is provided by FoodDrinkEurope (2021, available here).

PAL should not be misleading or confusing, and should be based on relevant scientific data (Regulation EU No 1169/2011 , Codex Committee on Food Labelling. CX/FL 19/45/8 2019) to ensure the safety of the product. An example of potentially misleading labelling is a product with a "blanket statement labelling for every possible allergen". Currently a harmonized phraseology to indicate unintentional allergen presence is lacking (see below for background information on phraseologies used). Internationally, there have been several best practice guidance documents developed by both industry (Australian Food and Grocery Council and Allergen Bureau 2021) and regulatory bodies (Livsmedelsföretagen 2015) to help guide manufacturers with the most appropriate phraseology to indicate unintentional allergen presence. These can be regionally different.

In addition to differences in wording, regional variations exist regarding for PAL and the interpretations/recommendations/regulations of:

- Place on label
- Font size
- Listing specific type of cereal and tree nut instead of general group names (gluten, nuts)
- Which Action Level / Reference Doses to use

- Upper limits for PAL
- Combination with 'free-from' claims

Users should update themselves regularly on this topic as it may be subject to (rapidly) changing views of local authorities.

As the provision of information regarding UAP is voluntary to date, there are numerous different types of phraseology used, which can often prove confusing for the allergic consumer. Based on a recent retail survey of 20,000 products in the U.S. found that 25 different types of phrases were used (Pieretti, Chung et al. 2009). Other studies (Hefle, Furlong et al. 2007, Pele, Brohée et al. 2007, Ford, Taylor et al. 2010, FSAI 2011) have indicated that the most frequently used phrases on pre-packaged foods are:

- May-Contain (name of allergen/s)
- May-Contain Traces of (name of allergen/s)
- Made on a line or equipment that also uses (name of allergen/s)
- Made in a factory that also uses (name of allergen/s)
- May Contain a risk of (name of allergen/s) from the supply chain (i.e., ingredients)
- Not Suitable for (name of allergen/s) allergic consumers

Research conducted with allergic consumers indicates a perceived difference in the level of risk associated with the different phrases used (Barnett, Leftwich et al. 2011, Holleman, van Os-Medendorp et al. 2021). A study conducted on 184 parents of peanut and tree nut allergic children found that a large number of parents ignored warnings or assumed that there was a gradation of risk depending on the wording of the statement (Noimark, Gardner et al. 2009).

The study found that the products most commonly avoided by the parents of allergic children where those which were labelled with 'not suitable for' precautionary statements. These statements were regarded as the most effective because they not only provide information but also proposes the decision for consumers/care-givers regarding the appropriateness of consumption (Noimark, Gardner et al. 2009). This type of phraseology is also the preferred option for both regulatory bodies and allergic patient support groups in the UK (FSA 2020). However it is noted that the phraseology for PAL should consider taking into account local linguistic nuances and these statements could be regionally different from English language ones (Flanagan 2015).

2. Communication across supply chains

This chapter describes best practices for obtaining and communicating reliable information on allergen risks across food production supply chains. It specifically looks at the interface between ingredient suppliers and the users of those ingredients so that residual ingredient allergen risks can be integrated into the food producer's QRA analysis. A 'best practice' data gathering system should be able to gather (and communicate) the necessary data for a QRA approach within the supply chain.

Some challenges for such a data gathering system are described as follows:

- The complexity of regulatory requirements across geographies. A QRA exercise may sometimes need to consider only one legislative framework, whereas on other occasions ingredients might be purchased from territories with a vastly different allergens regulatory framework from the one the finished product is retailed.
- The level of expertise of different people working in a given supply chain may be diverse, ranging from very knowledgeable to only limitedly informed, impacting the quality of the gathered data.
- The risk an ingredient poses to a finished product may be treated differently for bulk ingredients used at high percentages in a finished product, versus situations where ingredients are used in very low concentrations.
- Identified risks could be heterogeneous in nature, rather than homogeneous. How does one quantify a heterogeneous risk?
    - See section ***5.1.2.4*** for more information
- The chance of a certain risk occurring could be as important as its magnitude. Can the data gathering system also capture this type of information?

Giving guidance in this domain must strike a balance between what is the 'best in class approach' and what is feasible for businesses, taking into account that resources dedicated by companies to allergen management can be very different between large companies and small - / medium size enterprises (Remington, Baumert et al. 2022).

The current chapter aims to provide guidance around how to collect 'best practice' data for allergen QRA within complex supply chains. This section of the Guidance is intended to enable better communication between ingredient suppliers and users of those ingredients, as well as transparent communication of relevant allergen information for further use by those conducting assessments as part of their operational management, as detailed in the **Management of Operations** chapter.

This section of the Guidance does not seek to "reinvent the wheel", but rather to provide the type of information that needs to be gathered from suppliers. Detailed herein are the type of information that is needed, and to which level of detail, to work effectively in an allergen risk management framework that incorporates QRA.

To that end, there are three major elements that require consideration to facilitate communication across supply chains:

1. Make sure business partners are aware of the globally diverse legal framework for allergens, so that no mistakes are made by simply not knowing which allergens are relevant in a geographical territory one is importing from or exporting into.
2. Define the level of detail that needs to be obtained from an ingredient supplier, in order to later use this information as a part of risk-based decisions.
3. Understand the questions important to ask suppliers to obtain the required information for your allergen assessments and management program.

Finally, this Guidance provides a starting framework for communication and evaluations across supply chains. However, it is not intended to be a cut & paste system for the user and it is crucial to remember that a thoughtful process is needed to appropriately manage each supplier situation. Additionally, as the final product producer is responsible for ensuring that the label reflects what is in the product, it is important that suppliers are adequately evaluated (i.e., audits, contractual documentation, analytical checks).

2.1. Global regulatory aspects (including variation in allergen requirements)

Consistent and transparent communication, as well as being alert to changes (i.e., regulatory changes, production process changes, new origin of ingredient, etc.), is needed both internally and with external partners in order to avoid mistakes by simply not knowing which allergens are relevant in a geographical territory one is exporting into or sourcing from:

1. External, transparent chain of communication between producers and suppliers.
2. Internal, transparent communication between quality/regulatory affairs/procurement teams and product developers.

Open, transparent communication is needed due to the highly complex nature of supply chains (e.g., information flow is needed from farmer to commodity producer, to ingredient maker, and then to final product maker). Additionally, the likelihood that ingredients might be purchased from territories with a vastly different regulatory framework for allergens to the one where the finished product is sold requires effective chains of communication. Simply put, as a purchaser this means being clear about the information required to be provided by the supplier and taken into account during risk assessments. This means that forwards and backwards thinking is needed about which allergens are regulated in the destination jurisdiction and which have the potential to be present. As shown in the International Regulatory Chart for food allergens curated by the (University of Nebraska Food Allergy Research and Resource Program), the international regulatory situation for allergen labelling requirements varies by region.[3] Exemptions from mandatory allergen labelling also vary by region and as of June 2022 there is no freely available and updated summary of globally permitted exemptions.[4] One should be aware of the possibility that a supplier might not declare an allergen as it is exempted in the region of the supplier, even though it could be relevant to the region the final food is sold.

In summary:

1. Regulated allergens (those that are legally notifiable) can vary between regions.
2. Exemptions from mandatory allergen labelling can also vary regionally
3. this should be taken into account in communication between producers and suppliers.

Currently (as of June 2022) there are no generally recognized international regulatory frameworks for the application or management of precautionary allergen labelling (PAL). For more information regarding PAL, see ***Box PAL.***

In addition to the regulatory complexities across different jurisdictions, the complexity of suppliers' ingredient production and the level of expertise present in a given supply chain may be diverse. A simplistic overview of the situation is reflected in **Box 1**, with geographic complexity, ingredient/supplier complexity and the supplier/auditor technical capabilities all able to range from low to high. Based on the outcome of this simplistic review (i.e., orange outcome in **Box 1**), a company may want to consider different types of questions during an assessment/audit or trigger alerts requiring more information from their suppliers for a specific ingredient. Examples of detailed questions are given in **2.3.2** below.

When requesting a supplier to provide allergen information for your specific ingredient information form/questionnaire, it needs to cover the requirements of the final product in

[3] An additional, regularly updated online resource is available from Allergenen Consultancy and focuses on the situation for international regulation of allergens with interpretations for additional warning statements. This resource is available for a small yearly subscription fee.

[4] A summary of EU exemptions from Annex II labelling requirements is provided by FDE in the Allergen Labelling Annex of their Guidance on Food Allergen Management for Food Manufacturers

question. It is recommended that a company maintains a master reference list of what is acceptable for the finished product which a specified person is dedicated to maintain. Additionally, a company may find it easier to maintain a global form/questionnaire for their allergen requirements instead of a survey for each region. A global form is not necessarily required because information is needed on all allergens in every country of the world, but rather to understand the existing risks and to be ready to facilitate future expansion into other markets.

Box 1. *A simplistic overview of the global supply chain with regards to allergen specific situations and capabilities in view of geographic complexity, ingredient/supplier complexity and the supplier/auditor technical capabilities. Three illustrative examples are also provided.*

Geographic complexity	**Ingredient / supplier complexity**	**Supplier technical capability**
Low**:** Ingredients are being purchased from the same regulatory territory as the final product sales territory	***Low*****:** *Homogeneous* cross-contact risk 1. Low complexity environment 2. High complexity environment	***High*****:** Company with dedicated people and verified systems for allergen management
High**:** Ingredients come from a regulatory territory other than the final product sales territory	***High*****:** *Heterogeneous* cross-contact risk 1. Low complexity environment 2. High complexity environment	***Low*****:** Company with few to no people or systems dedicated to allergen management

Examples of situations which may trigger different alerts or different types of questions could include but are not limited to the following:

Example situation	**Global regulatory considerations?**
1. Regional, simple ingredient supplied by company with high technical capabilities for allergens: - Geographical complexity: LOW - Ingredient / supplier complexity: LOW - Supplier technical capability: HIGH	- External: Have the allergens for the region of this ingredient and final product been considered in the regulatory framework assessment? - External: Have other countries, that are included in the upstream supply chain, been considered in the regulatory assessment? - Internal: Is there a system to trigger an alert/reassessment if this ingredient is used in the future for a product sold outside of the initially approved market?
2. Regional, simple ingredient from a supplier with low technical capabilities for allergens: - Geographical complexity: LOW - Ingredient / supplier complexity: LOW - Supplier technical capability: ***LOW***	- In addition to Example 1: - Extra considerations or audits in the initial supplier verification may be warranted regarding the reliability of information if the SME has limited allergen knowledge/capabilities
3. Across regions, simple ingredient supplied by company with high technical capabilities for allergens: - Geographical complexity: ***HIGH*** - Ingredient / supplier complexity: LOW - Supplier technical capability: HIGH	- In addition to Example 1: - External: Have the allergen requirements for the two regions in the supply chain of this ingredient been considered in the regulatory assessment?

2.2. Information requirements to enable QRA across the supply chain

The declaration of allergens intentionally present within a food presented to consumers due to their use as an ingredient or processing aid, is mandatory in most jurisdictions. To facilitate appropriate labeling of finished foods, information on the presence (yes/no) or (y/n) of regulated allergens is provided to partners earlier in supply chains. Such information is also provided between trading partners in the case of allergens that are unintentionally present.

However, in order to enable QRA for allergens, information is needed regarding the total amount of protein from the allergenic source in the final product or ingredient. Ideally for allergen QRAs there should be disclosure of allergen specific derivatives/components of supplied ingredients, their proportions of use and their protein contents. Unfortunately, in practice this is not always the case and there is only an indication that the ingredient contains an allergen (y/n) or 'may contain' an allergen through cross-contact (y/n).

In addition to a 'contains allergen: a, b, c' (y/n) statement, sometimes only nutritional/nutrient information will be available in order to understand protein concentration, while other times there may be more detailed information available such as the composition/proportion of the formulation which is coming from a specific allergenic ingredient. Allergen QRAs, albeit conservative and with high levels of uncertainty, may still be conducted on the basis of such limited information.

It should be noted that the intention here is not to obtain ingredients that are free from allergenic components, given that ingredients do not necessarily need to be free from allergenic components for a food to be acceptable. For the purposes of allergen QRAs, providing more information across the supply chain is not with the aim to make ingredients/products allergen-free, but ensure QRA is possible. Thus, more detailed composition information on the proportion of an allergen within a formulation may allow for QRA to calculate cross-contact scenarios that do not warrant the need for PAL (depending on the risk management framework). This concept is illustrated below.

2.2.1. Nutritional/nutrient information

Nutritional/nutrient values per 100 grams (g) or milliliters (mL) should be provided to fulfill legal requirements in many jurisdictions, and reported values will include but are not limited to:

- Fats, total (g per 100g)
- Protein, total (g per 100g)
- Carbohydrates, total (g per 100g)
- Sodium (g per 100g)

Specific requirements and necessary reporting will vary per market/jurisdiction, but these basic requirements are relatively easy to obtain if missing. More detailed compositional information may be missing because of confidentiality or because the supplier simply doesn't know. However, at a minimum the basic composition should be provided during the procurement process.

If no other information is available, the total protein information available from nutritional/nutrient specifications for an ingredient could be used to enable QRAs. In the case of complex ingredients, total protein information available from nutritional/nutrient specifications would be conservative as the complex ingredient is made from multiple components, however this information could be used in a further risk assessment if no other information regarding allergen protein amounts is available.

Examples of basic risk assessment calculations can be found in **Table 2** of this Chapter with the following three scenarios:

1. Nutritional/nutrient values per 100 g used for allergen QRA
2. Allergen specific ingredients and proportions available as inputs in allergen QRA
3. Change in usage levels from previously approved inclusion rates

2.2.2. Allergen specific ingredients and proportions

For the purpose of risk management decision-making on finished product labeling an ideal scenario for enabling allergen QRA would be the disclosure of allergen specific derivatives/components supplied ingredients, their concentration and their protein contents. However, in practice this is not always communicated, often there is only an indication if the supplied product/ingredient contains a specific allergen (y/n) in its formulation or if it 'may contain' an allergen through cross-contact (y/n). Furthermore, for the purpose of risk management of appropriate production controls, such as sanitation, allergen QRA may also be related to minor components of supplied product/ingredient, that intentionally contain allergenic protein. Again, understanding the concentration and protein content of the allergenic food within the product/ingredient is required information to enable allergen QRA.

In order to enable allergen QRAs across the supply chain that are of good quality, the following information is recommended to be provided for intentionally added ingredients:

- What food allergens are present in the ingredient formulation?
- Composition/proportion (%) of all derivatives/ingredients in product formulation to be declared as allergens
- The protein content (%) in each of the derivates/ingredients in product formulation to be declared as allergens

Regarding potential allergen cross-contact, the following information is recommended to enable allergen QRAs across the supply chain:

- What food allergens are potentially present through cross-contact?
- Type of derivative/ingredient causing potential cross-contact?
- Is the potential cross-contact due to shared lines/equipment?
- Has or can the potential cross-contact be quantified/estimated?
- If quantifiable, please list concentration and reasoning behind estimation

Section **5.1** provides information on the characteristics of cross-contact that require understanding or reasonable assumptions to enable allergen QRA. These include the reality that cross-contact may occur (the chance of occurrence), and the form, distribution, and concentration and frequency of cross-contact. Some information on all these parameters is needed to perform allergen QRA, however it would be too complex to require this comprehensive information for all ingredients from all suppliers, and so a targeted risk-based approach is recommended that begins with the collection of basic information (what is in cross-contact, why and concentration). It should be noted that compared to the type of information historically made available across food chains, it is a step-change to have information on the amount or concentration of allergenic ingredients and protein content within product information.

In this context, another consideration is that the resources invested to investigate ingredients as per their allergens risk, could be prioritized based upon the amount of ingredient that is in a finished product.

Information on the specification of ingredients is often collected from suppliers via questionnaires that include an allergen component. For more information regarding available Product/Allergen Information Sheets, see **Box 2** below. These sheets should not be seen as a stand-alone document but are intended to start a dialogue between the supplier and the

purchaser. When requesting a supplier to provide information for your specific form/questionnaire, it is important that an individual with suitable knowledge in allergen management completes the request.

Box 2. *Containing links / references to different available supplier allergen information sheets*

Name	**Market**	**Comments**	**Link**	**Freely available?**	**Includes the proportion of allergen in the recipe?**
Allergenen Consultancy	EU	Comprehensive. 'Intentionally added' assessment and 'Cross-contact' assessment Sections for allergen claims and qualifications regarding production site management of allergens to be documented.	https://www.allergenenconsultancy.nl/questionnaire Download the 'Allergenen questionnaire' for English-language form	Yes	Yes
Australian Food & Grocery Council (AFGC) Product Information Form (PIF)	AUS-NZ	Comprehensive. Online only (v5 was previously PDF form). Intentionally added assessment and Cross-contact assessment	https://www.afgc.org.au/industry-resources Note, version 7 soon to be released.	No	Yes

Form with both Intentionally added assessment & Cross-contact assessment on one page (from Allergenen Consultancy B.V.):

1. Allergen information Regulation (EU) No 1169/2011 Annex II Including products thereof	**Recipe/ product formula = present** (added ingredients, additives, carriers, processing aids etc. derived from an allergenic source)						**Cross contamination = possibly present** (unintentional presence due to production on the same equipment, used utensils, personnel, airborne contact or by other means).					
	Used as ingredient?		Type of ingredient E.g. peanut oil, soy lecithin, wheat starch, celery seed	Com-position	Protein content from allergenic source (%) [1]	Exempt from allergen labelling? [2]	Used on same line?	Cross- contact possible?		Type of ingredient(s) which could cause cross contact. E.g. peanut oil, soy lecithin, wheat starch	Type of contamination Homogeneous: powder, liquid of paste. Inhomogeneous: particles. Provide detailed information of the contamination[4]	
	YES	NO		%	%		YES	YES	NO		Homogeneous	Particle
Cereals containing gluten												
Wheat	☐	☐	ingredient name	% recipe	protein %	☐	☐	☐	☐	yes->ingredient name	☐ ppm	☐ grams, protein %
Rye	☐	☐	ingredient name	% recipe	protein %	☐	☐	☐	☐	yes->ingredient name	☐ ppm	☐ grams, protein %
Barley	☐	☐	ingredient name	% recipe	protein %	☐	☐	☐	☐	yes->ingredient name	☐ ppm	☐ grams, protein %
Oats	☐	☐	ingredient name	% recipe	protein %	☐	☐	☐	☐	yes->ingredient name	☐ ppm	☐ grams, protein %

Feedback received from external auditors about information actually provided upon request: Please note that these remarks are applicable for every detailed allergen info sheet. See section **2.3** for additional support and questions to help address difficulties in gathering allergen related information in the supply chain:

- The percentage composition of intentionally and incidentally present allergens (eg process aids) is often not provided, and the protein content is almost never provided.
- The source of cross-contacting allergens is sometimes not provided even if cross-contact is indicated, and the characteristics of contamination (eg heterogeneous particle, homogeneous liquid) are almost never provided.

2.2.3. Usage levels of ingredient in final product under assessment

The risk an ingredient potentially poses to cause a UAP of concern to allergic consumers (due to cross-contact with subsequent products made on the same production equipment) can also depend on the amount of that ingredient in the product formulation (and eventually eaten). Therefore staple ingredients used at high percentages in finished products present a higher risk, versus situations where ingredients are used in very low concentrations.

In this context, another consideration is that the resources needed to investigate ingredients as per their allergens risk, could be prioritized based upon the amount of ingredient that is in a finished product.

As demonstrated in **Table 1** the percentage of inclusion (%) for an ingredient can be translated to ppm (mg/kg), or vice versa, and this information can be useful in allergen related discussions. For example, an ingredient included at 0.1% in the final product formulation is present at 1000 ppm (mg/kg) in the final product formulation (before any conversions to protein equivalents, etc.). Thus, while it seems that 0.1% is a small percentage of the recipe, it could be a surprisingly large value for some individuals when expressed as ppm (mg/kg).

Table 1. *Comparison of percentage (%) inclusion for an ingredient and the ppm (mg/kg) equivalents.*

Percentage of inclusion (%)	ppm (mg/kg) equivalents
100%	1 000 000 ppm
10%	100 000 ppm
1%	10 000 ppm
0.1%	1000 ppm
0.01%	100 ppm
0.001%	10 ppm
0.0001%	1 ppm
0.00001%	0.1 ppm
0.000001%	0.01 ppm

This information, in combination with any conversions to protein equivalents could be used in allergen assessments, for example a PAL assessment for determining, "Does the risk of cross-contact with other products made on the same line warrant a 'may contain' statement?" [for more information on conducting these assessments in production facilities see **Chapter 2** or see **Chapter 5** for more information on characterizing cross-contact].

An approach utilizing bands of inclusion in a formulation processed on shared equipment (i.e., <0.1%, <1%, <10%...) when conducting PAL assessments or PAL carry-over assessments could be useful to allow companies to focus resources, such as analytical resources. Additionally, if a previously approved ingredient is desired to be utilized differently in the future, (approved at <0.1% in a product formulation now being used up to <1% in the formulation) then an information system should be in place where an alert would be raised for this ingredient. For example, "*This ingredient has been approved for usage levels up to 0.1% in product formulations with allergen information listed as follows [example allergen text]; higher usage levels will require reassessment of the ingredient for allergen labelling purposes.*" Again, it is understood that any allergens included in the product formulation will need to be declared, but the change in inclusion rates for this ingredient could impact any associated PAL assessments or PAL carry-through assessments.

As shown in the **Table 2**, changes in inclusion/usage rates of an ingredient could lead to a different risk assessment outcome compared to the initial assessment for that ingredient. Systems should be in place to alert the relevant teams within a company if an ingredient is being used outside the scope of its initial approval. Essentially this comes back to following

good allergen management practice where there is a good exchange of information from product design through to production and labelling.

Table 2. *Examples for basic risk assessment calculations based on information provided in product or supplier information forms. The use of the VITAL® 3.0 Reference Dose (RfD) is for example purposes and not an endorsement or rejection of any specific risk management system. For more information and key references regarding the fundamentals of how allergen RfDs are derived from oral food challenge data and subsequent dose-distribution models, as well as what might constitute an appropriate RfD see* ***Box Reference Doses****.*

1. Nutritional/nutrient values per 100 g could be used for allergen QRA:
While more detailed composition information/proportion of formulation may be desired, nutritional values could allow for worst-case QRA to calculate cross-contact scenarios that do not warrant the need for PAL (dependent on the risk management framework). This concept is illustrated below.
Example scenario when nutritional/nutrient values per 100 g could be used for allergen QRA: • *Complex ingredient is used in formulation of Final Product 1 (FP1) at 1%* • Complex ingredient is known to contain wheat • Exact form of wheat is unknown • Proportion of wheat-based component(s) in complex ingredient unknown • Nutritional/nutrient information of complex ingredient lists protein at 0.5g per 100g • FP1 is only wheat containing formulation on production line
1. Proportion/composition of complex ingredient in FP1 = 1% (10,000 ppm) 2. Nutritional/nutrient information of complex ingredient lists protein at 0.5g per 100g (0.5%) 3. 50 ppm total protein from complex ingredient in FP1 formulation (10,000 ppm * 0.5%) 4. Worst-case assumption: 50 ppm total wheat protein in FP1 formulation
This information could be used in the PAL assessment for the other, non-wheat products made on the same line as FP1. Does the risk of FP1 cross-contact with other products made on the same line warrant a 'may contain wheat' statement? 5. Particulate pieces of FP1 of up 1g (worst-case of 0.05mg total wheat protein) can be infrequently found in the production line after cleaning 6. A single particulate of 1g (worst-case of 0.05mg total wheat protein) could be incorporated in a following product with a package size of 50g 7. Worst-case exposure of 0.05mg total wheat protein in a final package 7.1. VITAL® 3.0 Reference Dose for wheat is 0.7mg total wheat protein Worst-case 0.05mg total wheat protein in a final package if cross-contact occurs, which is well below the Reference Dose (i.e., 7% of the Reference Dose).
Conservative assessments could be done using only the conservative protein values listed in the nutritional/nutrient information. If a potential risk is indicated then further refinement of the risk assessment could be possible through discussions with the supplier to obtain more information regarding the wheat-based component or potentially through wheat-targeted ingredient analysis.

Table 2 contiued *Examples for basic risk assessment calculations based on information provided in product or supplier information forms. The use of the VITAL® 3.0 Reference Dose (RfD) is for example purposes and not an endorsement or rejection of any specific risk management system. For more information and key references regarding the fundamentals of how allergen RfDs are derived from oral food challenge data and subsequent dose-distribution models, as well as what might constitute an appropriate RfD see* ***Box Reference Doses***.

2. Allergen specific ingredients and proportions available as inputs in allergen QRA:
If there is more detailed information available, the assessment can be refined compared to Example 1.
Allergen specific ingredients and proportions available as inputs in allergen QRA: • *Complex ingredient is used in formulation of Final Product 1 (FP1) at 1%* • Complex ingredient is known to contain wheat • Wheat flour is used at 1% in formulation of complex *ingredient* • Wheat flour is listed at 10% protein in nutritional *information* • FP1 is only wheat containing formulation on *production* line
1. Proportion of complex ingredient in FP1 = 1% (10,000 ppm) 2. Proportion of wheat flour in complex ingredient = 1% (10,000 ppm) 3. Nutritional/nutrient information of wheat flour lists protein at 10 g per 100 g (10%) 4. 0.001% (10 ppm) total wheat protein from wheat flour in complex ingredient in FP1 (1% * 1% * 10%)
This information could be used in the PAL assessment for the other, non-wheat products made on the same line as FP1. Does the risk of FP1 cross-contact with other products made on the same line warrant a 'may contain wheat' statement? 5. Particulate pieces of FP1 of up 1g (estimated 0.01mg total wheat protein) can be infrequently found in the production line after cleaning 6. A single particulate of 1g (estimated 0.01mg total wheat protein) could be incorporated in a following product with a package size of 50g 7. Estimated exposure of 0.01mg total wheat protein in a final package 7.1. VITAL Reference Dose for wheat is 0.7mg total wheat protein Total wheat protein content is 0.01mg in final package if cross-contact occurs, i.e., 1.4% of the Reference Dose for wheat.
The prior assessment example was already conservative, with seemingly no need for further refinement. The conservatism of the prior assessment is confirmed when the assessment was redone with specific information regarding the wheat-based component.

Table 2 contiued *Examples for basic risk assessment calculations based on information provided in product or supplier information forms. The use of the VITAL® 3.0 Reference Dose (RfD) is for example purposes and not an endorsement or rejection of any specific risk management system. For more information and key references regarding the fundamentals of how allergen RfDs are derived from oral food challenge data and subsequent dose-distribution models, as well as what might constitute an appropriate RfD see* ***Box Reference Doses***.

3. Change in usage levels from previously approved inclusion rates:
The prior assessment example had a complex ingredient initially assessed and approved in formulation of Final Product 1 (FP1) at levels of inclusion up to 1%, while new inclusion rates of up to 75% are being explored by product development.
Allergen specific ingredients and proportions available as inputs in allergen QRA: • Complex ingredient initially assessed and approved in formulation of Final *Product* 1 (FP1) at levels of inclusion up to 1% • *Product development now wants to use this complex ingredient as a bulk component in a new product (FP1a) at usage levels up to 75% in product formulation* • Complex ingredient is known to contain *wheat* (see example 2 in this table)
1. Proportion of wheat flour in complex ingredient = 1% (10,000 ppm) 2. Nutritional/nutrient information of wheat flour lists protein at 10g per 100g (10%) 3. 0.1% (1000 ppm) total wheat protein from wheat flour in complex ingredient 4. Proportion of complex ingredient in FP1a = 75% (750,000 ppm) 5. 750 ppm total wheat protein from complex ingredient in FP1a (750,000 ppm * 0.1%)
This information could be used in the PAL assessment for the other, non-wheat products made on the same line as FP1a. Does the risk of FP1a cross-contact with other products made on the same line warrant a 'may contain wheat' statement? 6. Particulate pieces of FP1a of up 1g (estimated 0.75mg total wheat protein) can be infrequently found in the production line after cleaning 7. A particulate of 1g (estimated 0.75mg total wheat protein) could be incorporated in a following product with a package size of 50g 8. Estimated exposure of 0.75mg total wheat protein in a final package 8.1. VITAL Reference Dose for wheat is 0.7mg total wheat protein Total wheat protein content is 0.75mg in a final package if cross-contact occurs, i.e., above the Reference Dose
Change in inclusion/usage rates could impact the risk management decision for PAL on other products produced on the same equipment.

2.3. How do you obtain the required information (Guide for questioning/obtaining info from suppliers)?

2.3.1. Transparent communication[5]

Transparent communication between producers and suppliers is crucial during the initial allergen risk assessment for introduction of a new supplier or ingredient, as well as during the ongoing risk management of allergens in the producers' day-to-day operations. It is good practice to have a defined procedure to approve a food supplier and consider their ability to provide reliably safe, quality products. As such, a company should have procedures in place for the selection of a new supplier and the ongoing approval of any existing suppliers. For example, adherence to a third-party certification scheme to a recognized Hazard Analysis Critical Control Point (HACCP)[6] standard (such as the Food Safety System Certification [FSSC] 22000, the ISO 22000 Food Safety Management System, or a GFSI-recognised certification program) could be used as one of the criteria for supplier approval and continued monitoring.

On a basic level, information from suppliers can be gathered through a number of different methods (eg questionnaires, audits, regular receival checks) and such information gathering should be as effective as possible. The information received should include answers to the following allergen-related questions, but the specific questions asked may vary depending on the specific situation but can be asked in a number of different ways to ensure the desired information is gathered.

It should be noted that if you decide to continue business with a supplier without first acquiring the necessary required information, then your company may be taking the risk/responsibility (due diligence) and not the supplier. In addition to transparent communication, as a part of supplier qualification, it may be advisable to generate data on the content of an ingredient which may contain allergenic protein(see **5.2.1.2.2** for information if needed).

[5] This section is largely referenced and reproduced from: Food Allergy Research and Resource Program (FARRP), n.d. Controlling Peanut Ingredients in Food Processing Facilities.

[6] For more information regarding General Principles of Food Hygiene or Hazard Analysis and Risk-Based Preventive Controls for Human Food, see the guidance documents from the Codex Alimentarius and US FDA.

These questions (and the questions in **2.3.2**) provide guidance for users who are developing an ingredient questionnaire:

- What food allergens are present in the ingredient formulation?
- What food allergens are present on the facility/site?
- What food allergens are present on the same production line? Can these food allergens cause residues which can cause UAP from cross-contact in following product?
- What food allergens are present on the adjacent production line? Can these food allergens cause residues which can cause UAP from cross-contact in following product on the production line in question?
- How is the supplier managing food allergens?
- What allergen control programs are in place?
- How is the supplier managing their upstream supply chain? (See questions in **2.3.2** for more potential questions)
- How will any changes to formulation or allergen controls be communicated to customers?
- How will any changes to precautionary allergen labeling be communicated to customers?

In addition to seeking responses to these questions, it is recommended to add simple, open questions to the allergen questionnaire/survey/form, including to check if the correct person with food allergen knowledge completed the response.

2.3.2. Assessing potential cross-contact in supply chain

Suppliers can be separated into two broad categories:

- Primary producers/Commodity ingredient suppliers
- Secondary producers/Complex ingredient suppliers

For complex ingredient suppliers, depending on the nature of the ingredient being shipped, the processes for allergen assessment and management will be potentially the same as the process described in the Management of Operations chapter for other final product producers (see chapter **3** for more information).

However, when dealing with primary producers and other members of the agricultural/commodity supply chain, a different set of assessment goals and questions may be helpful. As detailed by the (Allergen Bureau's Unexpected Allergens in Food 2021), Food Business Operators (FBOs) who source commodities should have a vendor assurance program in place which ensures that the answer to questions such as the ones outlined below are known, recorded, and included in the allergen risk review of the commodity ingredient. If the FBO is a supplier of commodity ingredients, they should be able to provide a considered allergen specification to their customer, who is encouraged to also refer to this specification when reviewing their ingredient information.

Questions such as those listed below are expected to facilitate more transparent lines of communication within the supply chain and continued dialogue/discussions in these areas will improve the overall awareness and competence of allergen assessments.

The questions listed below are in many cases copied or influenced by examples provided by the Allergen Bureau in their comprehensive document Unexpected Allergens in Food (2021, available here). For more product supply chain specific questions and examples, and for general educational purposes, we encourage reference to this document.

Dependent on the degree of UAP concern associated with a supplied ingredient, and the details of the supply chain in question, some of the below questions may be relevant to any specific situation.

Geographic location based questions

- What is the geographical origin of the ingredient (e.g., the geographic origin of the tree nut or peanut)?
- Are any wheat, sesame, mustard or soy crops grown in the same geographical region?
- Is there any wheat, barley, oats, spelt, lupin or soy crops grown in the same geographical region?
- Are any peanuts grown in the same geographical region?
- Are any tree nuts grown in the same geographical region? (please specify nuts with Latin names)
- Have other countries, that are included in the supply chain, been considered?
- What other crops are being (or can be) grown nearby?
- What other crops are used for crop rotation by the grower (e.g., many allergens can be used in crop rotation)?
- How much of the previous year's crop is present and harvested with the current crop?

Facility based questions

- Are other allergens regulated in your sale jurisdiction processed in the same facility?
- Are allergens allowed on site by any means (i.e., in the canteen/vending machines)?
- What is the policy on the presence of certain allergens on site (e.g., not allowed in production area, or not allowed on full site – including canteen/vending machines)?
- Does the primary and/or secondary processor have allergen controls within their facility?
- What effective measures are in place to remove allergen cross-contact from the prepared (washed, diced, de-husked, peeled, podded etc.) vegetables?
- What effective separation processes are used by the processor?
- What effective separation processes are used by the grain processor (e.g., milling facilities for oats can be shared with wheat, barley etc.)?
- What effective separation processes are used by the primary and secondary processors (e.g., sorting facilities for dried vegetables can be shared with wheat, soy products or dried vegetables with an allergen cross-contact etc.)?
- What effective separation processes are used by the pulse processor (e.g., pea flour milling facilities can be shared with soy etc.)?

Farm, harvest, transport and storage based questions

- Are storage silos and harvest equipment shared across farms and seasons?
- Are the crops early or late season crops? Early or late season crops may be close to other plants of different maturity e.g., immature/mature wild wheat.
- Is there crop rotation with peanut?
- What crop measures are in place to effectively remove physical remains of other crops?
- What effective measures are in place to prevent or minimise potential allergen cross-contact from maintenance machinery and harvesting equipment, including both large fragments and minute particles of residue?
- What effective measures are in place to prevent or minimise potential allergen cross-contact from shared storage equipment (e.g. silos) and facilities and/or bulk transportation containers?
-
- What seasons are the crops harvested in? This provides information about other plants nearby, shared equipment and shared storage.

Food form/shape and purchasing/sourcing

- How are the [ingredients] traded/sourced (e.g., through general markets with lesser known controls; contracted farms; controlled Backward Integration programs)?
- What crops are purchased from contract farms or wholesalers?
- What is the form of the [ingredient] (e.g., readily dispersible [powder/dust], a liquid, a particulate [split, whole, seed, leaf, pod, grit, hull, pearl, kernel, flake, coarse ground, pieces, meal], or an intermediate product [piece of dough, etc])?
- Is the allergen similar in size, colour and/or appearance as the ingredient supplied (i.e., difficult to clean and separate)(Allergen Bureau's Unexpected Allergens in Food 2021)?

3. Management of Operations

Introduction to the chapter

This Chapter provides guidance for the management of allergen cross-contact in production facilities, it is focused on the application of allergen QRA. However while it is the focus of this Chapter, QRA is only one the available tools for allergen risk management. Regarding general allergen control and options for allergen risk management other than QRA, they are briefly touched upon in this Chapter but more information is present in the **ANNEX: Guidance documents**.

This Chapter concerns the application of QRA within the management of food production processes, with a focus on assessing allergen risk resulting from cross-contact. The objective of allergen risk management is to estimate the potential for allergen cross-contact, and only in cases where cross-contact cannot be avoided, a PAL may be applied (**ANNEX: Guidance documents**). The application of QRA may be considered as part of the risk management decision making process, as it may provide additional data on whether PAL is appropriate.

This Chapter also includes text regarding appropriate application of QRA as a part of the validation of production line cleaning. A graded process is often used for the application of allergen controls in a production facility as described in this Chapter, particularly with respect to approaches for cleaning validations. When conducting a cleaning validation, visual inspection of equipment after cleaning is primarily used to ensure allergenic proteins (and other residues) have been removed. Additionally, a visual inspection can be supported by analysis, when feasible and appropriate, but it is not necessary for all cases (Codex Alimentarius, 2020). For example, qualitative analysis of swabs of food contact surfaces or final rinse water samples using suitably sensitive and allergen-specific Lateral Flow Devices (LFDs) can support the visual assessment of the cleaning procedure.

Not described in this Chapter are incidents that may occur in food operations that potentially result in high risk to allergic consumers, such as wrong labeling, a wrong ingredient being used, or mispacking. The management of allergen incidents is described in Chapter **4**. In context to this Chapter, it is assumed that these issues, in addition to potential cross-contact of supplied ingredients described in Chapter **2**, are effectively managed. Concerning routine food operations, production processes are often validated within the context of normal HACCP and GMPs. As a part of these practices, when new processes, products or ingredients introduce or potentially alter the allergen cross-contact risks of a facility, allergen management planning should be reviewed and updated as needed as there may be changes to the allergen risk profile of products produced at the location.

In this Chapter, there is a review of how and where in the process of producing a food product it may be appropriate to apply allergen QRA. The described process is a systematic approach to assess risk in individual steps of the production process. The Chapter provides the key elements/areas in allergen management for site operations, indicates the appropriateness of applying a QRA (note that a qualitative approach should first be applied, and only when it is not possible to eliminate that there is a risk of cross-contact, should QRA be considered), indicates the requirements of the inputs for the QRA, and provides guidance on how to gather and qualify the required information. Examples are used to illustrate the approach and there is extensive reference to other sections of this Guide.

3.1. QRA within Allergen Control Programs

UAP in a food that does not contain the allergen in the recipe can result from cross-contact of that food with a food containing the allergen during the production process. Food allergen RA is the estimation of the frequency and nature of potentially adverse effects experienced by allergic consumers, due to a specific exposure scenario to an allergenic food substance in the consumed final product. In food production, the FBO commonly determines the risk of UAP

using a HACCP-based risk assessment approach which considers the hazard (allergen) and risk (likelihood of undeclared allergen due to failure of a control).

The HACCP-based risk assessment will identify allergen controls (risk management measures) which will require validation to determine their effectiveness. The type of validation undertaken will largely depend on the nature of the control and could involve either qualitative or quantitative measures as will be explained later in this section. This validation process will become the basis in deciding if PAL is needed. Only cases where the risk cannot be mitigated to the desired extent (i.e., the control measure is not adequate at eliminating cross-contact) should there be consideration of a PAL being applied onto the finished food product. The HACCP-based risk assessment is built on a foundation of GMPs which are there to ensure that risks related to the environment, processes and people are in control. It is common for FBOs to manage allergens as a Universal PRP as allergenic cross-contact risks may be present at multiple stages of the manufacturing process and need to be controlled more widely than through discrete process flows which are assessed within the HACCP process. All robust HACCP plans are based on a foundation of robust prerequisite programs.

3.2. Guide on QRA within Site Cross-Contact

The assessment of controls usually considers a combination of both qualitative and quantitative evidence to determine whether they are adequate at preventing cross-contact. Qualitative evidence includes factors such as knowledge of the production process, training, GMP audits or process / records checks and can incorporate a large amount of uncertainty, as under normal operating conditions you are trying to predict if the system will fail and give rise to UAP. Quantitative evidence may be based on estimates of carry-over and/or analytical data linked to a structured sampling plan which is also open to significant uncertainty such as robustness of analytical methods and sampling for heterogeneous cross contact. Important factors in managing allergen cross contact points are an understanding of the scenario. This encompasses: an understanding on the physical reasons for potential cross-contact, the chance that it will actually occur or not, and the characteristics of the resulting cross-contact (the form, distribution, frequency and concentration of cross-contact). A scheme for understanding and describing these parameters is provided in the Core Concepts section (see **5.1**).

In cases where a cross-contact point is identified, and the chance of occurrence cannot be eliminated with adequate confidence such that a QRA may be deemed appropriate, it should be explored whether the QRA is possible; in essence, the conduct of QRAs can be simple to perform. The QRA should aim to gather and incorporate all reasonable knowledge including uncertainty surrounding the UAP scenario for a characterization of risk that is as complete as possible.

To illustrate these steps and considerations, in this Chapter we use an example scenario developed for a ravioli manufacturing process. This example of a pre-packed ready meal manufacturing process illustrates how to assess where there is a risk of egg cross-contact due to shared manufacturing lines and equipment (Case Study 1). Also provided is commentary on other types of cross-contact and how these would differ from the first example for egg. There are two other examples developed for reference, Case Study 2 and Case Study 3, which can be found in **ANNEX Examples Management of Operations**.

Robust allergen risk assessment is the foundation of effective allergen management. The assessment of risk requires careful consideration of intentional allergen presence from ingredients used in the recipe and unintentional allergen presence through cross-contact. The scope of the risk assessment must cover all stages of the food production process from the primary production of raw materials through to the dispatch of finished products. As such this Chapter should not be used in isolation, **Chapter 2** on information from the upstream supply chain is a critical input into risk management decision making (Flanagan 2015).

Concerning food production systems that are under direct control, food allergen RA includes the identification of potential sources and points of cross-contact, the chance that these identified sources and points may actually lead to cross-contact (chance of occurrence), the characterization of cross-contact (including where possible an understanding of the form and distribution, frequency and concentration of cross-contact) and development of suitable controls. The final step before the update and implementation of the allergen management plan is the validation of those controls, and where cross-contact risk cannot be excluded with sufficient confidence, the assessment of residual risk to inform risk management decision making **Figure 4** provides an overview of this process flow which is further elaborated in **Box 3**.

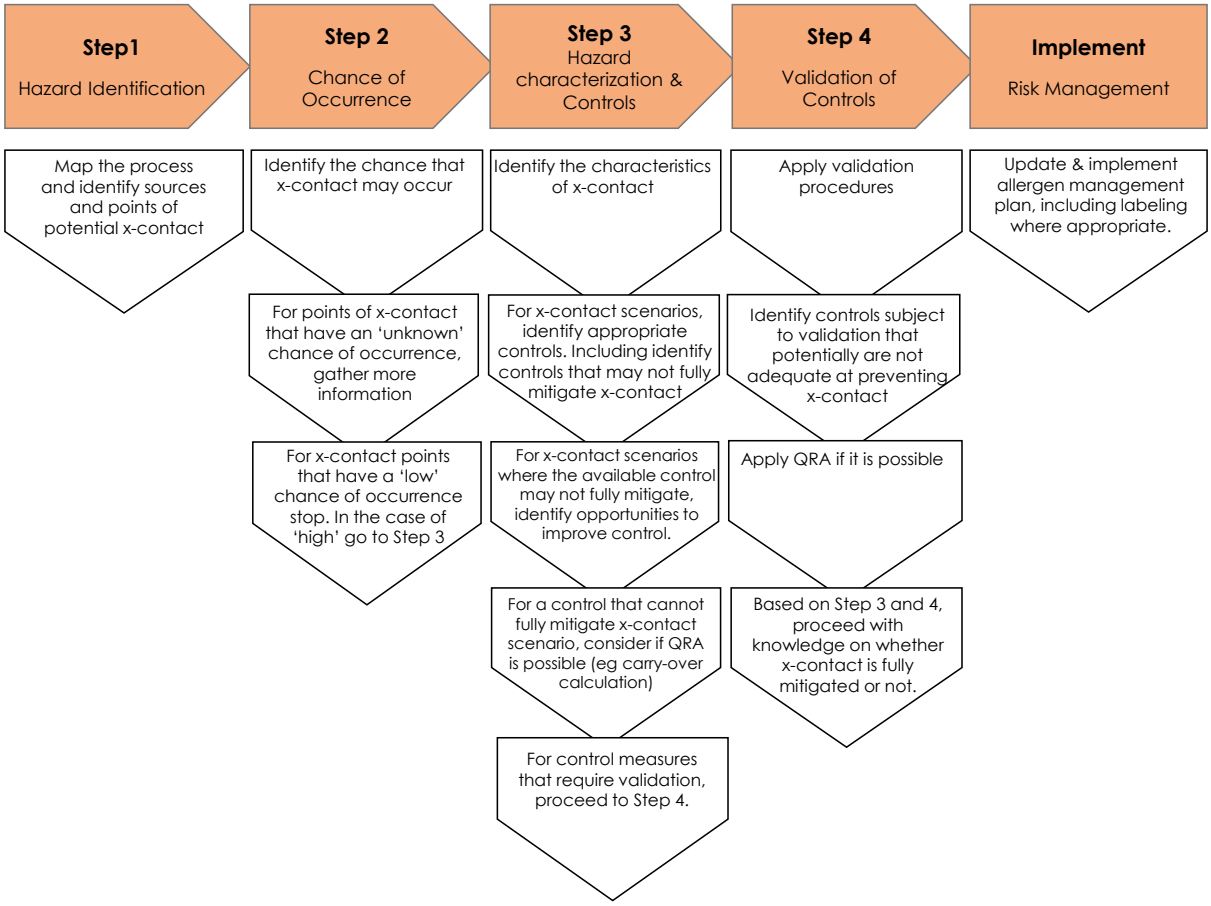


Figure 4*.Process for the management of allergen cross-contact within production processes under direct control, including the role of QRA within that process.*

Robust allergen HACCP-based risk assessments are used to identify where allergen hazards occur and whether the existing controls can manage the potential risk (chance of occurrence) under normal operating conditions and good manufacturing practice. Such risk analysis should be undertaken by appropriately trained experts, such as members of HACCP teams, as an integral part of the manufacturer's quality and food safety system (see **Box 4** for further information on the legal framework of HACCP). The Allergen HACCP-based risk analysis can be described in 4 steps which will be further explained in the following paragraphs using Case Study 1.

Box 3. *Allergen HACCP-Based Risk Analysis Steps*

Step 1. Hazard Identification
Identify sources of potential presence of Allergens as Unintentional components within the production system being evaluated; this should cover all stages of the production process (ingredients inputs, processing and change over procedures). This process usually comprises the following steps:
• Allergen Mapping – Ingredients, formulation/recipe, production flows, taking into account the following sources of cross contact risk:
- o Employees (people)
- o Method (production process)
- o Material (raw materials)
- o Machine (equipment)

Step 2. Chance of Occurrence
For the identified allergen hazards estimate the resulting chance of occurrence of UAP based on knowledge of the UAP scenario, including the potential for failure under normal operating conditions of control measures already present. When chance of occurrence is 'unknown' perform additional investigations to clarify status.

Step 3. Hazard Characterisation & Control
Where there is an identified chance of occurrence, establish the characteristics of cross contact for each UAP scenario, and develop control measures against those scenarios. Identify the expected success of the control measure, and in situations where there may not be full mitigation of x-contact, consider whether QRA is possible.

Step 4. Validate control measures
For control measures that are not able to mitigate the chance of UAP occurring, assess the allergen risk in finished product (including QRA where possible) and apply appropriate risk management measures (such as PAL).

Implementation involves the update of the allergen management plan, including routine verification and monitoring.

3.2.1. Case Study – Example Foods - Case description of a process with homogeneous cross-contact.

In this Case Study, fictional food business, Example Foods, makes fresh pasta, including ravioli. In their production site located at Exampleville, they make both the dough and the filling. Their products are sold exclusively in the European Union. They have determined that cross-contact is not reasonably likely to occur within either their supply chains or production site with crustaceans, peanuts, nuts, celery, mustard, sulphur dioxide, lupin, and molluscs. The HACCP team has found that allergenic food sources, including eggs, sesame, milk (including lactose) and fish may occur in the processing facility either as cross-contact with supplied ingredients or as cross-contact residues at the production site. For the further assessment, the focus will be on eggs in this example, wherein the concern is cross-contact between product made using eggs and subsequent product made on the same line that does not contain egg as an ingredient. Example Foods want to explore the use of QRA to see if PAL for egg is needed.

In the schematics below, we have the defined process flow (allergen mapping) for the manufacturing of egg-containing ravioli (**Figure 5**).

You can see from the boxes highlighted in orange the parts of the process flow where there is a potential risk of egg cross-contact. These are areas identified for further assessment to ascertain if current controls are effective. For additional reading the Annex **7.4** provides the process flow for two other Case Studies for reference. Case Study 2 outlines the risk associated with sesame seed cross-contact in a bakery, where some of the products manufactured have sesame seeds applied as a topping. Case Study 3 describes the risk associated with potential cross-contact of milk in a fruit drink production scenario.

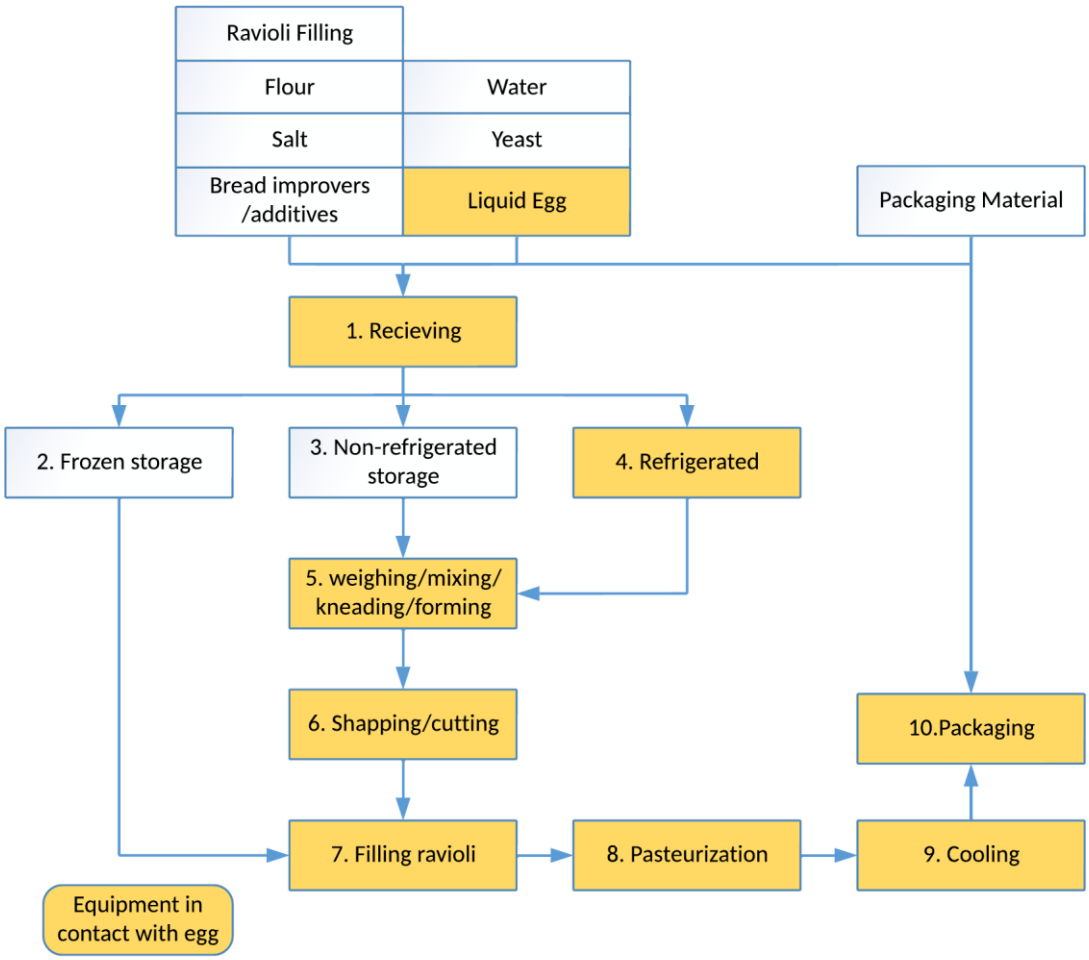


Figure 5. *Case Study 1 Example – Cross contact with egg. An example of wet and dry cleaning homogenous contamination. Orange boxes indicate equipment that comes into contact with egg; blue boxes indicate areas and equipment not in contact with egg. For a detailed description and explanation of this example, the user is referred to* ***Box 5****. Other case studies are described in the Annex* ***7.4****.*

As highlighted in the process flow for the Case Study (**Figure 5**), due to the nature of the process (blending/mixing/depositing), physicochemical nature of the allergen (liquid) and where the allergen is added in the process (at the start), the liquid egg cross-contact is likely to be homogeneously distributed within the subsequent product. If instead the allergen was sesame and it was applied at a later stage in the production process such as the 'filling' stage (7), then the allergen would likely be heterogeneously distributed in subsequent product, due to it being a particulate form of the allergenic ingredient (see Case Study 2 in Annex **7.4.1**).

3.2.2. The Role of GMP's in Allergen Management

Before continuing with the Case Study, the role of GMP's in allergen management should be explained. Food businesses should operate in line with GMP principles. This requires a commitment to ensuring that products meet food safety, quality and legal requirements, using appropriate manufacturing operations controls, including effective food safety and quality assurance systems. Adherence to existing GMP controls is essential for allergen management, for example, avoiding cross-contact by physical means such as an appropriate combination of available methods, including segregation of production lines with dissimilar allergens within a production facility, using allergen cleaning between products produced on the same lines that have dissimilar allergens, use of separate utensils for products with specific allergen

combinations, line dedication, equipment and storage dedication. These measures should effectively prevent allergen cross contact to a large extent. Although this is not an exhaustive list, the GMP activities described in **Figure 6** are usually covered within FBO's prerequisite programs.

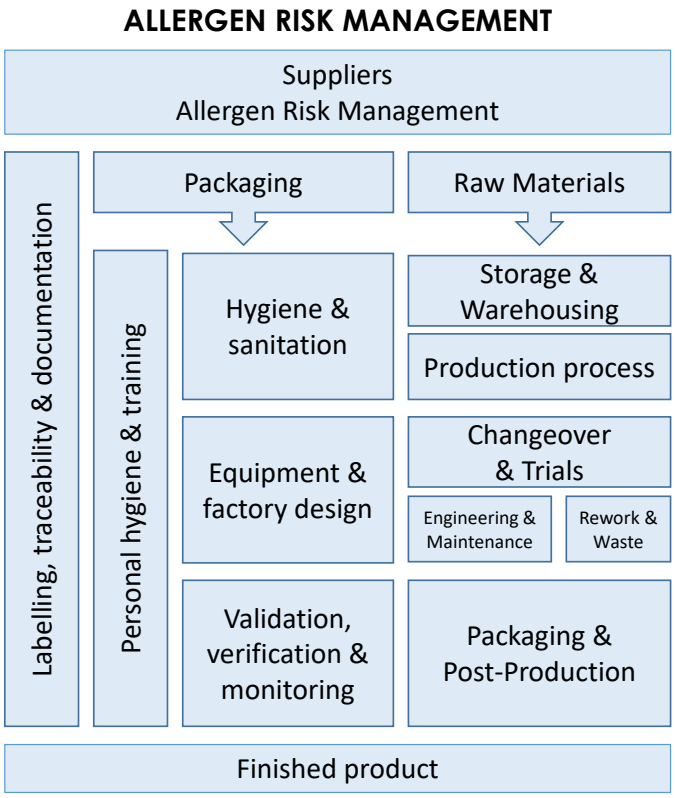


Figure 6. *The GMP activities usually covered within food business operator's prerequisite programmes integrated in allergen management.*

A failure in any one of these programs could potentially result in the risk of presence of an undeclared allergen. **Figure 6** illustrates the integration of GMP activities associated with effective allergen management. For an allergen control plan to be effective, it must be based upon an effective and functioning GMP programme. Only once these programmes have been demonstrated to be working effectively can the allergen risk assessment process be started, and ultimately effective allergen management is achieved. It is also worth noting that many of the issues associated with poor allergen control find their root cause in poor GMP practices and are potentially preventable. The management of allergens as a prerequisite programme is common place in the food industry due to the fact that allergen cross-contact risk can be present in many stages of the manufacturing process and therefore need to be controlled more widely than through just discrete process flows which are usually assessed within the HACCP study (Flanagan 2015). See **Box 4** for the legal framework.

Proper control of ingredients, recipes, and labelling of final products are key pre-requisites for food allergen management. Loss of label control, for example, is a leading cause of food allergen related recalls, and may result in higher public health risks than cross- contact.

From a QRA perspective, many of the GMPs listed above can only yield qualitative data and evidence (visual checks / challenge tests / records / audits, etc.), and as such need to be carefully assessed in order to support a quantitative assessment.

3.2.3. Step 1: Hazard Identification: Assessing Unintended Allergen Presence (Cross-Contact)

The first step in hazard analysis in a HACCP-based risk assessment for food allergens is the identification of relevant food allergens, which may be reasonably expected to occur at each process step (including production, acquisition, storage, transport and handling of raw materials and ingredients and impact of delays during manufacture on cross-contact). This is a complex task that involves documentation provided by suppliers (Chapter **2**), information about potential cross-contact in the supply chain from internal and external sources, and potential other sources (e.g., from personnel). As noted by Codex Alimentarius (2020), the list of recognised major food allergens varies in different countries; moreover, there is the potential for additional major allergens to be identified in the future (for example, sesame in the United States which was added to the list of priority allergenic sources with the passage of the FASTER Act in 2021). Account should be taken for all applicable legislation (allergens regulated in countries where the food will be marketed). The same is true for sourcing raw materials from countries with different regulated allergens. Products should not be considered compliant for a certain market unless the underlying risk assessment of the supply chain and production site has covered all relevant food allergens for that market (section **2.1**).

Potential cross-contact allergens may be those that are intentionally used in products that are run on the same equipment, or allergens that may be unintentionally present either due to cross-contact upstream (for example with ingredients) or that are present at the site with theoretical possibly of cross-contact. It is necessary to identify the key areas in manufacturing where cross-contact between allergen-containing and non-allergen ingredients and products can occur and identify the likelihood of undeclared allergen presence in the finished product.

This task is best completed by a multidisciplinary site HACCP team using the process flow diagram for the specific line/process being studied. Once the process flow has been determined it needs to be confirmed by the team by a physical inspection of the line / area under investigation which is best accomplished by using a flow diagram that describes the product and process flows of allergenic ingredients and how they come together to form finished products ("Allergen mapping"). A schematic of the manufacturing operation as described in **Figure 5** can be useful for this task. By using this approach, it will be easy to visualise potential points in the production process at risk of allergen cross-contact which can then be subject to a more detailed inspection as a part of Step 2. Mapping should be conducted for all the concerned products, ingredients, processes, and production lines with the goal of highlighting their respective allergen profiles, all potential carry-over zones, potential cross-contact allergens and rework added to the processes / lines. It is important to note that the standard flow chart required for HACCP may not be sufficiently detailed for allergen risk assessment as it does not encompass allergens specifically. For example, flow diagrams developed for HACCP have to be enriched with allergen flows using a schematic of the manufacturing plant layout (as in **Figure 5** and described in detail in **Box 5** for Case Study 1).

Using a HACCP-based risk assessment approach, all steps involved in the process should be examined in sequence and presented in detail. Operational/line downtime should be considered in terms of the resulting hazard, for example the opportunity for cross-contact if production lines are in close proximity or you have line cross-overs. Where possible, the flow diagrams should include a plan of the working premises, equipment layout and characteristics, and segregation of allergen zones (areas where allergens are/are not allowed).

In order to evaluate the chance of occurrence of allergen cross-contact, the allergen map (cross-contact flow chart) can be analysed as described in **Table 3**. This will lead to the identification of potential areas where allergen cross-contact (CC) risk is present. Color coding such as the orange highlight in our example visually highlights these cross-contact areas.

Table 3. *The possible risk for allergen cross-contact at Process steps 1- 10 of Case Study 1– Cross-contact with egg of* ***Figure 5****.*

	Process step	CC Risk	CC Risk
1	Receiving	Yes	breached egg bins (plastic containers)
2	Frozen storage	No	separate storage area
3	Non – refrigerated storage	No	separate storage area
4	Refrigerated storage	Yes	egg stored with other chilled raw materials -> breached egg bins
5	Weighing/mixing/kneading/forming	Yes	egg used in the dough mix –> sanitation issues
6	Shaping/cutting	Yes	egg shared shaping equipment –> sanitation issues
7	Filling ravioli	Yes	egg shared filling equipment –> sanitation issues
8	Pasteurization	Yes	shared cooking vessels –> sanitation issues
9	Cooling	Yes	shared cooling equipment -> sanitation issues
10	Packaging / Labelling	Yes	shared packaging equipment –> sanitation issues

It is important not to overlook the risk of cross-contact between different allergenic ingredients particularly if allergens are not stored in dedicated or segregated areas (i.e., allergen cage) or subject to specific handling requirements using dedicated equipment. In essence all three of the mapping exercises (ingredient, recipe and process flow) will reveal which products / processes need to be controlled to prevent allergens from getting from 'where they should be to where they should not'. During the final stage of mapping, it may also become apparent that certain allergens are used in the majority of recipes produced, therefore careful consideration should be given to those ingredients / recipes and processes where they are not used (i.e. the identification of vulnerable ingredients / finished products) (Flanagan 2015). Production scheduling can help to minimize the number of cleaning changeovers needed by adding to the allergen profile of subsequent products. For example, if we start with the egg-containing ravioli product that is produced first in the schedule, followed by an egg and milk containing ravioli product next, and finally the production of an egg-milk-sesame-containing ravioli product, before changeover to a product that does not contain these allergens. This production schedule allows the FBO to build upon the allergen profile while minimizing the number of allergen cleaning changeovers.

Having established the points in the process where cross-contact could potentially occur, the next step is to focus on specific risks arising from the following factors / activities:

- Employees
- Method (production process)
- Materials (raw materials)
- Machine (equipment)

When conducting a risk assessment, information must be accurately recorded in a standardised format using a proforma such as the one detailed in **Figure 5** (and in **ANNEX Examples Management of Operations**).

Specifically:

- Area of site / process step under consideration
- Area of concern of potential allergen cross-contact
- Allergen of concern in contact with which ingredient / product

Using the example of the ravioli manufacture scenario, some examples of allergen risks related to the specific factors and activities are presented in **Table 4**.

Activities such as shared storage, handling, mixing, transportation, cross-over / spillage points, shared cleaning equipment, shared production / packaging equipment and lines should also be considered during the assessment process.

Table 4. *Examples of allergen risks related to the specific factors and activities in the ravioli manufacture scenario.*

Employees - Assembly area	Operatives moving between different lines with different allergen profiles without washing soiled hands or changing soiled PPE (Personal Protective Equipment) where appropriate.
Method - Waste handling	Empty egg pallecons (containers) are moved through a sensitive production area.
Materials - Storage	Egg container stored on top of non-egg container (milk container for example)
Machine - Design	Hopper that feeds liquid egg into the mixer is not hygienically designed therefore difficult to clean.

3.2.4. Step 2: Chance of Occurrence

Having identified the specific points of potential allergen cross-contact through allergen mapping exercises, the next step is to determine the chance of UAP, i.e., under normal operating conditions is the risk high, medium, low or unknown (see Section **5.1.1**, **Table 11**), where others prefer to use the terms probable (likely to happen) or remote (unlikely but not impossible) which align with the general principles of HACCP and are commonly used within that context. We've chosen to list both options in this Chapter. What this step in the risk assessment seeks to do is determine the chance that allergen cross-contact will occur and therefore provides information for the next step concerning control measures used for the minimisation of the potential for cross-contact. These measures should be practical and sufficiently robust to be effective. The rationale for the evaluation should be documented. Sometimes this assessment of probability can be very objective, for example finding visible residue of an allergen on previously cleaned equipment and sometimes it is more subjective. When conducting this assessment, it is critical that the operatives involved with the specific process or activity are involved in the assessment process as they will have 'first-hand' knowledge of whether controls have failed in the past. From a risk assessment perspective, any risks which are deemed to be remote (i.e., of 'low' chance of occurrence) would not normally require further action; however, it is critical that the rationale for this decision is fully documented (Flanagan 2015).

For the ravioli scenario (Case Study 1), you can see the process steps where there is a chance of UAP and the controls which have been identified (**Figure 5** and **Table 5**). From a QRA perspective you will see that the availability of evidence / data is a mixture of both qualitative and quantitative data. In Annex **7.4**, these details are provided also for other processes in the scenarios for the bakery with sesame (Case Study 2) and the milk in a fruit drink (Case Study 3).

Table 5. *Case Study 1 Example – Cross contact with egg. Identification of the effectiveness of controls for process steps of* ***Figure 5*** *and the availability of qualitative and quantitative data to examine the effectiveness of these process controls. Chance of occurrence relates to the likelihood of UAP occurring at a given process step. Data refers to the quality of evidence required to conduct either a qualitative or quantitative risk assessment.*

	Process step	Control	Chance of occurrence*	Data
1	Receiving	Goods receipt checks	Not likely/ Remote*	Qualitative
2	Frozen storage	Physically segregated	Not likely/ Remote	Qualitative
3	Non-refrigerated storage	Segregated storage / racking in chill store	Not likely/ Remote	Qualitative
4	Refrigerated storage	Physically segregated	Not likely/ Remote	Qualitative
5	Weighing/mixing/ kneading/forming	Dedication of sieves	Likely/ probable	Qualitative
		Cleaning of mixing equipment	Likely/ probable	Quantitative
		Cleaning of kneading equipment	Likely/ probable	Quantitative
		Cleaning of forming equipment	Likely/ probable	Quantitative
6	Shaping/cutting	Cleaning of shaping equipment	Likely/ probable	Quantitative
		Cleaning of cutting equipment	Likely/ probable	Quantitative
7	Filling	Cleaning of filling equipment	Likely/ probable	Quantitative
8	Pasteurization	Cleaning of cooking vessels	Likely/ probable	Quantitative
9	Cooling	Dedication of cooling racks	Likely/ probable	Qualitative
		Cleaning of cooling racks	Likely/ probable	Quantitative
10	Packaging	Cleaning of packaging equipment	Likely/ probable	Quantitative

* The terms probable (likely to happen) or remote (unlikely but not impossible) can be related to the chance of occurrence in Section **5.1.1** as follows: probable (high or medium), remote (low).

3.2.5. Step 3: Hazard Characterisation & Controls

Once the chance of occurrence has been determined, the next stage is to characterise the hazard associated with all cross-contact risks identified in the previous step that are medium or high (probable). Section **5.1.2** of the Core Concepts section provides additional information on how to capture the known characteristics of a cross-contact hazard. There are a number of key factors which drive the amount of allergenic protein that a consumer may be exposed, and therefore can influence the ability of an ingredient / finished product to trigger an immune response in an allergic consumer and the severity of the hazard, and ultimately, the level of control needed to manage the risk / hazard. These factors include:

A. Allergen protein concentration
B. Physical form of the allergen
C. Amount likely to contribute to cross-contact

A. Allergen Protein Concentration

The protein component of the allergenic food is responsible for eliciting the allergic reaction in sensitised consumers. The lower the allergenic protein content, the lower allergenic potential of the foodstuff. Materials with very low levels of protein may present low or very low levels of risk potential but would still need to be labelled in the ingredient statement and handled as an allergen as per the country or region-specific regulations. Some derivatives of allergic foods have been exempted from mandatory allergen labelling on the basis of dossiers demonstrating the lack of allergic protein and therefore likelihood of reactions upon food challenge or other pertinent clinical criteria, these derivatives are listed in EU labelling legislation. Examples include highly refined oils derived from allergens such as refined soya bean oil, or highly processed allergen derivatives such as wheat maltodextrin. These all have extremely low protein concentrations, and therefore have low allergenic potential. As not all countries have exemptions, it is important to check local regulations if exporting outside of the EU (see section **2.1** for more information).[7]

B. Physical Form of Allergenic Ingredients

Particulates and food fragments (nuts, seeds, chunks, solid agglomerates etc.) will usually remain intact and could potentially appear as non-homogeneous (hot-spot) cross contact. This will potentially deliver higher doses of unintended allergenic material to the consumer (see section **Particulate allergen cross-contact**). Readily dispersible unintended allergenic material includes powders or liquids in homogeneous form (e.g., milk powder, soya flour). Assuming cross-contact occurs between similar phases (liquid into liquid or powder into powder), they are likely to appear evenly distributed throughout a product, especially if their process has mixing after the point of cross-contact. To illustrate this point, if the suspected UAP was 1L of cow's milk cross contact in 10,000L of soya milk, the cross contact would potentially be diluted 10,000-fold. If, however, the same volume of UAP solid peanut fragments were in contact with the same volume of soya milk, these would remain as discrete particulates and not be diluted. However, a cross-contact point that results in particulate cross-contact should not automatically lead to a precautionary label. As described above, the assessment of the probability of such cross-contact, combined with the factors described in earlier sections, should be used to identify the potential risk of the final product to the allergic consumer and therefore appropriate risk management action.

C. Concentration of Allergen Likely to Contribute to Cross-Contact

During the risk assessment process, it is important to try to roughly estimate the physical amount and form of the allergenic source likely to contribute to cross-contact of other products made on the same line or equipment that do not contain that allergen. This in theory could range from very small traces (milligram levels) to gross-level cross-contact (grams or whole units of residual product). This would obviously present different levels of risk to the allergic consumer and potentially require different controls. The user is referred to the Core concepts chapter UAP Scenarios and characteristics of cross-contact, and especially **section Concentration or Quantity** and **Carry-over calculation guidance**.

Two example scenarios could be:

- Lower risk – a small amount of allergenic residue hung up in a valve, which will be introduced into subsequent product as it flows into a large mixing tank
- Higher risk – either a finished product or a Work-in-Progress (WIP) containing an allergen not present in subsequent product, but still present on the line following a product changeover.

[7] A summary of EU exemptions from Annex II labelling requirements is provided by FDE in the Allergen Labelling Annex of their Guidance on Food Allergen Management for Food Manufacturers

3.2.6. Step 4: Validate control measures to minimise the risk of unintentional allergen presence

A. Assessment of Control Measures.
This next stage of the risk assessment process seeks to determine whether appropriate control measures are currently in place or need to be implemented to minimize the risk of allergen cross-contact. This is referred to as risk management and determined through a process of monitoring, validation and verification. Validation work should be carried out and documented for each control measure/combination of control measures. Cleaning is a commonly applied control measure as it usually provides the break between allergen-containing and non-allergen-containing products produced on a shared production line or component thereof. If the control measure has been implemented previously, the results from

this historical work can be used as an input into the validation study. Further details relating to cleaning validation are set out in the section **3.3** below.

It should be recognised that on-going verification of control measures will still need to be undertaken, after allergen risk assessment has been completed and the requirements implemented, using a variety of methods to ensure it is working effectively in practice. This may include audit, inspection for visual and physical clean status, data analysis and review, or in some instances additional sampling and analytical testing.

There are usually three discrete outcomes following the validation of existing allergen control measures that are commonly conducted during ongoing verification of controls:

1. Existing controls are sufficient to minimise risk of allergen cross-contact to an 'acceptable level' (see section **3.3** for additional information)
2. Existing controls need to be improved to minimise the risk of allergen cross-contact to an acceptable level (e.g., revised cleaning methods). Once changes have been made the control will need to be re-validated.
3. Existing controls cannot be modified to reduce the risk to an acceptable level due to technical constraints such as configuration of equipment, difficulty in cleaning or issues with supplied ingredients. In this outcome, risk assessment, including QRA when possible, can be undertaken to inform risk management (e.g., precautionary labelling)(Flanagan 2015).

Concerning point number 3, when there is a need to perform an assessment of residual risk arising from a cross-contact point and its control measure, there are both qualitative and quantitative inputs that can be considered. Note for the conduct of a QRA it is only the quantitative inputs that may provide sufficient data.

B. Qualitative Inputs into an Assessment of Residual Risk
Returning to the ravioli scenario, **Table 6** details the qualitative evidence that can be gathered in the risk assessment. As you can see, there is a heavy reliance on training and auditing. The assessment of training can be observational (checking that staff are following set procedures) or through questioning during line briefings. From a GMP auditing perspective, you need to ensure that all designated controls (qualitative/quantitative) are part of the audit schedule. These controls should also be weighted on the quality of evidence in order that these can be included in the quantitative assessment.

Table 6. *Qualitative evidence for consideration in the risk assessment and possible quality of data for quantitative risk assessment.*

Qualitative validation	**Controls**	**Quality of evidence for QRA**
Good receipts checks [P1]*	• Training • Visual aids • Visual inspection • Quarantine-challenge testing	Insufficient
Segregated storage [P2/3]	• Training • GMP audits • Spillage procedures	Insufficient
Dedicated storage [P4]	• Training • Signage • GMP audits	Insufficient
Dedication of equipment [P5/P9]	• Training • Labelling/color coding	Insufficient
Enclosed/packaged product [P10]	• Training • GMP audits • Packaging integrity checks	Insufficient

*[P1], [P2], etc. refer to the process steps described in **Box 5**.

C. Quantitative Inputs into the Assessment of Residual Risk

The quantitative inputs necessary for QRA rely either on estimations of carry-over or analytical data linked to a robust sampling plan. These will be described in more depth in the **Carry-over calculation guidance** and **Allergen Sampling and Analysis** sections. Concerning samples related to cleaning effectiveness, should they be available, there are different types of samples that can be gathered. **Table 7** summarises the different sample types and their relevance to establishing the robustness of allergen controls.

Table 7. *Sample types and their relevance to establishing robustness of allergen controls.*

Sample types	**Relevance to allergen control**
Finished products/Prepared foods	Direct measure of amount of allergen the consumer is exposed to
Ingredients used in finished products/Prepared foods	Direct measure of amount of allergen the consumer is exposed to (dilution needs to be considered)
Rinse waters	Indirect measure of effectiveness of automated cleaning systems, poor correlation with amount of allergen consumer is exposed to
Settle plates	Indirect measure of environmental cross-contact, can be used to calculate an estimated amount of allergen the consumer may be exposed to in a finished product, but includes significant assumptions and uncertainty
Environmental swabs	Indirect measure of effectiveness of cleaning of food contact surfaces, poor correlation with amount of allergen the consumer exposed is to

Returning again to the ravioli example, a cleaning validation is undertaken which is initially based on visual inspection of equipment to confirm that the food contact areas are visually and physically clean. Further, a validation is often comprised of a combination of swab samples and qualitative analysis, and if a QRA is utilized, finished product can be tested to provide quantitative information regarding potential residual allergen cross-contact. If residue is detected and it is not possible to improve cleaning procedures, the available data if it is considered to be suitably representative may be input into an allergen QRA.

As a general guide, if it is determined that equipment swabs should be taken, the use of a 'risk-based' approach that is developed during the allergen mapping should be utilized. In practice, the swab locations are determined by worst-case scenario (difficult to clean / access / hang-up points) and may comprise different types of contact materials which make up the line (stainless steel, rubber, PTFE, etc.) as these would have different adhesive properties. The results of the swabbing would be considered as 'semi-quantitative' as there is no direct correlation with the microgram (µg) quantity of protein from the allergenic source/swab and concentration of carry-over allergen in finished product. A positive swab would only highlight that further investigation is needed, cleaning/sanitation at that point on the line reviewed, and a more effective method of cleaning should be implemented as needed. Returning to the example, the acceptable limit determined by the ravioli manufacturer was 'below limit of quantification' µg/swab as an acceptable level analysed by a quantitative, sensitive, validated ELISA method (see **Allergen Sampling and Analysis**).

Testing of finished product post-clean is regarded as the gold standard as this is a direct measure of the amount of allergen the consumer is exposed to (this will be described more in the following section). However, finished product testing is not always done and is not always necessary. FBO's may choose not to test finished product in a limited number of scenarios, such as:

1A. Based on experience of similar equipment there is a high level of confidence in the standard of visually and physically clean

OR

1B. If there are dedicated lines / plants where the allergen is absent (or all products have the same allergen profile)

AND

2. There are high levels of confidence that allergen cross-contact is not present in raw materials.

Testing would be necessary if an FBO was making a positive label claim for the absence of an allergen (allergen free-from claim). In the ravioli example, the acceptable limit determined by the ravioli manufacturer was 'below limit of quantification' mg/kg as an acceptable level analysed by a quantitative, sensitive, validated ELISA method. The number of samples selected for analysis also used a risk-based approach linked to homogeneity or heterogeneity of potential allergen carry-over (see also **Allergen Sampling and Analysis** section for guidance on the number of samples).

In this Case Study because egg was predicted to be homogeneously distributed due to its physicochemical properties (liquid) and the manufacturing practices (mixing / blending / depositing), samples were taken at time intervals across the batch of production. If the cross-contact were sesame seeds, then more samples or an alternative approach may be needed due to the particulate nature of sesame seed cross-contact. Further, if it were determined that pieces of dough could hang up in the system, these could be considered particulates that would need to be assessed in the risk assessment (particulates are discussed further in the **Particulate allergen cross-contact** section).

To prove that cleaning as a control is repeatable and reproducible, it is normal practice to perform a validation exercise on 3 separate occasions (primarily swabs but also finished product if desired) covering different shifts and different sanitation teams. In Case Study 1, results from the 3 cycles of validation all came back that the equipment was visually and physically clean. However, due to a potential hang-up point that could not be adequately disassembled and therefore inspected it was determined to perform testing. When samples were taken for testing the results were below the limit of quantification. As such, this was deemed a successful validation of the cleaning procedure.

For Case Study 1, concerning the potential hang-up point due to a mixing blade that could not be fully inspected and given the known size of the mixing blade, a reasonable worst-case estimation was used of hang-up quantity for dilution into the batch of subsequent product. This theoretical QRA indicated that risk was within the range of agreed acceptability. PAL was not used for egg or for the other non-egg containing products manufactured on the line based upon the effectiveness of the allergen control program and validation of the cleaning procedure based on the visually clean standard and carry-over QRA.

There are other possible outcomes which could be considered in this sampling exercise:

1. All of the finished product sampled did not contain any detectable residue of egg, but there was low level detection of egg on two (3 & 5 µg egg protein/per swab) of the 18 swabs sent for analysis. As swab results are not directly correlated with the amount of allergen carry-over in the finished product, all this is indicating is that enhanced sanitation may be required related to the two sampling points which returned positive results and the validation repeated after the enhancements. Alternatively, as all finished products did not contain any detected residual egg protein, then the company may choose to regard this as a successful validation.
2. Another possible scenario is that 3 out of the 10 finished products contained levels of allergen at 0.3, 0.4 and 0.5 mg/kg egg protein, and no further enhancements in line cleaning could be made. By applying a QRA based on Reference Doses the company could calculate (section **5.4**) how this amount of residual egg allergen compares to the Action Level for egg in the following manner:

> Concerning the worst-case situation of 0.5 mg/kg egg protein in the finished product: with a serving size for pasta based on national dietary survey (75th percentile consumption) is 335 grams (**Table 23** in section **5.3**); an ED01 Reference Dose for egg protein is 0.2 mg.[8] The calculated exposure dose for egg protein for a 335 grams intake amount is 0.17 mg egg protein, and below the ED01. Therefore by using this approach this could also be considered a successful validation but of course would need to be repeated on two separate occasions ensuring that samples were representative of potential cross-contact and return results at or below 0.5 mg/kg egg protein in the finished product runs.

Table 8. Quantitative evidence for consideration in the risk assessment. details the quantitative evidence that can be gathered in the risk assessment. As you can see, there is a combination of qualitative and quantitative evidence that can be determined from a cleaning validation. These controls should also be weighted on the quality of evidence for consideration to be included in the quantitative assessment. To determine the risk for the allergic consumer population the user is referred to section **5.4** Basic calculations.

[8] For more information and key references regarding the fundamentals of how allergen RfDs are derived from oral food challenge data and subsequent dose-distribution models, as well as what might constitute an appropriate RfD see ***Box Reference Doses***.

Table 8. *Quantitative evidence for consideration in the risk assessment.*

Quantitative validation	**Controls**	**Quality of evidence for QRA**
Cleaning/changeover effectiveness	- Training - SSOPs - Application of visual clean standard	Insufficient evidence for QRA
	- Pre-operational start up checks against visual standard and KIP's	Insufficient evidence for QRA
	- Analysis of surface swabs of visually clean equipment (semi-quantitative and repeated on 3 separate occasions).	Limited evidence that QRA might be appropriate
	- Analysis of proceeding finished product (not containing egg)	Good evidence for QRA (input data)

If we were to contrast Case Study 1 with the example of sesame cross contact in baked bread rolls (Case Study 2 in Annex **7.4.1**), the risks, controls and validation may be different. This is due to the fact that sesame is a particulate, and due to its physical and electrostatic properties, would most likely result in heterogeneous cross contact. As sesame is applied at the end of the process pre-bake, then it is only equipment downstream of the sesame application that would need to be reviewed from an 'equipment risk' perspective. The risk assessment should, however, cover the area where sesame is stored and handled before it is brought to the line and placed in the hopper feeding the line.

As sesame is a particulate (and potentially visible to the naked eye), then a different approach would need to be applied when conducting the QRA using the principles outlined in the **Particulate allergen cross-contact** section of this Guidance. Rather than an analytical sampling program, it may be prudent if sampling is based entirely on a visual sampling inspection program to ascertain how many 'defective' lots are present post change over. If low level and intermittent cross-contact is identified that cannot be further mitigated, based on the number and frequency of sesame seeds visually detected, it is possible to calculate the protein concentration and dose of sesame protein to compare to established Reference Doses derived from clinical dose-distribution curves. However, again it is critical to note that sampling and knowledge of the amount of cross-contact has to be representative of what may happen on an on-going basis, this requires an understanding of the root cause of cross-contact and therefore its characteristics. Further guidance on how this is accomplished in practice can be found in the sections of the **Core concepts** of the document, including **Allergen Sampling and Analysis**, **Particulate allergen cross-contact**, **5.4** Basic allergen **QRA calculations** and also **2.2.3 Usage levels of ingredient in final product under assessment**.

3.3. Validation & Verification of Cleaning

3.3.1. Guide on the application of QRA in Cleaning Validation

Time separation with effective cleaning between production runs on shared equipment is a common form of segregation control used by manufacturing sites. Where production equipment or lines are not dedicated to specific products, effective cleaning is the main control measure to prevent cross-contact.

It is recommended that the method of cleaning implemented is designed using a risk-based approach and be validated by generating and documenting evidence that the cleaning is effective at removing contaminants (allergens in this instance) in a worst-case scenario for all relevant hazards. This means that the cleaning must be able to remove allergens (in this instance) from previous production under the following circumstances:

- For the most difficult to clean food that the site produces on the line in question.
- For the hardest to clean food matrix containing allergen(s) at the highest level (e.g., highest % allergen protein) on the line in question.
- Taking into account of the most difficult part(s) of the equipment to clean.
- Avoid leaving behind residues of cleaning chemicals that could transfer to food.
- And using the cleaning instructions that will be used as specified in the cleaning procedure for the line, or the lowest acceptable cleaning instructions specified by the chemical manufacturer (e.g., if a detergent is described as suitable for use at 3-4% dilution and 45-50˚C, validation should be at 3% and 45˚C to serve as the worst-case scenario). It is recommended to work closely with your chemical manufacturer to determine the appropriate detergent, concentrations, contact time and temperature for your particular equipment and food residue(s) to be removed.

FBOs should consider the physical form of any material that needs to be removed through cleaning, as some materials are naturally harder to remove due to their nature e.g., chocolate, oil-based products, certain proteins that tend to stick to equipment surfaces during heat processing (e.g., egg ovalbumin), etc.

To validate a cleaning process, it is recommended that FBOs demonstrate that cleaning is effective following three separate, consecutive production runs by evaluating equipment for residues of cross contact (equipment should be visibly and physically clean), in some cases it may also be appropriate to test equipment, rinse waters or finished product for residues of cross contact. The results of each run should be reviewed before repeating the process in the event that a modification to the cleaning procedure is required.

Once validated, there should be ongoing monitoring and verification in place to ensure that the validated cleaning is carried out correctly and continues to be effective. Often as a part of routine allergen cleaning there will be equipment breakdown to ensure equipment is visually and physically clean. It may be appropriate to use analytical testing as an additional verification that the cleaning control is effective. This can be reviewed at a frequency based on a risk assessment (related to the product, production equipment and marketing claims) for example annually. If any portion of the process or product formulation being manufactured change (e.g., a new product from the R&D team that is being considered for production on the line), it is important to make sure that the controls that are in place remain effective and appropriate to control the hazards and risks identified. This risk review process should also be initiated before and during the fitting of new equipment with considerations of hygienic design or the placement of alternate equipment on a production line as these factors may also have an impact on the overall allergen control program.

3.3.2. Approach to validation

The site-specific risk assessment will have identified the ingredients on site that need to be controlled, including those for which cleaning between production runs is the main control.

In order to achieve Step 1, the identification of points of possible cross-contact (hazard identification), equipment used as a part of a production process should be inspected to identify any points where traces of residue could remain due to limited access or difficulty in cleaning. This assessment must take into account parts of machinery that are hard to reach and clean as worst-case scenarios, for example, dead legs of pipes, complex moving parts of machinery, etc. These areas in the machinery need to be highlighted in the risk assessment so that the cleaning procedure can be designed to clean them thoroughly. If there is a decision to take samples for analysis and swabbing is conducted, these points should also be included as points for taking swab samples as part of the validation process and also when appropriate for the ongoing verification and monitoring.

In order to identify suitable cleaning methods, consideration should be given to the way that the processing machinery works, and the suitability of cleaning materials, chemicals and equipment or utensils that will remove UAP. The type of cleaning required might vary

depending on the nature of the food that was processed last, what will be processed next, and therefore, the ingredient(s) that needs to be controlled.

Once a cleaning procedure has been designed, there should be a validation to ensure that it is effective in practice, following the actual food processing and clean down procedure.

3.3.3. Steps to take to validate cleaning

The following provides an example of a step-by-step approach to carrying out a validation exercise (see also **Figure 7**). Validation of cleaning is needed to demonstrate that a proposed cleaning procedure will be effective, and it therefore needs to be completed prior to implementation of full-scale production. In the below, not all individual levels of assurance may be required in every instance. The stage that is performed depends on the risk of carry-over which is case-by-case related to equipment and product parameters, existing knowledge of similar production systems and product marketing claims relating to allergens.

- **Stage 1:** Design of change-over allergen cleaning/sanitation
    - The fundamental way in which cleaning/sanitation is assured is by the confirmation that equipment is visually and physically clean. Sampling and testing may be considered appropriate to provide additional data in situations where there is uncertainty on the ability to fully clean a system of residual allergen cross-contact hazard and may in these situations provide data on allergen absence (not detected), or presence either to inform on the need for further mitigation measures, or as an input into a QRA.
    - It should be noted that systems are cleaned to manage contaminant issues beyond allergen cross-contact. Systems that have undergone cleaning/sanitation may provide negative results with allergen testing but may still be unclean and present microbiological hazard. For this reason, the breakdown of equipment for confirmation of visual and physical clean is performed for hygienic purpose in addition to allergen management.
    - There are specific situations (e.g., confectionery) where the physical nature of the foodstuff and equipment used can prevent cleaning and equipment break down to confirm visual and physical cleanliness, and it is not needed to ensure hygienic safety. In these situations, analytical testing is generally used as a part of change-over measures.
    - For the purpose of validation of a system that processes multiple allergenic products, an appropriate combination of food matrix and ingredient should be chosen such that it is representative. In the case of systems that cannot rely on the confirmation of visual and physical cleanliness, and analytical testing will be used, the matrix selected should be amenable to such testing.
- **Stage 2**: Conduct of change-over allergen cleaning/sanitation
    - The cleaning method as designed and documented is used to clean the processing equipment.
    - Following cleaning, all food contact surfaces and areas where food debris/residue might enter future production are inspected to ensure there is no debris and surfaces are visually and physically clean. Inspection of equipment often necessitates its disassembly for inspection. In some production scenarios, this is considered sufficient as a stopping point, however if additional assurance on allergen absence is warranted or in cases where allergen presence cannot be further reduced and therefore allergen QRA may be appropriate, further testing may be required to provide either information on allergen absence (below LoQ) or quantitative information regarding the level of potential allergen residue that may remain and inform risk assessment and therefore risk management decisions.
    - In the instance of equipment that cannot be disassembled as a part of sanitation and there is a known potential hang-up point, further risk assessment measures should be undertaken (e.g. QRA based on carry-over estimate may be appropriate, possibly in addition to QRA based on analytical data). When this is not possible, for example due to high uncertainty, the potential for cross-contact should be reflected in the risk management measures employed (e.g. PAL).
- **Stage 3:** Consideration of allergen analysis (if determined to be needed)
    - In case of allergen testing, the below describes an ascending list of samples that may be taken based on the level of assurance considered appropriate:
        - First level of assurance: sampling for the effectiveness of the cleaning procedure

- Surfaces including, but not limited to, the points identified as hard to reach or hard to clean can be swabbed and tested. Consider swabbing food contact surfaces and food contact materials from lines, belts, tote bins and hoppers, etc. through to utensils such as scoops, trays, scales, etc.
- In instances where CIP systems are used, samples of final rinse water can be tested.
- Testing should be done using an appropriate test method that preferentially will only pick up the allergen of concern and is sensitive enough to detect and quantify low-level cross contact. If at all possible, it is recommended that testing is quantitative so that results are expressed with a level, uncertainty and limit of quantification.

▪ Second level of assurance: sampling of the subsequent product
  - After a subsequent production run that does not contain the allergen of concern, samples may be taken of the first produced products using the equipment that was cleaned and tested using a method that has been validated as effective at detecting the specific ingredient or allergen for that specific product matrix at a suitable level of analytical uncertainty and sensitivity.

▪ Third level of assurance: This may only be necessary for products that have a label claim to denote the absence of an allergen, e.g. free from claim. A tertiary sampling which is 2 production cycles after the allergen clean.
  - As per the first level of assurance, in the case of production systems that use CIP, samples of finished rinse water can be taken, after the subsequent production. Swabs may also be taken of the identified problematic areas of equipment.
  - Samples of finished product may also be taken from the beginning, middle and end of production.
  - As this is two production cycles after the cleaning to manage the allergen of concern, all samples should show absence (i.e., below the limit of quantification or below an established regulatory limit) of the allergenic ingredient of concern.

▪ Irrespective of the validation plan (whether it stops after Stage 2 or includes some level of testing as per Stage 3), it should be undertaken 3 times on consecutive change-over cycles with satisfactory results to demonstrate that the cleaning procedure is effective.

NOTE: if allergen testing is used (as recommended):

Product testing might not always be on a final finished product. Where the purpose is to validate the cleaning of a particular piece of equipment such as a dispenser, the intermediate that is dispensed could be sampled for testing if it becomes a component of the finished product.

If results show presence of the allergen that was supposed to be removed, the cleaning has not been effective. If this happens, there should be a thorough investigation to understand and correct the root cause of the failure. If a correction in the cleaning process is possible, the specific part of the production system impacted can undergo a re-clean and re-test as part of the subsequent validation run. If a correction in the cleaning process is not possible, the implication regarding risk to allergic consumers should be assessed to inform appropriate risk management measures. QRA as described in section **5.4** can be considered. Once the process has been reviewed, validation should be re-run to demonstrate that the cleaning procedure is effective in three consecutive production runs. FBOs should make sure that cleaning procedures do not result in residues of cleaning products or chemicals used that could contaminate subsequent batches of product.

Products made during the validation or verification process should only be released if it can be assured that they do not contain undeclared allergen residues beyond a level that presents unacceptable risk to allergic consumers.

FBOs will need to set their own control or acceptance limits, which in many cases will be based on absence (i.e., below the limit of quantification) when using the best or most appropriate test methods for the UAP in question. This generally means:

- The *most* sensitive and robust (suitable for the product matrix) method available should be used, as rule. This requires work in consultation with qualified analytical experts/laboratories to determine if there are issues with how specific the method is in the product matrix. This is also important to determine if there are risks of false positive results at low levels.
- Methods that are not very sensitive (i.e., methods that would not be able to pick up low levels of UAP) should not be used. A result showing 'not detected' when the limit of quantification of the method is too high could mean that there is still substantial amount of UAP left.

It is valuable to have evidence from the laboratory that the limit of detection is sufficiently low to be a reliable indication that the UAP has been removed through cleaning. Furthermore, in the instance that QRA is applied, if analytical data is used as an input, the method used should be capable of quantifying allergen below a calculated Action Level (Remington, Baumert et al. 2022).

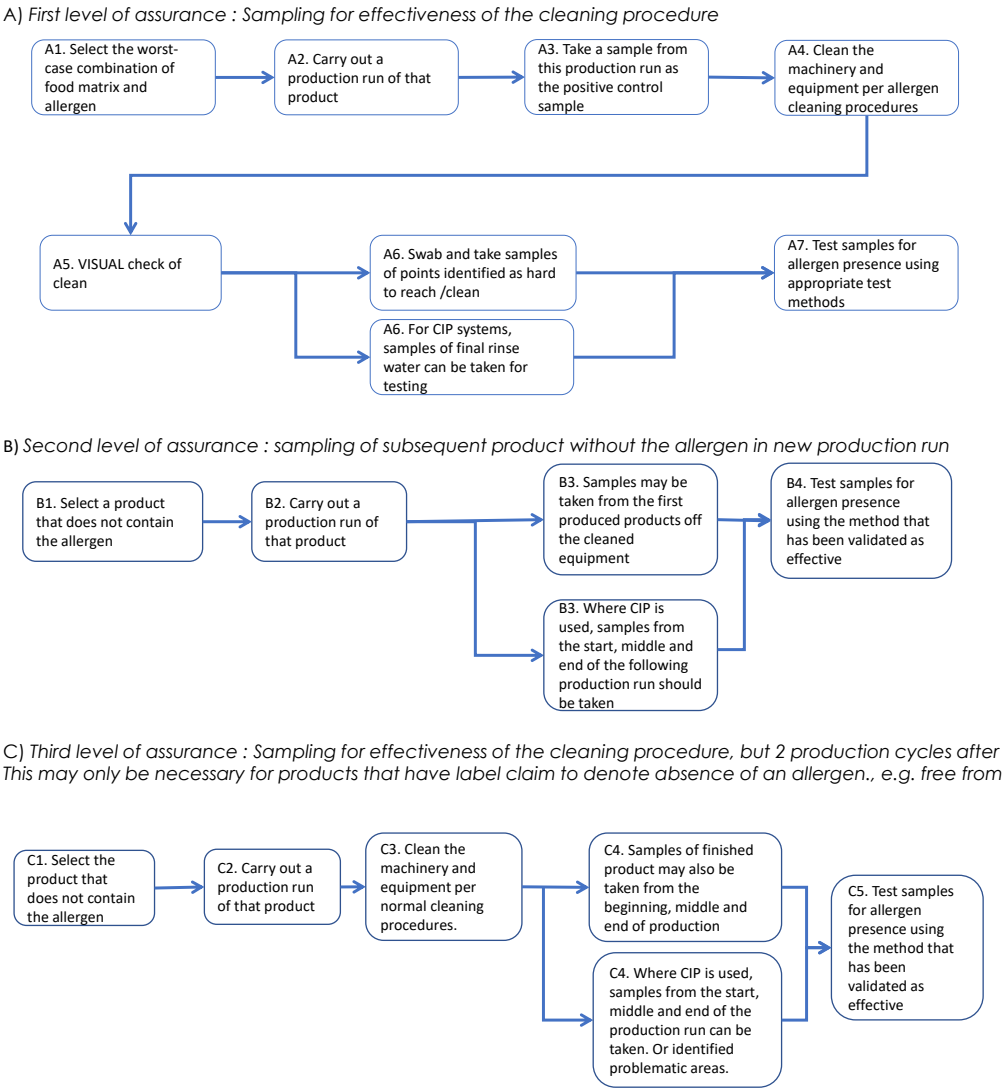


Figure 7**: Allergen analysis (sampling and testing) may be applied for additional assurance in cleaning validation and for demonstration that a cleaning procedure is effective. The flow chart shows a simplified scheme for the various steps when QRA is the purpose. In this situation various levels of sampling and testing for the allergen can be considered with different levels of assurance: (A) the basic sampling and testing scenario assuming worst-case combination of food matrix and allergen (first level of assurance) and (B) testing subsequent product without the allergen in a new production run (second level of assurance) or C) sampling for effectiveness of the cleaning procedure two cycles after the allergen cleaning (third level of assurance). Note: In other situations than QRA the validation can stop at any level for A5, A6 or A7, and not all steps will be needed every single time. The conduct of testing on samples after A5 should be based on a risk-based decision. Note, the third level of assurance may only be necessary for products that have a label claim to denote the absence of an allergen, e.g. free from claim. Further details and explanations are described in section **3.3.3.

Box 4. Legal frameworks for allergen management.

Many FBOs, are likely to operate under HACCP or similar principles (depending on the region), as described in Article 5 of Regulation No 852/2004, and more extensively discussed in the Commission Notice on the implementation of food safety management systems covering prerequisite programs (PRPs) and procedures based on the HACCP principles, including the facilitation/flexibility of the implementation in certain food businesses of 2008. The approach described in this chapter can be reconciled with HACCP as described in table below. Managing a food allergen QRA in the context of HACCP helps focus on validation and may integrate more effectively allergen management in food safety procedures.

For example, the hazard analysis typically (European Commission 2016) considers the likelihood of occurrence of hazards and severity of their adverse health effects, the qualitative and/or quantitative evaluation of the presence of hazards and the persistence in foods of allergens which are described in the approach.

The Codex Alimentarius Commission (2020) has recommended that "Allergen management practices should be part of good hygiene practices (GHPs), and, where appropriate, HACCP systems, in manufacturing, retail and food service" noting that "The unintentional presence of allergens in food is prevented or minimised by taking preventive measures through GHPs and HACCP-based controls at appropriate stages in the operation". Typically, food allergens are not managed via CCPs (HACCP principles 2-5 of the 7 HACCP principles described in (Codex Alimentarius 1969) although exceptions apply. The table below shows the link of food allergen risk assessment with HACCP.

In the U.S., the Food Safety Modernization Act (FSMA) requires the FBOs to develop risk-based preventive control programs for allergens as well as use of cGMPs to mitigate the potential for cross-contact of allergens in food processing facilities (21 CFR part 117.5). The FDA enforces the mandates set forth by FSMA with the expectation that a Hazard Analysis and Risk-Based Preventive Control Program (HARPC) is implemented to identify potential allergen hazards and implement risk-based controls to minimize the occurrence of allergen cross-contact in production facilities (FDA 2018).

HACCP	**Food allergen risk assessment**
Hazard analysis (Principle 1)	Assessing Unintended Allergen Presence (cross-contact).
Hazard analysis (Principle 1)	Estimation of likelihood of failure of the control measure under normal operating conditions (probable / remote)
Hazard analysis (Principle 1)	Hazard Characterisation. Assessment of the identified hazard to determine allergenicity / severity against a number of specific criteria.
Hazard analysis (Principle 1)/Verification (and validation) procedures (Principle 6)	Validation of existing control measures to minimise the risk of unintentional allergen presence.

Box 5 (continued on the next page). *Case Study 1. Example – cross contact with egg - detailed process description*

The product of interest is "Ben's Ravioli". The recipe for "Ben's Ravioli" dough does not contain any eggs. However, possible cross contact from other doughs produced on the same equipment containing (liquid) egg cannot be excluded; egg-containing ravioli are also produced at the site.

Example Foods could consider a precautionary allergen labelling (PAL) statement of 'may contain egg'; however, they want to explore the use of quantitative risk assessment to see if a PAL statement is needed. Example Foods follows with Codex Alimentarius recommendations for training, personal hygiene, maintenance, design and facilities.

For this assessment, the production process has been mapped and areas of possible allergen cross-contact have been identified. (See below)

The Example Foods factory line for ravioli has been put into a flow diagram with the different process steps highlighted. Process steps shared with eggs, or egg derived products, intermediates or ingredients are in orange. **It is assumed that Examples Foods has implemented procedures and controls to avoid raw material, ingredient, recipe or finished product mix-up or mislabeling.**

Additionally, the points of control where qualitative or quantitative data can be obtained have also been identified (in **Table 3**). Note: for review of potential risks in the supply chain for potential allergen cross-contact in the ingredients, such as for the ravioli filling, soy in wheat for the flour, the user is referred to the chapter 2 **Communication across supply chains**.

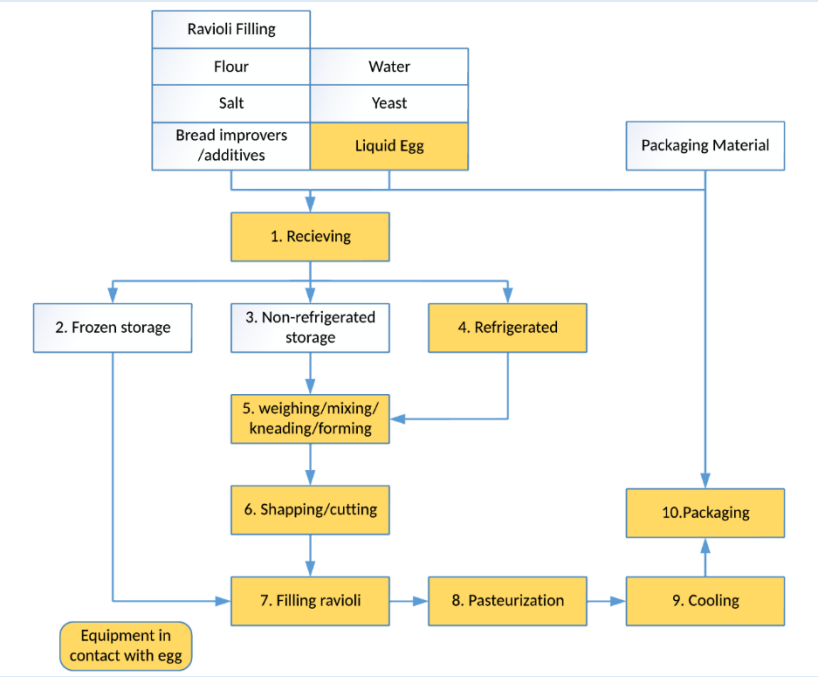


Figure 8.a *[Process steps 1-4]: All ingredients and materials except eggs are stored in areas where egg or egg derived ingredients are not reasonably expected to occur. Liquid eggs are delivered in 500 kg plastic bins and kept in stored refrigeration until use. They are mixed with other ingredients in a single vessel continuous pasta dough pre-mixer, this is followed by a further mixer with automatic dosage of water, eggs and flour which are fed from silos through hoppers and into dosing tanks. The automated feeding tanks for eggs have a capacity of 600 L, made in stainless steel, two speeds, and internal spray ball for cleaning.*

Figure 8.b *[Process step 5] Example of dough mixer. Images courtesy of Italpast srl of Fidenza, Italy.*

The dough is then transferred to an automatic sheeter, with shutters that can be opened for cleaning [Process step 5]. Rework is permitted but tightly controlled through an allergen control program. In line with the Codex Alimentarius Code of Practice on Food Allergen Management for Food Business Operators recommendations (Codex Section 5.2.1.2)(Codex Alimentarius 2020), dough for rework is stored in sturdy containers with secure covers in designated, clearly marked areas (within the refrigerated storage area). It is labelled with all food allergens specifically highlighted, including eggs, and logged into an (electronic) system for traceability of storage and use.

From the sheeter [Process step 5], the sheet pasta is transferred to a forming machine (in stainless steel, dismountable and washable) [process step 6]. Any rework generated at this stage will again be handled following Codex recommendations.

[Process step 7]: The filling device is independent and removable for washing operations. The forming machine also molds and cuts the ravioli; this part of the machine is entirely in stainless steel. The molding and cutting rollers are teflon-coated.

Figure 8.c *[Process step 8]: A conveyor belt carries the pre-dried ravioli to a pasteurizer, which uses overheated steam with two drying tiers so that the raviolis are ready for cooling and packaging. Hot air and steam are not recycled to avoid potential cross contact. Images courtesy of Italpast srl of Fidenza, Italy.*

Belts for pasteurization are made of a stainless steel net. Belts in polyester net are used in the drying area [process step 9]. The ravioli's are then sent to a cooling machine in stainless steel that allows the ravioli to reach 0°C.

[Process step 10]: A packaging machine is used to package the product. The machine is also in stainless steel. Individual units are labelled with mandatory food information via a sticker immediately following packaging; labels are selected from a label portfolio by the packaging area manager.

Metal detection takes place after packaging, before storage and distribution.

For the possible risk for allergen cross contact the steps can be analysed as described in Table 1. This will lead to the coloring boxes orange (as an example) if allergen CC risk is present.

4. Management of incidents

An allergen incident is when there is product that has been produced including that which may be at market, for which new information becomes available relating to its allergen profile. As such, incidents are the unexpected and previously unaccounted presence or potential presence of an allergen in a foodstuff that has been produced, distributed or retailed.

This Chapter provides a standard format for allergen incidents in order to enable the details of an incident to be captured, the risk to be assessed using the most appropriate method, and the outcomes of an assessment to be communicated. This Chapter is supported with information available in the Chapter on **Core concepts**.

4.1. Guidance on Incident Assessment[9]

4.1.1. How to use the Incident Form

The form consists of 4 parts

1. General Information, used to identify the incident and capture a high level summary.
2. A flow chart to guide the user through the process of incident assessment and the use of the Form.
3. The Form which includes an Assessment Matrix, which is designed to capture information relevant to the assessment, and to judge the quality of evidence.
4. Guidance on the Form, how to capture the information and perform the assessment.

The Form has 4 functions:

- to provide guidance on the process of risk assessment of incidents.
- to act as a living document to capture an incident as information evolves.
- to capture for communication the incident and its assessment.
- to serve as a record of the incident and its assessment.

It should be noted that in the case of most incidents not all parts of the Form will be completed. For most incidents only partial information is available at the time the assessment is performed. The Form is designed to capture information that is available, and based on the amount and quality of information, provide a guide on what type of assessment may be appropriate (e.g., no assessment possible or quantitative assessment).

In order to serve this function, the form has been designed to be 'semi-quantitative' in that the quality of available evidence is scored, including an overall quality concept we term 'tier of refinement', and this serves both to help decide what type of assessment should be performed and provides information on the quality of evidence to communicate alongside the outcome of the assessment.

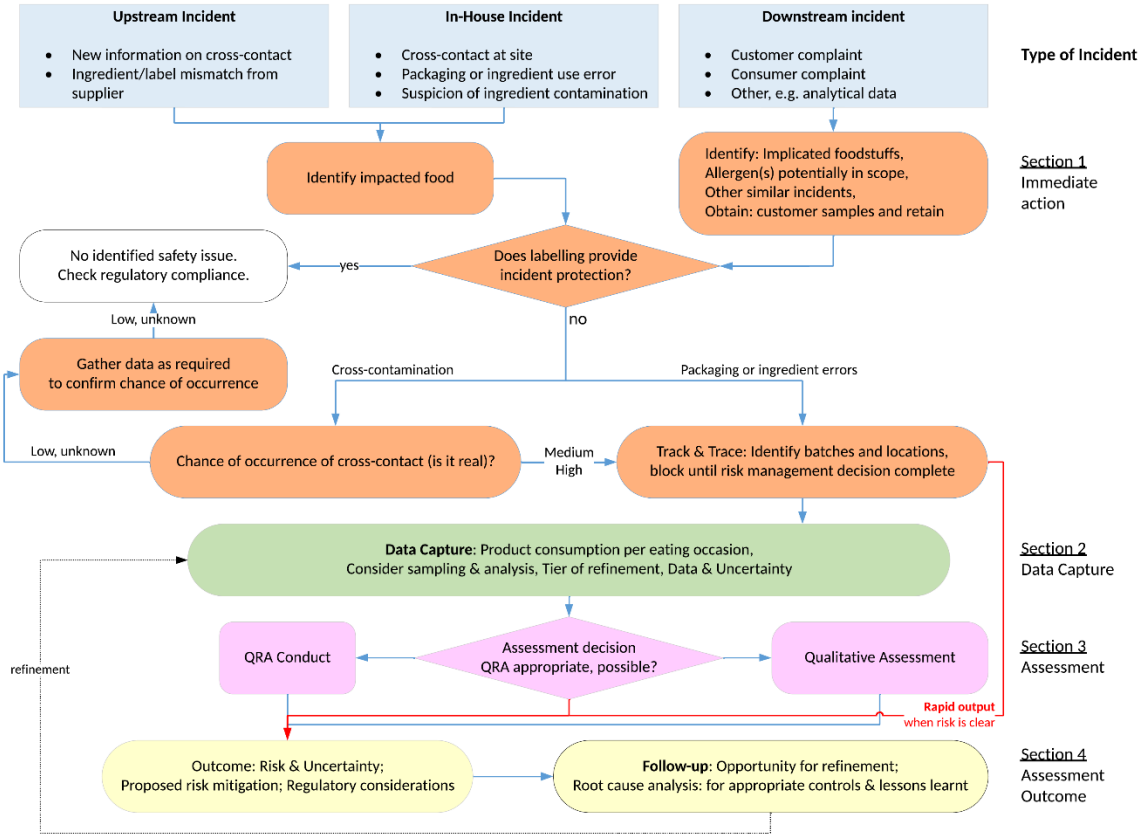
There are always opportunities to refine risk assessments, but in the case of an incident it is critical to know when an assessment outcome should be communicated. For some incidents there may be time restraints, possibly imposed by regulatory requirements, to report the outcome of the assessment. In some incidents the risk to consumers may become obvious at an early stage, due to information captured in the 'immediate action' or 'data capture' phases of the assessment. If this is the case there should be immediate communication on the risk identified.

[9] A training webinar will be made available for this Chapter here.

4.1.2. General Information & Assessment Summary (The following form may be downloaded and adapted for use)

Assessment Team			
Assessment Date			
Incident Dates			
Type of incident	Upstream In-house Downstream	Source of information ☐ ☒ ☐	Point of cross-contact ☐ ☐ ☒
Foodstuff and allergen(s):			
Market(s):	Country, region, retailer etc.		
Product disposition:	Number of consumer units on hold, in distribution, at market etc.		
Risk to consumers:	• There is a risk to allergic consumers • Risk within agreed limits of acceptability • Not currently possible to determine		
Quality of Evidence:	High, medium, or low		
Scale of risk:	e.g., does identified risk relate to ingredient / labelling error, or concerns incorrect PAL statement, or concerns allergen presence in a product that claims absence? What is the frequency of UAP? Is there an excessive and clear risk to consumers?		
Opportunity for refinement:	Next steps possible to improve the assessment		
Regulatory situation:	Description of any non-compliance		
Proposed mitigation & actions, next steps:	Proposed action plan, based on risk and quality of evidence, including recommendation to risk managers, contact with authorities or patient organisations etc.		

4.1.3. Incident Flow Chart



4.1.4. Assessment Matrix

Section 1: Immediate Action	
Identity of foodstuff implicated	
Allergen(s) implicated	
Supporting information	
Does labelling provide incident protection, or exacerbation ?	See explanation in 4.1.5
Summary if relevant of consumer complaints	Including any trend in consumer complaints
Chance of Occurrence of Cross-Contact	
See '**Core concepts**' Section **5.1.1** for a description of 'Chance of Occurrence'	
Chance of Occurrence	**Notes**
☐ High or known to have happened	
☐ Medium	
☐ Low or unknown	
Track & Trace	
Current status	
Degree of success of T&T	
Implicated batch no.s, production dates	
No. Packs (consumer units) implicated	
No. Packs Held	
No. Packs in distribution	
No. Packs at consumer market	
Shelf-life remaining	
Other supporting information	

Section 2: Data Capture			
Consumption			
See '**Core concepts**' section **5** for guidance on consumption estimates			
Pack size (consumer unit) (g)	Meal preparation	Portion size (g)	Quantity of implicated food eaten per consumption event (g)
	How pack is used	See explanation in **Core concepts** section	possible range & description of uncertainties
the 'Tier of Refinement'			
Tier	**Description**		**Source of Data**
☐ **Tier 1** 'Theoretical'	Concern has been raised on UAP but there is no physical evidence of cross-contact at the product site or supply chain in question.		No data available, only 'reverse' QRA possible (see **Core concepts**).
☐ **Tier 2** 'Informed'	Some physical evidence of UAP of the specific supply chain in question, high uncertainty in quantification.		The data available for QRA is based on 'reasonable worst case' assumptions, e.g., hang-up estimation (see **5.2.3** carry-over guidance).
☐ **Tier 3** 'Data-driven'	Physical evidence of UAP at the production site or specific supply chain in question, with indirect quantification possible.		The data available is from upstream in the supply chain, for example on a purchased ingredient.
☐ **Tier 4** 'Verified'	Physical evidence of UAP at the production site or specific supply chain in question, with direct quantification possible.		data is available on finished product as presented to consumer, or in case of mis-labeling or ingredient error there is clarity on the allergen content of the food.

<table>
<tr><th colspan="4">Characteristics of UAP: Data & Uncertainty</th></tr>
<tr><td colspan="4">See 'Core concepts' Section 5.1.2 for a description of UAP Characteristics and Uncertainty</td></tr>
<tr><th colspan="2">Characteristics</th><th>Uncertainty</th><th>Data & Notes</th></tr>
<tr><td rowspan="3">A
Form of UAP</td><td>☐ Amorphous</td><td>1 ☐ High</td><td rowspan="3">---
---
Note: If 'unknown', assessment should be based on both amorphous and particulate, until refined information is available.</td></tr>
<tr><td>☐ Particulate</td><td>2 ☐ Medium</td></tr>
<tr><td>☐ Unknown (please mark uncertainty as 'high')</td><td>3 ☐ Acceptable</td></tr>
<tr><td rowspan="3">B
Distribution of UAP</td><td>☐ Homogeneous</td><td>1 ☐ High</td><td rowspan="3">---
---
Note: If 'unknown', assessment should be based on both hetero' and homogeneous, until refined information is available.</td></tr>
<tr><td>☐ Heterogeneous</td><td>2 ☐ Medium</td></tr>
<tr><td>☐ Unknown (uncertainty is always 'high')</td><td>3 ☐ Acceptable</td></tr>
<tr><td rowspan="4">C
Frequency of UAP
(how often the cross-contact is happening)</td><td>☐ Isolated</td><td>1 ☐ High</td><td rowspan="4">---
---
Note: If 'unknown', assessment should assume UAP is 'regular'.</td></tr>
<tr><td>☐ Intermittent</td><td>2 ☐ Medium</td></tr>
<tr><td>☐ Regular</td><td rowspan="2">3 ☐ Acceptable</td></tr>
<tr><td>☐ unknown (uncertainty is always 'high')</td></tr>
<tr><td>D
Concentration of UAP</td><td colspan="2">1 ☐ Unknown or Estimate (not analytical). Note: see carry-over guidance 5.2.3
2 ☐ Analytical, point data
3 ☐ Analytical, data range.

In the case of mis-labeling or wrong ingredient used, where there is knowledge on amount of allergen present, mark as 3.</td><td>Provide data:

Describe suitability of analytical data:

Note: If 'unknown', assessment can only be qualitative. More information is needed before QRA can be performed.</td></tr>
<tr><td colspan="2">Overall data uncertainty (sum of A-D)</td><td>4-7 ☐ High
8-10 ☐ Medium
>10 ☐ Acceptable</td><td>Notes</td></tr>
</table>

Section 3: Assessment		
Assessment Decision		**Notes: rationale for selected option**
It is beyond doubt that there is an unacceptable risk, no further assessment required	☐	
Uncertainty is too large to enable an assessment, further information required	☐	
QRA is appropriate but not possible without further information, qualitative assessment only	☐	
QRA is appropriate and possible	☐	
QRA Metrics (for 'screening' and 'deterministic' QRA)		
See '**Core concepts**' section **5.4** for calculation guidance		
Description of the exposure scenario		
In case an Action Level (ppm) was calculated to compare to concentration in food (ppm), what was is the Action Level ?	Action Level =	Conc in food =
In case exposure of allergic consumer was calculated (mg) to compare to RfD (mg), what was the exposure ?	Appropriate RfD[10] =	Consumer exposure =
Description of the calculation		
In case of higher level calculations, eg probabilistic, population level, provide details		

[10] For more information and key references regarding the fundamentals of how allergen RfDs are derived from oral food challenge data and subsequent dose-distribution models, as well as what might constitute an appropriate RfD see ***Box Reference Doses*.**

Section 4: Assessment Outcome			
Key Output	**Evidence**		
Risk Assessment Outcome	There is a risk to allergic consumers Risk within agreed limits of acceptability Not currently possible to determine	☐ ☐ ☐	
Proposed risk mitigation (in case of risk to allergic consumers)			
Need to contact external agencies	Eg authority, patient org ?		
Method of assessment	Qualitative Quantitative (QRA) Not currently possible to assess	☐ ☐ ☐	
Regulatory implications			
Product Presentation			
Describe aspects of product presentation that may modify the risk	For example, partial risk mitigation due to existing PAL warning, or exacerbation due to use of a free-from claim. Frequency of contamination as an indicator of scale of risk.		
Quality of Evidence Framework			**score**
Tier of refinement	Tier 1 – theoretical Tier 2 – informed Tier 3 – data-driven Tier 4 – verified	☐ ☐ ☐ ☐	1 2 3 4
Chance that cross-contact is occurring	Low or unknown Medium High or known to have happened	☐ ☐ ☐	1 2 3
Overall data uncertainty	High uncertainty Medium uncertainty Acceptable uncertainty	☐ ☐ ☐	1 2 3
Quality of Evidence	9 – 10 : high quality evidence 6 – 8 : medium quality evidence 5 and below : low quality evidence	☐ ☐ ☐	
Opportunities for Refinement			
If there is sufficient time available for refinement, describe data needed and next steps			
Root Cause Analysis & Corrective Action			
Describe root cause, corrective action			

4.1.5. Guidance on the Assessment Matrix

Type of Incident

*At the initiation of an assessment of an allergen incident there is usually an understanding of the type of incident that has possibly occurred. Knowledge on the type of incident is fundamental to data gathering to enable the assessment, and in performing a root cause analysis (see section **5.1.1** on root cause analysis). Allergen incidents can be the result of various types of errors or oversights that can occur at any point across a supply chain, as illustrated by **Figure 9**. For example, an error in production scheduling (or unaccounted for change in scheduling at a co-packer) without appropriate change-over sanitation may result in an 'incident' where unlabeled cross-contact is possible. A generalized scheme to capture the type of incident from the perspective of any position within a supply chain is illustrated in **Figure 10**.*

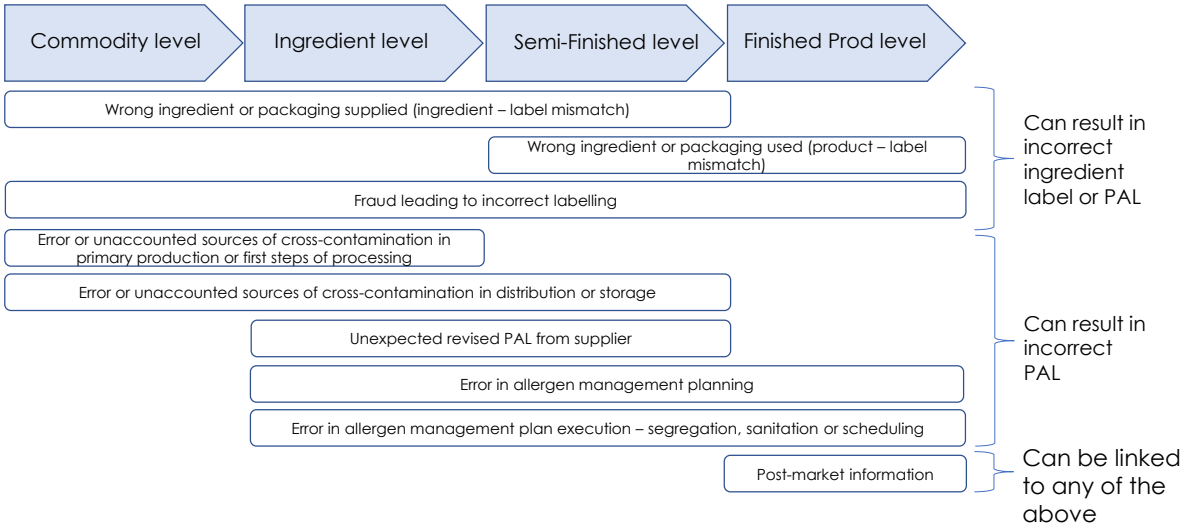


Figure 9. *Types of incidents that can occur across different parts of a supply chain.*

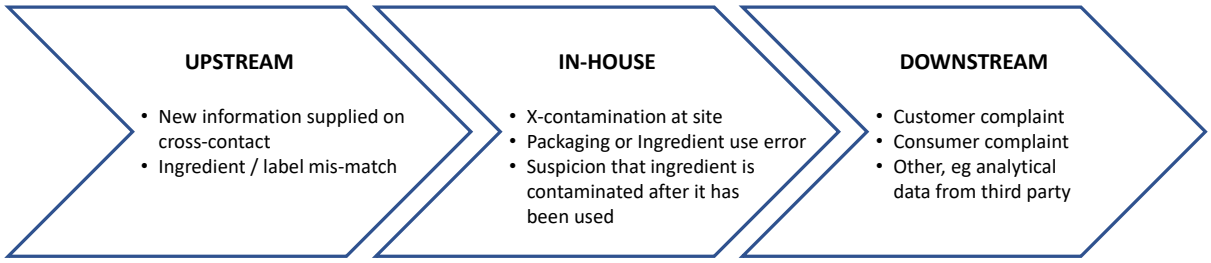


Figure 10. *General scheme to capture the type of incident irrespective of the position in the supply chain.*

In many cases the point at which a cross-contact has occurred is different from where the incident is reported. For example, the source of information on possible cross-contact may come from downstream information, such as a consumer complaint, however the actual point of cross-contact may be upstream in the ingredient supply chain. The incident process described here captures this information simply within the Assessment Summary.

4.1.5.1. Section 1: Immediate Action

Incident fundamentals
Immediate actions invariably start with clarifying the potentially impacted foodstuff, in the case of most incidents fundamental information will be known, such as the product(s) and allergen(s) involved, however in some incidents such as a consumer report of allergic reaction, investigation may be needed to clarify these fundamentals. To facilitate an investigation, it may be necessary to obtain samples of implicated product.

Existing labelling
Once the potential impacted product(s) are clarified it should be checked whether the labelling could provide mitigation, if this is not the case the assessment should proceed. As illustrated in the **Table 9** existing labelling on the implicated product may already carry the allergen of concern within the ingredient list or precautionary statement. If this is the case, the degree of mitigation should be determined. If the existing labelling provides full mitigation, no further action is required other than to review and update preventive controls. Note however that there may be regulatory concerns with labelling errors that are beyond the scope of this Guidance. If existing labelling provides only partial mitigation, the assessment should continue to assess the risk to consumers and the details of the partial mitigation incorporated into the assessment outcome.

It should be noted that in some cases labelling may increase the level of concern related to an allergen incident, if there is a claim that increases the product attractiveness to allergic consumers (e.g. 'free from' claim).

Table 9. *The degree to which existing product labeling may mitigate the incident under assessment*

Incident concerns	**Existing labelling states the implicated allergen**	
	as Ingredient	**as cross-contact PAL**
Allergenic ingredient mistakenly present	Full mitigation	Partial mitigation
Cross-contact	Full mitigation	Partial to full mitigation*

**it may be the case that allergic consumers consider a product acceptable for consumption despite the presence of PAL based on their experience of the product, therefore if there is a significant change of allergen presence it may result in risk to these consumers even though PAL is present.*

Consumer complaints
When there is information on incidents that comes from upstream in a supply chain, usually fundamental information is available, such as the allergen and food item that is in scope. However, information coming from downstream sources, such as consumer complaints can be different in terms of the immediate action required compared to either information from authorities or from upstream and in-house incidents. In the case of consumer complaints there may be a lack of fundamental information which as far as possible should be gathered as a part of immediate actions, and includes the following:

Understanding the type of reaction experienced.
- Nature of the reaction experienced, symptoms, treatment and recovery.
- Knowledge of pre-existing food allergy.
- Knowledge of previous reactions (if any), severity, frequency.
- Knowledge of other types of allergy (dermal, inhalation).
- Who experienced the reaction (age).

In many cases allergic consumers will be aware of their condition and how they react, however if this is not the case information can be provided to understand allergic reactions.

There are a number of useful sources of information on food allergy for consumers, for example from Food Allergy Canada (see here)

Understanding the link to the food in question.

- Time period between the consumption and symptoms.
- Amount of the food consumed.
- Whether the food item has been consumed previously, with any issues experienced, if so, how often the food item is consumed.
- Whether the food item lists the allergen of concern as an ingredient.
- Whether the food item lists the allergen of concern in precautionary labeling.
- Whether other foods were consumed at the same time as the implicated food.
- Whether the product bad been previously opened and may have been subject to cross-contact.

Identifying the food item in question, and its availability for testing.

- Type of food and brand name (if the pack is available photographic evidence is helpful, in addition if there remains some uneaten product to gain a sample).
- Information on where purchased (e.g., internet, grocery store, country and area).
- Bar code.
- Best before and use by date.
- Production code.
- Pack size.
- Whether there is any of the product remaining and if the consumer still has the product if it can be made available for testing.

In most cases only a limited portion of the above information will be available from the consumer. The objective should be to gather as much of this information as possible to inform the investigation should a decision be taken that a consumer complaint should be treated as an allergen incident that merits an investigation.

The evaluation of whether a consumer complaint is likely to be an allergen incident (and therefore requires investigation) should be based on a number of parameters. If there is a reasonable likelihood that the reaction was an allergic reaction, and that it was linked to the food item in question which does not label the allergen(s) possibly in scope, then it would be recommended to conduct an incident investigation. The first part of which should be to identify the specific food item in question such that the supply chain risk of cross-contact or mis-labeling can be investigated.

If there is any uncertainty as to what constitutes an allergic reaction, please review suitable sources of information such as (https://bit.ly/3OwyK8O) or contact an allergen expert for more information.

All consumer complaints, whether they are considered as a possible allergen incident or not, should be set in the context of all other complaints for the same and similar product types and within similar time frames, such that any emerging trends in complaints can be identified as early as possible for investigation. Within a food business there should be an awareness of the normal number and type of complaints received and if there is any change against the normal pattern this should trigger an interrogation of the complaints to understand if any potential risk is emerging with a product.

Chance of Occurrence
See section **5.1UAP Scenarios and characteristics of cross-contact** for a description of how to understand and capture the chance that UAP is or can occur.

Note that in many cases when the chance of occurrence is considered as 'low or unknown', it will not be appropriate to continue with an assessment, until further information is available. In such cases it may however be useful to perform a 'reverse QRA' as input on whether the potential UAP merits further investigation. This is where there is a lack of knowledge on whether a cross-contact incident is possible and if so the amount of allergen cross-contact involved, but by calculating an Action Level for a product (based on consumption amount and Reference Dose) and knowing the volume of product produced, it is possible to calculate the amount of cross-contact that would be of concern. This amount can then be evaluated in terms of whether such a cross-contact is likely to happen or not given the production scenario (see section **5.4.1.1**).

Track & Trace
If an incident is likely to have occurred, initiate the organisation track and trace procedure, and document the results including success.

4.1.5.2. Section 2: Data Gathering

In order to both decide on whether a risk assessment is appropriate and feasible, and to provide inputs into the assessment, the characteristics of UAP need to be captured. As a part of capturing the characteristics themselves, it is necessary to understand the uncertainty in the available information (see section **5.1.2**). This information can be used together with a high-level expression of the quality of available evidence (which we term the 'tier of refinement') to express the overall quality of evidence as a part of the risk communication described in section **4.1.5.4**.

Food Consumption
The likely and reasonable worst case of the quantity of food per consumption event should be determined such that the exposure to the allergen can be calculated – see section **5.3**.

Tier of Refinement
A 'tier of refinement' is a common concept in toxicological risk assessment and describes the quality of available data and therefore quality of the assessment output. In this Guidance we apply the concept of 'tiers' to capture key information for allergen assessment. The Tier of Refinement as applied to allergen assessment is defined within the form above, it provides a high-level description of the available quality of evidence. Note that this is different from the 'chance of occurrence' which provides an earlier decision point on whether it is appropriate to proceed with an assessment, based on whether UAP is actually occurring.

It may be the case that as knowledge of an incident unfolds, the available tier of refinement increases, as per the below diagram **Figure 11**.

***Figure 11**: illustration of the development of the Tier of Refinement as more information becomes available concerning an allergen incident.*

Characteristics of UAP: Data & Uncertainty
The Core Concepts section **5.1.2**, describes the 4 types of data which together are the characteristics of cross-contact and UAP, that are required to perform an allergen risk assessment. These data requirements are common to all allergen risk assessments whether performed in the context of incidents or operational controls.
1. UAP form
2. UAP distribution
3. UAP frequency
4. UAP concentration

Associated with each of these data types should be a description of the uncertainty in the available knowledge. Captured within the Incident Form is a simple system for scoring the uncertainty as a part of determining the quality of available evidence.

4.1.5.3. Section 3: Assessment

Assessment Decision
There are 4 possible outcomes of the assessment decision:

- When the available evidence makes clear that there is a risk beyond doubt to consumers, it is not appropriate to spend further time undertaking a risk assessment before communicating the risk (section 4.1.5.4). Notwithstanding, after the initial risk communication, in some circumstances, such as when affected product is at market, there may be value in performing a QRA to estimate the impact at market (so-called 'public health QRA' to determine the probability of expected reactions) as a follow-up measure to inform risk management.
- Uncertainty is too large to enable an assessment, further information is required.
- QRA is appropriate, but not possible without further information, qualitative assessment only.
- QRA is appropriate and possible.

Concerning these last 3 decisions, the **Table 10** is an illustration of how the Quality of Evidence Framework can be used to help the decision in a systematic fashion. If it is assumed that cross contact and UAP is occurring, the Tier of Refinement can be compared to the Overall Data Uncertainty (calculated in the Form) to provide direction on the appropriate assessment method. It should be noted that the table is for illustrative guidance only as each UAP scenario is unique.

Table 10. *Quality of Evidence Framework*

Tier of Refinement	**Overall Data Uncertainty**		
	High	**Medium**	**Acceptable**
1*	Uncertainty too large, more data required	Uncertainty too large, more data required	Uncertainty too large, more data required
2*	Uncertainty too large, more data required	Qualitative assessment only	Qualitative or Quantitative assessment
3	Qualitative or Quantitative assessment	Quantitative assessment	Quantitative assessment
4	Quantitative assessment	Quantitative assessment	Quantitative assessment

**A 'reverse' QRA may be useful to understand the amount of UAP that would present concern, to enable evaluation of whether that amount is feasible given the UAP scenario.*

Quantitative Assessment Calculation
See section **5.4** for calculation methodology.

4.1.5.4. Section 4: Assessment Outcome

The purpose of this section is to capture the outcome of the assessment and the quality of evidence by combining the chance of occurrence, tier of refinement, and overall data uncertainty. Possible next steps in terms of refinement of the assessment should be captured, and an understanding of the cause of the incident to facilitate corrective action. Key parts of this template are intended to be summarised at the head of the form in the section 'summary of the Assessment Matrix'.

4.1.6. Communication templates

The Allergen Incident Form is intended to be used by those involved in the risk assessment and for the purpose of communication with risk managers. It is not intended to use the Form with other audiences. For external communication to a general audience concerning an allergen incident, reference should be made to templates that may be suitable such as those made available by the US FDA:

Allergens Model Press Release:

https://www.fda.gov/safety/industry-guidance-recalls/allergens-model-press-release

Different templates from different model press releases:

https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts/industry-guidance-recalls

4.1.7. Use of food allergy prevalence data in public health QRA

The QRA calculations (section **5.4**) described in the Core Concepts section below enable an assessment of the whether an exposure is above or below a set Reference Dose. In the case of allergen incidents, it may also be helpful to estimate the public health impact of UAP by calculating the number of reactions likely to be experienced at market.

These public health allergen assessments can range from being simple to perform to being more sophisticated when refinement is needed (via the use of probabilistic methods). The inputs needed that are additional to those for regular QRA (which includes the amount of UAP in the implicated food) are:

- Data from the relevant publication on the full dose distribution for the allergen in question (not just a specific Reference Dose). This enables an estimation of the percentage of allergic consumers who may react to a particular dose of allergen.
- Knowledge on the number of implicated products at consumer market, and therefore using the approach described in **5.4.5** below, the number of consumption events possible of the implicated product.
- Estimation of the prevalence of specific food allergies within a population, as detailed in **0** below. Note, a conservative estimate for the prevalence of allergy to a particular allergenic food for further use in a 'rough' public health risk assessment can be estimated at 3%.

If it is assumed that the allergic consumers within a population are as likely to consume the implicated product as are non-allergic consumers, a simple calculation can be performed to predict the number of reactions that may be expected due to the presence of an UAP at market.

Although these 'public health' calculations are useful in the context of providing additional risk evidence, due to the uncertainties involved they should be considered as providing ancillary information only into a wider assessment. Risk management action should be informed by all available evidence.

For a more refined assessment of food allergy prevalence data please consult the **ANNEX Food allergy prevalence data** for more information or contact an external allergen risk assessment expert.

5. Core concepts

In this section, the concepts which are common to all allergen risk assessments including QRA are discussed. These overarching core concepts detailed below are:

- UAP scenarios and characteristics of cross-contact
- The amount of UAP in food
- Guidance on food intake data for allergen risk assessments
- Exposure assessment inputs and basic QRA calculations

These core concepts will be used to a large extent in any allergen (Q)RA, but the specific core concepts utilized will vary with the needs of each assessment. Finally, some of the core concepts that follow are quite brief and are intended to be used in combination with other sections of the Guidance. Links in those sections will direct the user to the appropriate core concept section.

5.1. UAP Scenarios and characteristics of cross-contact

5.1.1. UAP Scenario & Chance of Occurrence

In many UAP situations the presence of an allergen will be a theoretical possibility based on supply chain or production process knowledge but there will be doubt on whether the UAP has or can actually occur. The likelihood, or probability, of UAP in a food product is an important component of a food allergen RA and needs to be taken into account during both the decision on whether it is appropriate to perform a risk assessment given the amount of information available or whether it is first necessary to investigate the UAP scenario to understand if it may actually occur. Information on chance of occurrence is also an important input into the risk management decision making process. Strategies for estimating the chance of occurrence of UAP are currently limited and were identified as an area where further guidance would be welcomed (ILSI Europe Digital Event report). The chance of occurrence is a component of likelihood. Likelihood is in many ways the most complicated of the major food allergen RA inputs to estimate or measure. It combines two distinct parameters, the chance of an event ever occurring, and when it does, the frequency at which it occurs. For the purpose of allergen QRA in this Guidance we separate these distinct elements into 'chance of occurrence' and 'frequency of occurrence'. In this Guidance, the chance of occurrence is treated as a fundamental parameter which needs to be investigated first within an assessment, whereas the frequency of occurrence forms part of the parameters which we term 'characteristics of UAP'.

Chance of occurrence is the probability of whether the incident has occurred or not. In the **Table 11** we propose 3 categories to describe chance of occurrence.

Table 11. *Categories to describe chance of occurrence*

Chance of Occurrence	**Description**	**Recommended Action for the assessment of incidents**
High	It is more likely than not that UAP has/will occur: The factors that cause UAP are known, and there is acceptable uncertainty that those factors have/will happen.	Proceed with the assessment (next step Track & Trace).
Medium	It is possible that UAP has/will occur, but also equally likely it has/ will not: The factors that may cause UAP are known, and there is significant uncertainty on whether those factors have/will happen.	Gather data to decrease uncertainty on whether the incident has occurred. or If due to level of concern or time constraints proceed with the assumption that UAP has/will happen and the assessment, when/ if data becomes available repeat assessment of chance of occurrence.
Low	It is not likely that UAP has/will occur, but based on the uncertainty of the factors that cause UAP, it remains a possibility albeit unlikely.	Gather data to decrease uncertainty before progressing with an assessment.
Unknown	There is circumstantial evidence only that UAP has/will occur: Whether the UAP occurs or not cannot be estimated with acceptable level of certainty.	Gather data to decrease uncertainty before progressing with an assessment.

In order to determine what is the chance that UAP is occurring and therefore results in an incident, a root cause analysis can be useful. The **Table 12** is an example of a simple systematic process of performing root cause analysis. Incidents happen to supply chains that are already under allergen management controls, as such a review of those controls can be helpful in prioritizing potential sources of cross-contact when the source of an incident is not known.

Table 12. *A simple systematic process of performing root cause analysis*

Stage of Root Cause Analysis	Example of Information Gathered
Statement of the problem	Emerging consumer trend of reaction, commonality is that consumers are allergic to milk protein. A number of products are in-scope.
Hypothesis as to the cause of the problem	No milk protein is present in your own production processes for the product in question. Therefore, focus shifts to milk protein that may be present as UAP in one or more of ingredients received from suppliers.
Information and Investigation designed to test the hypothesis	Sample and test implicated finished product, in addition identify which supplied ingredients are likely to be at most risk of milk cross contact, liaise with the suppliers on any changes to cross-contact risk and conduct targeted ingredient testing.
Interpretation of the information gained	No milk protein identified in implicated finished product or at-risk ingredients.
Conclusions (and new hypothesis if required)	Either implicated finished product is not related to the reactions at market, or the contamination is intermittent and not identified in the above. Develop new hypotheses to test.

5.1.2. Characteristics of UAP

Based on an understanding of the cause of an UAP, if it is determined that there is a sufficient chance that UAP is or can occur, there are 4 types of data that are required to perform an allergen risk assessment. Together these data define the characteristics of UAP:

1. UAP form
2. UAP distribution
3. UAP frequency
4. UAP concentration

Uncertainty will be associated with each of these types of data, for example uncertainty on whether a cross-contact allergen is fully distributed within a finished product or whether it is clumped in a smaller quantity of finished product.

The characteristics of cross-contact in combination with the level of uncertainty determine the way in which an assessment is performed including the assumptions used. For example, the approach to sampling for analysis, and the way in which the QRA calculation is performed such as whether to perform a calculation for isolated particles or assume evenly distributed cross-contact.

Here we present simple tables to capture the characteristics of cross-contact including the uncertainty.

5.1.2.1. Form & Distribution (e.g., particulates vs homogeneous)

The form of UAP, with respect to how it is present in the finished consumer product can be one of the following:

- Amorphous
- Particulate
- Unknown

The form of UAP is linked to its distribution, which can be either:

- Homogeneous
- Heterogeneous
- Unknown

The **Table 13** illustrates the relationship between form and distribution and the **Table 14** the uncertainty of available information.

Table 13. *Relationship between form and distribution*

	Amorphous	**Particulate**
Homogeneous	UAP does not have a discrete structure, and is uniformly distributed within the sensitive product.	UAP has a discrete structure, those discrete structures are uniformly distributed within the sensitive product at a particular density per unit volume.
Heterogeneous	UAP does not have a discrete structure, but it is isolated (clumped) in one or more regions of the sensitive product.	UAP has a discrete structure, those discrete structures are not uniformly distributed within the sensitive product (discrete UAP structures vary in number in different samples of the sensitive product).

Table 14. *Uncertainty of available information*

Form & Distribution: quality of evidence	**Description**
High uncertainty	There is insufficient information to describe the form and/or distribution of UAP in the sensitive product.
Med uncertainty	The form and/or distribution of UAP in the sensitive product can be inferred based on knowledge of materials and process, but has not been confirmed.
Acceptable uncertainty	The form and/or distribution of UAP in the sensitive product has been confirmed (observation and/or measurement).

5.1.2.2. Frequency of cross contact

Frequency of Occurrence

Frequency of UAP can be stated as either:

- isolated
- intermittent
- regular
- unknown

An estimate of the frequency that cross-contact is occurring (eg the fraction of batches or consumer products that are subject to cross-contact) is important information as together with the other characteristics it provides an indication of the scale of concern. It is also a key input if QRA calculations are performed that estimate the probability and number of reactions at market, so-called 'public health' QRA (see **4.1.7** & **5.4.5**).

Table 15 provides the description of the above terminology of UAP frequency and **Table 16** describes the uncertainty.

Table 15. *Description of the different levels of UAP frequency.*

Frequency of UAP	Description
Isolated	The UAP has or will occur once only or very infrequently due to a set of unusual circumstances.
Intermittent	The UAP has or will occur more than once.
Regular	The UAP has or will occur systematically, it is more likely than not to happen.
Unknown	No data upon which to base judgement (always associated with high uncertainty).

Table 16. *Different statements of uncertainty of UAP frequency.*

Frequency of UAP: quality of evidence	Description
High uncertainty	The available information does not allow frequency of UAP to be estimated with acceptable level of certainty.
Med uncertainty	The factors that drive frequency of UAP are known. and there is significant uncertainty on whether those factors will happen.
Acceptable uncertainty	The factors that drive frequency of UAP are known. and There is acceptable uncertainty on whether those factors will happen.

5.1.2.3. Concentration or Quantity

Knowledge on quantity of UAP could be characterized as:

- Unknown
- Estimate, not based on analytical data, such as carry-over estimate (Carry-over calculation guidance)
- Analytical - point data
- Analytical - data range

The **Table 17** describes the meaning of these phrases, and the usual degree of uncertainty that may be associated using a simple scheme.

Table 17. *Different level of knowledge on quantity of UAP and the usual associated degree of uncertainty.*

Parameter	Meaning	Description of the usual associated uncertainty
Unknown	No basis for estimating concentration.	Results in high uncertainty.
Estimate	Concentration estimate is not based on analytical data but is based on understanding of amount of residue that may be available to enter sensitive product.	The basis for calculation has high probability of not being representative. May under-predict or over-predict concentration.
Point data	Single datum or limited analytical data available.	It is not known whether sample(s) analyzed are representative.
Data range	Multiple analytical data available.	The samples(s) analyzed are anticipated to be representative.

5.1.2.4. Particulate allergen cross-contact

5.1.2.4.1. Introduction

For practical purposes, allergen cross-contact can take place in two distinct physical forms: non-particulate and particulate. Particulate cross-contact refers to the situation where the allergen is in the form of discrete pieces, chunks, fragments or lumps, visible to the naked eye. Currently, quantitative approaches to assessment of allergen risks are rarely applied in relation to unintended allergen presence (UAP) in particulate form, in part because particulate UAP often occurs infrequently, in uneven numbers and nonuniform distributions in the product and as such, particulate allergen cross-contact continues to pose arduous risk assessment challenges. Guidance on managing particulate cross-contact remains extremely limited (FSA 2006, Madsen, Crevel et al. 2014) and a fear of severe outcomes has led the risk to be managed through the use of precautionary allergen labelling, without any attempt to apply quantitative methodologies.

In order to properly characterize the risk of particulate cross-contact, three different particulate variables are of potential importance:

- Size (volume) and Mass
- Composition
- Distribution

These parameters permit a characterization of the risk incurred by an allergic individual being exposed to one or more particles in a (portion of) product. Evaluation of the public health dimension of the risk will require estimates of how frequently the product in question contains one or more particles, and how frequently the product will be consumed by an allergic individual reactive to the amounts present.

These aspects will be discussed further below.

5.1.2.4.2. Definitions

For the purposes of this Guidance, *particulate allergenic ingredients* are materials in which the physical form of the allergen consists of pieces visible to the naked eye. They can be retained if passed through an appropriately sized sieve. In contrast, n*on-particulate allergens* are materials such as powders, etc., the individual components of which cannot be distinguished by the naked eye and which are not retained by a sieve. The current Guidance describes this type of cross-contact as 'homogeneous amorphous' and the Australia-New Zealand VITAL 2.0 scheme described this type of cross-contact as 'readily dispersible'.

Particulates could originate entirely from an allergenic source, such as pieces of nuts or could contain the allergenic source in a mixture of other components, such as pieces of dough with a percentage of the recipe coming from allergenic sources. The principles for assessing and quantifying the amount of protein from an allergenic source for these two types of particulates is similar and is described below.

5.1.2.4.3. Methodologies to assess particle size and mass

Information regarding potential protein exposure from particles of different sizes can be derived relatively simply from the particle size and mass, and possibly indirectly by using appropriate assumptions about its shape, volume and density (**Figure 12**).

Figure 12. *Visual illustrations of main tools needed for estimating particle size and subsequent potential protein exposures.*

Particle characteristics to a certain extent may be specified by the ingredient supplier. For

instance, the range of sizes for nut pieces may differ per product and nut suppliers will offer ingredients in certain millimeter (mm) size ranges. However, the weight of particles is rarely given and in some cases the sizes specified by the supplier have been shown to underestimate the actual size of supplied particles [***Table 18***, Meima, Remington et al. (2021)]. This limitation of potential information available can be solved by manually measuring the size and mass of individual particles with a ruler and a scale. The process is not time-consuming and a set of individual particles can quickly be measured and weighed. Characterization of a set of 30 or 50 particles will provide a range of data for the particle in question, but that may not always be practicable. It is recommended that a set of at least 10 particles is characterized. Quick outputs from this particle characterization will include:

- Mean size (mm, average ± standard deviation)
- Size range (min mm – max mm)
- Mean mass (mg, average ± standard deviation)
- Mass range (min mg – max mg)

These results can be summarized in a table or box plot, similar to the examples in ***Table 18*** and ***Figure 13***.

Bulk weighing procedures (i.e., weighing 100 particles at the same time) could give a quick estimate of the average particle weight, though this method is less precise than weighing and measuring a number of individual particles and it will not provide the upper range of individual particle size or mass.

Table 18. *Characteristics of model particles [reproduced from Meima, Remington et al. (2021)].*

Particle type	Mean size (mm)[c]	Size range (mm)	Mean mass (mg)	Shape
Mustard seeds laboratory experiments (n = 50)	2.1 ± 0.2	1.7 – 2.6	7.2 ± 1.2	Spherical
Mustard seeds test facility	2.1 ± 0.3[a]	1.5 – 3	8.2 ± 1.7 (n = 30)	Spherical
Sesame seeds (n = 10)	3.5 ± 0.5	3 – 4	2.9 ± 0.3	Irregular
Hazelnuts pieces (n = 30)	4.6 ± 1.1[b]	2 - 6	20.0 ± 7.4	Irregular
Walnut pieces	5 – 10 mm, n = 53, 10 – 15 mm, n = 44, 15 – 20 mm, n = 3	5 - 20	223 ± 148 (n = 30)	Irregular
Decoration pearls (n = 30)	4.9±0.2	4.3-5.4	65.7 ± 8.3	Spherical

[a] 0.5 – 1 mm, specified by supplier.
[b] 2 - 4 mm, specified by supplier.
[c] Manually measured. In some cases, the supplier provided information.

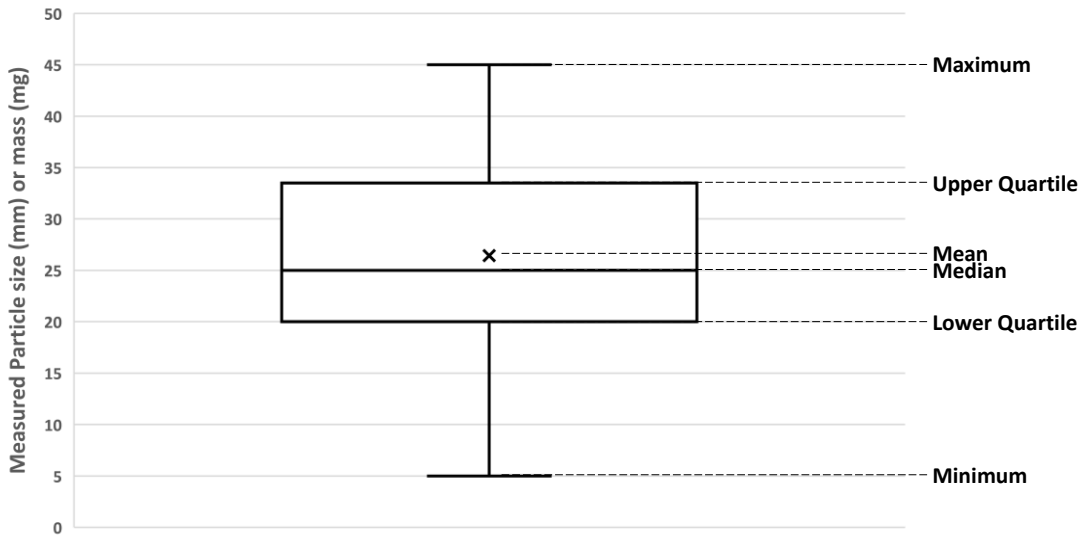


Figure 13. *Visual display for particle size (mm) or mass (mg), with the mean and range displayed*

Where to find particles for measuring?

- From supplied particulate ingredients known to be in prior recipes on production line
- From remaining final product on production line before cleaning / sanitation procedures
- From final product remaining on / in equipment after cleaning / sanitation procedures
- Sanitation filters or other filters used in the production process

5.1.2.4.4. Particle Composition

Particulates could be composed entirely of material from an allergenic source, such as pieces of nuts. Or conversely, particulates could contain the allergenic source in a mixture of other components, such as pieces of dough with a percentage of the recipe coming from allergenic sources. Once the particulate has been characterized, total protein from an allergenic source exposure amount can be calculated based on the recipe information available and using the formulas below:

Example: Particulate, 100% allergen composition $Exposure\ due\ to\ particulate\ (mg\ protein) = Particle\ mass\ (mg) \times \%\ total\ protein\ from\ allergenic\ source$	
- Particulate mass	= 100 mg
- % composition of total protein from allergenic source	= 26 % = 0.26
➔ Exposure due to particulate	= 100 * 0.26 = 26 mg peanut protein

Example: Particulate, Recipe composition is < 100 % from the allergenic source $Exposure\ due\ to\ particulate\ (mg\ protein) =$ $Particle\ mass\ (mg) \times \%\ Composition\ from\ an\ allergenic\ source\ \times \%\ total\ protein\ from\ allergenic\ source$	
Dough pieces with skimmed milk powder or nonfat dried milk (NFDM) ingredient used at 5%	
- Particulate mass	= 50 mg
- % ingredient composition from allergenic source	= 5 % = 0.05
- % total protein from allergenic source in ingredient	= 35 % = 0.35
➔ Exposure due to particulate	= 50 * 0.05 * 0.35 = 0.875 mg milk protein exposure

5.1.2.4.5. Using density to calculate the mass of allergen per particle

If data are available on the density of an allergenic material, they can be used to calculate the mass of particles instead of undertaking a physical measurement of weight.

It is often the case that UAP particles vary in size, as such a reasonable worst case of particle dimensions should be taken as input into a calculation of particle volume (which can be performed using either an equation for sphere or cube volume depending on most appropriate particle shape). The volume of the particle should be converted into a weight (mg) using the density of the particle in question, for example the density of peanut (from an authoritative source, such as, the FAO density database, available here) or if information is not available density can be assumed the same as water (1 g/mL or 1 g/mm^3). The weight of a particle should then be divided as per above, by the proportion of the particle that is protein to derive a value for the protein content of the particle.

5.1.2.4.6. Particle Distribution

As discussed in other sections of this Guidance, risk is the probability that a reaction will occur, with a consideration of the possible nature of that reaction. This requires an estimation of the frequency with which one or more particles will occur in the product, combined with the frequency with which that occurrence will exceed the Reference Dose for the allergen of interest and finally the frequency with which the product is consumed by a person with an allergy to that allergen. Particle distributions and likelihood of cross-contact can be difficult to characterize, but not in all cases.

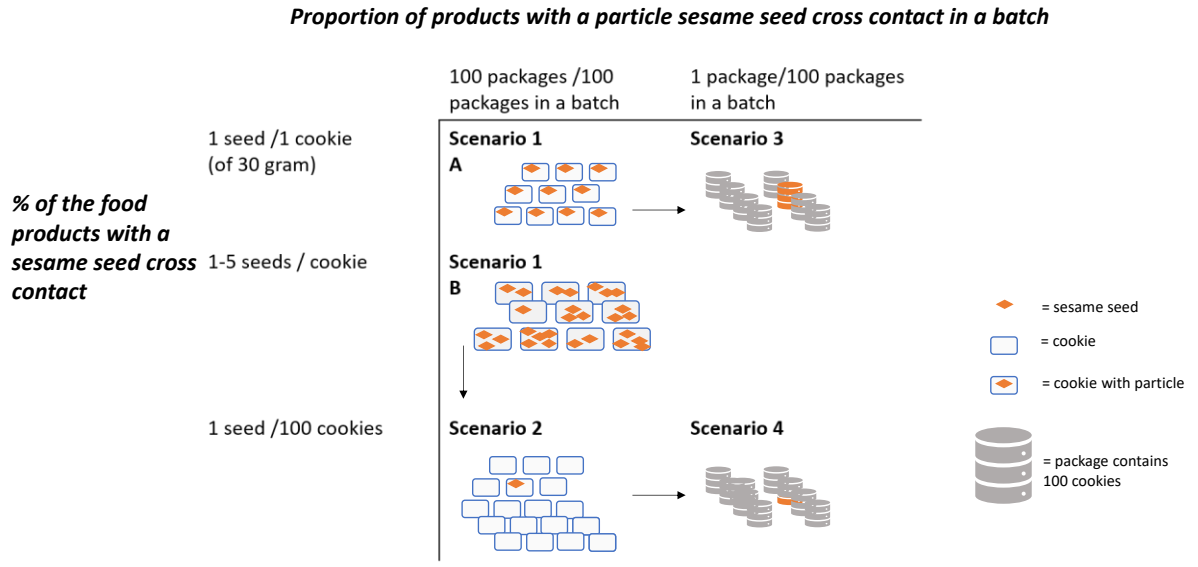


Figure 14. *Diagram of different potential particle cross-contact scenarios. The scenarios illustrate where particulates are present in every cookie (1A, 1B) or 1 out of 100 cookies (2) in every package for a batch, or when 1 of 100 packages in a batch contains UAP (3, 4).*

Figure 14 illustrates a number of different particulate cross-contact scenarios. If the outcome of a particulate distribution assessment points to a situation similar to **Figure 14's** scenario 1A or 1B, the allergen control measures within the Management of Operations should be reviewed and further risk mitigation options should be identified and implemented.

If the outcome of a particulate distribution assessment seems more likely to be similar to **Figure 14's** scenario 2, this could be similar to a situation where there has been a changeover and the first few units are known to still be heavily contaminated. In these cases other risk mitigation actions, such as discarding the first products produced, should be taken in place of sampling and analysis. If the particulate distribution assessment is similar to scenarios 3 or 4 then a sampling and analysis plan could be warranted, although preliminary calculations of worst-case frequency should be made to determine whether sampling is a realistic approach to actually detect particulate cross-contact (see Example 3, **5.1.2.4.7.3** for more information). Sampling plans clearly play a critical role in establishing valid estimates of particulate numbers, distribution and the probability that a particle will be present in the product. Sampling for particulates is discussed more broadly in the **Allergen Sampling and Analysis** section of this Guidance.

Based on the situation and the equipment layout and configuration, it may be recommended that either 1) incremental sampling or 2) sampling from the production section(s) of concern would be conducted to help characterize the distribution of particles in following products due to cross-contact.

> 1) Incremental samples are a quantity of material (intermediate or final product) taken from a single place in the consignment, lot, sublot or batch. As far as possible incremental samples are taken at various preselected (and recorded) random places distributed throughout the consignment (etc). For example, if the sampling point is at the final packed product, then it is as simple as pulling every Xth packet off the line before it goes into further packaging (boxes, pallets). Any departure from such procedure must be recorded. It may be relevant to consider a homogenized aggregate/composite sample when possible, however take into account whether UAP was homogenously or heterogeneously distributed.

2) Sampling from identified sections of concern may be possible if it is possible to identify specific time stamps of concern (first production off the line, etc) or other areas of concern for the specific production.

Sampling plans of this nature could better characterize the distribution of particle cross-contact in following product, but it must be noted that sampling & analysis will not solve particulate risk assessment and risk management issues by themselves, but only in the context of an overall risk assessment, which takes wider factors into account, such the acceptability of the risk. The concept of acceptable risk (both exposure amount and probability of exposure) is still under debate and viewed differently by various stakeholders (Madsen, van den Dungen et al. 2020) see ***Box Reference Doses***.

One example which considers the context of the overall risk assessment is as follows:

- Particulate cross contact may occur in the form of a single, large piece of the allergenic material, such as half of a peanut cotyledon (half of a peanut approx. 200mg i.e., 50mg peanut protein).
- Such an amount is predicted to provoke a reaction in 25% of people with a peanut allergy and is of an order of magnitude associated with severe or fatal reactions.
- In this context, even a very low frequency of occurrence (e.g., >1:1,000,000), may be considered unacceptable and a precautionary approach, using a PAL statement may still be recommended.

5.1.2.4.7. Illustrated examples

5.1.2.4.7.1. Product contaminated by doughy piece from prior product

A manufacturer produces egg-containing cookies and cookies without eggs present in the recipe on the same production lines. The manufacture has observed in some instances, a few pieces of dough remain in certain parts of equipment after cleaning and could be incorporated into the first cookies produced in the following batch.

The manufacturer conducted an exposure assessment for potential egg in cookies made on the same equipment that led to a decision to not state egg on the label.

Collection and weighing of pieces found dough particles of 1-10mg in weight. In the case of the egg containing cookie, the product formulation contains 3% liquid whole egg. Liquid whole egg is ~13% protein (section **7.8**). Using the upper limit of the observed dough particle sizes (10mg), a maximum per particle exposure was determined to be 0.04 mg protein from egg (0.04 = 10 * 0.03 * 0.13, see **Particle Composition** for information).

Based on the number of residual particles found and their ability to enter the subsequent product, it was considered a worst case that no more than 2 individual particles might cross-contact individual cookies.

5.1.2.4.7.2. Hazelnut on sanitation filter

An error occurred in the sanitation of an ice cream line producing ice cream containing almond (Incident example 2 in annex **7.5.2** for full details). Briefly, a filter was not removed and cleaned (2 mm circular pores). On inspection this filter is not normally found to hold residual particles, but on occasion a limited number of particles have been found. These potential particles on the sanitation filter could be hazelnut due to it being used in other recipes on the same production line.

The almond-containing ice cream carries PAL (may contain tree nuts) and an assessment was requested to determine whether the risk to hazelnut allergic individuals was increased due to lack of adequate cleaning of the filter.

It is not known if any 2 mm diameter particles were actually present, however, even with no analytical data available, the worst-case size of particle(s) is known and the mass can be calculated.

Example: Particulate, 100% allergen composition	
- Hazelnut particulate diameter (∅)	= 2 mm
➔ Particle volume (V)= Π * $\varnothing^3$ / 6	= 3.14 * 8 / 6 = 4.18 mm^3
- Density of hazelnut (d)	= 0.6 mg/mm^3
➔ Particulate mass (m) = V * d	= 4.18 * 0.6 = 2.5 mg peanut protein
- % composition of total hazelnut protein from allergenic source (hazelnut)	= 15 % = 0.15
➔ potential exposure = m * %composition =	= 2.5 * 0.15 = 0.38 mg *hazelnut protein in hazelnut particulate*

* See **Using density to calculate the mass of allergen per particle** for more information

Based on experience of the production line, it was considered highly unlikely that any cross-contact would result in more than one particle in subsequent product. Therefore the allergen risk profile of the product was not considered to be altered.

5.1.2.4.7.3. Sampling and estimating Cross-contact by nut pieces in a water-soluble ingredient

A manufacturer produces a water-soluble ingredient, itself in the form of particles, on machinery which also processes nut pieces. A full wet clean and inspection of equipment is undertaken after nuts are used and before the ingredient is produced. As the ingredient manufacturer is unwilling to 'guarantee the absence of nut pieces' in the ingredient, the manufacturer of the final product needs to consider whether a precautionary 'may contain nuts' label is necessary.

The ingredient is water soluble, while the nut particles are not. It is produced in batches of several hundred kilograms. The approach to establishing the frequency of nut particles was therefore to dissolve 1kg samples of the ingredient in water and pass the contents through a sieve (0.25 mm) to retain any particles, which would then be counted. Nut particles are assumed to be evenly distributed in the ingredient. In this instance, 21 independent samples (1 Kg) of ingredient each, were tested and no particle was found. As no frequency could therefore be established, the Poisson distribution was used to calculate the probability of finding 1 to 10 particles (10 separate scenarios) in the (untested) 22nd sample. This means 1 to 10 pieces in 22 kg of ingredient, while the final product contains 1.5 g of the ingredient per serving.

An initial assessment, based on sampling data, suggested a maximum frequency of cross-contact of 1 nut piece per 14705 products.

5.2. Amount of UAP in food

This core concept section includes guidance on sampling and analysis, the conversion of data such that it is suitable for use in QRA, and options for carry-over estimation.

5.2.1. Allergen Sampling and Analysis

5.2.1.1. Introduction

This section is intended to help with harmonisation of good practices in allergen sampling and analysis across the food chain. This section includes flow charts, a standard template (Annex **7.7**) and examples to aid clarity and record keeping. We discuss mainly IgE mediated food allergy but touch on other conditions within the wider ambit of food hypersensitivity such as Coeliac condition (gluten) and target analytes such as sulphites and food intolerance parameters such as lactose.

Allergen testing is just one component of information that can be used as input into a QRA. When testing is considered, there are parameters discussed in this section, that require consideration in order to plan and perform testing that provides useful information, see **Figure 15.**

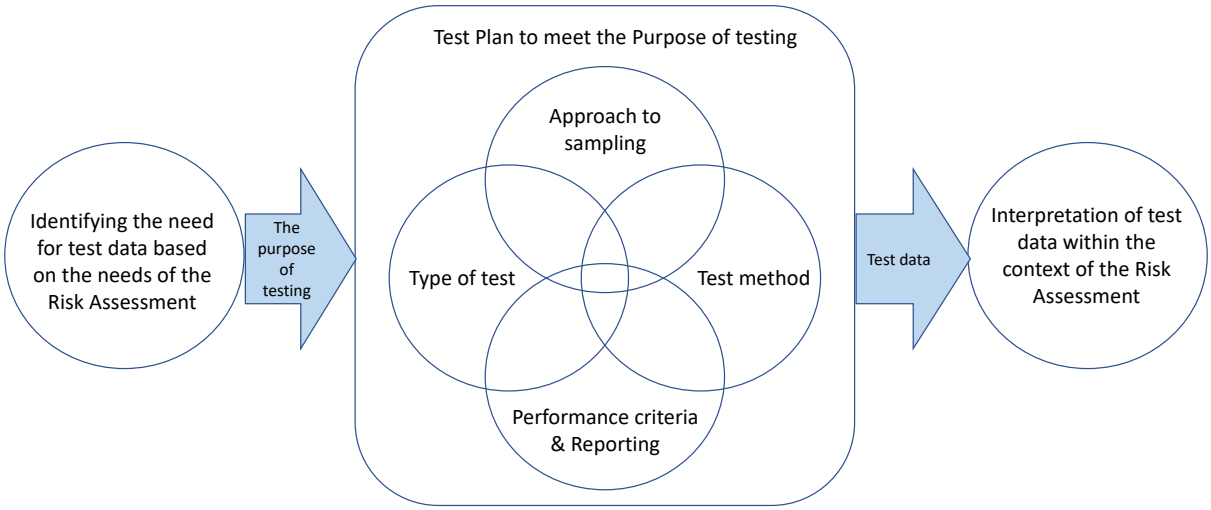


Figure 15. *The basic process of testing for the presence of allergens.*

This section on allergen analysis is divided into the purposes of analyses, available methods and result interpretation. It is important to define why analysis is needed and the required outcome in order to set up an appropriate sampling plan, including type of sample(s), frequency of testing, methods and even which specific test kits to use. The choice of method highly depends on the purpose and any limitations of methods or test kits. Due to the multiple parameters involved in every sampling and analysis scheme there is no general one-fits-all solution. Analysis is offered by a wide range of laboratories for all the 14 food groups in Annex II (Substances or Products Causing Allergies or Intolerances) of Regulation (EU) No 1169/2011 *on food information to consumers*. However, no single analytical approach can be applied to all the possible targets.

Analysis for allergens may be more problematic than other types of analysis with more care needed to guard against false negatives, false positives or incorrect quantification. False negatives may arise from failure to extract the relevant allergen marker or surrogate owing to matrix interferences (see below). In ELISA, false positives may arise from cross-reactivity to multiple allergens. The quality of the results depends on the sampling process, the sample preparation and the subsequent analysis including its reporting and interpretation. This

Guidance does not provide information on how to perform analysis, such information is available from laboratories, standard methods of analysis, the literature and test kit manufacturers / suppliers, this section provides information on how food businesses can appropriately apply sampling and analysis within their operations.

Allergen analysis should be used to provide objective evidence of the effectiveness of allergen controls and never to be used in isolation for allergen management. Even the most robust sampling and analysis plan will have limitations in the ability to accurately reflect the true situation of allergen content within a sample.

5.2.1.2. Type of samples

Table 7 in the section **3** on Management of Operations describes the common types of samples, which can be taken as a part of a sampling plan. Depending on the purpose of testing a choice can be made between one or more sample types to answer a question or challenge an assertion/assumption. Section **5.2.1.3.5** describes the possible purposes of sampling, the suitable sample types and sampling methods that may be used.

5.2.1.2.1. Finished products/ prepared foods or intermediate products

When taking samples careful consideration is necessary on the form of the product and where possible cross-contact may have happened in the preparation of the product.

The first packs produced after a product-change over from a product with the highest allergenic protein content to a product without this allergen, are often the best choice of situations for sampling to provide information on the capability of the change-over process. However, when it is known that cross-contact may occur at a specific point in a process, this should be taken into account in terms of the location for sampling.

For example, when a product consists of different components such as a ready-to-eat meal with rice, meat, vegetables and a sauce, a composite or aggregate sample can be prepared by homogenising the whole meal from which a test portion is taken for analysis. However, because of the dilution effect of the other components of this meal, possible cross-contact may be more challenging to identify analytically. Thus for example, if it is known cross-contact with a specific allergen may occur at the filling station of the sauce, preferably only the sauce could be sampled.

In the case of dry granulated or coarse products, like dried pulses or sprinkles, packed into a carton, depending on the density and particle size cross-contact UAP may be more likely at the bottom or the carton instead of the top, for example allergen-containing particles that are smaller or more dense than the intentional foodstuff will migrate to the bottom of the carton. If this is suspected, stratified sampling down the pack, or thorough mixing before taking a test postion for analysis should be practised.

5.2.1.2.2. Ingredients/ raw materials

For raw materials, it is often the case that heterogeneous contamination can be expected, which makes finding a cross-contact UAP more difficult.

The form of the ingredient and previous handling of the ingredient in the supply chain should be taken into account.

For example, when receiving materials like grains or pulses, which have undergone minimal processing, agricultural co-mingling with allergens are the main concern. Such allergens come

in the form of seeds (e.g. mustard seeds) or parts of other crops like wheat kernels, soy and lupin beans. In these cases a visual check of a suitable quantity of the raw material may yield as much or more information as allergen analysis since a larger quantity of raw material can be inspected. Careful assessment of reject materials from cleaning processes like windsifting, colour-separation or sieving can give insight in cross-contact from the primary production.

In case of large solid products like primal cuts of meat, samples of the external surfaces are recommended. The inside of the product should not contain cross-contact allergens. Only the slaughtering process or cutting/ packaging can lead to cross-contact, and if that occurs it will invariably be on the surface of the food.

For powders, liquids and ingredients that are more highly processed with mixing steps a more homogeneous cross-contact should be expected. For such products random sampling throughout a bulk-consignment is recommended.

5.2.1.2.3. Rinse waters

In closed systems surface sampling by means of swabs is often difficult. It is likely that many areas cannot be reached or even that production lines should not be opened due to sterility reasons. Such installations are often cleaned by CIP-systems. Rinse water can be tapped somewhere at the returning CIP- line to the rinse tank or just before the drain, though always at the end of the rinse stage. However, dismantling of equipment should always be considered (for visual inspection and/or surface swab testing) and is preferred above rinse water testing. Rinse water testing has numerous limitations, including that protein/mineral residues may still remain adhering to cleaned surfaces. Moreover, due to the huge dilution effect of many litres of water used for rinsing, a cross-contact allergen is likely to be too diluted to detect with routine analytical methods. Also, the presence of chemicals like cleaning and disinfecting agents can interfere with analysis, particularly when rinsing has been ineffective (e.g. due to pump failure).

When there is no alternative to rinse water testing, complete the rinse cycle and tap the last water (stagnant water) from different parts of the equipment after some minutes. This water has been in contact with the equipment for a longer time compared to free-flowing water drained from the installation so in theory remaining product residues might dissolve in this smaller amount of water.

Turbidity testing of rinse water can be considered as a surrogate or additionally to allergen testing. Water containing food particles in the lower ppm range, which increase turbidity, may appear clear and can not be distinguished from clean water by eye. Section **5.2.1.4.8** describes the use of turbidity testing as an alternative method for rinse waters.

5.2.1.2.4. Airborne dusts or aerosols - Settle plates / air sampling

Airborne dusts from powdered ingredients, including but not limited to flour, (skimmed) milk powder or powdered egg, or aerosols may give rise to UAP if they settle in open bins, on uncovered product or 'clean' surfaces not intended for the airborn ingredient. A relatively straightforward way to investigate allergens in airborne dusts is to allow the dust to settle on a petri dish or similar container of known surface area (settle plate). The location, time of exposure and the activities taking place alongside need to be considered. If there is a possibility for allergen-containing dust cross-contact this can be quantified with increasing levels of refinement. Firstly, by collecting settled dust and using assumptions to calculate the concentration in unlabeled product, and if necessary, refining the calculation with analytical data on the protein content of settled dust (For methods to collect and analyse dust see CFS (Commercial Food Sanitation 2015) and for an example see (Remington, Baumert et al. 2022).

If needed, airborne dust that accumulates on the settle plate can be analysed for allergens by swabbing the plate or dissolution of its contents with a known volume of extraction buffer. The exposure time may relate to the actual time of a dust-generating activity, or longer if a lower detection capability is required or the exercise is untargeted surveillance for environmental monitoring purposes. Passive air sampling such as this may be used to validate the effectiveness of segregation or air flow systems or investigate potential cross-contact due to the production of dusts or aerosols e.g. when cleaning.

The advantages of using settle plates include low costs and operator time. Active air sampling is more comprehensive approach. A calibrated pump is used to draw air at a known flow rate for a given time through a filter which is extracted and analysed for allergens.

Bear in mind that airborne dusts may settle on, or become attracted by static charge, to such things as wrapped pallet forklift loads and be transported to unexpected places in the plant.

Reference: (Brown and Arrowsmith 2015). Sampling for food allergens. In Handbook of Food Allergen Detection and Control (pp. 181-197). Woodhead Publishing, https://doi.org/10.1016/C2013-0-16428-8

5.2.1.2.5. Environmental swabs

Environmental swabbing is used to test for the presence of allergens on surfaces. This type of sampling can be applied to the surfaces of food manufacturing, people's hands and protective clothing of food factory operators.

The locations to take swab samples from, are selected to challenge the efficacy of cleaning, targeting difficult to clean areas such as specific "trouble spots' where allergen residue may accumulate.

Sampling is ideally done with prewetted swabs or on moistened surfaces to transfer any residue from the surface of interest to the swab. Dry swabs used on dry surfaces will not pick up all residues.

A defined area is swabbed in a consistent manner and dimensions, e.g., 10 cm x10 cm =100 cm^2, in order to compare results to each other. In practice, it is often the crevices and seams of equipment that are challenging to clean and therefore need to be sampled. In such cases, ensuring that the well-practiced sampling technique used during cleaning validation is used when verifying cleaning, in order to ensure comparability of results.

Swabs for microbiological testing should never be used for allergen testing. Often media/ broth is used which contains milk, egg, soy or other allergens (such as in peptone buffer). In that case false positives are obtained by using those swabs. Specific swabs with a suitable swabbing solution are available from different ELISA kit manufacturers. Lateral flow kits are often supplied with swabs.

For alternative methods like protein testing or ATP (Adenosine triphosphate), the swab is supplied with the test kit.

5.2.1.3. Purposes for testing

Food allergen analysis is useful for a number of purposes including:

- Confirm free-from claims
- Establish allergen status of raw materials / ingredients
- Provide additional data as a part of validation of the effectiveness of cleaning / allergen changeovers
- Identify equipment that is difficult to clean
- Inform the risk assessment
- Provide additional data to verify the risk of allergen carry-over in finished product.
- Monitor effect of critical changes
- Provide evidence in root cause analysis e.g. with incidents like product recalls or complaints.
- Provide objective evidence for internal or customer audits and certification schemes.

Choosing the most appropriate type of analysis and selecting the test method (test kit) can be complex. The selection of tests and designing a sampling plan consists of four steps:

1. Define the question that needs to be addressed and purpose of testing. This should provide an overview of type of samples that would be fit for purpose.
2. Decide what allergens, alternatives (indirect testing) or food intolerance targets to test for.
3. Choose the method suitable for the selected analytical targets and matrices. Based on the previous steps a type of test can be chosen. Within the type of test, many methods/ kits may be available.
4. Define the location, frequency and numbers of samples to take for testing. (See also section 5.2.1.3.5 Sampling: Key aspects).

Table 19 illustrates types of tests and their applicability. The Table includes analytical approaches for both IgE mediated allergens and other foods, such as lactose and sulphites which elicit hypersensitivity reactions which are not true allergies.

5.2.1.3.1. Types of tests and their applicability

***Table 19**. Types of tests and their application*

Type of test	General remarks	Confirming claim	Confirming composition	Quantification risk	Validation of cleaning	Verification of cleaning
Immunoassay (e.g.) ELISA						
Products (end)	Product testing provides quantitative results. Settle plates and swabs are qualitative or semi-quantitative.	X	X	X	X	(X)
Raw materials		X	X	X		
Rinse waters					X	(X)
Settle plates					X	
Swabs					X	(X)
PCR						
Products (end)	Only quantitative real-time PCR can be used for comparison with Action Level. Method speed can restrict some applications.	X	X	X	X	
Raw materials		X	X	X		
Rinse waters					X	
Settle plates					X	
Swabs					X	
LC-MS/MS						
Products (end)	Screening method, currently mainly qualitative. No cost-effective option of verification purposes. Method speed can restrict some applications.	X	X		(X)	
Raw materials		X	X			
Rinse waters		(X)				
Distillation, ion-chromatography	Sulphites only (not protein based)					
Products (end)	* Concentration of sulphite-containing products are often too low to choose as previous product in a validation/ verification (note1). * Not part of QRA	X	X			
Raw materials		X	X			
HPAEC-PAD HPLC, enzymatic	Lactose only					
Products (end)	* Lactose-free claims should not be confused with milk-free or dairy-free. * QRA should not be performed on lactose, but based on milk proteins * Only when products contain pure lactose, see section 5.2.1.4.10 * LSw = Lactose swab	X	X		(x)	
Raw materials		X	X		(x)	
Rinse waters					(x)	LSw
Settle plates					(x)	
Swabs						LSw
Next Generation Sequencing						
Products (end)	Only for confirmation or screening purposes to look for presence of DNA from multiple allergens.	(x)	(x)			
Raw materials		(x)	(x)			
Lateral flow/ flow-through immuno assays						(#)

Type of test	General remarks	Confirming claim	Confirming composition	Quantification risk	Validation of cleaning	Verification of cleaning
Products (end)	Rapid allergen testing, only qualitative (#) Flushing product, first start-up product		X			X
Raw materials			X			X
Rinse waters			X			X
Settle plates			X			
Swabs			X			X
Turbidity	Only for water					
Rinse waters					(X)	X
Protein swabs						
Raw materials	Only for surface testing as a general marker for protein containing foods (**5.2.1.4.9**).		(X)			
Swabs						X
Glucose/ lactose swabs						
Swabs	Only for surface testing as a general marker for non/ low-protein containing foods (dried fruits, supplements)					X
ATP swabs						
Swabs	Only for surface testing as a general marker for non-processed foods (fresh vegetables, fruit, meat)					X
UV inspection	Only for improved visual inspection					X

(X) not first choice method, but can be used in specific circumstances
Note 1: *For example in a change over from product containing sulphites (say, potato powder ~100 mg/kg SO_2) to another product without sulphites potato powder cross contact will not be detected below ~10% (typical method LoD 10 mg/kg as SO_2)and 10% isan unrealistically high amount of cross-contact.*

5.2.1.3.2. Choice of type of test

5.2.1.3.2.1. Confirming the status of an allergen claim (e.g., "free-from")

In the case of "confirmation of claim" it may be the case that only finished product testing is appropriate, as legal limits, and commercial standards are set in mg/ kg in the finished product. It is also important that any test is considered appropriate for the specific scenario (allergen and matrix combination, etc) as an incorrect choice of test may result in an incorrect result (see **5.2.1.3.4**). Calculated Action Levels, based on RfDs, should not be used or with caution as a limit for 'free-from' claims.

Testing of ingredients is likely to be an important part of validation and on-going verification but should not be seen as a surrogate for finished product testing, not least as cross-contact can occur downstream of ingredient receipt. Testing of air particles, swabs or rinse water cannot directly be linked to the concentration of allergen in the product. Such techniques are only suitable for checks on verification of allergen controls, not for allergens in the final product.

Depending on the type of claim, specific test methods are available. **Table 19** indicates that different techniques can be used for the determination of lactose (enzymatic or HPLC). More information can be found in section **5.2.1.4.5**. A lactose-free claim should be carefully distinguished from milk-free or "dairy"-free claims. Those are often misunderstood, leading to erroneous selection of unsuitable methods or drawing incorrect conclusions.

A gluten-free claim has to be confirmed by testing of the finished product, however, additional testing of high-risk ingredients is recommended. The latter gives more insight into possible fluctuations in gluten cross-contact in the incoming raw materials and possible issues of cross-contact in the supply chain. Because raw materials are used as a proportion of an end product, a 'dilution' effect occurs when only testing end products. Depending on the percentage used in a finished product formula certain amounts of cross-contact in the raw materials may be able to be ignored.

For gluten testing special attention is needed for fermented foods like beer, soy sauce or malt extracts. For those products a competitive ELISA must be used instead of a sandwich ELISA. Fermented hydrolysed products such as some beers present particular difficulties in calibration and interpretation of assays. Due to this issue labelling requirements may differ between Europe and the USA. These issues are discussed fully here by Coeliac UK. (section **5.2.1.3.4.2**).

5.2.1.3.2.2. Confirmation of composition / cross-contact

Testing can provide information on the potential for UAP by analysis of raw materials when suppliers cannot provide suitable cross-contact information or during a monitoring program to tackle unknown/ unexpected cross-contact within a supply chain.

When testing products with a kit, special attention is needed for possible cross-reactions (**5.2.1.3.4.4**) leading to false positive results due to presence of similar proteins. For example, an ELISA for almonds may also detect apricot kernels, and return a positive result when proteins from either source is present. In the bakery industry apricot kernels can be used as a substitute for almonds for production of marzipan (persipan). ELISA is not suitable for testing almonds in marsipan, while other techniques (e.g. PCR or LC-MS/MS) can distinguish proteins from different species. In addition, LC-MS/MS and multiplex real-time PCR is a cost-effective multi-analyte technique able to screen and identify the multiple allergens present.

5.2.1.3.2.3. Quantification of risk: comparison QRA/ Action Level

Special attention is needed regarding the sensitivity (detection capability) of a method when generating data for a quantitative risk assessment. Is the chosen method sensitive enough to provide useful information for the risk assessment? This is also relevant when comparing the amount of exposure to an allergen with a Reference Dose or a concentration of an allergen in food to an Action Level. Detection capability is assessed by the method limit of detection, LoD and limit of quantification, LoQ (see below section **5.2.1.3.4.1.**).

5.2.1.3.2.4. Validation of cleaning/ allergen control measures

The section concerning the **Management of Operations** describes a validation and verification setup. The goal of cleaning step is to remove food residues (e.g. allergens) from a previous production. A cleaning process is validated only when it is proven to be effective. One approach is to test product and equipment for residues of contamination following consecutive – often 3 - production runs.

A validation test protocol may combine different test types. For example: equipment swabs combined with testing finished product at the first start-up. Also, in-depth testing of air movement of airborne allergens by settle plates or rinse water testing can be applied.

When multiple allergens are present on-site it may not be necessary to test for every allergen, instead a worst-case approach may be used. This type of approach involves making a choice for a specific allergen based the lowest RfD or Action Level, highest protein content, suitably low method detection capability and assessment of the possibility of allergen cross contact owing to the difficulty of cleaning the line. This can be considered as the worst-case. If this allergen cannot be detected after a product-change over, other allergens are most likely to be absent as well.

The use of a riboflavin solution, which is sprayed on areas or surfaces to be cleaned, may be a practical alternative for validation of CIP or cleaning. Riboflavin (vitamin B2) is fluorescent under UV light and small residual amounts may be visible (section **5.2.1.4.12**).

5.2.1.3.2.5. Verification of cleaning/ allergen control measures

Once validated, ongoing verification and monitoring must be in place to ensure that the validated cleaning is carried out correctly and continues to be effective. Periodic assessment *after* cleaning of an allergen containing product is recommended and can include testing as a part of the process.

Verification may also be needed to ensure adequate sanitation *before* processing a product, for example when producing a product with a free-from claim. A test may be used as part of a line clearance procedure.

Verification can include using a specific type of test or a combination of test types, and other non-test methods including visual inspection. In addition to a powerful normal light, visual inspection can be enhanced by use of UV light (section **5.2.1.4.12**).

When using test methods as a part of verification it is necessary to set control or acceptance limits based on absence (i.e., below the limit of quantification) when using the best or most appropriate test methods for the contaminant in question. However, this does not always mean:

- The *most* sensitive method available must always be used (for example if there are issues with how specific it is or if there are risks of false positive results at lower levels), or
- Methods that are not very sensitive (i.e., methods that would not be able to pick up low levels of contamination) should not be used because a result showing "not detected" if the limit of quantification is too high could still mean there is significant contaminant remaining.

5.2.1.3.3. Choice of allergen to test

Table 19 provides an overview of type of test and their suitability for different purposes (discussed in detail in **5.2.1.3**). The next step is to make a choice within or between the remaining different type of tests. This depends on many factors including practical considerations like test speed or costs and analytical characteristics (see **5.2.1.3.4**). But the choice is also limited by the availability and suitability of a test for specific allergens or targets. Some test type/ target combinations are not available on the market, or testing based on certain allergenic ingredients may not be meaningful. Taking the product-based limitations into account, it further limits the options.

For some foods, such as celery, no ELISA method is available but real time polymerase chain reaction (PCR) methods for the species DNA are. Conversely PCR methods are not appropriate for milk and egg as the same DNA is amplifiable from bovine or chicken (Gallus gallus domesticus) muscle and other tissues.

The analytical target protein may need careful consideration. If the ELISA antibodies target egg white protein, as many do, egg yolk proteins (e.g. in mayonnaise) will not be detected. However, commercial egg yolk is often blended with substantial amounts of egg white to adjust the product viscosity and if this is known ELISAs using antibodies to either are feasible. On the other hand, lysozyme, a protein found in egg white at about 6% of the ovalbumin content, can be used as an ingredient in cheese, sausages and wine. An egg white protein ELISA kit may not detected lysozyme and a specific egg lysozyme ELISA should be used.

More information on Specificity of test method to the target protein(s) can be found in section **5.2.1.3.4.3**.

Annex II (Substances or Products Causing Allergies or Intolerances) of Regulation (EU) No 1169/2011 contains a number of exemptions; ingredients derived from a listed allergen which are exempt from mandatory allergen labelling (section **2.1**), as they have been shown not to contain sufficient protein to elicit a response in consumers with allergies. These include wheat based maltodextrins, fully refined soybean oil and fat, fish gelatin, distillates from nuts and other

substances.[11] Additionally, highly refined oils are exempt from mandatory allergen labelling in the USA. Testing for the presence or even cross-contact of such exempted substances is not meaningful. Other ingredients can be very low in protein, such as soy lecithin or sesame oil so testing may not be useful.

Highly processed products, e.g. high temperature/prolonged heating, may cause protein to degrade to the point where it is not detectable (but unless demonstrated otherwise may still present some allergy hazard). Examples include egg in rusks, charred products or frying oil and highly fermented products such as soy sauce or HVP. Testing for the parent allergens in such products by routine methods is not (yet) meaningful. See also section **5.2.1.3.4.6.**

When multiple allergens are included in a product, testing for each individual allergen may be replaced by testing for a 'worst case' single marker allergen (section **5.2.1.3.2.4**). This approach, if properly validated and verified is more economic and can help avoid testing for allergens for which the analytical options are more problematic.

Testing for non-specific surrogates may also be an option. For example, a general test for protein e.g. the Biuret or Coomassie based colour reactions, are less susceptible to processing effects (section **5.2.1.4.9**) may be used when absence of protein needs to be verified (e.g. after wet cleaning on surfaces). Markers that are not allergenic in themselves such as glucose or lactose may be useful when present in an allergenic ingredient in amounts significant with regard to protein (eg. dried fruits). In most cases indirect methods based on non-specific surrogates are only used for verification purposes.

5.2.1.3.4. Selection of testing methods

When choosing a method for allergen analysis, it is critical that there is a clear understanding of the intended use of the analytical outcomes and the appropriate application of results for each allergen detection scenario. There are many factors that can affect the accuracy of analytical testing.

An inappropriate technique can produce a false negative result (the allergen is present, but the test is not capable of detecting the allergen) or a false positive result (the allergen is not present, but the test incorrectly identifies it as being present). An incorrect choice of analysis test may result in an incorrect result.

The various analytical techniques produced by different manufacturers may produce diverging results. When choosing a test method, ensure that the limitations of the test and the required outcome for the test are considered. Ask your laboratory for known information on false negative or false positive (eg. Cross-reactivity) results.

Method performance characteristics are the criteria that can be used to assess if a method is fit for purpose. They include accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), recovery, selectivity (specificity), sensitivity, linearity and measurement uncertainty. More guidance is available in an IFST Information Statement "*How to choose and instruct a*

[11] A summary of EU exemptions from Annex II labelling requirements is provided by FooDrinkEurope in the Allergen Labelling Annex of their Guidance on Food Allergen Management for Food Manufacturers

laboratory for chemical food analysis" and in particular see "*Analytical method performance characteristics*".

Analytical method performance must be evaluated during method validation and must be adequately covered when the method is accredited to ISO/IEC 17025:2017 '*General requirements for the competence of testing and calibration laboratories*'. In addition to such accreditation a laboratory reporting allergen analysis must be aware of all the issues and be able to guide a food business through the complexities of allergen analysis so that the analytical results are meaningful and informative (Allergen Bureau's Unexpected Allergens in Food 2021). Such knowledge and experience is costly to acquire and maintain which will be reflected in the laboratory cost structure.

The analyses of known negative and positive samples should be included in the method validation study carried out by the kit manufacturer and the user. This ensures that the most frequent occurring detection issues are understood and mitigated, whether they are due to matrix, processing, or cross-reactivity.

5.2.1.3.4.1. Sensitivity (detection capability) / LoD and LoQ

The detection capability, often informally called 'sensitivity[12]', of an analytical method is an attempt to indicate the smallest amount of analyte in a sample that can be accurately measured by an analytical method. Detection capability is assessed by calculating the method Limit of Detection, LoD, and Limit of Quantification, LoQ. These two metrics are not the same.

- LoD is a concentration below which a method is unable to distinguish an analyte signal from the 'blank' response, or 'noise';
- LoQ is a concentration at and above which the target analyte can be quantified, with a given statistical probability;
- The LoQ is usually higher than the LoD.

There are several ways in which LoD and LoQ can be estimated.

- From analyses of 'blank' or 'near-to-blank' samples
  - Blank: a test sample in which the analyte is absent
  - Near-to-blank: a test sample in which the analyte is present at a concentration level close to, but not exceeding five times, the expected LoD. Can be naturally occurring or spiked. Also called a 'pseudo-blank.
- From calibration curves prepared from spiked blank or near-to-blank samples.
- From signal-to-noise ratios.

Each approach has advantages and disadvantages and depends on various assumptions that may not be fully met. Hence LoD and LoQ data should be treated with some caution.

[12] Sensitivity is formally defined as the slope of the calibration curve, i.e. the change in analytical response, as a function of a change in analyte concentration.

There are practical considerations that should be borne in mind.

- From the definitions above it is clear that the LoQ should be used in QRA, e.g. comparing a result of analysis against an Action Level.
- The LoQ must be *below* the calculated Action Level.
- Check the stated measurand before comparing an LoQ to the Action Level. When the reported unit of the result is stated in mg food/ kg product apply a protein conversion factor as indicated by the manufacturer (section 5.2.2) to obtain mg of total allergenic protein per kg food.
- It is accepted practice that some ELISA kit manufacturers quote LoD and LoQ 'in buffer' rather than in a real-life matrix where they would be expected to be higher, you are advised to check.
- Method developers, and subsequently laboratories may set their LoQ as the minimum of their working range or the lowest non-zero point on their calibration curve. In that case use this LoQ to make a choice for a specific kit and compare this customised 'reporting limit' to the calculated Action Level.
- Test kits manufacturers often quote a single LoQ for many matrices, which should be taken into consideration when determining whether a method is likely to be sufficiently sensitive in the matrix of interest. You may also want to check whether the method developer or laboratory have data to verify the performance expected at the calculated Action Level of interest and in your matrix of interest.

For further reading see:

- Wenzl, T., Haedrich, J., Schaechtele, A., Robouch, P., Stroka, J., *Guidance Document on the Estimation of LOD and LOQ for Measurements in the Field of Contaminants in Feed and Food*; EUR 28099, Publications Office of the European Union, Luxembourg, 2016, ISBN 978-92-79-61768-3; doi:10.2787/8931; https://publications.jrc.ec.europa.eu/repository/handle/JRC102946
- Barwick et al., 2011, Terminology in Analytical Measurement – Introduction to VIM 3, Eurachem Section 4.4 Detection Limit, https://eurachem.org/images/stories/Guides/pdf/TAM_2011_Final_web.pdf

5.2.1.3.4.2. Qualitative or quantitative

When applying QRA and Action Levels, an important consideration is whether the method is qualitative or quantitative. ELISA methods provide quantitative results.

LC-MS/MS is capable of yielding quantitative results however contract laboratories, if they offer LC-MS/MS at all, may provide only qualitative data although this may be very useful to assure the absence (to an appropriate concentration) of an allergen which should not be present. In the case of DNA detection qualitative and quantitative (real-time) PCR methods are available.

When using PCR or ELISA methods for swabbing, results are often (i) provided as a qualitative result (detected or not detected) or (ii) expressed as µg/swab, µg/ml swabbing solution or µg/ 100 cm². Because swabs require a different extraction and dilution protocol, the LOQ for swabs is lower than the LOQ in a real (matrix) sample. Depending on the kit, the difference can be 2 to 20 times. When swabbing results are quantified, they are not given in mg (milligrams) but in µg (micrograms).

Please remember that swabbing or air sampling results cannot be compared to Action Levels. Swabbing is used to analyse for allergens on surfaces (per square centimetres, rather than food (mg per kg; the units are not comparable, nor can swab results be converted to mg/kg (ppm).

The amount of allergen residue detected on a surface by swabbing may not aways pose a risk to the health of consumers with allergies. When using swabs, it is therefore recommended to combine with finished product testing to be able to determine the amount of residues that may result in the food.

Most rapid tests provide a qualitative result only.

In quantitative methods a calibration curve is used to relate the measured signals to the relevant units (mg of total allergenic protein per kg food) of standards containing known concentrations of the analyte. The concentration of the analyte in an "unknown" sample can be determined by comparing the measured signal of the sample with the calibration curve. The food product or extract used for the calibration standards is called the calibrator.

When the calibrator differs from the allergen ingredient to be detected, differences in calculation of concentrations can be expected. Some examples:

- The protein content of peanut products differs, e.g. defatted peanut flour (typically 50% or more protein), full fat peanut flour (26% protein) and peanut butter 22% protein).
- Soy ELISAs often use soy flour as calibrator, which may be full fat or defatted with differing known protein levels. Soy isolates contain much higher protein levels but have usually undergone considerable processing, which alter proteins and thus recovery significantly. This leads to underestimation of the actual soy protein content in most kits;
- In both cases unless the calibration curves are set up based on mg/kg peanut protein or soy protein respectively using appropriate conversion factors (section **5.2.2**) for protein equivalents differing results will arise;

In such cases, and when a concentration needs to be determined, it is recommended to construct a calibration curve using the allergenic ingredient to be detected. However, it is recognized that this is not common practice, as the production of an own standard for the calibration at the low mg/kg (ppm) range is challenging.

Generally, most ELISA test kits use a 5 to 7 calibration points to generate the calibration curve covering the concentration range of interest. In most cases the lowest standard is set as the limit of quantification (LoQ). Quantitative results must not be extrapolated beyond the highest calibration point; if an analytical signal is recorded above the highest calibration point the sample or extract must be diluted and run again. In particular use only the the linear range of the ELISA test kit (**Figure 16**). Above the ELISA linear range the kit antibodies are saturated with a plateaued or downward turning calibration curve (the hook effect) and differing results can be obtained for the same analytical signal.

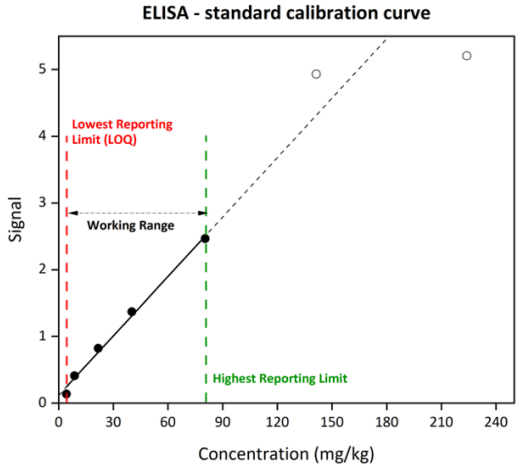


Figure 16. *Standard calibration curve ELISA.*

5.2.1.3.4.3. Specificity of test method to the target protein(s)

It is important to select a test kit that targets the correct protein. While allergen testing may be highly specific for a particular target, it is important to establish whether the marker targeted by the test is relevant for the product investigated. For example, kits which detect ß-lactoglobulin (BLG) are specific to those milk proteins. If the cross-contact allergen comes from the casein fraction of milk (not containing BLG) then a BLG kit would provide a false negative result for milk. A casein ELISA or total milk ELISA kits should be selected instead, where antibodies against BLG and casein are combined. Thus regard must be made to the 'measurand'. See section **5.2.1.5** for more information about the measurand transparent reporting and interpretation of analytical results.

5.2.1.3.4.4. Cross-reactivities

Cross-reaction is based on the binding of antibodies used in immunological tests to homologous allergen structures (epitopes) that may be present on non-target proteins. Such structures may be conserved among proteins with similar functions or members of the same botanically families. As seen before for almond and apricot kernels (Walker, Burns et al. 2018), cross-reactivity is quite common in ELISA kits. Other examples are peanut and fenugreek, soy and other legumes such as peas but also in non-related plants like linseed (flax) and mustard. Furthermore, non-specific binding can lead to false positive results, for example in a specific ELISA casein kit when soy protein concentrate is present.

Although PCR techniques do not detect proteins but specific DNA sequences, sometimes the chosen DNA strand (base pair sequence)is not specific enough for the plant or animal species being evaluated. The almond and prunus mahaleb case (Walker, Burns et al. 2018), and more recently apparent detection of mustard in wheat which proved to arise from a different plant (*Sinapis arvensis*) or rapeseed, means care must be taken even with PCR to avoid misleading results.

Cross reactivity must be evaluated for all ELSA and PCR test kits and if in doubt the supplier should be asked about this aspect.

5.2.1.3.4.5. The effect of matrices

Different matrices will affect the validity of allergen testing. Some matrices, such as cleaning or flush solutions, may destroy target proteins and give a negative result which does not accurately reflect the allergen status of the product or flush. Extreme pH levels, high levels of sugars, fats, salts and polyphenol rich foods (e.g., blueberries, blackberries, vegetables including onion, cabbage, and legumes) or darkly coloured matrices – that can interfere with protein extraction and/or colorimetric detection – may all present challenges for analysis and require specialised extraction protocols to provide correct results. Similarly, some matrices, such as chocolate and meat, do not readily liberate proteins and can produce false negatives (Allergen Bureau's Unexpected Allergens in Food 2021).

If DNA based methods are used, acid matrices like salad dressings may lead to false negative results due to DNA degradation at low pH levels. LC-MS/MS is less prone to matrix interference.

5.2.1.3.4.6. The effect of processing

It is possible that target proteins can be adversely affected by processing in terms of the ability of test methods to detect and quantify their presence accurately. High temperatures or prolonged heating and/or chemical or enzymic hydrolysis can alter the structure of proteins, making them more difficult to detect. Antibodies used in test kits might not be able to detect the altered proteins. It should be noted that the ability of a test kit to detect processed protein does not necessarily correlate with the allergenicity of that processed protein. It may actually be the case that protein changes that reduce the detectability of an allergen could enhance allergenicity. And although, for example some egg allergic individuals can tolerate baked egg it should not be assumed this is the case for all egg allergic consumers .

As another example, soy trypsin inhibitor is a target used by some soy allergen test kits. If the tested product contains processed or hydrolysed soy (soy trypsin inhibitor destroyed) the presence of soy will not be detected by the test kit leading to a false negative result.

Cleaning products may hydrolyse or otherwise change the tertiary structure of allergenic proteins, so it is particularly important that this is considered when testing after cleaning.

5.2.1.3.4.7. Test turnaround times

Results are not immediately available when sending out samples to a laboratory. Depending on transport time, turnaround time of the method of analysis, work schedule planning in the laboratory and urgency options results can be expected typically in one to ten days.

When applying analysis on allergens as a positive release for received raw materials or finished end products, the use of rapid tests or the inhouse performance of ELISA kits can be considered. For verification purposes rapid tests are often used, in contrast to validation where laboratory-based techniques are the preferred approach.

5.2.1.3.4.8. Method complexity

Verification of cleaning is performed before new production starts, Results are required to be known quickly for line clearance and start of production. Therefore often rapid tests are used. Such tests could also be done during all production hours. These tests should be simple, easy to perform and easy to interpret by the (trained) operators.

As for the advanced laboratory- analyses other aspects should be considered, e.g. investment of effective equipment, trained practitioners and amount of samples.

5.2.1.3.4.9. Measurement uncertainty

It will not come as a surprise that chemical and bio-analytical results cannot be perfect. We use the term measurement uncertainty to describe this lack of perfection. Reporting the measurement uncertainty of allergen results is currently not a common practice, for various reasons. However, for other applications in certificates of analysis and scientific articles an expression of the following form can be found:

$$\begin{matrix} & & Result\ of\ analysis \\ Measurand\ (e.g.\ Aflatoxin\ B_1) & \ldots & x \pm U\ (k=2)\ (followed\ by\ an\ appropriate\ unit) \end{matrix}$$

In an expression of this form 'x' is the mean result, 'U' *(the number after the* $\pm$*)* is the expanded measurement uncertainty and k is the coverage factor. The estimated measurement uncertainty is reported with the result of a measurement in order to characterize the dispersion

(spread) of values that could reasonably be attributed to the measurand. An appreciation of the range of values between '$x - U$' and '$x + U$' gives a risk assessor a much better basis to make a decision.

The expanded uncertainty, U, is obtained from the standard uncertainty (u), which represents about 68 % of the possible spread of results in a 'normal' (Gaussian) distribution (the familiar 'bell-shaped curve' See Walker 2021 IFST, How to Choose a Lab, Apprendix 1 for more details). Laboratories should report the expanded uncertainty where 'u' is multiplied by a 'coverage factor', 'k'. This is typically set as equal to 2, which gives an expanded uncertainty representing over 95% of the possible spread of results. Best practice for laboratories is to include expanded uncertainties on their certificates of analysis, and these should be accompanied by a statement of the level of confidence and the coverage factor used. In practice, laboratories, if they report it at all, may report uncertainty in a number of ways. The uncertainty can be a standard deviation 's', the standard uncertainty 'u', or the expanded measurement uncertainty 'U'. It is important that you understand which they use. The practical implications of this are:

- if you want to know the uncertainty, you may need to ask the laboratory to state the measurement uncertainty as it may not be routinely reported;
- if you want to understand the uncertainty, you will need to check with the laboratory which of the above measures has been reported: standard deviation (s), standard uncertainty (u), or expanded measurement uncertainty (U);
- if you want to compare results from different laboratories, or from different methods of analysis, you can only do so with a knowledge of the measurement uncertainty;
- the lower, or occasionally the upper, bound of the measurement uncertainty may be the datum of interest to establish whether the result is compliant or otherwise (Institute of Food Science Technology).

Various sources of measurement uncertainty can be identified, e.g. sampling, number of samples analysed, and various stages of analysis such as extraction and instrumental factors as well as concentration levels influence measurement uncertainty. Measurement uncertainty is one of the performance characteristics of a method or kit that is usually covered in method validation, and must be covered if the method is accredited to ISO/IEC 17025 '*General requirements for the competence of testing and calibration laboratories*'. Target expanded measurement uncertainties are often set at or about 20% of the measured value. In some applications, such as pesticides residues analysis, it is traditional to assume the expanded measurement uncertainty is 50% of the measured value. For food allergen analysis a value of between 20% and 50% would be usual, and often the expanded measurement uncertainty is closer to 50% of the measured value. According to the recommendations of the European Commission, the value obtained by subtracting the expanded uncertainty (U, with coverage $k = 2$) from the reported mean concentration, x, is used for compliance assessment. When that value is greater than the maximum level (ML) specified in legislation ($x - U >$ ML), the sample is considered "beyond reasonable doubt" to be non-compliant.

Further Reading

- Williams and B. Magnusson (eds.) Eurachem/CITAC Guide: Use of uncertainty information in compliance assessment (2nd ed. 2021). ISBN 978-0-948926-38-9. 2694
- Breidbach, A., Nørgaard, J.V., Cubero-Leon, E. and Martinez Esteso, M.J., 2022. Assignment of a Reference Value of Total Cow's Milk Protein Content in Baked Cookies Used in an Interlaboratory Comparison. *Foods*, *11*(6), p.869.

5.2.1.3.4.10. Accreditation

In the context of this document 'Accreditation' is the independent evaluation of laboratories against recognized standards to carry out analyses to ensure their impartiality and competence. Through accreditation customers and users can have confidence in the test results. The recognized standard for testing laboratories is ISO/IEC 17025 General requirements for the competence of testing and calibration laboratories. For further information see https://ilac.org/ . ELISA and PCR test kits are validated by the manufacturers. These and LC-MS/MS method validations are often published in the scientific literature. However, all analytical methods used should be properly validated by the laboratory using them in order to demonstrate that they are fit for the intended use, e.g. for the determination of a specific allergen substance in a given food matrix.

Further reading: "B. Magnusson and U. Örnemark (eds.) Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics, (2nd ed. 2014). ISBN 978-91-87461-59-0."

Accreditation of an allergen method within a laboratory may not ensure that the method is suitable for the product or can be used due to specific circumstances during processing, the laboratory may not be aware of these factors and it is recommended that there is dialogue with the laboratory on the purpose of the test. The external contract laboratory to be selected must provide the following information:

- Relevant accreditation (or compliance) to ISO17025. The scope of accreditation should be carefully scrutinized.
- Validated methods for the determination of the measurand of interest (allergen/matrix);
- Participation in appropriate proficiency programs (when available);
- Robust quality control processes
- Comprehensive reports (including method description, definition of the measurand, results and associated expanded uncertainty).
- Proven expertise in the field of allergen analysis and awareness of the scientific context of allergen analysis.

5.2.1.3.5. Sampling: key aspects

The selection of timing, frequency and location of sampling should be to maximize the possibility that if cross-contact has occurred then it will be present in a sample that is taken for analysis. When allergen is not detected in a thorough sampling regime, it should not be expected in other situations.

Most food products which are mixtures will depend on homogeneous mixing of all constituents in order to meet standards including quality and nutritional value. A starting point for FBOs might be to consider whether any reasonable assumptions of homogeneity could be made based on knowledge of their processes. It is possible to use that knowledge to describe the worst-case scenario. For example, in a batch produced dry powder product, the highest level of carryover could be in the first e.g., 50-100 kg of the batch. This is the range where most of the samples should be collected. When repeat mixing of the bulk batch occurs, any unintended allergen contaminant could be redistributed throughout the final product and random sampling will be more appropriate.

Cross-contact of raw materials is often inhomogeneous (heterogeneous), and it may be unknown which part of a batch will be affected. In that case random sampling is needed, where more samples should be taken to provide an accurate overview of a batch.

When testing raw materials and end products to verify free-from claims, information gained over time can be used to modify the sampling approach, such as an increase of specific raw material testing and a decreased numbers of end product testing or the other way around.

When determining an appropriate sample number for analysis, consider how the cross-contact allergens may be distributed.

Even an extensive sample programming may not detect all cross-contact allergens. Take also in consideration the nature of the allergen (e.g., finely ground powder, paste, meal, whole or segmented nuts); and the nature of the process. Is it a single/continuous flow? Does it use multiple depositor heads? Running through heat exchange columns? Is a single batch produced? What is the impact on the distribution of any cross-contact allergen?

Factors that may affect the frequency of testing:

- Known problems with suppliers or in the production process. Building history/ trend analysis underpins increased or reduced numbers of samples.
- Any claims made regarding the allergen status of a product. A stringent, risk based and ongoing sampling plan should be applied to support pack claims.

The number of samples is discussed in the next paragraph. Be aware that the amount or volume of sample taken should be:

- enough material to be homogenized by laboratory to obtain a representative sample
- roughly half of the sample taken by FBO is laboratory sample and half of it is retain sample kept by laboratory (eg for re-analysis)
- minimum of 100 grams, but more can be feasible

Two recent studies (Sharma, Pereira et al. 2020, Sharma, Wang et al. 2021), on sampling and analysis for gluten in oats have shown that measurement dispersion and consequently consumer and producer risks can be reduced by a number of actions. These include either increasing laboratory sample size (e.g. to 5000 g), increasing the number of samples sent to the laboratory, or within the laboratory increasing test portion size or increasing the number of test portions (full replicates).

References:

Sharma, G. M. *et al*. (2020) 'Evaluation of sampling plans for measurement of gluten in oat groats', *Food Control*, 114(March), p. 107241. doi: 10.1016/j.foodcont.2020.107241.

Sharma, G. M. *et al.* (2021) 'Sampling plan designs for gluten estimation in oat flour by discrete and composite sampling', *Food Control*, 129(November 2020), p. 107943. doi: 10.1016/j.foodcont.2021.107943.

Two further sources of guidance on sampling are of interest. Both are general, rather than devoted to allergen analysis. They provide readable guidance that is scientifically sound and practically oriented with good examples.

The first 'GOOD Samples' is an 82-page guidance document that outlines the scientific and systematic approach to ensure that analytical data generated as a result of a sampling process is representative of the 'decision unit' and is defensible. The 'decision unit' is the material from which a sample is collected and to which an inference (e.g. the concentration of the analyte of interest) is made.

The second 'GOOD Test Portions' is a 78-page guidance document aimed at laboratory activities focused on the selection of the test portion, and the processes necessary to achieve a representative test portion.

Good Samples: Guidance on Obtaining Defensible Samples. Sampling and Sample Handling Working Group, FDA, AAFCO, AFDO, APHL and Industry. © 2015 Association of American Feed Control Officials (AAFCO). Freely downloadable from https://www.aafco.org/Publications/GoodSamples (Accessed 03.06.2022)

Good Test Portions: Guidance On Obtaining Defensible Test Portions. Laboratory Sampling Working Group, , AAFCO, AFDO, APHL. © 2018 Association of American Feed Control Officials (AAFCO). Freely downloadable from GOODTestPortions (aafco.org) (Accessed 03.06.2022)

5.2.1.3.6. Recommended number of samples

Foodstuffs and ingredients are often heterogeneous making it difficult to obtain a single representative sample. Owing to the costs and practical difficulties of full statistical sampling, food analysis is often carried out using a small number of random samples. If the product is homogenous or can be mixed (a free-flowing powder or liquid) a small number of samples may be representative.

Inhomogeneity, with 'hot spots' of unintended allergen or particulate allergens at unpredictable places in the bulk or batch, poses greater difficulty in finding the true allergen status of the product. Inevitably more samples will be required both to give a representative idea of the allergen concentration and some indication of how unintended allergens might be distributed.

When sampling from a large number (*N*) of packaged units, several 'rules of thumb' have emerged which provide consistency. These do not have any known underlying basis in statistical sampling theory but have been widely adopted with supporting publication. Examples include the square root of N plus 1 ($\sqrt{N} + 1$) rule (Muralimanohar and Jaianand 2011) and the cubed root of N ($\sqrt[3]{N}$,or *N*^(1/3)). As an illustration, for *N* = 10,000 packaged units, ($\sqrt{N} + 1$) = 101 samples or increments and $\sqrt[3]{N}$ = 22 samples or increments. Clearly the former may result in a large number of individual samples and may not be practical. The choice of which to apply may depend on the level of concern and other factors.

In order to reduce the cost of analysis, the analysis of composit or aggregate samples may be envisaged, instead of the analysis of all the individual samples or increments collected. A composite sample is formed by mixing each sample or increment thoroughly and combining half of each to form the composite samples. The remaining half of each sample or increment is retained in the event that these may need to be analysed individually or in a different combinations. The relevance of composite sampling depends on the limit of quantification (LOQ) of the method. And may be misleading. Composite samples may dilute the high allergen concentration in a small number of increments (i.e. a 'hot spot') in the batch. This risks a reported value that does not represent the UAP level in the individual 'hot spot' samples. When composite or incremental sampling are used the detection capability (LoD and LoQ) of the analytical methods must be adequate. In practice, LoD should be significantly below (e.g. 10 times less) the critical risk concentration or the action limit.

As an alternative, the samples may be composited in batches, e.g., in sets of 10 samples, or any other combination, commensurate with the level of concern and other factors, that reduces the number of samples sent to the lab. These factors are important to discuss with the laboratory.

If the way in which the concentration of unintended allergen varies across the batch needs to be known all the samples or increments should be analysed. When defining numbers to test, a batch or lot can be characterized in outline **(see also Table 20 below):**

1. Agricultural commodities, where the allergen is expected to be distributed randomly and often appear as particles, require higher numbers to test. (See Allergen Bureau resources, https://allergenbureau.net/resources/allergen-bureau-resources/
2. Testing can also be supported by a first visual screening.
3. Raw materials or products undergone processing in a previous step in the foodchain, where the allergen is not expected to be distributed randomly and may appear as both heterogeneous and homogeneous cross-contact, require medium numbers to test.
4. (semi) Finished products in the production facility, where a better understanding of cross-contact often allows targeted sampling. This may result in a lower number of samples.

Example

Batch size: N = 10,000 units, rule of $\sqrt[3]{N}$ applied, = 22 samples taken. The risk assessment produces a critical concentration (Action Level) for allergen cross contact of 1.5 mg/kg as protein. If the product does not contain unintended allergen above 1.5 mg/kg as protein PAL is not required. The laboratory analytical method has a LoQ of 0.1 mg/kg as protein. If only one of the 22 samples contains unintended allergen at 1.5 mg/kg as protein a composite of all 22 samples will contain 1.5/22 = 0.07 mg/kg as protein, too low for the method reliably to quantify it. However, if two sets of 11 samples are composited one set will contain the single positive sample and the composite sample will contain 1.5/11 = 0.14 mg/kg as protein, which the method is expected reliably to quantify.

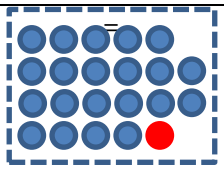
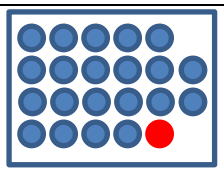
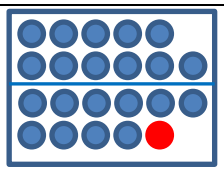
Critical concentration = 1.5 mg of total allergenic protein per kg food (see red dot) LOQ = 0.1 mg of total allergenic protein per kg food		
		
22 samples	1 composit sample of 22 samples	2 composite samples of 11 samples each
22 analyses	1 analysis	2 analyses
Costly	➔ 1.5/22 = 0.068 mg/kg Not quantified (< LOQ) No PAL required but opinion **not** justified by analysis	Sample 1 < LOQ Sample 2: 1.5/11 = 0.14 mg/kg No PAL required, opinion justified by analysis

Table 20 illustrates a general scheme that may be applied to determine an appropriate number of samples based on the level of concern relating for the cross-contact risk. It summarises the various approaches to sampling based on the level of concern about the consequences and various other factors. Having decided such questions as how and where to sample, by whom, how much, what container to use, how to transport to the lab and what instructions to give the lab, the sampling plan and how it was carried out must be documented. A proforma example is provided in the Annex **7.7** to help you plan and record the sampling and analysis exercise.

***Table 20**: Strategy to determine the appropriate number of samples*

Supporting conditions to be considered	☐ Regular frequency of allergen presence ☐ Low availability of material samples ☐ Homogeneous distribution of allergens ☐ Limited time & resource available	☐ Homogeneous distribution of allergens ☐ Limited time & resource available ☐ Sufficient or abundant material available to sample from ☐ Urgent and more resources made available	☐ Sufficient or abundant material available to sample from ☐ Urgent and more resources made available
Level of concern	**LOW** Routine verification of ingredients without claim	**MEDIUM** Routine verification of ingredients without claim	**HIGH** Quantification needed for risk assessment, claim validation or incident
Number of Samples recommended See also Allergen Bureau https://allergenbureau.net/resources/allergen-bureau-resources/	A single or small number of samples	Two up to six samples, particularly if allergen presence may be intermittent Sample size is also important	• Allergen presence is regular and homogeneous: take at least six samples or two from every batch (risk based for claim validation). • Allergen presence is NOT regular and/or NOT homogeneous: (i) consider the size of the batch and take "$\sqrt[3]{N}$" [or N^(1/3)] samples, where N is the number of units available; or (ii) consider incremental sampling (see main text).

5.2.1.3.7. Sampling from a production line

So far we have dealt with sampling from static batches. What about sampling from a production line? It goes without saying that production lines differ considerably from plant to plant. The optimum approach would be to design out opportunities for cross contact on shared lines by avoiding dead end pipework, snags and sharp bends. Nevertheless, sampling from production lines is almost inevitable and we offer an approach that hopefully will map onto the particular needs of many businesses

Using Case study 1, the manufacture of egg-containing ravioli, 'cleaning assessment for cross contact'. In **Figure 17** the production line diagram is annotated with coloured circles that represent consensus among the expert group as the most appropriate points at which to sample. Each 'circle' indicates equipment that if not properly cleaned has a potential to lead to cross contact and hence cross contact of subsequent products with egg.

Cross contact may arise from remaining residues of egg-containing product, more complex equipment that is difficult to clean (dead areas etc.). The size of the 'circle' indicates a subjective estimate of the likelihood of cross contact as **L**ow, **M**edium or **H**igh possibilities. These are examples and **you must make this determination yourself for your own production line** e.g. by observing the state of the equipment prior to clean down on a representative number (not less than 6) of occasions, (e.g. on different days, different operators or shifts, different batch sizes…) (Ask the engineers who designed the equipment for diagrams and to comment). If pre-clean inspection is not possible all points should be sampled from.

Samples (swabs or rinse water) should be taken from all indicated equipment after it has been cleaned to visually clean with more samples or more emphasis on equipment badged with larger circles. Analysis must be by a method with an appropriate detection capability (sensitivity).

If the data indicate cleaning is not adequate this does not necessarily imply a risk. QRA and, if required, end-product testing are needed to assess the risk.

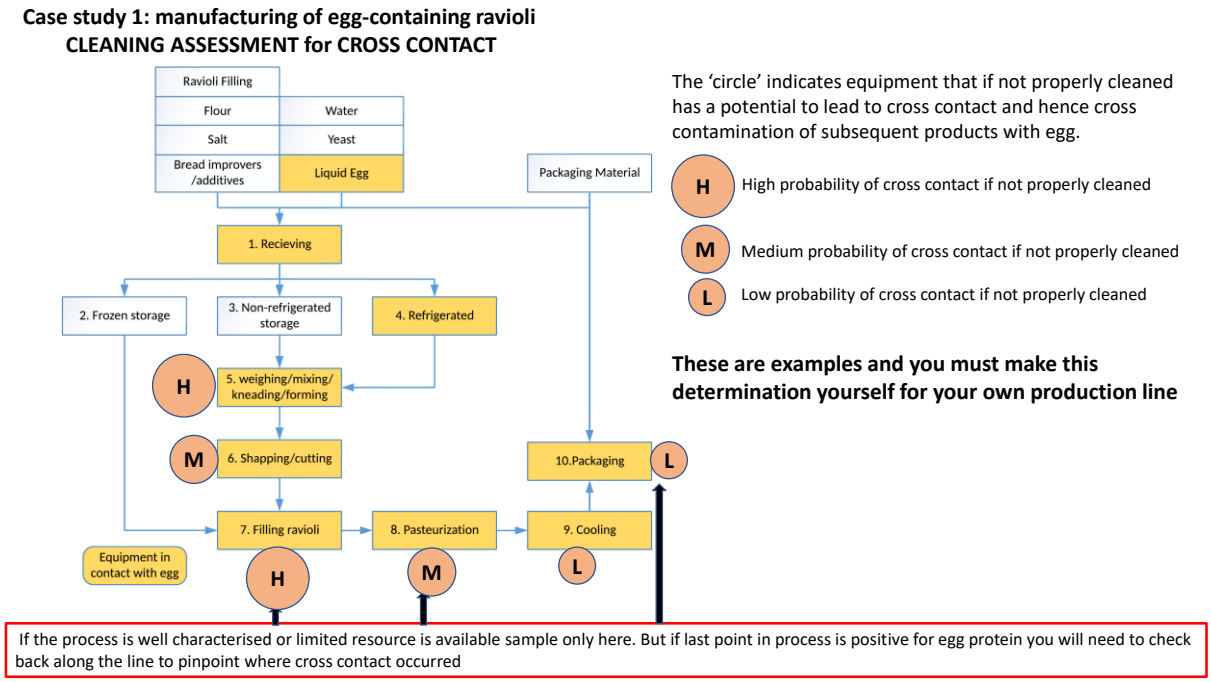


Figure 17: *Sampling from a production line*

5.2.1.4. Test methods

5.2.1.4.1. ELISA

IgE mediated food allergens are large protein molecules for which currently the most frequent approach is enzyme linked immunosorbent assay (ELISA). ELISA test kits are the most commonly used for routine food analysis. There is an increasing range of immunoassays available as kit manufacturers respond to industry needs.

ELISA test kits generally focus on specific 'marker' proteins. They should be (i) specific, to ensure minimal false positives); (ii) quantitative, to provide an allergen content); and sensitive, to detect low levels (mg/kg, ppm) of the protein (see **5.2.1.3.4.1**). Although cost and time effective, and relatively easy to use, result interpretation requires some technical expertise. No single ELISA kit allows the detection of all the relevant priority allergens at once. See the European Commission Regulation 1169/2011 - Annex II; or the the US FDA "Food Allergen and Labelling Consumer Protection Act", 2004.

The biggest challenge for ELISA analysis is due to the food matrix to be tested. Processed food can reduce the ability of the kits to detect the allergens (see **5.2.1.3.4.6**). This may be due to the fact that ELISAs are biological assays prone to matrix and process interference. Antibodies can also cross-react (false positives) with other proteins having similar structural elements to the allergen proteins to be investigated.

ELISA test kit manufacturers routinely assess cross-reactivity (see **5.2.1.3.4.4**) against a wide range of food commodities. Particular care is taken to include foods that are genetically similar to the target because of the higher probability of expression of proteins with amino acid sequence homologies in the target analyte protein. Nevertheless cross-reactivity remains an issue that must be investigated during the single (in-house) validation.

Detection antibodies are often raised against different targets for the same allergenic food and the form and processing history of the food may alter the target proteins. Hence different ELISA platforms often give different quantitative results and there are as yet few reference materials to harmonise the analysis (Littleton P 2021).

5.2.1.4.2. PCR

Polymerase Chain Reaction (PCR) methods amplify and detect DNA sequences of the allergenic species (not the allergenic protein). They can be used to detect multiple allergens at once (multiplex). They are specific, sensitive, qualitative and, for Real Time PCR, quantitative. They can confirm an ELISA result but can also detect potentially allergenic products. PCR is currently a laboratory-based method that requires a skilled analyst. However, like many other detection methods, it is impacted by food processing. Although DNA is a more robust molecule than many proteins, some processing methods (e.g. hydrolysis, heating) can destroy DNA causing false negative results. Some food matrices may inhibit DNA extraction, and co-extracted inorganic salts, organic molecules (e.g. tannins) and proteins may inhibit amplification of DNA. The use a DNA sequence that is not specific enough for the species to be detected can lead to false positives (see **5.2.1.3.4.3** & **5.2.1.3.4.4**). DNA methods are not suitable for the detection of certain allergens such as egg and milk since the species DNA is found also in beef and or chicken tissues.

A calibration curve is required for quantification. It is important to check that the calibrator corresponds in terms of DNA prevalence to the tissue fraction from which the DNA of the allergenic ingredient to be detected is sourced. For example, celery PCR kits often use a celery seed calibrant. When testing for the presence of celery originating from celery stems in a juice blending facility, the underestimated concentrations do not match the actual concentration in juice, since the amount of DNA in juice is much lower than in the seeds.

5.2.1.4.3. LC-MS/MS

Tandem mass spectrometry (MS/MS) which is very successful for small molecule identification and quantification can also be applied to food allergen proteins. Quantitative results are not often currently offered routinely however this may change in the future.

Liquid chromatographic separation coupled with tandem mass spectrometry (LC-MS/MS) is a costly technique and requires complex instrumentation and skilled operators, but for critical problems it may be the best approach. The basis of routine LC-MS/MS for allergens is that extracted protein is broken down into its constituent peptides using enzymatic digestion. The peptides are chromatographically separated and presented to the tandem mass spectrometer for identification and quantification. The analysis can selectively identify very closely related proteins that cannot be distinguished by other techniques. Allergen proteins that have been altered or broken down by food processing and which may not be detectable by antibody-based techniques may still be detectable by MS. In addition, the technique has the ability to analyse simultaneously multiple allergens in a single analysis, offers a wide dynamic range and high sensitivity can be achieved with optimised sample preparation.

Difficulties can include under recovery due to poor extraction of protein from the food matrix, although LC-MS/MS MS allows harsher extraction conditions than can be used with ELISA or PCR. Challenges include achievement of adequate enzymatic digestion to release peptides in an equimolar fashion, clean-up and extraction of the released peptides and Instrument memory effects. Quantification of the peptide concentrations is relatively straightforward but conversion to protein concentration can be challenging as is the use of conversion factors to translate the amount of measured proteins into total allergenic protein. Guidelines have been published by (Johnson, Baumgartner et al. 2011) dealing with criteria for the selection of target protein analytes, peptides, optimization of digestion, quantification (e.g. through standard addition or isotopically labelled peptide standards), and effective validation of methods and harmonization of results through the use of naturally-incurred reference materials spanning several types of food matrix. Further guidance aimed at unambiguous data analysis and reporting to improve the evaluation, comparability, and transferability of LC-MS/MS methods has been published by (Johnson and Downs 2019).

LC-MS/MS approaches are the only means of obtaining metrologically traceable reference methods to anchor allergen protein measurements between different laboratories across the globe. An important step forward was the publication (Martinez-Esteso, O'Connor et al. 2020) of a LC-MS/MS reference method for the measurand 'mass of total allergen protein per mass of food', in this case 'mg of total milk protein per kg of food'. The method is based on a selection of eleven representative allergenic peptide markers from the total milk protein, establishes the metrological traceability of the measurement results to the SI, and is able to quantify the milk protein content in cookies at relevant clinical levels (low mg/kg). Estimation of the uncertainty contributions as well as of the combined uncertainty of the final result was achieved. The publication is an exemplar of the analytical workflows, calculations, validation and performance characteristics of an LC-MS/MS allergen protein method.

Key references:

Johnson, P.E et al., 2011. Current perspectives and recommendations for the development of mass spectrometry methods for the determination of allergens in foods. Journal of AOAC International, 94,1026-1033, https://doi.org/10.1093/jaoac/94.4.1026

Johnson, P.E. and Downs, M., 2019. From Signal to Analytical Reporting for Allergen Detection by Mass Spectrometry, Journal of AOAC International 102, 1255-1262. https://doi.org/10.1093/jaoac/102.5.1255

Martinez-Esteso MJ, et al. A reference method for determining the total allergenic protein content in a processed food: the case of milk in cookies as proof of concept. Anal Bioanal Chem. 2020;412(30):8249-8267. doi:10.1007/s00216-020-02959-0

5.2.1.4.4. Distillation Ion-chromatography (sulphites)

Many methods have been developed for the detection and determination of sulphur dioxide, including enzymatic, HPLC and several variations of the Monier-Williams procedure. The Monier-Williams method - considered as the Reference Method - is known to be interfered with by foods such as dried garlic and soy proteins. These foods contain volatile sulphur-containing compounds that induce false positive or overestimated results, and lead to the common misunderstanding that sulphites naturally occur in those foods.

Ion-chromotographic methods detects only free sulphites. Since sulphites can bind to other molecules, they are no longer detected, resulting in false negative (underestimated) sulphite results.

5.2.1.4.5. Chromatographic and Enzymatic methods (lactose)

For many years enzymatic tests have been used for the determination of low lactose concentrations in foods. Most enzymatic assays are based on a different measurements for lactose/galactose or lactose/glucose. Presence of other sugars like maltose, galactose (in lactose-free milk) or GOS (galacto-oligosaccharides) should be taken into account when choosing an enzymatic method. Not every method is suitable for such matrices, unless a specific pre-treatment is applied to remove the excess of sugars. In addition, the LoD of the different methods should be taken into account. While enzymatic lactose tests often have high detection limits, the maximum level of lactose allowed in "lactose-free" products has decreased during the last years to 100 mg/kg (ppm), which requires a more sensitive analysis method.

A high performance anion exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD) can be applied to detect different sugars, including lactose at sufficiently low concentrations, of relevance to confirm "free from" claims. Also high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS) and LC-MS/MS techniques are used to detect low quantities of lactose in food products.

5.2.1.4.6. Next Generation Sequencing

For confirmation purposes or in case of doubt, Next Generation Sequencing (NGS) can be used. As with PCR, DNA is extracted and amplified but rather than a targeted approach seeking a pre-determined DNA sequence NGS is untargeted. DNA sequences in the sample are determined and compared to a database with known sequences. If the sequence can be found in the database, it can be identified. However, limited information is available on sensitivity of NGS.

5.2.1.4.7. Lateral flow and flow-through rapid immune-assays

There are generally two types of rapid methods that are immunologically based, similar to ELISA. They are used to screen for the presence of allergens. They are suitable to monitor surfaces and rinse waters and should only be used for these matrices and for raw materials or finished products after proper validation. Blanks and positive controls should be analysed together with the relevant samples. The tests are qualitative, rapid, portable and relatively easy to use with suitable (although usually minimal) training.

Lateral Flow Devices (LFDs) are commonly used and are often called strip tests. The sample is wicked through a reagent zone and then running through a strip of porous membrane by passive, capillary flow, ending up in an absorbent pad. Captured antibodies in defined areas on the strip forming coloured lines, making visible interpretation possible. LFDs are intended for detecting small quantities of an allergen. However some technical limitations need to be taken into account. For example, the saturation of the signal 'hook effect' may occur in the

presence of a high allergen load, leading to under estimated (false-negative) results. A hook effect occurs when a highly concentrated specimen is mixed directly with the assay antibodies. Therefore, the LFDs should never be tested using a highly concentrated (or even pure) allergenic food in order to avoid any overload/saturation which would cause a false negative. In this context, some manufactures included an additional line on the strip, the so-called "hook line", "overload line" or "control line" to indicate (if a line appears)that the LFD is working correctly. If the control line is not visible after the LFD is used the absence of a positive in the 'test' line is a false negative caused by antibody saturation.

Passive flow-through assays generally consist of the same materials used as in LFD's, but in a stacked, vertical arrangement. The reagent pad of a LFD is comparable with the membrane on top of a flow-through device. Membranes with captured antibodies and absorbent pad are layered underneath. Advantage of the flow-through format is the absence of the 'hook effect'.

5.2.1.4.8. Turbidity

Turbidity in water or an aqueous solution is caused by the presence of particulate or colloidal material which scatters light. The extent of scatter can be measured as the loss of intensity of a light beam relative to a reference solution. Inexpensive, easy to use and reproducible turbidity meters are available, hand held, or can be mounted in-line with continuous readout. It is a simple, cheap and sensitive method to detect the presence of food particles in rinse waters, but of course is non-specific and not selective for allergens. Validation of this method as a screening tool is needed by testing a series of dilutions with known product concentrations.

5.2.1.4.9. Protein swabs

Since allergens are proteins, a general test for protein can be used for verification purposes. After wet cleaning, surfaces are expected to be completely free of product residues. No protein, of any origin, should be found. Protein swabs use a simple colour indicator to identify the presence of protein on surfaces. The more protein present, the more pronounced the colour. Commercial protein tests are swab tests using the Biuret or Coomassie reagent. A wide range of Biuret based tests is available on the market with different sensitivities. Higher sensitivity can be obtained in this type of test by incubation (various times and temperatures).

In many cases composite foods consist of proteins from different sources, not only from allergenic ingredients, thus there is a greater probability of detecting proteinaceous food residues. However, protein swabs cannot distinguish and/or identity allergens, and are not suitable to use of cleaning verification after producing products containing no or small amounts of protein (e.g. oils, fats, food supplements, fruits or candies).

All swabbing may be impacted by sanitizers or residues and swabbing in general is qualitative and can only be used in easily accessed areas, so results may not accurately reflect the risk associated with hang up or closed systems.

5.2.1.4.10. Glucose/ lactose swabs

As described in the previous section, glucose/lactose swabs can be used as rapid tests to indicate general hygiene of equipment. Such swabs are suitable to detect residues of products consisting mainly of lactose or other sugars (e.g. testing for possible cross-contact from tablets, food supplements, or dried fruits). These products could also contain allergens in small quantities. When sugars are the main components of the products, demonstration of their absence indirectly implies minor allergenic ingredients should also not be present.

5.2.1.4.11. ATP swabs

Adenosine Triphosphate (ATP) is found in all living cells and in food products produced from a living source. ATP tests are used to determine whether a surface has been cleaned effectively. Any food residue or microbial cells that remain on the surface will contain ATP and will react with the reagents contained in the test (luciferin and luciferase enzyme) to produce light. Once an area has been swabbed, the test is activated and placed inside the luminometer test device. The light produced will be measured and expressed in Relative Light Units (RLU). Every manufacturer of luminometers and ATP swabs uses its own calculation of RLU, hence results obtained by devices cannot be compared.

As the ATP molecule breaks down easily when the cell membrane is destroyed, the use of ATP test for detecting food residues is limited to fresh (unprocessed) products. The ATP in heated or processed foods is mainly attributed to ATP of micro-organism (not to the product itself). While ATP tests are effective indicators of sanitation, they have limited value for allergen testing.

5.2.1.4.12. UV inspection

Many impurities or contamination particles (e.g. fats, proteins or dust and chalk) fluoresce under an UV lamp or torch. Hence, UV inspection can be used as an enhanced visual inspection tool, to cover bigger areas or to determine certain difficult spots to clean.

For UV inspection, light of 365 nm gives the best results. 365 nm wavelength UV belongs to a type of ultraviolet known as "UV-A", and also known as blacklight . A high power UV lamp is needed to inspect at large distances, and can be used without any additional materials (care may be required with regard to eye protection).

Also the so-called riboflavin (vitamin B2) test requires UV lighting. This test can be used for CIP or wet cleaning validation. Riboflavin glows brightly green-yellow under 365 nm light. A solution of riboflavin is sprayed onto the equipment and then cleaned. The critical surfaces can then be illuminated with a UV inspection lamp. Any residues of riboflavin present will light up brightly, indicating an insufficiently cleaned surfaces.

5.2.1.5. Reporting and interpretation of analytical results

Transparent reporting and interpretation of analytical results are crucial elements enabling proper use to be made of the analytical data by customers, especially risk assessors. A central concept is the term ‘measurand’. Measurand is less well known than ‘analyte’ and the terms are not interchangeable. Of the two, only ‘measurand’ includes the concept of quantity and because of this it drives coherent reporting of results with quantity units. Hence the measurand is of primary relevance to allergen QRA. allergens in airborne dusts is to allow the dust to settle

Formal definitions are:

Measurand: the quantity intended to be measured, [1]

Analyte: the component measured by the measurement system, where 'component' may be a chemical or molecular entity which is marker(s) or surrogate(s). [2]

The following **Table 21** illustrates measurands, analytes and measuring systems. The units relevant to allergen analysis are included in the descriptions of the measurand. As can be seen from the Table, for allergen QRA food allergen concentrations must be expressed as **mg total allergenic ingredient protein per kg food** [3]**.** It is critical all results are expressed in the same unit.

Table 21: *Examples of measurands, analytes and measurement systems for allergens*

Measurand	**Analyte (*Note 1*)**	**Matrix**	**Measurement system**
Mass fraction (e.g. mg/kg) of total protein in a foodstuff	Nitrogen	Foodstuff	Kjeldahl or Dumas
Mass fraction (e.g. mg/kg) of total milk protein (or a specific protein such as casein) in (for example) cookies	Peptides	Foodstuff, but usually needs to be more specific as to the actual food matrix	LC-MS or LC-MS/MS
Mass fraction (e.g. mg/kg) of total milk (or total milk protein) in (for example) cookies	Protein (Relevant epitopes)		ELISA
Mass fraction (e.g. mg/kg) of total peanut in (for example) cookies	DNA		PCR

Note 1: A conversion between the analyte and the measurand is required and, since many ELISA kits report the food commodity, e.g. peanut, a conversion factor between the food commodity and its protein content is also required, (section **5.2.2**). There remains work to be done to harmonise such conversion factors. If the kit or method reports as a food commodity there is a need to specify the food commodity and its characteristics in sufficient detail to be of value to the customer and/or a risk assessor. For example, if the quantity value obtained (measurand) depends on the nature of the calibrator, details of the calibrator should be given. The units (mg/kg allergen commodity or preferably allergen protein) should be specified every time a result is *cited*. To be of benefit to risk assessors there should be clear reporting of the method of analysis (ELISA, PCR, LC-MS/MS …) and labs should consider reporting measurement uncertainty, any (suspected) method cross reactivity if applicable, recovery and whether or not the result is corrected for recovery.

Thus the laboratory report must include:

- A description of the analytical method used;
- A clear definition of the measurand and unit e.g. total amount (mg) of the (specified) allergenic protein per kg of food).

As appropriate, the laboratory report should include

- The results and associated expanded measurement uncertainty (coverage k = 2) properly rounded;
- Any conversion factor(s) used;
- The accreditation status and accreditation scope.
- The working range, LoD and LoQ;
- Identified cross-reactivity or interferences.

When a laboratory reports a result "greater than (>)" this means the result is higher than the upper limit of the working range of quantification (ROQ). The result can be moderately or far above this value. Quantitative results should never be reported by extrapolating above the highest calibration standard measured. The sample should be diluted to bring the measured

concentration within the working range. This additional step needs to be included in the uncertainty calculation and may extend the turnround time of the analysis. It is also prudent to discuss and agree with the laboratory sufficient time to allow "greater than (>)" results to be diluted and re-analysed if required.

A single test result is often insufficient, whereas patterns and trends from multiple samples and techniques are more informative.

Interpretation of food allergen analysis results is often complex. Having a well thought out and documented sampling plan is a good start. For antibody based methods the 'technology' resides in the antibodies and calibrators. For PCR methods the primer and probe sequences, the instrument-specific amplification calling thresholds and the calibrators are crucial to the results. For LC-MS/MS methods the uniqueness of the peptides used to identify and quantify the protein and the relationship between peptides and protein and key factors. Thus a pre-established relationship with an experienced analytical testing service provider (laboratory, ELISA kit manufacturer or LFD supplier), where the above expertise resides, will pay dividends in productive dialogue when assessing results.

References

[1]. Joint Committee for Guides in Metrology, *International Vocabulary of Metrology* (VIM). [VIM3] 2.3 measurand (bipm.org) Accessed 13.01.2022]
[2] *(AOAC Guidance on Food Allergen Immunoassay Validation 2022)*; AOAC International: Gaithersburg, MD, USA, 2022 (in development).
[3] G. O'Connor, M. Haponiuk (DG SANTÉ), F. Ulberth, Joint DG SANTÉ and DG (JRC 2017) Workshop - Harmonisation of Approaches for informing EU allergen labelling legislation, JRC108259, https://www.efanet.org/images/2017/Newsltter10_2017-10_DG_Sante_DG_JRC_Workshop_report_Geel_June_2016.pdf

Further reading

- IUPAC Gold Book, 'analyte' https://goldbook.iupac.org/terms/view/A00331
- Eurachem 2011
- Terminology in Analytical Measurement: Introduction to VIM 3, https://www.eurachem.org/index.php/publications/guides/terminology-in-analytical-measurement
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5.2.2. Data conversion guide

A critical element of a food allergen QRA is the "amount (in mg) of total protein from an allergenic source per kg food". This includes all proteins of a single allergenic source like milk or peanut. Results of analytical testing can only be compared to the output or action levels of an QRA when both are stated in the same unit. Unfortunately, results are often reported in different units, e.g. allergenic product or single a specific protein, and need to be converted to the agreed unit.

Two types of conversions are described hereafter:
- Conversions of analytical results
- Calculation of the allergen concentration in a food product

5.2.2.1. Conversions of analytical results

Results of analytical testing can only be compared to a Reference Dose or a calculated Action Levels in a QRA when both are stated in the same unit. Analytical results are often stated as 'ppm (parts per million or mg/ kg). 'Mg' can refer to total protein from an allergenic source, the whole allergen material, a single protein or a specific allergenic ingredient. The reporting unit needs be clarified in a report of analysis. The internationally agreed unit "mg total

allergenic protein per kg food" (JRC 2017, Joint FAO/WHO Expert Consultation on Risk Assessment of Food Allergens 2021) is the preferred reporting unit for harmonization of food allergen risk assessments, as clinical information regarding thresholds of allergic reactions are also reported in total protein from an allergic source (JRC 2017, Joint FAO/WHO Expert Consultation on Risk Assessment of Food Allergens 2021).

Three scenarios are presented below where results may need to be converted:

a) mg total protein from an allergenic source/kg product
b) mg whole food or ingredient/kg product
c) mg sub protein/kg product

Details for each scenario are as follows:

a) mg total protein from an allergenic source/kg product

The result is stated as total protein from an allergenic source, for example:

- total peanut protein/kg product;
- amount of total cow milk protein (mg) per kg baked cookie.

No conversion is needed.

b) mg whole food or allergen ingredient name/kg product

The result is stated as whole food product. Convert the result to mg total protein by multiplying the result by a suitable conversion factor.

For a table of general assumptions of the total protein content of common food ingredients see Annex **7.8**. This table provides the percent protein content of several allergens (% = g / 100 g) in different raw materials. Note, when using this or other similar tables it is important to choose the right product to read the protein content.

Concerning data that has been generated by a quantitative analysis such as ELISA, a calibration curve is used and this calibration line is set up with an allergenic ingredient (calibrator). The protein content of this calibrator shall be used for the conversion factor. For example, soy ELISA kits often use soy flour as calibrator and therefore results are often expressed in soy flour and not as fresh soy beans. For peanut, results could be reported as peanut or whole peanut, and not reported in total peanut protein. Please contact your laboratory if the calibrator or reporting unit is not clear in order to ensure use of the correct conversion factor.

Example: Peanut results reported as "peanut", instead of "total peanut protein". Contact laboratory for more information about the conversion factor used.	
- Certificate of analysis	= 10 mg peanut / kg
- % peanut protein content in peanut bean (see Annex **7.8**)	= 26 % = 0.26
➔ Converted result	= 10 * 0.26 = **2.6 mg peanut protein/ kg product**

IMPORTANT! In case the protein ratio in the product detected differs from the calibrator, the conversion factor needs to calculated case by case.

Example: Egg results reported as "whole egg powder", instead of "total egg protein". Egg is detected in a product. Cross-contact occurred with liquid egg white (not containing egg yolk). The calibrant of the egg analysis is whole egg powder, containing proteins from egg white and yolk. Conversion is required.	
- Certificate of analysis	= 10 mg whole egg powder / kg
- % egg protein content in whole egg powder: (see Annex **7.8**)	= 47 % = 0.47
➔ Converted result 1: (to be used for Risk Assessment[13])	= 10 * 0.47 = **4.7 mg total egg protein/ kg product**
- % egg white protein in all egg proteins: (see Annex **7.8**)	= 83 % = 0.83
➔ Converted result 2:	= 4.7 * 0.83 = 3.9 mg total egg white protein / kg product

c) mg sub protein/ kg product

The result is stated as one of the proteins of the allergenic product. To obtain the total protein content from the allergenic source, the ratio sub protein: total protein is needed.

Commonly used sub proteins for allergen analysis and their ratios to total protein:

Sub protein	Allergen source	Ratio	Conversion factor to total protein from allergenic source
ß-lactoglobulin	Milk	10:100	x 10 (= 100/10)
Casein	Milk	80:100	x 1,25 (= 100/80)
Gliadin	Gluten	50:100	x 2 (= 100/50)
Tropomyosin	Crustaceans	Varies by species (Eurofins)	
Lysozyme	Egg	Do not convert to total egg, use other egg test when total egg result is needed. Use this method only when lysozyme is used in its pure form (wine, cheese)	

5.2.2.2. Calculation of the allergen concentration in a food product

When a QRA does not start with analytical testing but rather with a calculation intended to determine the maximum concentration of an allergenic ingredient that could occur due to processing, some input parameters are needed (e.g. batch size, amount and composition of hang-up/carry-over product residues). See section **5.2.3** for more information on tools

[13] The concentration reported in total protein from an allergenic source should be used for any allergen risk assessments as clinical information regarding thresholds of allergic reactions are also reported in total protein from an allergic source.

available for estimating the amount and composition of hang up/carry-over product residues that could not be eliminated at a product changeover.

The composition of defined hang ups is a result of each allergenic ingredient in the previous formula and the protein level of that allergen ingredient. The protein level of allergen containing ingredients should be provided by the supplier and can be found in the product specification. When supplier information is lacking, the approaches described below can be used in the case of (a) single allergenic ingredients or (b)compound products.

a. Single allergenic ingredients

When all proteins in the product originate from the same source, the protein content provided in the nutritional table of the product specification is equal to the allergen protein. If no nutrition information is available use Annex **7.8** or another suitable similar reference.

Example: Part 1 - First estimate

A biscuit manufacturer identifies a hang-up in the processing line that may consist of egg containing biscuits. See section **5.2.3** for more details regarding estimating the actual g or kg amounts of hang up/carry-over product residues.

- % egg protein content in fresh whole egg (see Annex **7.8)**:	= 13 % = 0.13
- Egg biscuit formulation (% in recipe)	= 5 % = 0.05
➔ concentration of egg protein in hang-up* (to be used for Risk Assessment)	= 0.13 * 0.05 * 1 000 000 = 6500 mg total egg protein/ kg product

* Starting from a maximum of possible of 100% or 1 000 000 parts per million (mg/kg)

IMPORTANT! Make sure the purchased ingredient is exactly the same as mentioned in Annex **7.8**. For example, a product in powdered form is more concentrated and therefore contains more protein than in liquid form. When the specific type of ingredient is not clear from the product specification (e.g., only 'milk proteins' mentioned or 'nuts') first clarify with the supplier.

Example: Part 2 – refined information

The supplier informed the manufacturer that the egg ingredient was not whole egg but egg white only.

- Egg white composition (specs nutrition panel)	= 10.5 %
- % egg white protein content in total egg	= 83.3 %
➔ conversion factor (egg white to total egg)	= 1 / 0.833 = 1.2
➔ Converted result	= 10.5 * 1.2 = 12.6 % total egg protein
➔ refined concentration of egg protein in hang-up* (to be used for Risk Assessment)	= 0.126 * 0.05 * 1 000 000 = 6300 mg total egg protein/ kg product

* Starting from a maximum of possible of 100% or 1 000 000 parts per million (mg/kg)

b. Compound products

The product is made from two or more ingredients, each of them (may) contain proteins. The protein content provided in the nutritional table of the product specification may overestimate the allergenic protein content.

Example: Ingredients from different allergenic sources A blend is bought as a compound ingredient, containing wheat flour and skimmed milk powder amongst other ingredients. Cross-contact of this mix to other products is possible. Hence, an QRA on milk is performed. The supplier could not provide the concentrations of total milk protein and total wheat protein, but information about % compositions in the recipe is available.	
- % wheat flour in the recipe	= 80 %
- % skimmed milk powder in the recipe	= 10 %
- % wheat protein content in cereals (see Annex **7.8)**	= 11 %*
- % milk protein content in skimmed milk powder (see Annex **7.8)**	= 36 %

* use total wheat protein. Do not convert to gluten.

When using some risk calculation tools, such as VITAL® Online, only one figure for % allergenic ingredient and the protein level of that allergen ingredient (%) can be entered in the tool. Therefore, the data should be combined and converted before being entered into the tool, an example of this conversion is shown in the following table. In other tools, such as the iFAAM cross-contact estimate worksheet (see Contamination estimate calculator here), it is possible to enter each allergenic ingredient separately and the tool will perform the calculation automatically. It is important to know which information and conversions are needed for the specific tool being used to calculate a carry-over estimate.

Example: Two or more ingredients from the same allergenic source A blend is bought as a compound ingredient, containing anhydrous milk fat and skimmed milk powder amongst other ingredients. Cross-contact of this mix to other products is possible. Hence, an QRA on milk is performed. The supplier could not provide the concentrations of total milk protein, but information about % compositions in the recipe is available.	
- % anhydrous milk fat in the recipe	= 10 % = 0.1
- % skimmed milk powder in the recipe	= 5 % = 0.05
➔ total ingredients	= 15 %
- % milk protein content in anhydrous milk fat (see Annex **7.8**)	= 0.01 %
- % milk protein content in skimmed milk powder (see Annex **7.8**)	= 36 %
➔ % protein (from anhyd. milk) in recipe	= 0.01 % * 0.1 = 0.001%
➔ % protein (from skim. milk) in recipe	= 36 % * 0.05 = 1.8 %
➔ total % protein in recipe from milk ingredients	= 1.8 + 0.001 = 1.801 %

5.2.2.3. Discussion of protein equivalents

It is understood that for every derivative-based ingredient that there will potentially be the need to introduce a conversion factor to express the results as 'Mg total protein from an allergenic source/kg food product'. The examples of conversion factors presented above are certainly not exhaustive. However, the guidance in this section should provide the user with enough information to allow an informed conversation with their analytical partners regarding comparable results expressed in the same units and potential conversion factors needed to express results as "Mg total protein from an allergenic source/ kg food product."

5.2.3. Carry-over calculation guidance

For a number of reasons it may be appropriate to perform an allergen QRA based on an estimate of carry-over of allergen-containing product into subsequent product. These reasons include the uncertainty inherent to sampling cross-contact that may be intermittent, the availability of appropriate analytical methods, and that carry-over calculations can be performed easily, quickly and cheaply and usually with a knowledge that they over-estimate potential cross-contact. In some cases it may be appropriate to combine a carry-over QRA with one that is based on analytical data in order to provide additional assurance. It is possible to estimate potential carry-over through knowledge of the line(s) and equipment, together with easy and cheap measurements, such as the mass of preceding product that may be left within the equipment based on the size and number of potential hang-up points. The steps leading to this estimate are summarised in **Table 22**.

A spreadsheet was developed by the EU project iFAAM to estimate the UAP in the product. This tool is made publicly available by TNO (see here) as the Contamination estimate calculator. The calculator guides the user through entry of data relevant for calculating and documenting UAP. The approach uses a simple calculation and can be one of the inputs used for risk management decision making. Similarly the Allergen Bureau (https://allergenbureau.net/) provides training and resources such as the VITAL® Online

Calculator (https://vital.allergenbureau.net/) to record ingredient and processing profiles to enable hang-up estimation and calculation of potential carry-over into the following products made on the same equipment.

In order to calculate the concentration of potential UAP due to carry-over from the previous batch, information needed from these calculators can include, but are not limited to:

- % of composition for each ingredient from an allergenic source in the preceding recipe
- % protein in each ingredient from an allergenic source.
- Any conversion factors needed to combine different ingredients from the same allergenic source in order to express results in total protein from the allergenic source.
- Quantity (kg) of hung-up material that could be carried-over into subsequent production, with documentation for basis of this estimate. This can be estimated based on the mass of material that theoretically could be present at known hang-up points.
- Batch size of subsequent production that will be mixed with or exposed to the hang up (kg).
- Form and distribution of the carried-over material, based both on the characteristics of the material and subsequent production, and process steps in the line that are subsequent to the point of cross-contact (e.g. mixing with a certain quantity of subsequent product, or no mixing etc).

These tools provide the user a first understanding of the possible risk based on conservative inputs and provide the basis for further refinement or investigation if warranted.

Note that examples in the section **5.2.2** provide more information regarding potential steps needed for converting different ingredients from the same allergenic source into an expression equivalent to total protein from the allergenic source.

Table 22. *Description of the steps to estimate the potential UAP carried over into subsequent food production.*

a) Carry over from preceding to next product.	
What is the allergen content as ingredient in the preceding recipe?	QA/product manager knows the allergen ingredients and concentration in preceding product on the production line.
What is the mass of product that could be carried over into next product?	This step needs estimation of the mass of carry over by the QA/product manager. QA/product managers may have a good idea on what is the cross contact/carry over potential related to equipment design and cleaning capability.
What is the mass of subsequent product that is potentially in cross-contact?	Available from production scheduling data. Note that this step should consider not just the total amount of product subsequently run, but if there is a fraction of the run which is more likely to contain the cross-contact material (eg the first part of the production run).
Concentration of cross-contact.	Based on the above, calculate the concentration of cross-contact.
b) Concentration of cross-contact allergen	
Concentration of allergen of concern in the hung-up product	This is based on the percentage of ingredient(s) within the hung-up product that contain the allergen of concern and their concentration(s) within those ingredient(s).
Concentration of cross-contact in the affected product. * Note that if there is information regarding UAP or concentrations of allergen cross-contact in intentionally added ingredients then that should be accounted for reflected in the final calculations	This is based on dividing the concentration of allergen in the hung-up product by the concentration of cross-contact in the subsequent product (from step a).
c) Form & Distribution of cross contact.	
Amend the above concentration, by considering the form and distribution of potential allergen cross-contact.	A knowledge of the physical characteristics of the hung-up and affected products, in combination with knowledge of the production process subsequent to the point of cross-contact will enable a determination of whether the cross-contact should be considered as homogeneous or more concentrated etc.
Estimated UAP in the product after refinement in steps a, b and c	This summarizing estimate of UAP is input for further RA

5.3. Guidance on food intake data for allergen risk assessment

This section will provide guidance on how to make estimations of food intake for use in allergen risk assessment.

Food allergic reactions generally develop within a very short time frame, within 30 minutes and thus the intake amounts should reflect what is consumed at a single eating occasion. Taking bread as an example, one slice of bread is often described as a "portion size" and is the basis of nutritional values for bread. However, people typically do not consume one slice of bread at an eating occasion, and the actual consumption during a meal can range between less than one slice up to more than 5 slices of bread, but the average is 2-3 slices of bread per meal (Birot, Madsen et al. 2018, Meima, Blom et al. 2021).

The optimal percentile per eating occasion for use in deterministic allergen risk assessment and Action Level calculations is currently considered to be the 75th percentile of the food consumption distribution (P75). This P75 is sufficiently conservative and provides a good balance between compliance with the predefined food safety objective and feasibility and practicality of management measures. A list of population P75 intakes at single eating occasions for a broad range of food groups[14] is provided **Table 23**, reprinted from Birot, Madsen et al. (2018). In fact, the P50 intake has also been shown to be sufficiently conservative for 99% of food groups studied (Blom, Remington et al. 2019). However, since then a considerable amount of additional dose-distribution data have been made available and improved dose-distribution modelling methods were developed. Therefore, Blom et al. (in preparation) have updated their sensitivity analyses and showed that Reference Amounts based on the p50 to p65 of the general population distribution of the single eating occasion intake of foods result in compliance with the safety objective intended by using the Reference Doses established in the 2nd WHO/FAO consultation meeting (FAO/WHO 2021) without being over-conservative. Based on these results, it may be possible that in the near future the p50 will be recommended as a Reference Amount of food intake for use in food allergen deterministic risk assessment and calculation of Action Levels. If the p50 is not available, the mean would be a good alternative, as analyses of the intake data showed that the mean generally is between the p50 and p65.

It is expected that intake data for other countries should become available in the future, see also **Box 6**.

[14] Multiple similar food items are grouped into approximately 60 food groups considering the similarity in food use and consumption patterns.

Table 23. *A list of population P75 intakes at single eating occasions for a broad range of food groups, from (Birot, Madsen et al. 2018) Food consumption summary statistics per food group (in g)*

Name	Country	Mean	SD	P75	P90
Chestnut paste and coconut milk	Combined	63.7	55.3	100	116.5
Peanut butter	Combined	27.4	17.5	35	45
Cheese	Combined	39.8	31.3	48	80
Milk powder and Cocoa powder	Combined	18.5	14.1	26.4	33.6
Coffee creamer	Combined	4.3	4.2	6	8
Cream and coffee milk	Combined	22.5	26.1	30	40
Ice cream	Combined	88.2	47.3	100	150
Milk and milk products for drinking	Combined	264.5	163.7	317.5	432
Milk and milk products consumed with a spoon	Combined	156.9	76.4	200	250
Peanuts, nuts and dried fruits	Combined	33.3	29.5	40	60
Potato and other starch based chips (including salty sticks/pretzels))	Combined	43.4	38.2	59	79
Fried/warm snacks	DK	162.8	103.1	180	270
Fried/warm snacks	FR	109	89.2	140	210
Fried/warm snacks	NL	77.4	50.5	85.5	140
Meal replacements and meat imitates	Combined	105.1	111.6	113	250
Supplements	Combined	1.7	2.6	2	3
Pancakes and waffles	DK	151.5	104.6	200	300
Pancakes and waffles	FR	152.7	102.3	200	300
Pancakes and waffles	NL	87.1	100.1	100	210
Soups	Combined	318.9	161	400	500
Small sweets - sweet confectionary unspecified/Combined	Combined	47.9	42.7	60	100
Small sweets - sweet confectionary specified	Combined	25.5	31	28	60
Sugar	Combined 1	21.4	12.9	24	33
Chocolate and chocolate products	Combined	32.1	33.4	40	60
Sweet confectionary (jam, marmalade)	Combined	33.4	25	35	60
Cereal bars	Combined	31.7	27.1	32.1	50
Chewing gum	DK	10.6	7.8	10	20
Chewing gum	FR	5.9	7.4	6	10
Chewing gum	NL	2.9	2.5	4	5
Mashed potato powder	Combined	177.3	84	200	300
Potato product (excl. powder)	Combined	172.2	108.2	225	300
Vegetable oils and animal fat	Combined	14.8	11.9	20	30
Butter/halvarine/margarine	Combined	14.3	10.6	20	25
Sauces used as condiments and dessert sauces	Combined	22.3	20.7	30	46.5
Sauces , savory, chutneys and pickles	Combined	57.1	47.7	75	105
Fish products - mean 35 g such as fish fingers, fish paté	Combined	34.2	29.7	40	62.6
Fish products - mean 75 g such as smoked salmon, canned fish in oil	Combined	74.2	49.5	100	136.2
Fish products - mean 115 g such as fish cake, fish balls	Combined	115.6	75	150	190
Meat products - mean 65 g such as bacon, salami, paté	Combined 2	64.5	46.2	75	125
Meat products -mean 105 g such as meat loaf, sausages	Combined	107.4	63.1	126.3	178
Crackers, crisp bread, rusk and toast	Combined	22.9	19.3	28	45
Bread, bread rolls and bread doughs	Combined	90.9	51.3	120	150
Herbs and spices mixes, bouillon cubes, yeast extract	Combined 1	18.1	32.4	20	20
Spices and salt	Combined	2.9	2.5	3	4
Alcoholic drinks, alcohol ≤15%	Combined	222.1	144.5	282.5	420
Alcoholic drinks, alcohol above 15%	Combined	68.9	69.6	83.8	120
Beer	Combined	567.1	520.3	660	990
Syrups	Combined	26.6	30.8	33.8	60.4
Drinks without alcohol (excl. syrup)	Combined	362.3	252.1	483.3	600
Cookies (biscuits)	Combined	32.8	27.8	42	60
Cakes (including pastry)	Combined	144.4	78	180	250
Breakfast products eaten unprocessed (e.g. muesli, oat and maize flakes)	Combined	46.9	28.1	60	83.2
Breakfast products, porridge	Combined 1	168	163.2	202	257
Pasta, rice, couscous and other grains	Combined	155.4	91.2	200	270
Legumes	Combined	132.2	67.3	175	215
Fruit and vegetables, processed	Combined	139.1	86.7	190	238
Eggs	Combined	40.9	29	55	80
Egg based dishes such as omelet	Combined	123.8	69	180	200
Sandwich and pizza	Combined	270.4	209.9	335	500
Composite dishes such as lasagna, quiche, vegetable casserole	Combined	238.2	155.5	320	450

1 The group is combined but the consumption data used are the Danish
2 The group is combined but the consumption data used are the French

Box 6. *Consumption estimate tables for use in allergen risk assessment.*

DESIGNED SPECIFICALLY FOR ALLERGEN RISK ASSESSMENT

Mean and P75 reported using maximum consumption on a single eating occasion

Northwest Europe (NL / FR / DK)

- Birot, Madsen et al. (2018). https://doi.org/10.1016/j.fct.2018.05.042

NOT DESIGNED SPECIFICALLY FOR ALLERGEN RISK ASSESSMENT

Average consumption / mean / reference amounts per eating occasion

United States

- US Intake Tables: Reference amounts customarily consumed per eating occasion from the Code of Federal Regulations Title 21
  This document lays out the "general principles and factors that the Food and Drug Administration (FDA) considered in arriving at the reference amounts customarily consumed per eating occasion (reference amounts)" and includes tables of the resulting values.

Canada

- Health Canada: Table of Reference Amounts for Food
  "This document sets out reference amounts for different categories of foods. Reference amounts: [1] represent the amount of food typically consumed in one sitting, [2] are used to determine what is considered to be a single-serving container, [3] serve as the basis for determining the serving size to be shown in the nutrition facts table of multiple-serving packages of foods, [and 4] serve as part of the criteria for making nutrient content claims. Also provided are instructions on how to determine the serving size for the nutrition facts table."
- Canadian Food Inspection Agency: Information within the Nutrition Facts table - Serving sizes and reference amounts, including a decision tree for determination and declaration of serving size for a prepackaged product

Other datasets are publicly available but were analyzed on a per day basis. We have not placed links to those documents here as allergen risk assessments should be done "per eating occasion" and any "per day" estimates need a detailed evaluation regarding potential usefulness of the data. See **7.9 ANNEX for food intake section** for more information.

Exceptions for not taking the P75 but a different food intake value to calculate the exposure can be:

- When you as a company have good data on how much is consumed of your specific product these data are useful to consider.
- For some products the exact quantity can be expected to be consumed (airplane foods).
- If the package size of your specific product is close to the average or P75 consumption estimates, then the entire package could be expected to be consumed*.
- Use of another regional database although currently not providing the P75 values, the P50 has been shown to be conservative in 99% of assessments and might be considered as better reflecting local specific consumption amounts of a product

Figure 18 incorporates these considerations and can support in selecting food intake data.

If more information is desired regarding explanations and considerations that can provide support in deciding the food intake value for your specific product, please see ANNEX **7.9** with more information for the following sections.

- Portion or serving size?
- Data provided in national food consumption databases
- Can I use data from the acute daily intake for a single day?
- Can I use data from one country for another country?
- General population vs Population with food allergies
- Frequency of consumption
- References for further reading

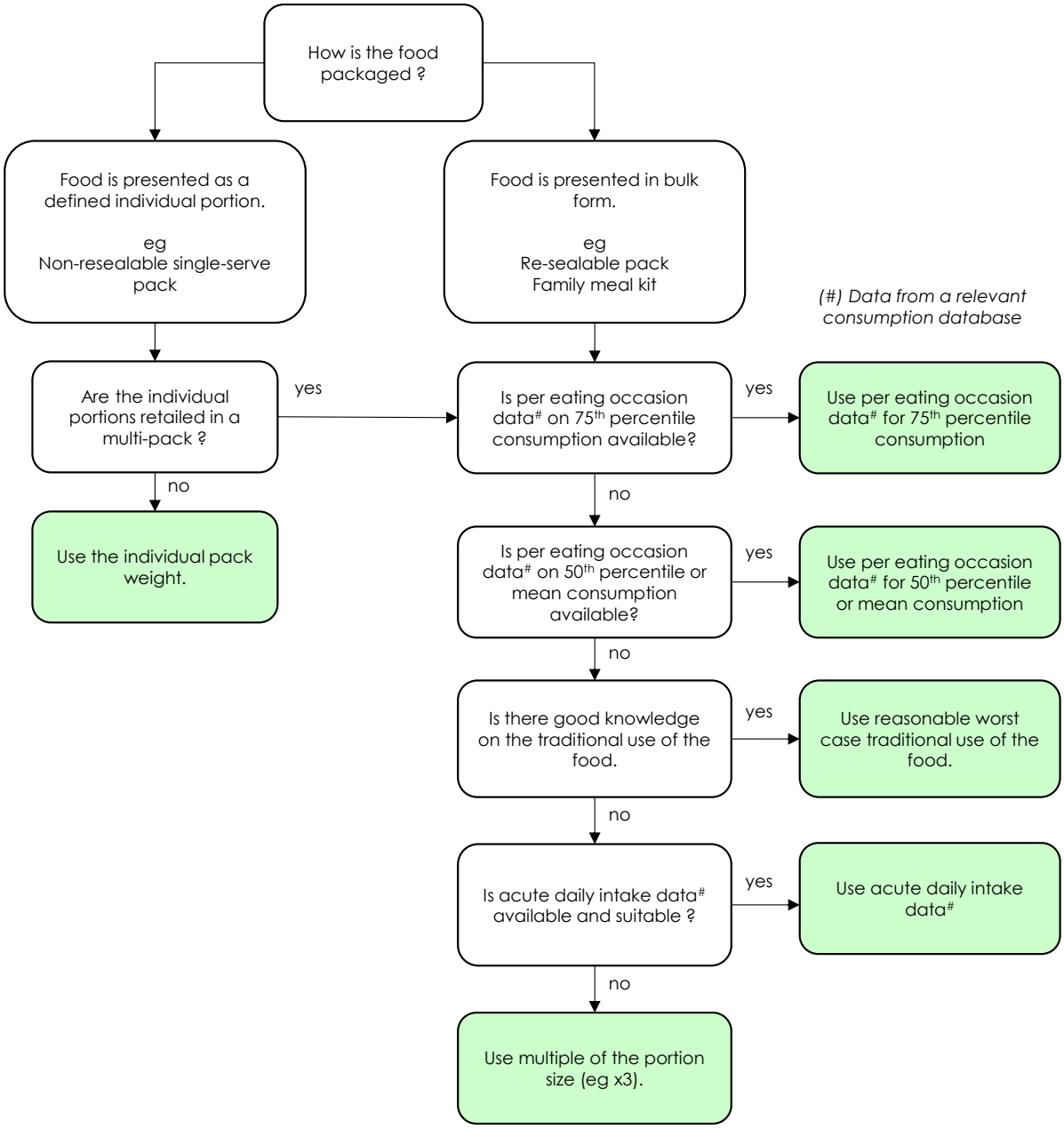


Figure 18. *Scheme supporting the selection of food intake data*

5.4. Basic allergen QRA calculations

Risk management action aims to mitigate risk, when there is a chance that UAP has occurred, risk is a function of hazard, exposure, and the frequency of occurrence, further refined by a consideration of the nature of the effect. As such, an allergen RA including QRA should incorporate the different inputs of frequency of occurrence, exposure [concentration x intake], and sensitivity of individuals with food allergy or the allergic population. In practice, information for all of these inputs is not always necessary and not always available when performing the RA.

Food allergen (Q)RA at the most basic level will compare the exposure to allergen to an appropriately protective Reference Dose for that allergen based on the relevant allergic population. For exposure assessments in a food allergen QRA, two main variables will determine the calculation:

- the concentration of total protein from an allergen in a consumed product [either found through sampling and analysis (**Allergen Sampling and Analysis)** or estimated from carry-over (section **5.2.3**)**.**
- the intake amount of the specific product eaten by the individual or distribution of intakes by the at risk population, which is also known as the Reference Amount (**Table 23**).

As a part of the above, the form and distribution of the allergen within the affected food is a key consideration to understanding exposure from a portion of that food. A third input, the frequency of occurrence should also be considered when assessing risk even though it is not included in the simple calculation formulas shown in this chapter. It is usually characterized as a more categorical consideration (isolated / intermittent / regular / unknown; section **5.1.2.2**).

A number of calculations may be applied by the user of this Guidance to perform their QRAs, such as:

- Exposure calculations;
- Action Level calculations;
- Intake amounts required to reach a specific exposure level;
- Basic risk calculations;
- Public health impact assessment.

These calculations are discussed hereafter.

Additionally, it is possible to create a simple spreadsheet to perform these calculations such as the one linked here and provided as a part of this Guidance. Such a spreadsheet can be created that contains information on the appropriate Reference Doses for allergens with fields for the input of allergen concentration and portion size, for the calculation of whether the Reference Dose or Action Level is exceeded.

*Disclaimer: Any example Reference Doses used in the calculations below does not mean that ILSI Europe or the authors of this Guidance endorse or recommend/require the use of a specific risk management system. They are used only for example calculation purposes. For more information and key references regarding the fundamentals of how allergen Reference Doses are derived from oral food challenge data and subsequent dose-distribution models, as well as what might constitute an appropriate Reference Dose see ***Box Reference Doses.***

5.4.1. Exposure (mg) calculations

The exposure calculations that follow assume the user has already converted an available analytical result to desired units of ppm (mg/kg) of total protein from the allergenic source. If the analytical result is reported in a different unit, a conversion must be applied (see Section **5.2.2**).

$$Exposure\ \ dose\ (mg) = Ref.Amount\ (food\ intake, kg)\ x\ protein\ content\ (mg/kg)$$

Example: Exposure dose Milk powder and cocoa powder with 2.5 mg/kg of peanut [Parts per million,ppm] detected by ELISA. This product has a pack size of 250g.	
- Reference Amount (P75) of milk and cocoa powder (**Table 23**)	= 26.4 g = 0.0264 kg
- Concentration of whole peanut in milk and cocoa powder, detected by ELISA	= 2.5 mg whole peanut/kg food
- Conversion to total peanut protein (**ANNEX 0**– peanut is 26% protein)	= 2.5 * 0.26 = 0.65 mg peanut protein/kg food
➔ Exposure dose	= 0.65 * 0.0264 = **0.017 mg peanut protein**

The calculated exposure dose can be compared to a suitable Reference Dose (RfD) to determine whether there is a potential risk to peanut allergic consumers ***Box Reference Doses***.

5.4.1.1. Reverse calculation: Quantity of food consumed available.

Reverse calculation What concentration needs to be present before an exposure equal to a "hypothetical" Reference Dose would be reached? $$UAP\ concentration\ (mg/kg) = Ref.Dose\ (mg)/Known\ food\ intake\ (kg)$$	
Example: Known food intake of 10 g	
- Reference Dose[15] for peanut	0.2 mg peanut protein
- Known food intake (kg)	= 10 g = 0.010 kg
➔ Concentration of peanut protein required at known food intake amount to reach the Reference Dose	= 0.2 / 0.010 = **20 mg peanut protein/kg food**

[15] This example Reference Dose does not mean that ILSI Europe or the authors of this guidance endorse or recommend/require the use of a specific risk management system. It is used only for example calculation purposes. For more information and key references regarding the fundamentals of how allergen RfDs are derived from oral food challenge data and subsequent dose-distribution models, as well as what might constitute an appropriate RfD see ***Box Reference Doses***.

5.4.1.2. Reverse calculation: analytical result is available:

Reverse calculation How much product needs to be consumed before an exposure equal to a "hypothetical" Reference Dose would be reached? $$Food\ intake\ (kg) = Ref.Dose\ (mg)/protein\ content\ (mg/kg)$$	
Example: Package size of 250g. Concentration of 2.5 mg whole peanut/kg food detected.	
- Conversion to total peanut protein (**ANNEX 0**– peanut is 26% protein)	= 2.5 * 0.26 = 0.65 mg peanut protein/kg food
- Reference Dose[16] for peanut	0.2 mg peanut protein
➔ Amount required to be consumed to reach the Reference Dose	= 0.2 / 0.65 = 0.307 kg = 307 g ➔ **Larger than an entire package size**

In this scenario, in order to achieve an exposure equal to or greater than the example Reference Dose, an individual would need to consume an amount greater than the entire package size.

[16] This example Reference Dose does not mean that ILSI Europe or the authors of this guidance endorse or recommend/require the use of a specific risk management system. It is used only for example calculation purposes. For more information and key references regarding the fundamentals of how allergen RfDs are derived from oral food challenge data and subsequent dose-distribution models, as well as what might constitute an appropriate RfD see ***Box Reference Doses***.

5.4.2. Calculation of an Action Level (mg/kg, ppm)

Action Levels are concentrations of protein which can be used as a cutoff for determining different outcomes of a risk assessment. Action Levels are determined using an appropriate Reference Dose (RfD) and portion size, or Reference Amount.

$$Action\ Level\ \ (mg/kg)\ = \frac{Ref.\ Dose\ (mg\ protein\ from\ allergenic\ source)}{Ref.\ Amount\ (kg)}$$

For help with Reference Amount: see intake guidance (see section **5.3**)

Example: Action Levels Milk and cocoa powder with 2.5 ppm peanut (0.65 ppm peanut protein) detected by ELISA Package size of 250g. Concentration of 2.5 mg whole peanut/kg food detected.	
- Conversion to total peanut protein (**ANNEX 0** – peanut is 26% protein)	= 2.5 * 0.26 = 0.65 mg peanut protein/kg food
- Reference Dose[17] for peanut	0.2 mg peanut protein
- Reference Amount (P75) of milk and cocoa powder (**Table 23**)	= 26.4 g = 0.0264 kg
➔ Action Level	= 0.2 / 0.0264 = **7.6 mg/kg total peanut protein**

This example Action Level can be compared to data available on cross-contact. In this case the Action Level (7.6 mg/kg total peanut protein) is greater than the concentration of peanut protein detected (0.65 mg/kg), therefore it may be appropriate to state that risk to allergic consumers is within agreed limits of acceptability.

[17] This example Reference Dose does not mean that ILSI Europe or the authors of this guidance endorse or recommend/require the use of a specific risk management system. It is used only for example calculation purposes. For more information and key references regarding the fundamentals of how allergen RfDs are derived from oral food challenge data and subsequent dose-distribution models, as well as what might constitute an appropriate RfD see ***Box Reference Doses***.

5.4.3. Sensitivity related to the uncertainty of assessment

There are a number of factors which dictate that for allergen risk assessments it is often useful to perform a simple sensitivity analysis:

- The uncertainty inherent to the data collected.
- The variability of allergen presence and concentration in cross-contact scenarios.
- The consumption scenarios that may be reasonably foreseeable.
- The fact that QRA usually compares exposure to set hazard characterization values in the form of fixed Reference Doses (sometimes known as 'bright line' safe exposures).

If there is any choice in input data available, it is recommended to first perform an assessment using input data that is considered to be 'reasonable worst case', what is meant by this is data that is reflective of the cross-contact scenario being studied but is not likely to underestimate potential exposure. If this results in a potential consumer concern or is close to an exposure that may present concern, the input data can be tested to understand what differences in the data would result in an exposure that results in the opposite outcome when compared to the 'bright line' safe exposure.

Whether it is feasible or not, given the cross-contact scenario in question, for an opposing risk outcome to be reached (compared to the 'reasonable worst case' calculation), using realistic input data which is around the data of the initial assessment should be reported with the risk assessment outcome. A risk assessment that remains unchanged irrespective of sensitivity testing should be considered as carrying greater weight compared to a risk assessment outcome that can vary depending on reasonably foreseeable variation in input data.

5.4.4. Basic risk calculations

Instead of reporting "a concentration above the Action Level " or "an exposure above the Reference Dose," it may be desirable to express the results as a calculated risk.

The simplest form of a risk calculation for the "population at-risk" consists in comparing an Action Level to an allergen concentration or an exposure to a Reference Dose (RfD).

$$Risk = \frac{Allergen\ Concentration\ (mg/kg)}{Action\ Level\ (mg/kg)} \quad \text{or} \quad Risk = \frac{Exposure\ (mg)}{Ref.Dose\ (mg)}$$

In both cases a ratio larger than 1 indicates that there is potential risk greater than the tolerable risk defined by the Reference Dose. The larger the ratio, the greater the potential risk.

The input data available determine which equation can be used. When analytical results are available (allergen content in food (mg/kg)), the risk will be calculated using the "Action Level". Otherwise, when exposure or carry-over estimates are available, the risk will be calculated using the Reference Dose.

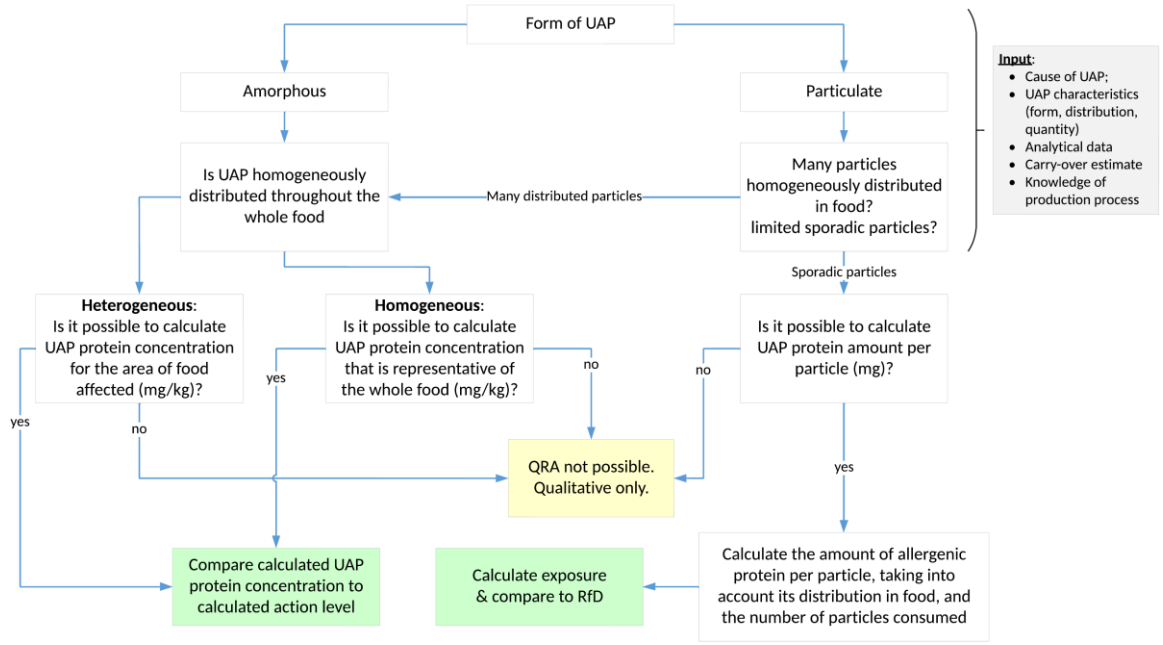


Figure 19. *QRA flow diagram: calculations possible with differing forms of UAP*

Figure 19 summarizes the simple QRA process, for a given allergen scenario. Different paths are to be followed depending on the form (amorphous or particulate) and distribution of UAP (homogeneous or heterogeneous), to calculate (i) the concentration of allergen within the whole foodstuff or an affected proportion of it, or (ii) the amount of allergenic protein per particle.

In the case of calculating a concentration across a food or for an affected area of a food, in many cases it will be preferable that input data consists of both an understanding of the source of UAP and a concentration estimate, in conjunction with analytical data. The use of data without taking into context other evidence can be misleading particularly in the case of infrequent UAP or heterogeneous allergen distribution.

In the case of particles, it is relevant to calculate the amount of allergenic protein per particle in the case of particles that are distributed in such a way that a consumer portion of the food would result in a low number of particles being consumed or indeed if there is a limited probability of actually consuming one particle. The number of particles likely to be consumed should be estimated based on their distribution in the food and the respective mg of allergenic protein multiplied proportionally. In the case of particles that are uniformly distributed across a foodstuff, for example large numbers of small particles, it is more relevant to calculate exposure as a concentration across the whole foodstuff. For a discussion on calculations for particulate contamination see section **5.1.2.4**.

5.4.5. Public health risk assessment (basic)

In the case of incidents where there is implicated product at market, an estimate of the public health impact of the "contaminated" food could be desired. This basic calculation requires several parameters, namely the amount of product on the market, available to consumers, which should be known to the relevant food business and the prevalence of allergy to the allergen in question. In this type of basic calculation it is assumed that allergic consumers have the same preference to choose to eat the implicated food as per non-allergic consumers.

The following template could be used to conduct the basic calculation for the public health risk assessment:

Step 1: calculate the amount of allergenic protein consumed		
Input	concentration of cross-contact allergenic protein (mg/kg)	
	amount of affected product that is eaten by consumer (g)	
Output	➔ amount of allergenic protein consumed (mg)	
Step 2: compare the amount of allergenic protein consumed (above) to the associated ED value from an appropriate dose-distribution model, to calculate estimated number of reactions at market.		
Input	number of portions that have reached the consumer (assuming 1 portion per consumer)	
	prevalence of allergy within the consumer population (%)*	3%
	estimated ED value related to amount of allergenic protein consumed (%) (Compare to tables in (Houben, Baumert et al. 2020))	
Output	➔ estimated number of reactions that could be experienced at market	

** Default of 3%. See section **4.1.7** for more information on prevalence of food allergy within the consumer population.*

Example calculation:

Step 1: calculate the amount of allergenic protein consumed		
Input	concentration of cross-contact allergenic protein (mg/kg)	= 60 mg/kg
	amount of affected product that is eaten by consumer (g)	= 250 g = 0.250 kg
Output	➔ amount of allergenic protein consumed (mg)	= 0.250 * 60 = 15 mg protein
Step 2: compare the amount of allergenic protein consumed (above) to the associated ED value from an appropriate dose-distribution model, to calculate estimated number of reactions at market.		
Input	number of portions that have reached the consumer (assuming 1 portion per consumer)	= 500 000
	prevalence of allergy within the consumer population (%)*	= 3% = 0.03
	estimated ED value related to amount of allergenic protein consumed (%) (Compare to tables in (Houben, Baumert et al. 2020))	= 15% = 0.15
Output	➔ estimated number of reactions that could be experienced at market	= 500 000 * 0.03 * 0.15 = 2 250

** Default of 3%. See section **4.1.7.** for more information on prevalence of food allergy within the consumer population.*

6. Concluding remarks and future perspectives

The purpose of this Guidance document is to provide tools and approaches to harmonize the data gathering process and execution of food allergen quantitative risk assessments, including how such assessments can complement existing allergen management practices. The Guidance is intended to facilitate the preparation and communication of allergen QRA. The risk assessment itself and any decisions and measures based on it (such as product recalls, application of PAL) remain the responsibility of the user.

With that in mind, it must be noted that at the time of writing this Guidance (June 2022):

There are no requirements that are generally accepted and implemented in any jurisdiction on the mechanisms that should underly decision-making on the application of PAL. There are various risk management strategies in use, in particular the application of a zero-tolerance approach to allergen risk management that does not best serve those with food allergies. Such an approach has limited capability in minimizing risk and maximizing quality of life for allergic consumers. When used in isolation without consideration of QRA, a zero-tolerance approach inevitably leads to an increasing number of product recalls or products bearing PAL with little, if any risk reduction. These are areas where QRA can especially be of interest and potentially benefit all stakeholders.

Although allergen QRA has started to achieve some maturity, there remain hurdles to overcome before it can achieve its full potential as a part of a system that is harmonized across food business operators. In the meantime, any effort to develop a more transparent risk management approach, such as in the management of allergen incidents or supporting the rationale for PAL declaration on the label, would already be an improvement for consumers with food allergies. As described in this Guidance, the application of allergen QRA requires systematic information gathering and documentation, and as such will help with efforts for increased supply chain transparency and better understanding of the reality of risk and its mitigation.

The Guidance document is not static, it has been and will remain based on the input of a community of practitioners. We look forward to future knowledge gained from, but not limited to:

- The Ad hoc Joint FAO/WHO Expert Consultation on Risk Assessment of Food Allergens
    - Summary and Conclusions: Part 1, Part 2, Part 3
- The Allergen Bureau's Agricultural Allergen Cross Contact Working Group
    - Full guidance will be made available here
- Allergen management guidelines for food manufacturers produced by the Université Laval, Food Allergy Canada and Canadian food industry stakeholders
    - Full guidance will be made available here
- ILSI Europe next EG – Safe upper limits for PAL and risk communication **https://ilsi.eu/scientific-activities/food-safety/food-allergy/**

To summarize, this Guidance is intended to provide the allergen management practitioner with an introduction to allergen QRA, an overview of different QRAs including their inputs and methods, and importantly an understanding of when QRA is appropriate and possible. We trust you will find it helpful, and value your comments.

7. Annexes

7.1. ANNEX: Definitions / Glossary

Allergen incident: the unexpected and previously unaccounted presence or potential presence of allergen, in a foodstuff that has been produced, distributed or retailed.

Clean-in-place: a method of automated cleaning the interior surfaces of pipes, vessels, equipment, filters and associated fittings, without major disassembly

Cross-contact occurs when an allergenic food, or ingredient, is unintentionally incorporated into another food that is not intended to contain that allergenic food. *Source* CODEX

Epitope: the amino acid sequence and its three-dimensional structure of an allergenic protein (antigen) molecule to which an antibody binds.

Good manufacturing practice: a system for ensuring that products are consistently produced and controlled according to quality standards. *Source:* WHO

Hazard Analysis Critical Control Point: a management system in which food safety is addressed through the analysis and control of biological, chemical, and physical hazards from raw material production, procurement and handling, to manufacturing, distribution and consumption of the finished product. *Source:* FDA

Particulate allergenic ingredients: materials in which the physical form of the allergen consists of pieces visible to the naked eye. They can be retained if passed through an appropriately sized sieve. In contrast, **non-particulate allergens** are materials such as powders, etc., the individual components of which cannot be distinguished by the naked eye and which are not retained by a sieve

Precautionary Allergen Labeling: Voluntary allergen advisory statements, like "may contain (allergen)" or "produced in a facility with (allergen)," have been used by food manufacturers to alert consumers about the possible presence of allergens due to allergen cross-contact. *Source:* FDA

PreRequisite Program: steps or procedures, including GMPs and SSOPs, which control the operational conditions within a food establishment and promote environmental conditions that are favorable for the production of safe food. Prerequisite programs are the foundation of a Food Safety/HACCP system. *Source:* FDA

Sanitation Standard Operating Procedures: the specific, written procedures necessary to ensure sanitary conditions in the food plant.

Universal Prerequisite Program: including the seven principles of HACCP has been universally accepted by government agencies, trade associations and the food industry around the world.

Unintended Allergen Presence: May be due either to cross-contact or mistakes in food processing or labelling.

7.2. ANNEX: List of abbreviations

ATP	Adenosine Triphosphate
CC	Cross-Contact
CIP	Clean-In-Place
ELISA	Enzyme Linked Immunosorbent Assay
FBOs	Food Business Operators
GMPs	Good Manufacturing Practices
HACCP	Hazard Analysis Critical Control Point
HPAEC-PAD	High Performance Anion Exchange Chromatography-Pulsed Amperometric Detection
HPLC	High Performance Liquid Chromatography
IgE	Immunoglobulin-E, which are a type of antibody produced by the immune system
LC-MS/MS	Liquid Chromatography-Tandem Mass Spectrometry
LFD(s)	Lateral Flow Device(s)
LoD	Limit Of Detection
LoQ	Limit Of Quantification
MoO	Management Of Operations
MS	Mass Spectrometry
NGS	Next Generation Sequencing
RLU	Relative Light Units
RoQ	Range Of Quantification
PAL	Precautionary Allergen Labelling
PCR	Polymerase Chain Reaction
PPE	Personal Protective Equipment
PRPs	PreRequisite Programs
(Q)RA	(Quantitative) risk assessment
RfD	Reference Dose
SSOP	Sanitation Standard Operating Procedures
UAP	Unintended allergen presence
UPRP	Universal Prerequisite Program
y/n	yes/no
WIP	Work-in-Progress

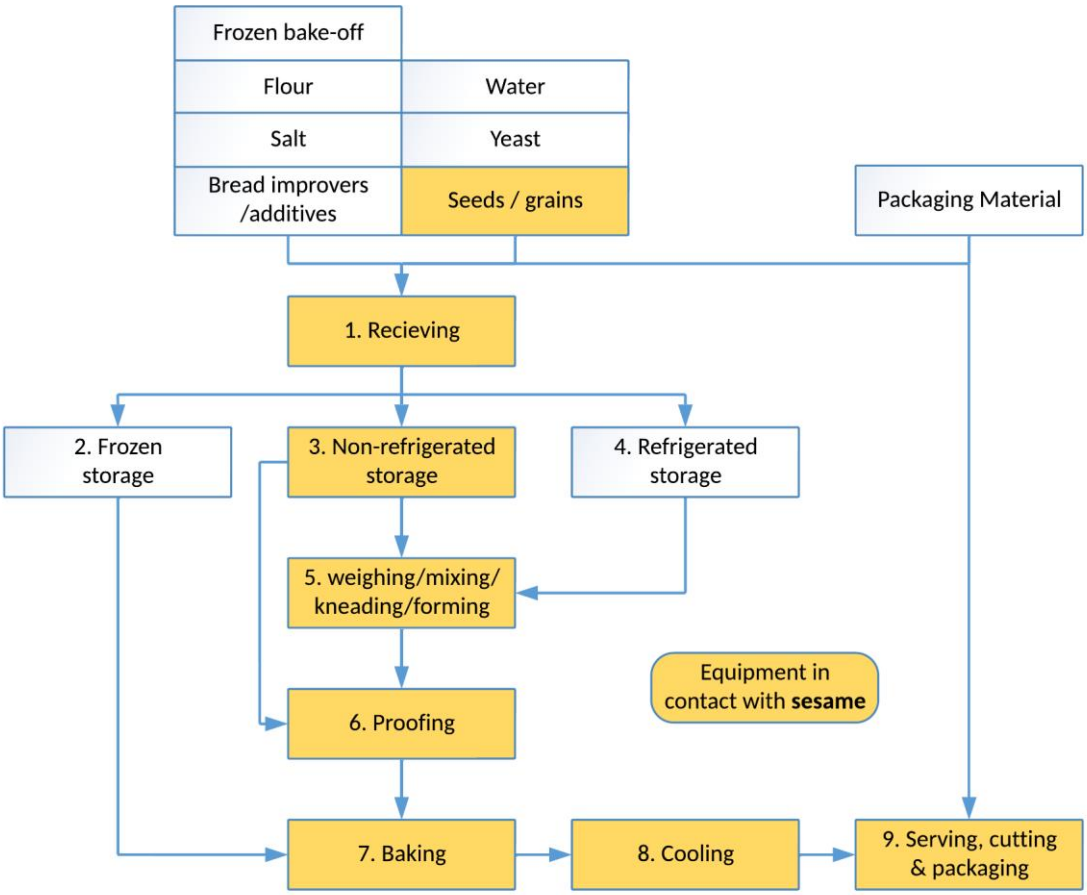
7.3. ANNEX: Guidance documents

Guidance	comments
Allergen Bureau Resources	The **Allergen Risk Review Website**, A freely available interactive factory map that shows where allergen risks can occur in different areas of a food manufacturing facility. Work your way through the interactive factory map, by clicking on each icon, to learn about the allergen risks and discover ways to address these challenges. Guidance on **Unexpected Allergens in Food** Guidance on **Agricultural Allergen Cross Contact**, including sampling and analysis **Food Industry Guide to the Voluntary Incidental Trace Allergen Labelling (VITAL®) Program Version 3.0** Additional guidances and resources are also available through their website.
ISO 22000	ISO 22000:2018 Allergen Management - III
CODEX ALIMENTARIUS	Code of practice on food allergen management for food business operators Discussion Paper on Allergen Labelling (CX/FL 19/45/8)
Swedish Food Sector Guidelines	For: Management and labelling of food products with reference to Allergy and other Intolerance dd 2015
Food Drink Europe	Guidance on Food Allergen Management for Food Manufacturers (2022) Precautionary Allergen Labelling (PAL): a science-based approach based on Quantitative Risk Assessment
IFS Food 7	IFS Standards
FSA-UK	Allergen labelling for food manufacturers
The Industry handbook for Safe processing of nuts (2020)	Handbook was developed for shellers, processors, and manufacturers in the United States. The addendum, Industry Handbook for the Safe Shelling of Peanuts, was developed for peanut shellers in the United States and references food safety guidelines for peanut shellers as well as current Good Manufacturing Practice (cGMP) guidelines for peanut buying points and Good Agricultural Practices (GAPs) guidelines for growers and farmer stock warehouses. These practices could be applied internationally, but the focus of this information resource is on meeting U.S. regulatory requirements. Industry members may want to consider the food safety programs referenced in this document as the foundation for a successful system designed to minimize the potential for product adulteration and cross contact
IFST Food Allergens Knowledge Hub	Links to resources and guidances for Medium & Large Businesses Links to resources and guidances for Small Businesses & Caterers

7.4. ANNEX Examples Management of Operations

Disclaimer: all examples in this annex are fictitious and created for illustrative purposes.

7.4.1. Case Study 2: Example Sesame in a Bakery



Annex Figure 1. *An example of wet and dry cleaning heterogenous cross contact. Orange boxes indicate equipment that comes into contact with sesame; blue boxes, equipment not in contact with sesame.*

FBO Best Bakes makes bread and bread rolls. In their production site in Anotherville, they make both plain and seeded rolls. Their products are sold exclusively in the European Union. After careful assessment, they have determined that cross-contact with soya, nuts, milk, wheat and barley are not reasonably expected to occur. On the other hand, their HACCP team has found that sesame, may occur in the processing facility either as ingredients or as cross-contact residues. For the further assessment, the focus will be on sesame as an example.

In the example in **Annex Figure 1** due to the nature of the process (weighing/kneading/ /depositing/ baking), physicochemical nature of the allergen (seed - particulate) and where the allergen is added in the process (at the end), the particulate sesame cross contact is likely to be heterogeneously distributed, being a particulate form of the allergenic ingredient.

Step 1. Assessing UAP (Cross-Contact).

	Process step	CC Risk	Cross-Contact (CC) Risk
1	Receiving	Yes	breached packaging sesame bags
2	Frozen storage	No	separate storage area
3	Non – refrigerated storage	Yes	sesame in store for other ambient RM's
4	Refrigerated storage	No	separate storage area
5	Weighing/mixing/kneading/forming	No	sesame not used in the dough mix
6	Proofing	Yes	shared proofing trays – sanitation issues
7	Baking	Yes	shared ovens and trays – sanitation issues
8	Cooling	Yes	shared cooling racks – sanitation issues
9	Serving, cutting, packaging	Yes	shared cutting and packing equipment sanitation issues

Step 2. Identification of the effectiveness of controls for process steps under normal operating conditions. Chance of occurrence relates to the likelihood of UAP occurring at a given process step (probable / remote). Data refers to the quality of evidence required to conduct either a qualitative or quantitative risk assessment.

	Process step	**Control**	**Chance of occurrence***	**Data**
1	Receiving	Goods receipt checks	Not likely/ Remote*	Qualitative
2	Frozen storage	Physically segregated	Not likely/ Remote	Qualitative
3	Non – refrigerated storage	Segregated storage / racking in chill store	Not likely/ Remote	Qualitative
4	Refrigerated storage	Physically segregated	Not likely/ Remote	Qualitative
5	Weighing/mixing/ kneading/forming	Dedicated sieves	Likely/ probable	Qualitative
		Cleaning of mixing equipment	Likely/ probable	Quantitative
		Cleaning of kneading equipment	Likely/ probable	Quantitative
		Cleaning of forming equipment	Likely/ probable	Quantitative
6	Proofing	Dedicated proofing trays	Likely/ probable	Qualitative
		Cleaning of proofing trays	Likely/ probable	Quantitative
7	Baking	Burn-out, brushing and scraping oven belts	Likely/ probable	Quantitative
8	Cooling	Dedicated cooling trays	Likely/ probable	Qualitative
		Cleaning of cooking trays	Likely/ probable	Quantitative
9	Serving, cutting, packaging	Cleaning of the cutting and packing equipment	Likely/ probable	Quantitative
		Packaging checks	Likely/ probable	Quali/Quantitative

* The terms probable (likely to happen) or remote (unlikely but not impossible) can be related to the chance of occurrence in Section **5.1.1** as follows: probable (high or medium), remote (low).

Step 3. Hazard Characterization. Estimate significance of the identified hazard to determine allergenicity / severity against a number of specific criteria.

See main chapter on **Management of Operations** for criteria.

Step 4. Validation of existing control measures to minimize the risk of unintentional allergen presence. Qualitative evidence for consideration in the risk assessment and possible quality of data for quantitative risk assessment.

Qualitative validation	Controls	Quality of evidence for QRA
Good receipts checks [P1]	• Training • Visual aids • Visual inspection • Quarantine – challenge testing	Insufficient
Segregated storage [P2/3]	• Training • GMP audits • Spillage procedures	Insufficient
Dedicated storage [P4]	• Training • Signage • GMP audits	Insufficient
Dedicated equipment [P5/P9]	• Training • Labelling/color coding	Insufficient
Enclosed/packaged product [P10]	• Training • GMP audits • Packaging integrity checks	Insufficient

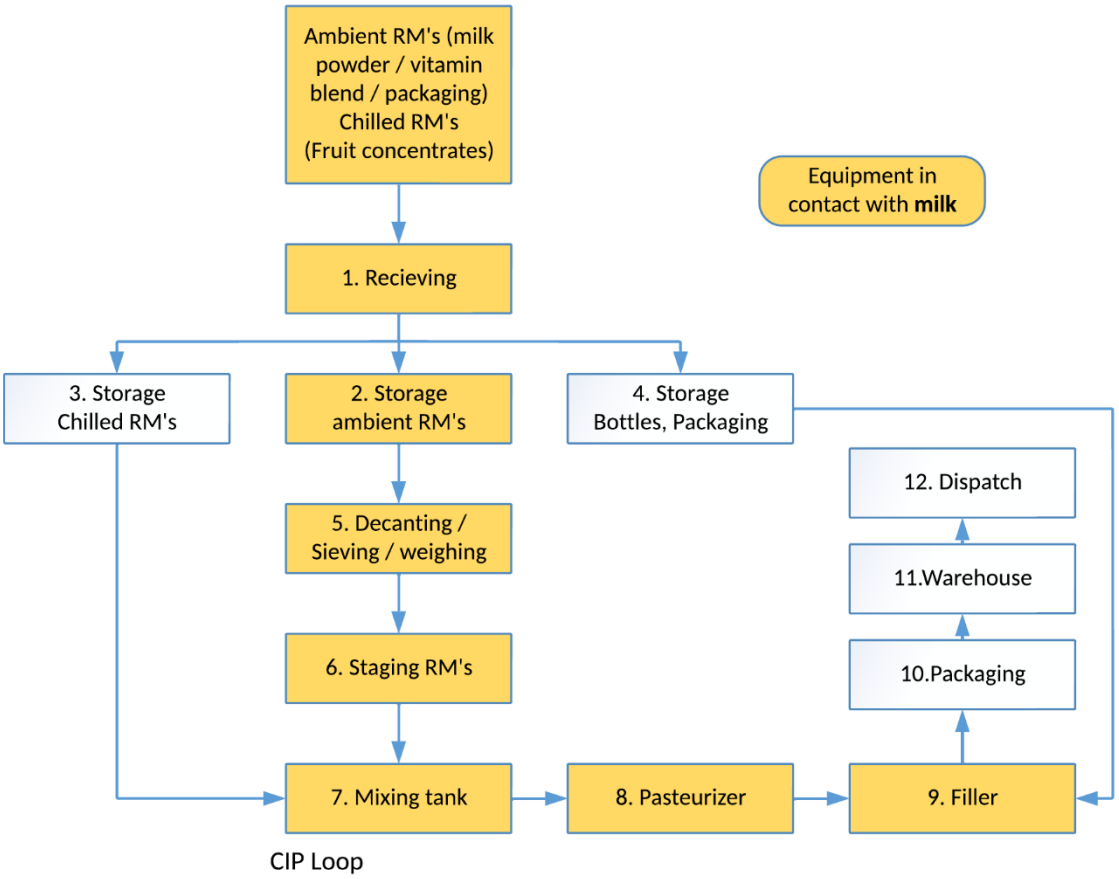
Quantitative evidence for consideration in the risk assessment.

Quantitative validation	Controls	Quality of evidence for QRA
Cleaning/changeover effectiveness	• Training • SSOPs • Application of visual clean standard	Insufficient
	• Pre-operational start up checks against visual standard and KIP's	Insufficient
	• Analysis of surface swabs of visually clean equipment (semi-quantitative and repeated on 3 separate occasions).	Limited
	• Analysis of proceeding finished product (not containing egg)	Good

7.4.2. Case Study 3 Example – Milk in a Fruit Drink

FBO 'Juices are Us' make predominantly pasteurized fruit juices. They have 2 SKU's made on shared equipment that have a milk derived ingredient at their production site in Everyville. Their products are sold exclusively in the European Union. After careful assessment, their HACCP team has found that there is a risk that cross-contact with milk, may occur as cross-contact residues. For the further assessment, the focus will be on milk as an example.

In the example in **Annex Figure 2**, due to the nature of the process (weighing/ mixing /pasteurizing/ filling), physicochemical nature of the allergen (liquid milk) and where the allergen is added in the process (at the start), that the milk cross contact is likely to be homogeneously distributed, due to mixing into similar physicochemical phases (liquid in liquid).



Annex Figure 2. *An example of wet and dry cleaning homogenous cross contact. Orange boxes indicate equipment that comes into contact with milk; blue boxes, equipment not in contact with milk.*

Step 1. Assessing UAP (Cross-Contact).

	Process step	CC Risk	Cross-Contact (CC) Risk
1	Good receipt	Yes	breached packaging
2	Storage - Ambient raw materials	Yes	vitamin mix / milk powder
3	Storage - Chilled raw materials	No	fruit concentrate steel drums
4	Storage - Bottles/ packaging	No	Separate storage
5	Decanting/sieving/weighing	Yes	vitamin mix / milk powder
6	Staging raw materials	Yes	shared totes with dry RM's
7	Mixing tank	Yes	ineffective CIP clean
8	Pasteurize	Yes	ineffective CIP clean
9	Filler	Yes	ineffective CIP clean
10	Packaging	No	product enclosed in packaging
11	Warehouse	No	product enclosed in packaging
12	Dispatch	No	product enclosed in packaging

Step 2. Identification of the effectiveness of controls for process steps under normal operating conditions. Chance of occurrence relates to the likelihood of UAP occurring at a given process step (probable / remote). Data refers to the quality of evidence required to conduct either a qualitative or quantitative risk assessment.

	Process step	Control	Chance of occurrence*	Data
1	Receiving	Good receipt checks	Not likely/ Remote*	Qualitative
2	Ambient raw storage	Segregated storage	Not likely/ Remote	Qualitative
3	Chilled raw Storage	No allergens in storage area + packaging	Not likely/ Remote	Qualitative
4	Refrigerated storage	No allergens in storage area	Not likely/ Remote	Qualitative
5	Decanting/sieving /weighing	Dedicated sieves	Not likely/ Remote	Qualitative
6	Staging raw materials	Cleaning of totes	Not likely/ Remote	Quantitative
7	Mixing tank	Validated CIP clean following dairy drink	Likely/ probable	Quantitative
8	Pasteurize	Validated CIP clean following dairy drink	Likely/ probable	Quantitative
9	Filler	Validated CIP clean following dairy drink	Likely/ probable	Quantitative
10	Packaging	Packaging checks	Not likely/ Remote	Quantitative
11	Warehouse	Product enclosed in packaging	Not likely/ Remote	Qualitative
12	Dispatch	Product enclosed in packaging	Not likely/ Remote	Qualitative

* The terms probable (likely to happen) or remote (unlikely but not impossible) can be related to the chance of occurrence in Section **5.1.1** as follows: probable (high or medium), remote (low).

Step 3. Hazard Characterization.

Estimate significance of the identified hazard to determine allergenicity / severity against a number of specific criteria.

See main chapter on **Management of Operations** for criteria.

Step 4. Validation of existing control measures to minimize the risk of unintentional allergen presence. Qualitative evidence for consideration in the risk assessment and possible quality of data for quantitative risk assessment.

Qualitative validation	Controls	Quality of evidence for QRA
Good receipts checks [P1]	• Training • Visual aids • Visual inspection • Quarantine – challenge testing	Insufficient
Segregated storage [P2/3]	• Training • GMP audits • Spillage procedures	Insufficient
Dedicated storage [P4]	• Training • Signage • GMP audits	Insufficient
Dedication of equipment [P5]	• Training • Labelling/color coding	Insufficient
Enclosed/packaged product [P10,P11,P12]	• Training • GMP audits • Packaging integrity checks	Insufficient

Quantitative evidence for consideration in the risk assessment.

Quantitative validation	Controls	Quality of evidence for QRA
Tote cleaning effectiveness	• Training • Visual clean standards	Insufficient
	• Analysis of surface swabs of visually clean equipment (semi-quantitative and repeated on 3 separate occasions).	Limited
CIP Cleaning effectiveness	• Training	Insufficient
	• Analysis of final rinse waters from CIP (semi-quantitative) following milk containing product	Limited
	• Analysis of proceeding finished product not containing milk (quantitative)	Good
	• Defined systems parameters post validation (including defined dump of caustic) & SSOP's	Good

7.5. ANNEX Examples of 'incidents' and their assessment

This Annex contains the following examples of incident assessments:

- Example 1: a tier 4 upstream incident assessment concerning homogeneous UAP
- Example 2: a tier 2 in-house incident assessment concerning particulate UAP
- Example 3: a tier 2 in-house incident assessment concerning homogeneous UAP
- Example 4: a tier 4 downstream incident assessment concerning homogeneous UAP

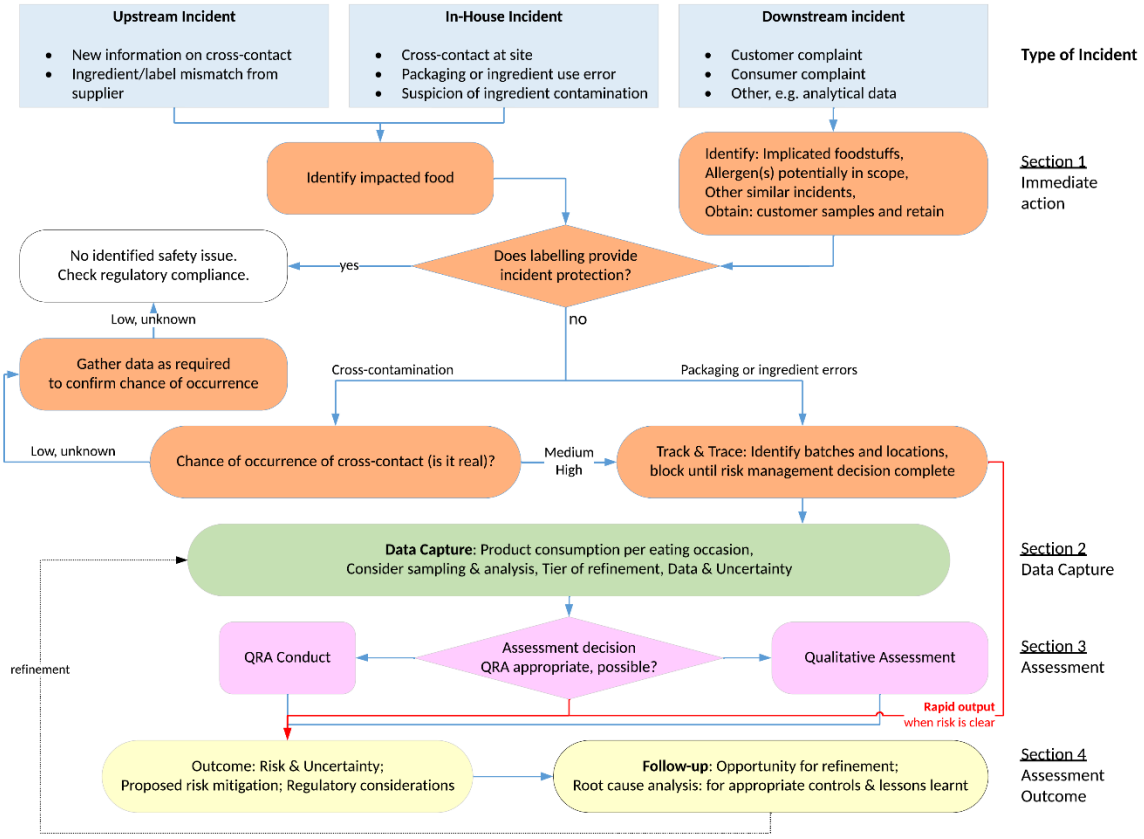
Disclaimer: all examples in this annex are fictitious and created for illustrative purposes.

7.5.1. A Tier 4 upstream incident assessment concerning homogeneous UAP

1 - General Information & Assessment Summary

Assessment Team			
Assessment Date			
Incident Dates			
Type of incident	Upstream In-house Downstream	Source of information ☒ ☐ ☐	Point of cross-contact ☐ ☐ ☒
Foodstuff and allergen(s):	Non-dairy creamer in white sauce and other products formulated with the same ingredient. Milk protein.		
Market(s):	EU & Nordic		
Product disposition:	At market		
Risk to consumers:	There is a risk to allergic consumers		
Quality of Evidence:	High		
Scale of risk:	Reactions at market would be anticipated.		
Opportunity for refinement:	Not considered as necessary		
Regulatory situation:	Product not considered as safe under general food laws		
Proposed mitigation & actions, next steps:	As product presents risk to allergic consumers, market action recommended (recall). Recall product on sites and distribution, notify retailers supplied impacted products of recall		

2 - Incident Flow Chart



3 - Assessment Matrix

Section 1: Immediate Action	
Identity of foodstuff implicated	White sauce and soups
Allergen(s) implicated	Milk protein
Supporting information	An internal investigation into an adverse event report associated with white sauce revealed that one of the ingredients (a creamer) which should not contain milk protein contained significant amounts (~6650 ppm milk protein measured in the most recent batches of ingredient). Beyond the white sauce, this ingredient is also part of the formulation of various soups, which do not declare the presence, or potential presence, of milk.
Does labelling provide incident protection ?	No, some products in which the ingredient is used do not label milk as ingredient or via PAL.
Summary if relevant of consumer complaints	A 7-year old girl, who is apparently very allergic towards egg and milk protein, had an allergic reaction after eating a meal at school that consisted of breaded fish and White Sauce. A sample of white sauce powder was sent to the Swedish authorities for analyses of the presence of casein and egg protein. The results indicated that the white sauce contained 553 mg casein / kg (ppm). No casein or egg protein was found in the breaded fish.

Chance of Occurrence of Cross-Contact	
See '**Core concepts**' Section **5.1.1** for a description of 'Chance of Occurrence'	
Chance of Occurrence	**Notes**
☒ High or known to have happened	Analytical data is assumed to be correct, that there is significant UAP in the supplied ingredient, as also evidenced by reported reaction at market and test result on finished product.
☐ Medium	
☐ Low or unknown	

Track & Trace	
Current status	At market, implicated products within distribution on hold
Degree of success of T&T	
Implicated batch no.s, production dates	
No. Packs (consumer units) implicated	
No. Packs Held	
No. Packs in distribution	
No. Packs at consumer market	
Shelf-life remaining	
Other supporting information	

Section 2: Data Capture

Consumption

See '**Core concepts' 5.3** for guidance on consumption estimates

Pack size (consumer unit) (g)	Meal preparation	Portion size (g)	Quantity of implicated food eaten per consumption event (g)
		Assumptions: worst-case use of creamer in implicated formulations, and 250g finished product consumed.	Assumed that one sachet would be consumed at an eating occasion.

the 'Tier of Refinement'		
Tier	**Description**	**Source of Data**
☐ **Tier 1** 'Theoretical'	Concern has been raised on UAP but there is no physical evidence of cross-contact at the product site or supply chain in question.	No data available, only 'reverse' QRA possible (see **Core concepts**).
☐ **Tier 2** 'Informed'	Some physical evidence of UAP of the specific supply chain in question, high uncertainty in quantification.	The data available for QRA is based on 'reasonable worst case' assumptions, eg hang-up estimation (see **Core concepts**).
☐ **Tier 3** 'Data-driven'	Physical evidence of UAP at the production site or specific supply chain in question, with indirect quantification possible.	The data available is from upstream in the supply chain, for example on a purchased ingredient.
☒ **Tier 4** 'Verified'	Physical evidence of UAP at the production site or specific supply chain in question, with direct quantification possible.	data is available on finished product as presented to consumer, or in case of mis-labeling or ingredient error there is clarity on the allergen content of the food.

Characteristics of UAP: Data & Uncertainty			
See '**Core concepts**' Section **5.1.2** for a description of UAP Characteristics and Uncertainty			
Characteristics		**Uncertainty**	**Data & Notes**
A Form of UAP	☒ Amorphous	1 ☐ High	Based on knowledge of ingredients, process and product. --- Note: If 'unknown', assessment should be based on both amorphous and particulate, until refined information is available.
	☐ Particulate	2 ☐ Medium	
	☐ Unknown (please mark uncertainty as 'high')	3 ☒ Acceptable	
B Distribution of UAP	☒ Homogeneous	1 ☐ High	Likely to be homogeneous within the supplied ingredient, but may be variable across batches depending on change-over with ingredient manufactured with milk protein. --- Note: If 'unknown', assessment should be based on both hetero' and homogeneous, until refined information is available.
	☐ Heterogeneous	2 ☐ Medium	
	☐ Unknown (uncertainty is always 'high')	3 ☒ Acceptable	
C Frequency of UAP	☐ Isolated	1 ☐ High	Identified in all batches after ingredient supplier's scheduling changes. --- Note: If 'unknown', assessment should assume UAP is 'regular'.
	☐ Intermittent	2 ☐ Medium	
	☒ Regular	3 ☒ Acceptable	
	☐ unknown (uncertainty is always 'high')		
D Concentration of UAP	1 ☐ Unknown or Estimate (not analytical). Note: see carry-over guidance **5.2.3**		Provide data: up to 6650 mg/kg milk protein in ingredient (non-dairy creamer) Describe suitability of analytical data: robust data set --- Note: If 'unknown', assessment can only be qualitative. More information is needed before QRA can be performed.
	2 ☐ Analytical, point data		
	3 ☒ Analytical, data range. In the case of mis-labeling or wrong ingredient used, where there is knowledge on amount of allergen present, mark as 3.		
Overall data uncertainty (sum of A-D)		4-7 ☐ High 8-10 ☐ Medium >10 ☒ Acceptable	Notes

Section 3: Assessment		
Assessment Decision		**Notes: rationale for selected option**
It is beyond doubt that there is an unacceptable risk, no further assessment required	☒	The amount of milk protein per portion substantially exceeds the ED01, ED05 and ED10, and is around the ED20 based on the best data available.
Uncertainty is too large to enable an assessment, further information required	☐	
QRA is appropriate but not possible without further information, qualitative assessment only	☐	
QRA is appropriate and possible	☒	
QRA Metrics (for 'screening' and 'deterministic' QRA)		
See '**Core concepts**' section **5.4** for calculation guidance		
Description of the exposure scenario		
In case an Action Level (ppm) was calculated to compare to concentration in food (ppm), what was is the Action Level ?	Action Level =	Conc in food = 4 mg/kg milk protein (dry mix)
In case exposure of allergic consumer was calculated (mg) to compare to RfD (mg), what was the exposure ?	Appropriate RfD[18] = 0.2 mg (Vital 3.0)	Consumer exposure = 21 mg milk protein (based on 250 ml portion)
Description of the calculation	6650 mg/kg milk protein in the ingredient at 1.3% in the finished product = 86 mg/kg. 250ml(g) consumed = 21 mg milk protein exposure.	
In case of higher level calculations, eg probabilistic, population level, provide details		

[18] This example Reference Dose does not mean that ILSI Europe or the authors of this guidance endorse or recommend/require the use of a specific risk management system. It is used only for example calculation purposes. For more information and key references regarding the fundamentals of how allergen RfDs are derived from oral food challenge data and subsequent dose-distribution models, as well as what might constitute an appropriate RfD see ***Box Reference Doses***.

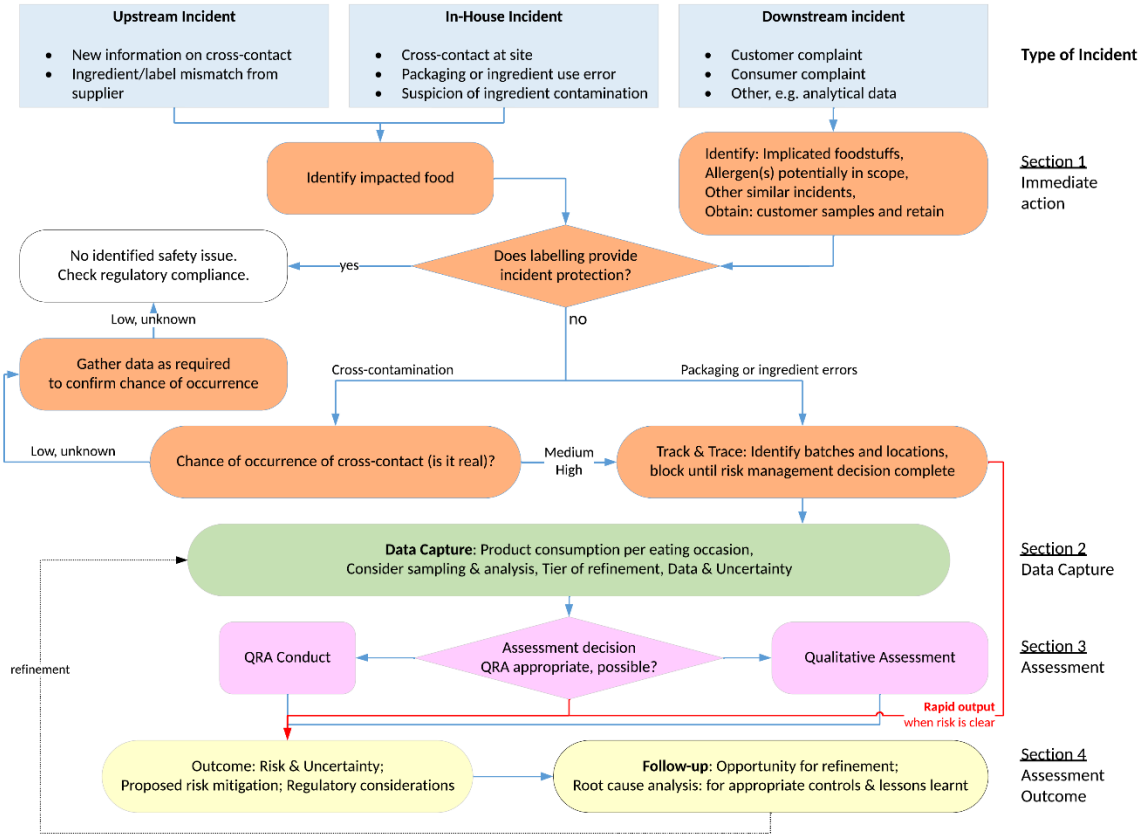
Section 4: Assessment Outcome			
Key Output	**Evidence**		
Risk Assessment Outcome	There is a risk to allergic consumers Risk within agreed limits of acceptability Not currently possible to determine	☒ ☐ ☐	
Proposed risk mitigation (in case of risk to allergic consumers)	Market action recommended. Supply chain controls will be amended to prevent on-going contamination.		
Need to contact external agencies	Relevant authorities and patient organisations		
Method of assessment	Qualitative Quantitative (QRA) Not currently possible to assess	☒qualitative backed up with quantitative ☒ ☐	
Regulatory implications	Product not safe due as per general food law due to risk to allergic consumers		
Product Presentation			
Describe aspects of product presentation that may modify the risk			
Quality of Evidence Framework			**score**
Tier of refinement	Tier 1 – theoretical Tier 2 – informed Tier 3 – data-driven Tier 4 – verified	☐ ☐ ☐ ☒	1 2 3 4
Chance that cross-contact is occurring	Low or unknown Medium High or known to have happened	☐ ☐ ☒	1 2 3
Overall data uncertainty	High uncertainty Medium uncertainty Acceptable uncertainty	☐ ☐ ☒	1 2 3
Quality of Evidence	9 – 10 : high quality evidence 6 – 8 : medium quality evidence 5 and below : low quality evidence	☒ ☐ ☐	
Opportunities for Refinement			
If there is sufficient time available for refinement, describe data needed and next steps	No further refinement of the assessment is considered necessary.		
Root Cause Analysis			
Describe root cause, corrective action	Corrective action to be implemented in supply chain. Supplier should be de-listed or audited against an updated and validated change over schedule and sanitation controls within their allergen management plan.		

7.5.2. A Tier 2 in-house incident assessment concerning particulate UAP

1 - General Information & Assessment Summary

Assessment Team			
Assessment Date			
Incident Dates			
Type of incident		Source of information	Point of cross-contact
	Upstream	☐	☐
	In-house	☒	☒
	Downstream	☐	☐
Foodstuff and allergen(s):	Ice cream containing ice cream stick bar, hazelnut		
Market(s):	Taiwan		
Product disposition:	Product was held, recommended for release		
Risk to consumers:	Risk is within agreed limits of acceptability		
Quality of Evidence:	Medium		
Scale of risk:	Product carries PAL for hazelnut, the new information on potential presence of a sporadic hazelnut particle does not exacerbate risk to hazelnut allergic consumers		
Opportunity for refinement:	Not considered as necessary		
Regulatory situation:	No regulatory implication		
Proposed mitigation & actions, next steps:	Sanitation controls updated so that filter is always cleaned as a part of future changeovers.		

2 - Incident Flow Chart



3 - Assessment Matrix

Section 1: Immediate Action	
Identity of foodstuff implicated	Ice cream containing almond pieces
Allergen(s) implicated	Hazelnut
Supporting information	An error occurred in the sanitation of an ice cream line producing ice cream stick bars containing almond. A filter was not removed and cleaned (2 mm circular pores). On inspection the filter is not normally found to hold residual particles, but on occasion a limited number of particles (5-10) have been found. The almond-containing ice cream bar carries PAL (may contain tree nuts). Assessment was requested to determine whether the risk to hazelnut allergic individuals was increased.
Does labelling provide incident protection ?	Partial mitigation (may contain tree nuts)
Summary if relevant of consumer complaints	None, product on hold
Chance of Occurrence of Cross-Contact	
See '**Core concepts**' Section **5.1.1** for a description of 'Chance of Occurrence'	
Chance of Occurrence	**Notes**
☐ High or known to have happened	Not known if any hazelnut fragments were residual on the filter, it has been known to happen.
☒ Medium	
☐ Low or unknown	

Track & Trace	
Current status	
Degree of success of T&T	
Implicated batch no.s, production dates	
No. Packs (consumer units) implicated	
No. Packs Held	All implicated product held.
No. Packs in distribution	
No. Packs at consumer market	
Shelf-life remaining	
Other supporting information	

Section 2: Data Capture

Consumption

See '**Core concepts**' **5.3** for guidance on consumption estimates

Pack size (consumer unit) (g)	Meal preparation	Portion size (g)	Quantity of implicated food eaten per consumption event (g)
		70g stick bar	One stick bar

the 'Tier of Refinement'

Tier	Description	Source of Data
☐ **Tier 1** 'Theoretical'	Concern has been raised on UAP but there is no physical evidence of cross-contact at the product site or supply chain in question.	No data available, only 'reverse' QRA possible (see **Core concepts**).
☒ **Tier 2** 'Informed'	Some physical evidence of UAP of the specific supply chain in question, high uncertainty in quantification.	The data available for QRA is based on 'reasonable worst case' assumptions, e.g., hang-up estimation (see **Core concepts**).
☐ **Tier 3** 'Data-driven'	Physical evidence of UAP at the production site or specific supply chain in question, with indirect quantification possible.	The data available is from upstream in the supply chain, for example on a purchased ingredient.
☐ **Tier 4** 'Verified'	Physical evidence of UAP at the production site or specific supply chain in question, with direct quantification possible.	data is available on finished product as presented to consumer, or in case of mis-labeling or ingredient error there is clarity on the allergen content of the food.

Characteristics of UAP: Data & Uncertainty			
See '**Core concepts**' Section **5.1.2** for a description of UAP Characteristics and Uncertainty			
Characteristics		**Uncertainty**	**Data & Notes**
A Form of UAP	☐ Amorphous	1 ☐ High	Not known if a limited number of 2 mm diameter particles entered product. --- Note: If 'unknown', assessment should be based on both amorphous and particulate, until refined information is available.
	☒ Particulate	2 ☒ Medium	
	☐ Unknown (please mark uncertainty as 'high')	3 ☐ Acceptable	
B Distribution of UAP	☐ Homogeneous	1 ☐ High	If particle(s) were present, based on experience there would be very few (less than 10), this would enter a subsequent product mass of 7,000 kg. --- Note: If 'unknown', assessment should be based on both hetero' and homogeneous, until refined information is available.
	☒ Heterogeneous	2 ☒ Medium	
	☐ Unknown (uncertainty is always 'high')	3 ☐ Acceptable	
C Frequency of UAP	☒ Isolated	1 ☐ High	Incident is due to known sanitation error. --- Note: If 'unknown', assessment should assume UAP is 'regular'.
	☐ Intermittent	2 ☐ Medium	
	☐ Regular	3 ☒ Acceptable	
	☐ unknown (uncertainty is always 'high')		
D Concentration of UAP	1 ☐ Unknown or Estimate (not analytical). Note: see carry-over guidance **5.2.3**		Provide data: although no analytical data available, worst case size of particle(s) is known. Describe suitability of analytical data: in the absence of a range of values, there is uncertainty in analytical data. --- Note: If 'unknown', assessment can only be qualitative. More information is needed before QRA can be performed.
	2 ☐ Analytical, point data		
	3 ☒ Analytical, data range. In the case of mis-labeling or wrong ingredient used, where there is knowledge on amount of allergen present, mark as 3.		
Overall data uncertainty (sum of A-D)		4-7 ☐ High 8-10 ☒ Medium >10 ☐ Acceptable	Notes

Section 3: Assessment		
Assessment Decision		**Notes: rationale for selected option**
It is beyond doubt that there is an unacceptable risk, no further assessment required	☐	Tier 4 scenario High likelihood of occurrence Overall data uncertainty is medium This supports QRA, which is appropriate to conduct as the risk to consumers is no obvious.
Uncertainty is too large to enable an assessment, further information required	☐	
QRA is appropriate but not possible without further information, qualitative assessment only	☐	
QRA is appropriate and possible	☒	

QRA Metrics (for 'screening' and 'deterministic' QRA)		
See '**Core concepts**' section **5.4** for calculation guidance		
Description of the exposure scenario		
In case an Action Level (ppm) was calculated to compare to concentration in food (ppm), what was is the Action Level ?	Action Level =	Conc in food =
In case exposure of allergic consumer was calculated (mg) to compare to RfD (mg), what was the exposure ?	Appropriate RfD[19] = ED01 is 0.1 mg ED05 is 3.5 mg	Consumer exposure = 0.4 mg hazelnut protein/particle
Description of the calculation	A particle with diameter 2 mm is 4.2 mm3. The density of hazelnut is 0.6 g/cm3 or 0.6 mg/mm3. The weight of a particle is 2.5 mg. Hazelnut is 15% protein, so protein per particle is 0.4 mg Hazelnut protein exposure per particle is 0.4 mg. The risk scenario is that a hazelnut, but not almond allergic, individual consumes a product that contains one of the few contaminant pieces, and that consumer is not avoiding the product due to labelling 'may contain tree nuts'. Against this unlikely scenario, the exposure is greater than the ED01 but less than the ED05. Due to mixing and density of product, it is considered extremely unlikely that a single product would contain more than one hazelnut fragment. Risk is considered within the bounds of acceptability. No substantive exacerbation of risk to hazelnut allergic consumers.	
In case of higher level calculations, eg probabilistic, population level, provide details		

[19] This example Reference Dose does not mean that ILSI Europe or the authors of this guidance endorse or recommend/require the use of a specific risk management system. It is used only for example calculation purposes. For more information and key references regarding the fundamentals of how allergen RfDs are derived from oral food challenge data and subsequent dose-distribution models, as well as what might constitute an appropriate RfD see ***Box Reference Doses***.

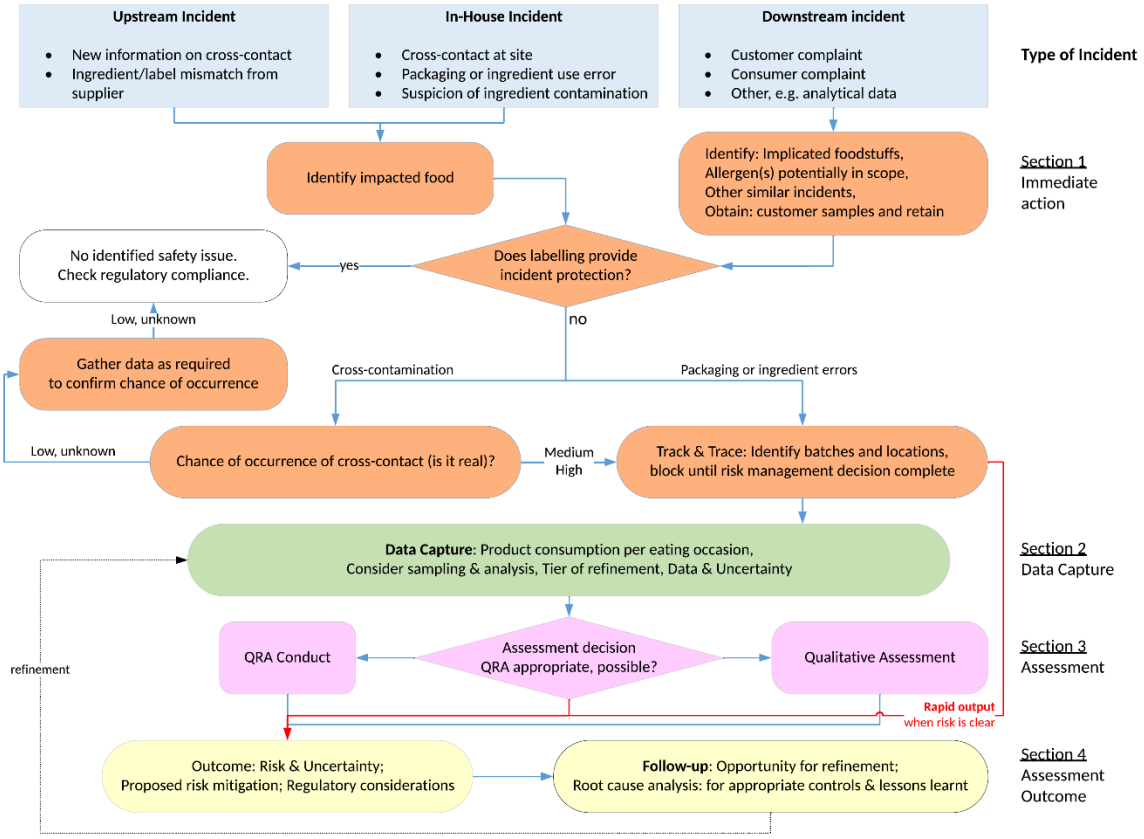
Section 4: Assessment Outcome			
Key Output	**Evidence**		
Risk Assessment Outcome	There is a risk to allergic consumers Risk within agreed limits of acceptability Not currently possible to determine	☐ ☒ ☐	
Proposed risk mitigation (in case of risk to allergic consumers)	Sanitation control has been enforced to ensure filter is cleaned.		
Need to contact external agencies	No		
Method of assessment	Qualitative Quantitative (QRA) Not currently possible to assess	☐ ☒ ☐	
Regulatory implications	None.		
Product Presentation			
Describe aspects of product presentation that may modify the risk	Implicated product carries PAL for tree nuts. This assessment provides information that no acerbation of risk to hazelnut allergic consumers is presented by the sanitation error.		
Quality of Evidence Framework			**score**
Tier of refinement	Tier 1 – theoretical Tier 2 – informed Tier 3 – data-driven Tier 4 – verified	☐ ☒ ☐ ☐	1 2 3 4
Chance that cross-contact is occurring	Low or unknown Medium High or known to have happened	☐ ☒ ☐	1 2 3
Overall data uncertainty	High uncertainty Medium uncertainty Acceptable uncertainty	☐ ☒ ☐	1 2 3
Quality of Evidence	9 – 10 : high quality evidence 6 – 8 : medium quality evidence 5 and below : low quality evidence	☐ ☒ ☐	
Opportunities for Refinement			
If there is sufficient time available for refinement, describe data needed and next steps	No further refinement of the assessment is considered necessary. Likely there are no particles in the implicated product.		
Root Cause Analysis			
Describe root cause, corrective action	Sanitation controls updated. Product can be released.		

7.5.3. A tier 2 in-house incident assessment concerning homogeneous UAP

1 - General Information & Assessment Summary

Assessment Team			
Assessment Date			
Incident Dates			
Type of incident		Source of information	Point of cross-contact
	Upstream	☐	☐
	In-house	☒	☒
	Downstream	☐	☐
Foodstuff and allergen(s):	Spice blend, UAP is milk protein		
Market(s):	US		
Product disposition:	At market		
Risk to consumers:	Risk is within agreed limits of acceptability		
Quality of Evidence:	Medium quality of evidence. Risk assessment is based on reasonable worst-case assumptions		
Scale of risk:	Incident concerns incorrect PAL (PAL for milk is missing).		
Opportunity for refinement:	Samples of implicated finished product could be tested analytically.		
Regulatory situation:	No regulatory implication		
Proposed mitigation & actions, next steps:	Not considered as necessary other than correction to sanitation and change over practice.		

2 - Incident Flow Chart



3 - Assessment Matrix

Section 1: Immediate Action	
Identity of foodstuff implicated	Multiple spice blends
Allergen(s) implicated	Milk protein
Supporting information	A spice blend produced on shared equipment may have had cross-contact with milk protein from milk powder. The spice blend normally carries PAL for milk, however due to a labelling error the PAL was not included. Issue was identified after the product was at market.
Does labelling provide incident protection ?	No
Summary if relevant of consumer complaints	None received, consumer contact database reviewed no relevant complaints identified.
Chance of Occurrence of Cross-Contact	
See '**Core concepts**' Section **5.1.1** for a description of 'Chance of Occurrence'	
Chance of Occurrence	**Notes**
☐ High or known to have happened	There is no data on the finished product, spice blend. There is a theoretical possibility of carry-over.
☒ Medium	
☐ Low or unknown	

Track & Trace	
Current status	At market
Degree of success of T&T	
Implicated batch no.s, production dates	
No. Packs (consumer units) implicated	
No. Packs Held	
No. Packs in distribution	
No. Packs at consumer market	
Shelf-life remaining	
Other supporting information	

Section 2: Data Capture

Consumption

See '**Core concepts**' **5.3** for guidance on consumption estimates

Pack size (consumer unit) (g)	Meal preparation	Portion size (g)	Quantity of implicated food eaten per consumption event (g)
100 g pack of spice blend		5 – 20 g	25 g Extremely unlikely more than this would be consumed.

the 'Tier of Refinement'

Tier	Description	Source of Data
☐ **Tier 1** 'Theoretical'	Concern has been raised on UAP but there is no physical evidence of cross-contact at the product site or supply chain in question.	No data available, only 'reverse' QRA possible (see **Core concepts**).
☒ **Tier 2** 'Informed'	Some physical evidence of UAP of the specific supply chain in question, high uncertainty in quantification.	The data available for QRA is based on 'reasonable worst case' assumptions, e.g., hang-up estimation (see **Core concepts**).
☐ **Tier 3** 'Data-driven'	Physical evidence of UAP at the production site or specific supply chain in question, with indirect quantification possible.	The data available is from upstream in the supply chain, for example on a purchased ingredient.
☐ **Tier 4** 'Verified'	Physical evidence of UAP at the production site or specific supply chain in question, with direct quantification possible.	data is available on finished product as presented to consumer, or in case of mis-labeling or ingredient error there is clarity on the allergen content of the food.

Characteristics of UAP: Data & Uncertainty			
See '**Core concepts**' Section **5.1.2** for a description of UAP Characteristics and Uncertainty			
Characteristics		**Uncertainty**	**Data & Notes**
A Form of UAP	☒ Amorphous	1 ☐ High	Any hang-up will be fully distributed in subsequent product, due to batch process and mixing post any possible hang-up. --- Note: If 'unknown', assessment should be based on both amorphous and particulate, until refined information is available.
	☐ Particulate	2 ☐ Medium	
	☐ Unknown (please mark uncertainty as 'high')	3 ☒ Acceptable	
B Distribution of UAP	☒ Homogeneous	1 ☐ High	--- Note: If 'unknown', assessment should be based on both hetero' and homogeneous, until refined information is available.
	☐ Heterogeneous	2 ☐ Medium	
	☐ Unknown (uncertainty is always 'high')	3 ☒ Acceptable	
C Frequency of UAP	☒ Isolated	1 ☐ High	Product codes with PAL labelling error is known. --- Note: If 'unknown', assessment should assume UAP is 'regular'.
	☐ Intermittent	2 ☐ Medium	
	☐ Regular	3 ☒ Acceptable	
	☐ unknown (uncertainty is always 'high')		
D Concentration of UAP	1 ☒ Unknown or Estimate (not analytical). Note: see carry-over guidance **5.2.3**		Provide data: cleaning does happen between batches of dissimilar spice blends, however theoretical possibility of small hang-up, 500g, on top of mixer blade in primary vessel. This was the reason for PAL normally being applied (which was omitted in error). Describe suitability of analytical data: --- Note: If 'unknown', assessment can only be qualitative. More information is needed before QRA can be performed.
	2 ☐ Analytical, point data		
	3 ☐ Analytical, data range. In the case of mis-labeling or wrong ingredient used, where there is knowledge on amount of allergen present, mark as 3.		
Overall data uncertainty (sum of A-D)		4-7 ☐ High 8-10 ☒ Medium >10 ☐ Acceptable	Notes
Section 3: Assessment			
Assessment Decision			**Notes: rationale for selected option**
It is beyond doubt that there is an unacceptable risk, no further assessment required		☐	Although it is a 'tier 2' incident, and the quality of evidence is medium, there is sufficient knowledge on the worst-case hang-up to enable a simple QRA.
Uncertainty is too large to enable an assessment, further information required		☐	
QRA is appropriate but not possible without further information, qualitative assessment only		☐	
QRA is appropriate and possible		☒	

QRA Metrics (for 'screening' and 'deterministic' QRA)		
See '**Core concepts**' section **5.4** for calculation guidance		
Description of the exposure scenario		
In case an Action Level (ppm) was calculated to compare to concentration in food (ppm), what was is the Action Level ?	Action Level = 8 mg/kg (for 25 g portion of spice)	Conc in food = 6.15 mg/kg
In case exposure of allergic consumer was calculated (mg) to compare to RfD (mg), what was the exposure ?	Appropriate RfD[20] = 0.2 mg (Vital 3.0)	Consumer exposure = 0.15 mg milk protein (assuming 25 g spice mix consumed)
Description of the calculation	The mixer might contain 500g of previous product despite sanitation. The previous product contained 3.85 % milk protein, so that is 19.2 g milk protein. Lowest batch quantity of subsequent product is 3130 kg. That means there is a possible 6.15 mg/kg milk protein in the spice mix. The 100g pack of spice blend is intended to be used over multiple servings, If a worst-case is assumed that a quarter of the pack is consumed in an eating event (25g) by a single person, this would result in exposure of 0.15 mg milk protein.	
In case of higher level calculations, eg probabilistic, population level, provide details		

[20] This example Reference Dose does not mean that ILSI Europe or the authors of this guidance endorse or recommend/require the use of a specific risk management system. It is used only for example calculation purposes. For more information and key references regarding the fundamentals of how allergen RfDs are derived from oral food challenge data and subsequent dose-distribution models, as well as what might constitute an appropriate RfD see ***Box Reference Doses***.

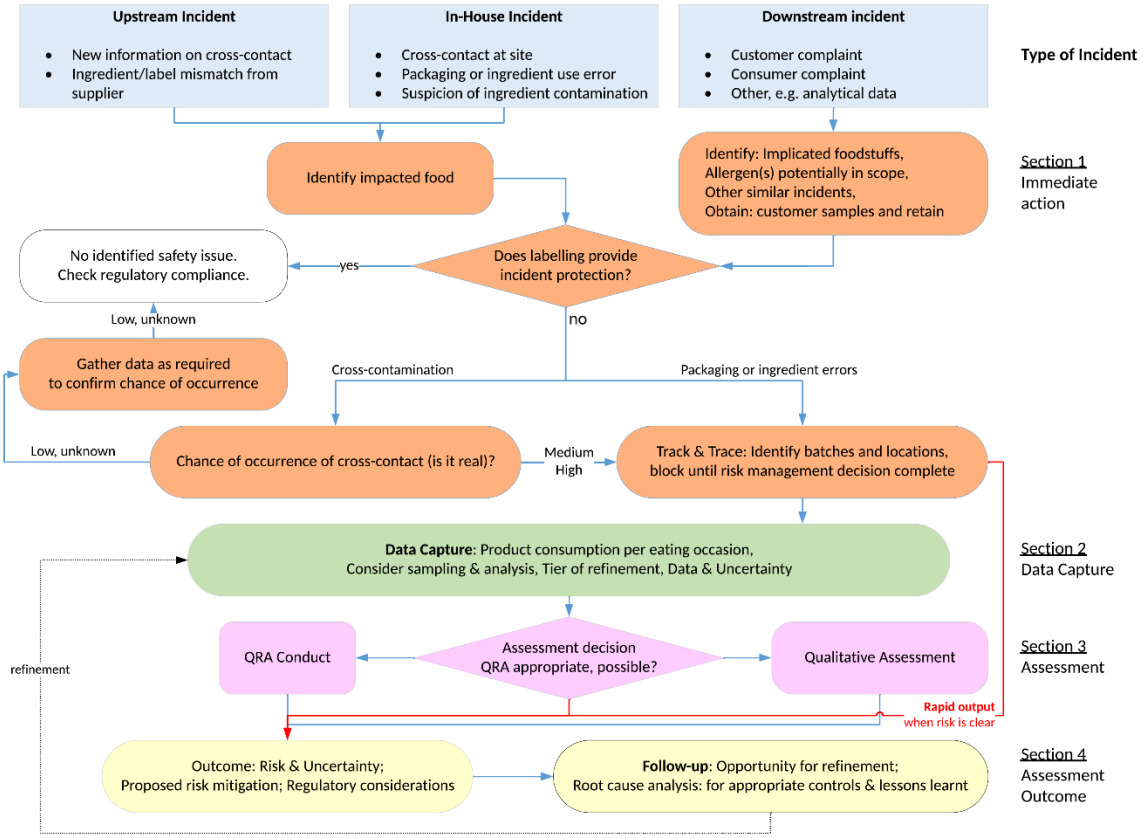
Section 4: Assessment Outcome			
Key Output	**Evidence**		
Risk Assessment Outcome	There is a risk to allergic consumers Risk within agreed limits of acceptability Not currently possible to determine	☐ ☒ ☐	
Proposed risk mitigation (in case of risk to allergic consumers)	None considered necessary, however production and sanitation controls will be amended to prevent future contamination.		
Need to contact external agencies	no		
Method of assessment	Qualitative Quantitative (QRA) Not currently possible to assess	☐ ☒ ☐	
Regulatory implications	None.		
Product Presentation			
Describe aspects of product presentation that may modify the risk			
Quality of Evidence Framework			**score**
Tier of refinement	Tier 1 – theoretical Tier 2 – informed Tier 3 – data-driven Tier 4 – verified	☐ ☒ ☐ ☐	1 2 3 4
Chance that cross-contact is occurring	Low or unknown Medium High or known to have happened	☐ ☒ ☐	1 2 3
Overall data uncertainty	High uncertainty Medium uncertainty Acceptable uncertainty	☐ ☒ ☐	1 2 3
Quality of Evidence	9 – 10 : high quality evidence 6 – 8 : medium quality evidence 5 and below : low quality evidence	☐ ☒ ☐	
Opportunities for Refinement			
If there is sufficient time available for refinement, describe data needed and next steps	Confirmation that risk is within agreed limits of acceptability, might be gained from analysis of samples of finished spice blend.		
Root Cause Analysis			
Describe root cause, corrective action	Absence of PAL does not unduly increase the risk to milk allergic consumers. Risk assessment illustrates that PAL is not required. However sanitation practice will be amended to ensure mixer blade does not present risk of carry over.		

7.5.4. A Tier 4 downstream incident assessment concerning homogeneous UAP

1 - General Information & Assessment Summary

Assessment Team			
Assessment Date			
Incident Dates			
Type of incident	 Upstream In-house Downstream	Source of information ☐ ☐ ☒	Point of cross-contact ☐ ☒ ☐
Foodstuff and allergen(s):	Whole egg cross-contact in dry mix soups		
Market(s):	Greece		
Product disposition:	At market		
Risk to consumers:	Risk is within agreed limits of acceptability		
Quality of Evidence:	High		
Scale of risk:	Risk is considered as within limits of acceptability		
Opportunity for refinement:	Not considered as necessary		
Regulatory situation:	No regulatory implication		
Proposed mitigation & actions, next steps:	No action required		

2 - Incident Flow Chart



3 - Assessment Matrix

Section 1: Immediate Action	
Identity of foodstuff implicated	Onion soup mix and Minestrone soup mix
Allergen(s) implicated	Whole egg
Supporting information	The Food Control Authority found small amounts of non-ingredient egg when they analysed samples of Minestrone soup mix (1.3 mg/kg whole egg protein in 77g sachets intended for 3 portions). The batches tested were manufactured in November and December of the preceding year and have been on the market since that time. No adverse reactions in consumers have been reported
Does labelling provide incident protection ?	No, egg is not in the list of ingredients or precautionary statement for either onion or minestrone soup.
Summary if relevant of consumer complaints	
Chance of Occurrence of Cross-Contact	
See '**Core concepts**' Section **5.1.1** for a description of 'Chance of Occurrence'	
Chance of Occurrence	**Notes**
☒ High or known to have happened	Analytical data is assumed to be correct, that there is occurrence of contamination. Root cause analysis has identified one source of cross-contact is feasible, carry-over of previous production of the dry finished product mix.
☐ Medium	
☐ Low or unknown	

Track & Trace	
Current status	At market
Degree of success of T&T	
Implicated batch no.s, production dates	
No. Packs (consumer units) implicated	
No. Packs Held	
No. Packs in distribution	
No. Packs at consumer market	20760 packs of Onion soup and 27648 packs of Minestrone soup were produced and distributed.
Shelf-life remaining	Product has long shelf life (2 years), so worst-case assumption is used that all units affected are still on the market.
Other supporting information	

Section 2: Data Capture

Consumption

See '**Core concepts**' section **5.3** for guidance on consumption estimates

Pack size (consumer unit) (g)	Meal preparation	Portion size (g)	Quantity of implicated food eaten per consumption event (g)
Onion as 60g sachets (4 portions) and the Minestrone as 77g sachets (3 portions)	Make sachet (or contents of sachet) to 750 ml with water.	250g, equivalent to mass of powder: onion 15g and minestrone 25.7g.	P75 for consumption of soup is 400 mL. Very conservative estimated assumed that one sachet (77g) in 750 mL water would be consumed at an eating occasion.

the 'Tier of Refinement'		
Tier	**Description**	**Source of Data**
☐ **Tier 1** 'Theoretical'	Concern has been raised on UAP but there is no physical evidence of cross-contact at the product site or supply chain in question.	No data available, only 'reverse' QRA possible (see **Core concepts**).
☐ **Tier 2** 'Informed'	Some physical evidence of UAP of the specific supply chain in question, high uncertainty in quantification.	The data available for QRA is based on 'reasonable worst case' assumptions, e.g., hang-up estimation (see **Core concepts**).
☐ **Tier 3** 'Data-driven'	Physical evidence of UAP at the production site or specific supply chain in question, with indirect quantification possible.	The data available is from upstream in the supply chain, for example on a purchased ingredient.
☒ **Tier 4** 'Verified'	Physical evidence of UAP at the production site or specific supply chain in question, with direct quantification possible.	data is available on finished product as presented to consumer, or in case of mis-labeling or ingredient error there is clarity on the allergen content of the food.

Characteristics of UAP: Data & Uncertainty			
See '**Core concepts**' Section **5.1.2** for a description of UAP Characteristics and Uncertainty			
Characteristics		**Uncertainty**	**Data & Notes**
A Form of UAP	☒ Amorphous	1 ☐ High	Based on knowledge of ingredients, process and product. --- Note: If 'unknown', assessment should be based on both amorphous and particulate, until refined information is available.
	☐ Particulate	2 ☐ Medium	
	☐ Unknown (please mark uncertainty as 'high')	3 ☒ Acceptable	
B Distribution of UAP	☒ Homogeneous	1 ☐ High	Based on knowledge of ingredients, process and product. --- Note: If 'unknown', assessment should be based on both hetero' and homogeneous, until refined information is available.
	☐ Heterogeneous	2 ☐ Medium	
	☐ Unknown (uncertainty is always 'high')	3 ☒ Acceptable	
C Frequency of UAP	☐ Isolated	1 ☐ High	Based on knowledge of ingredients, process and product. --- Note: If 'unknown', assessment should assume UAP is 'regular'.
	☐ Intermittent	2 ☒ Medium	
	☒ Regular	3 ☐ Acceptable	
	☐ unknown (uncertainty is always 'high')		
D Concentration of UAP	1 ☐ Unknown or Estimate (not analytical). Note: see carry-over guidance **5.2.3**		Provide data: 1.3 ppm egg protein (in dry mix), may vary across batch based on scheduling. Describe suitability of analytical data: in the absence of a range of values, there is uncertainty in analytical data. --- Note: If 'unknown', assessment can only be qualitative. More information is needed before QRA can be performed.
	2 ☒ Analytical, point data		
	3 ☐ Analytical, data range. In the case of mis-labeling or wrong ingredient used, where there is knowledge on amount of allergen present, mark as 3.		
Overall data uncertainty (sum of A-D)		4-7 ☐ High 8-10 ☒ Medium >10 ☐ Acceptable	Notes

Section 3: Assessment		
Assessment Decision		**Notes: rationale for selected option**
It is beyond doubt that there is an unacceptable risk, no further assessment required	☐	Tier 4 scenario High likelihood of occurrence Overall data uncertainty is medium This supports QRA, which is appropriate to conduct as the risk to consumers is no obvious.
Uncertainty is too large to enable an assessment, further information required	☐	
QRA is appropriate but not possible without further information, qualitative assessment only	☐	
QRA is appropriate and possible	☒	
QRA Metrics (for 'screening' and 'deterministic' QRA)		
See '**Core concepts**' section **5.4** for calculation guidance		
Description of the exposure scenario		
In case an Action Level (ppm) was calculated to compare to concentration in food (ppm), what was is the Action Level ?	Action Level =	Conc in food = 1.3 mg/kg whole egg protein (dry mix)
In case exposure of allergic consumer was calculated (mg) to compare to RfD (mg), what was the exposure ?	Appropriate RfD[21] = 0.2 mg (Vital 3.0)	Consumer exposure = 0.1 mg
Description of the calculation	The minestrone soup (worst case) is sold as 77g sachets (3 portions, 25.7g each of powder). The amount of egg protein detected in the dry mix was 1.3 mg/kg. 77g at 1.3 mg/kg = 0.1 mg exposure.	
In case of higher level calculations, eg probabilistic, population level, provide details		

[21] This example Reference Dose does not mean that ILSI Europe or the authors of this guidance endorse or recommend/require the use of a specific risk management system. It is used only for example calculation purposes. For more information and key references regarding the fundamentals of how allergen RfDs are derived from oral food challenge data and subsequent dose-distribution models, as well as what might constitute an appropriate RfD see ***Box Reference Doses***.

Section 4: Assessment Outcome			
Key Output	**Evidence**		
Risk Assessment Outcome	There is a risk to allergic consumers Risk within agreed limits of acceptability Not currently possible to determine	☐ ☒ ☐	
Proposed risk mitigation (in case of risk to allergic consumers)	None considered necessary, however production controls will be amended to prevent on-going contamination.		
Need to contact external agencies	Eg authority, patient org ?		
Method of assessment	Qualitative Quantitative (QRA) Not currently possible to assess	☐ ☒ ☐	
Regulatory implications	None.		
Product Presentation			
Describe aspects of product presentation that may modify the risk			
Quality of Evidence Framework			**score**
Tier of refinement	Tier 1 – theoretical Tier 2 – informed Tier 3 – data-driven Tier 4 – verified	☐ ☐ ☐ ☒	1 2 3 4
Chance that cross-contact is occurring	Low or unknown Medium High or known to have happened	☐ ☐ ☒	1 2 3
Overall data uncertainty	High uncertainty Medium uncertainty Acceptable uncertainty	☐ ☒ ☐	1 2 3
Quality of Evidence	9 – 10 : high quality evidence 6 – 8 : medium quality evidence 5 and below : low quality evidence	☒ ☐ ☐	
Opportunities for Refinement			
If there is sufficient time available for refinement, describe data needed and next steps	No further refinement of the assessment is considered necessary.		
Root Cause Analysis			
Describe root cause, corrective action	Allergen planning including change over and sanitation will be reviewed.		

7.6. ANNEX Food allergy prevalence data

Data on prevalence of allergy to a particular allergenic food is needed to conduct a 'public health' risk assessment wherein the number of probable allergic reactions at market can be estimated for a particular exposure scenario. Data on prevalence varies considerably in amount and quality, and includes:

- Self-reported prevalence, usually based on questionnaire surveys of consumers and varying considerably in quality, depending on the questionnaire design, for instance whether they specify self-report or only physician/health care professional diagnosis. Unsurprisingly, these produce the highest estimates.
- Prevalence of sensitisation to the allergenic food, based on serological diagnosis (specific IgE measurements) and/or skin prick testing, enumerating individuals who have produced a relevant immune response to the food.
- Prevalence of allergy, where the clinical relevance of sensitisation is validated by a positive food challenge.

Prevalence of food-challenge confirmed allergy is the parameter relevant to estimates of public health impact but is the most scarce of all the above forms of data, particularly given the resources required to generate them. To complicate matters even further, it varies considerably across populations depending on many factors, not least dietary and culinary habits.

A conservative estimate for the prevalence of allergy to a particular allergenic food for further use in a "rough" public health risk assessment can be found in **Annex Table 1**. For a more refined assessment or in cases where country specific data may be of interest, please consult an external risk assessment expert.

Annex Table 1. *These are general, conservative estimates for public health risk assessments by the food industry. As a conservative estimate, these err on the side of caution and may overestimate the actual prevalence of food allergy in a specific country or age group. If more detailed, country or age group specific data is desired please see Baseggio Conrado/Patel/Turner or the recent FAO/WHO report for more information, or consult an external risk assessment expert.*

Allergenic source	**Children** **2-18 years** **(reasonable upper bound estimate, up to X%)**	**Adults** **>18 years** **(reasonable upper bound estimate, up to X%)**
Cow's milk	2.0%	0.5%
Hen's egg*	1.0%	0.5%
Fish (as codfish)	0.5%	1.0%
Crustacean shellfish	1.0%	2.0%
Molluscan shellfish	Insufficient data	Insufficient data
Wheat-IgE mediated food allergy	0.5%	0.5%
Other grains (Barley, Rye, Oats)-IgE mediated food allergy	Insufficient data	Insufficient data
Celery / Celeriac (geographically limited)	0.3%	0.5%
Lupin	Insufficient data	Insufficient data
Peanut	3.0%	2.0%
Soybean	0.5%	0.2%
Mustard (EU data)	Insufficient data	0.03%
Sesame	0.5%	0.5%
Tree nuts***		
Almond	0.3%	Insufficient data
Brazil nut	0.3%	Insufficient data
Cashew nut / Pistachio**	1.5%	0.5%
Hazelnut (geographic variation seemingly higher in EU)	1.0%	2.0%
Macadamia nut	Insufficient data	Insufficient data
Walnut / Pecan**	0.6%	0.7%

Estimates are reasonable upper bounds, based on data published by Baseggio Conrado, Patel et al. (2021).

**this estimate may not apply for Australia*
*** estimate for pistachio based on very high cross-reactivity between pistachio and cashew*
**** listed tree nuts in in Annex II*

7.7. ANNEX for sampling and analysis

7.7.1. Sampling and Analysis flow chart & data capture form

A flow chart **(Annex Figure 3)** and data capture form are provided below to assist in the decision making and record keeping around sampling and analysis. For more information see the main text section **5.2.1 Allergen Sampling and Analysis**.

If analytical data are in fact needed to support an allergen risk assessment the samples may be from:

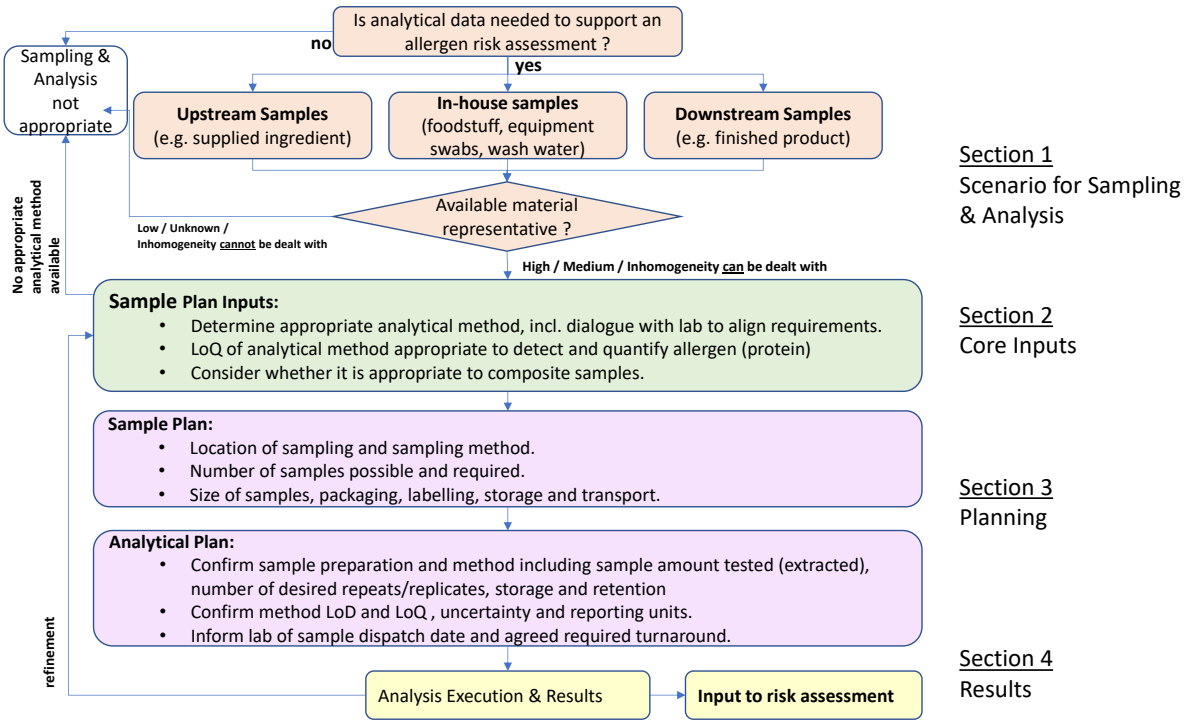
- Upstream in the supply chain, e.g. a supplied ingredient,
- In-house in which case they may be foodstuffs, equipment swabs, or wash water, or
- Downstream, e.g. finished product.

It is not always certain that sufficient material is available in the right condition to sample from. Is it a sufficient amount, representative, traceable (batch code) and has been appropriately handled (e.g. packaging undamaged)? Above all, is it known or suspected that any unintended allergen present (UAP) is homogenously distributed in the product, or are there 'hot spots' of UAP? If there is any doubt, sampling should be deferred until appropriate material is available to sample from. Homogeneity may be known from historical data and/or parallel analyses e.g. for nutritional data. Medium to high inhomogeneity can be dealt with by increasing the random sampling rate (see **5.2.1 Allergen Sampling and Analysis**).

It is important to discuss with the lab (or your supplier if you are using on-site rapid lateral flow device analysis) and agree on an appropriate analytical method, including turnaround time. The detection capability (sensitivity) of the method must be appropriate and it's limit of quantification (LoQ) must be appropriate to quantify allergen (protein). In other words the LoQ must be below any critical Action Level or allergen (protein) concentration calculated from a Reference Dose in allergen QRA, from 3 – 10 times below is satisfactory. This is especially true if you are considering whether it is appropriate to composite samples. See **5.2.1 Allergen Sampling and Analysis** for more details, specifically sections:

A documented sample plan is very useful and the actual actions taken during the sampling should be documented (who, what, where, when, and the sampling method (spear, cone and quarter, riffle, grab …). The number of samples possible and required should be decided beforehand and documented along with documentary records of how many were actually taken. The size (amount) of samples, their packaging, labelling so as to be traceable in the lab and from the lab report, their storage and transport must be agreed documented and the actual information from the sampling exercise recorded at the time it is carried out.

The Analytical Plan must also be decided and documented. Sample preparation is usually carried out in the lab and homogenisation is usual. If you require some sample to be left intact for subsequent visual examination this must be discussed beforehand. The method must reduce the particle size and mix the sample sufficiently, a kitchen-type blender if often an excellent choice so long as it is sufficiently powerful. Milling may be an alternative and there are specialist milling apparatus and techniques available to reduce heating the sample and to produce a given particle size. Some consideration should be given to sample size and the sample amount tested (extracted), number of desired repeats/replicates, as this may influence the uncertainty of the results. The storage and retention of the samples should be agreed on with the lab. The method LoD and LoQ, uncertainty and reporting units (preferably as mg of total allergenic protein per kg food) must be agreed on. It is wise to inform the lab of sample dispatch date and check agreed required turnaround.



Annex Figure 3. *Flow chart to assist in the decision making around sampling and analysis*

The following form may be downloaded and adapted for use in documenting the above and the details described in section **5.2.1 Allergen Sampling and Analysis**.

General Information & Summary		
Sampling & Analysis Team		
Sampling & Analysis to Support which Allergen Risk Assessment ?		
Sample description		
Date of sample(s) taken and date of analysis performed		
Sample reference no.s		
Type of samples	☐ Upstream ☐ In-house ☐ Down-stream	Description of sample type: E.g., no. samples from batch of compound food
Sample preparation	e.g., individual samples, composite of 3 finished products, test portion and aliquot	
Sample retention		
Result(s) and Interpretation		
Quality of sampling evidence	Acceptable, medium or low quality of evidence	

Sampling & Analysis Matrix

Section 1: Immediate Action	
Why is analytical data needed to support the risk assessment ?	e.g. data needed to verify carry-over calculation
Availability of material to sample	e.g. a production lot (volume) is available for sampling, or a single product returned from market is available
Representativeness of Material	
A - Representativeness	Notes
3 ☐ High	Describe how the available material to sample is representative of the material for which the risk assessment is being conducted, e.g. material from the batch with potential UAP via carry-over (high). Finished product for which claim is being made (high). The same finished product associated with an on-market incident, but not the same lot (medium). Ingredient from one supplier being tested as representative of all suppliers (low).
2 ☐ Medium	
1 ☐ Low or unknown	
Section 2: Core Inputs	

Suitability of the analytical method (food matrix and sensitivity)	Can the analytical method detect / quantify the allergen in proposed samples at a sufficient sensitivity to facilitate risk assessment ?
Based on the sensitivity required for the risk assessment, and analytical capability, are single samples or composites appropriate and possible ?	
Section 3: Planning	
Sampling Plan	
Form of sample(s)	e.g., liquid ingredient, swabs, finished product
Location of sampling and sampling method.	
Number of samples possible and required.	See guidance on **5.2.1.3.6**
Size of samples, packaging, labelling, storage and transport.	
Analytical Plan	
Sample preparation and method including sample weight tested, number of desired repeats/replicates.	
Sample retention and storage.	
Sampling & Analysis: Quality of Evidence (To a large extent this is a subjective judgement based on the adequacy of sampling, the numbers of samples, and the analytical data, but always act in a precautionary manner (e.g. with a spread of results such as <LoQ, <LoQ, 2.1, 7.9 25.8 mg/kg as allergen protein, proceed on the basis of the highest).	
B - Likelihood of sampling an UAP that is present.	3 ☐ High 2 ☐ Medium 1 ☐ Low or unknown
Method sensitivity and uncertainty.	
Section 4: Results	
Data (± uncertainty)	
Overall score You may want to use this simple scoring system as an aid to decision making however be aware that this is not a rigid approach and individual circumstances must over-ride this.	
Overall sampling quality (sum of A + B)	5-6 ☐ Acceptable quality of evidence 4 ☐ Medium quality of evidence 2-3 ☐ Low quality of evidence
Number of individual samples taken	
Laboratory used	
Signed/dated	

7.7.2. Sampling and Analysis References and useful links

a) Food Drink Europe, 2013, Guidance on Food Allergen Management for Food Manufacturers, section 4. Analytical Methods and their Application, although written some years ago this remains a useful reference https://www.fooddrinkeurope.eu/wp-content/uploads/2021/06/Guidance-on-Food-Allergen-Management_FINAL_MARCH-2014.pdf (Accessed 18.10.2021)
b) Allergen Bureau, Food Allergen Analysis, https://allergenbureau.net/food-allergens/food-allergen-analysis/ (click on each subject to access additional information) (Accessed 10.05.2021)
c) S Flanagan, Ed., Handbook of Food Allergen Detection and Control https://www.elsevier.com/books/handbook-of-food-allergen-detection-and-control/flanagan/978-1-78242-012-5
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Additional references are available in Section **5.2.1**.

7.8. ANNEX for protein content table used in data conversion

Annex Table 2 (special thanks to Allergenen Consultancy)

Allergen	Raw material	Protein content (g per 100g)
Gluten containing cereals	Barley flour	11
	Barley malt syrup	4,8
	Oat meal[1]	13
	Rye flour	16
	Spelt flour	15
	Wheat gluten	77
	Wheat starch[2]	0,3
	Wheat flour	11
	Wheat bran	16
Crustaceans	Shrimps	14
	Crab	18
	Lobster	17
Egg	Whole egg powder	47
	Egg protein powder	81
	Egg yolk powder	34
	Egg (fresh whole)	13
Fish	Anchovy (can)	29
	Herring	18
	Codfish	18
	Saithe[1]	18
	Mackerel	19
	Pangasius	18
	Flatfish	12
	Sardines (can)	24
	Tuna	22
	Salmon	20
Peanut	Peanut bean	26
	Peanut oil	<0,005[4]
Soy	Soy flour	35
	Soy bean (fresh)	13
	Soy bean (dried)[1]	36
	Soy protein isolate	88
	Soy lecithin	<0,3
	Soy sauce[1,*]	3,5

Fresh unprocessed raw materials are listed, unless stated otherwise.
Source USDA National Database for Standard Reference.
Figures are indicative literature values, check with supplier for actual content.

Allergen	Raw material	Protein content (g per 100g)
Milk	Butter[1]	0,7
	Cheese (48+)	24
	Lactose	0,2
	Skimmed milk powder	36
	Milk protein isolate[3]	90
	Milk permeate[3]	3-5
	Whipped cream	3,2
	Whole milk (pasteurized)[1]	3
	Whole milk powder	26
	Whey protein isolate[3]	90
	Whey permeate[3]	2-7
	Whey powder	13
	Skimmed yoghurt[1]	4,1
Nuts	Almond	21
	Hazelnut	15
	Walnut	15
	Cashew nut	18
	Pecan nut	9
	Para nut	14
	Pistachio nut	20
	Macadamia nut	8
Celery	Celery seed (dried)	18
	Celeriac (fresh)	2
	Celery (fresh)	1
	Leaf celery (fresh)	1
	Leaf celery (dried)	11
Mustard	Mustard seed	26
Sesame	Sesame seed	18
Lupine	Lupine flour	40
	Lupine bean (fresh)	36
Molluscs	Calamari (squid rings)	9
	Mussels	15
	Oysters	23
	Snails[1]	16

[1] Source: Nederlands Voedingsstoffenbestand (NEVO).
[2] Source: Starch Europe
[3] Source: American Dairy Product Institute
[4] Source: EFSA-Q-2004-122
[*] The proteins are hydrolysed to smaller fragments, which elicit less often allergic reactions.

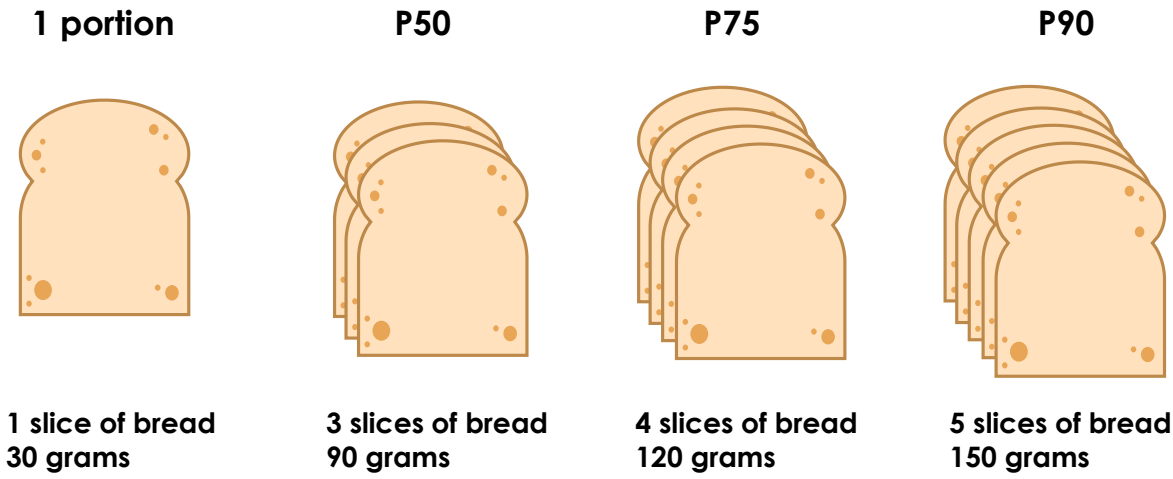
7.9. ANNEX for food intake section

For more information regarding explanations and considerations that can provide support in deciding the food intake value for your specific product, please see the following sections.

- Portion or serving size?
- Data provided in national food consumption databases
- Can I use data from the acute daily intake for a single day?
- Can I use data from one country for another country?
- General population vs Population with food allergies
- Frequency of consumption
- References for further reading

7.9.1. Portion or serving size?

Taking into account the single eating occasion that is required for allergen risk assessment, it is tempting to choose a portion size or a recommended serving size, which is typically provided on the label or present in nutritional guides. As explained in **Annex** Figure 4 , these refer to single eating occasions, but should not be used, because they generally are provided for nutritional purposes, such as to describe the values of one piece, or to discourage the use of large amounts of high caloric foods, and usually do not reflect actual intakes.



Annex Figure 4 *The consumption of a food product in the population varies and is a distribution of consumption estimates. A portion size of a food product is used for describing the nutritional values of one piece, in this example a slice of bread. On average a population eats 2-3 slices of bread during the eating occasion*. The figure is based on the highest consumption per eating occasion. Data on values reported in Birot, Madsen et al. (2018). The US population consumed less bread during a meal and thus the 50th percentile in a US population deviates from that determined elsewhere, see Meima, Blom et al. (2021).*

7.9.2. Data provided in national food consumption databases

Data on actual food intake are derived from national food consumption surveys (EFSA 2011, De Keyzer, Bracke et al. 2015). The databases present a variety of intake data and should be carefully considered as to their utility in allergen QRA.

- Acute and chronic consumption data. The acute values seems suitable for allergen risk assessment, but it is good to realize that the statistics calculating the acute exposure are based on the single day and sum up the different eating moments and may result in conservative numbers.
- Consumers of the product (so called 'eaters-only') or for all subjects. Data for all subjects means that the intake values include those participants that do not consume

the particular food and the average value will be lower than the intake calculated for the grouped consumers. You should use the values for the consumers of the product (eaters-only).
- Various percentiles of the distribution are provided. The average intake per single day, and also other percentiles, like the 95th, 97.5th percentile of the distribution (P95, P97.5) or even maximum intake values.

7.9.3. Can I use data from the acute daily intake for a single day?

As a first approximation, data on the acute intake for a single day for the consumer population as present in for instance the EFSA database (available here) may seem a pragmatic and conservative alternative for the optimal food intake. However, there are some limitations or considerations for these data. Still some food products are typically consumed at only one eating occasion and it may be valuable to look at the acute consumption when looking for data for particular countries.

Acute data are based on the single day and sum up the different eating moments and may result in conservative numbers. Using high intake values result in more conservative risk assessments that may overestimate the actual risks.

Although this appears safe from a risk management perspective, using too high intake values may miss the right balance between being protective on one hand and being practical and feasible to implement for example an Action Level on the other hand and thus may not optimally inform risk managers. In certain situations, such as an incident these data might provide first indications of the risk associated with the allergen concentrations in food products.

It is therefore not recommended to use acute intake data for a single day without prior validation of the suitability for allergen risk assessment.

7.9.4. Can I use data from one country for another country?

The EU iFAAM study by Birot, Madsen et al. (2018) showed that the intake amounts were very similar for 3 countries in North West Europe (Denmark, France and Netherlands) but not for all food groups. Also, a recent study systematically comparing the food intake data of the United States and the Netherlands concluded that differences between the two countries existed and that it is therefore not possible to just apply data from one country to another (Meima, Blom et al. 2021). It was recommended to develop and use a food intake dataset based on the highest intake levels for each food group of the involved countries to facilitate risk management efforts and harmonization across different countries (Meima, Blom et al. 2021).

7.9.5. General population vs Population with food allergies

Food intake data from the general population are suitable for use in risk assessment for allergic subpopulations. There is no consumption database available solely for food-allergic consumers. However a recent study showed that when allergic individuals choose to eat a food product, then the amounts eaten at the highest eating occasion will be of the same order as those eaten by non-allergic individuals (Blom, van Os-Medendorp et al. 2020).

7.9.6. Frequency of consumption

Good data on frequency of consumption of products by the food allergic population are lacking and risk assessors must assume that allergic and non-allergic individuals consume a product at the same rate. Country specific food surveys have data on frequency, but it is good to keep in mind that it is possible that the choice to eat a certain product can be different, that is, an allergic consumer may completely avoid certain products or eat alternative similar products depending on their allergy profile, e.g., replace a milk product for drinking with a soy-containing product.

While uncertainty exists regarding the product choices allergic consumers will make (including the frequency of consumption), the option to use the consumption data (how much is consumed) of the overall population are considered as a suitable input for risk assessment.

Summarizing, it is crucial to carefully consider the selection of the most appropriate food intake figure when performing a deterministic risk assessment:

- Data on real intake
- Highest single eating occasion
- The P75 of the food intake distribution, or surrogates as explained in section **5.3**.

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